Release History

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Release</th>
<th>Publication Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>N515-6024</td>
<td>B</td>
<td>December 2004</td>
</tr>
</tbody>
</table>

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Chapter 15
Reprocessing Files In Batch

After you have acquired data and stored it in a raw data file, you can process the data repeatedly. This is called reprocessing the data. This chapter explains how to reprocess data files on your local computer and on a network computer using the Batch Reprocessing function in TotalChrom.

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How Batch Reprocessing Works

After you have created a method and have analyzed data using the parameters in that method, you might want to reprocess the raw data for a variety of reasons:

- To generate a new report after recalibration.
- To change the format of the report.
- To reanalyze the raw data files listed in a sequence.

Reprocess the data stored in raw data files with the Batch Reprocessing function in TotalChrom. This function is also referred to as Batch.

As in the original analysis, a sequence provides processing parameters in a series of cycles, one for each raw data file. Because there is virtually no limit to the number of cycles you can include in a sequence, you can reprocess a large number of files in succession. Batch reprocessing enables you to reprocess multiple files without interruption. This differs from the Reprocess Results function in the Navigator, which lets you re-analyze only one file at a time.

Batch reprocessing lets you re-use the existing sequence or create a new sequence that contains only those files you want to reprocess. However, you are not limited to reprocessing files from a sequence. You can reprocess a group of files that have the same root name, or you can reprocess an individual data file.

There are important differences between reprocessing a sequence and specifying individual files or files with the same base name. In the latter case, the default is to reprocess the files using the current copy of the method(s) referenced in the file unless a new method is explicitly entered. In contrast, in the case of a sequence, the files are reprocessed using the current copy of the method(s) specified in the sequence row. If you are reprocessing result files, in either case you can use the original method parameters stored in the result file itself.

Another important feature of sequence reprocessing is that it is the only way to specify new sample values (such as internal standard amounts or dilution factors) for calculating component amounts. When you specify individual files or files with the same base name, the sample values used to calculate component amounts are always those from the original data file.
Using Batch Reprocessing

When you reprocess a data file or sequence file, you can include all the steps included in the original analysis or you can include selected parts of the analysis. The steps from which you can choose are:

- Peak detection
- Component identification
- Calibration
- Quantitation
- Report generation
- Replot generation
- Post-analysis program execution

You can start and end the reprocessing operation at any point, depending on the data you want to evaluate.

➢ To open the Batch Reprocessing window:

- In the Navigator, click the Batch button, or choose Batch from the Reprocess menu.

The Batch Reprocessing window will be empty when you open the application.
Reprocessing a Sequence or Index File

When you use the functions in the Graphic Method Editor, you can reprocess the data from only one file at a time. Batch Reprocessing allows you to reprocess an entire sequence or index file. This includes recalibrating the data. Therefore, the most common starting point for reprocessing multiple files is to use the Sequence command in the File menu.

For any succession of runs, from any type of setup, TotalChrom creates a file that contains information about each run acquired. This file is called an index file. The base file name is used, and the suffix .IDX is added. Index files have the same structure as sequence files, but the index file contains the actual name of each file collected, whereas the sequence file contains only the base file name and the cycle number. The actual file name might not be simply the combination of these two parts, because if that name already exists at the time of the run, TotalChrom adds a time stamp at the end to create a unique file name.

Use the index file to reprocess the data from multiple runs that are generated from Quick Start or from a method setup.

➢ To reprocess a sequence or index file:

You cannot reprocess a sequence that contains tokenized file names, since these can only be expanded at acquisition time and not during reprocessing. To reprocess data acquired using a sequence that contained tokenized file names, use the index file created during acquisition.

1. Choose Sequence from the File menu, then click the Sequence File folder icon.
2. To change the default file type from index to sequence, select from the Files Of Type list.

3. Select the name of the Index or Sequence file that you want to reprocess.

4. If necessary, enter or select a Data path to override the data path specified in the selected sequence file.

   Select or enter a Data path if the Sequence file you selected does not specify a data path and/or the data files are not in the same directory as that Sequence file.

5. In the Starting Row text box, enter the row number with which you want to start reprocessing.

6. In the Ending Row text box, enter the row number with which you want to end reprocessing.

7. If the sequence specifies dual-channel acquisition, select the channel or channels whose files you want to reprocess under Dual Channel.

   If the sequence specifies single-channel acquisition (either A or B), you can leave the Dual Channel options selected.

8. Select a starting point for this reprocessing operation from the Start Analysis list. The options are:

   **Peak Detection** — Starts the analysis with peak detection and integration using data from the raw data file. This procedure creates a new result file or overwrites the existing result file. The Replace Existing Result Files option is described later in this section.

   **Component ID** — Starts the analysis with component identification using data from the result file. This procedure creates a new result file or overwrites the existing result file. The Replace Existing Result Files option is described later in this section.

   **Calibration** — Starts the analysis with calibration using data from the result file. This procedure creates a new result file or overwrites the existing result file. The Replace Existing Result Files option is described later in this section.

   **Quantitation** — Starts the analysis with quantitation using data from the result file. This creates a new result file or overwrites the existing result file. The Replace Existing Result Files option is described later in this section.

   **Report Generation** — Starts the analysis by printing a report using data from the result file. This procedure can change the existing result file. The Use Method In Result File option is described later in this section.

   **Replot Generation** — Starts the analysis by printing the replot for a chromatogram using data from the result file (and raw file). This procedure...
can change the result file. The Use Method In Result File option is described later in this section.

**Post-Analysis Programs** — Starts the analysis by loading and executing the user programs listed as post-analysis programs in the method file.

9. Select an ending point for this reprocessing operation from the End Analysis list. The options are:

- **Peak Detection** — Ends the analysis after peak detection and integration.
- **Component ID** — Ends the analysis after TotalChrom identifies the peaks in the new result file.
- **Calibration** — Ends the analysis after updating the calibration information in the calibration section of the method.
- **Quantitation** — Ends the analysis after all the component amounts have been updated, but no reports have been generated.
- **Report Generation** — Ends the analysis after printing the report.
- **Replot Generation** — Ends the analysis after the replot is printed.
- **Post-Analysis Programs** — Ends the analysis after executing all user programs listed in the method as post-analysis programs.

10. To process files locally, select Local from the Batch Execution list.

To process files on a server, select the server name in the Batch Execution list.

11. To change your printer from one that is specified in the selected file, select one from the Batch Printer list.

See your application manager for information about default and other printers.

12. To use a plotter, select one from the Batch Plotter list, if available.

13. To suppress optional reports that have been specified in the method, deselect the Enable Optional Reports In Method check box.

14. Choose a Raw File Treatment option:

- Select Use Existing Raw File Sequence Info if you want to use the original sample information from the raw file to calculate results.
- Select Update Existing Raw File With New Sequence Info to store the new sequence information in the original raw data file and overwrite the existing information. Only the raw file header information will be modified.
• Select Create New Raw File With Updated Sequence Info if you want to update the sample information in the raw data file from the sequence being used for reprocessing and create a new raw data file with a modified name.

This option is available only if you start reprocessing with peak detection because it is the only option that processes the raw data file.

15. To reprocess the data using the parameter values in the result file, select the Use Method In Result File check box.

Each result file contains a copy of the method (or sections from different methods) that was used to generate it. If you do not select this option, TotalChrom takes values directly from the method file itself. Do not select this option if you are reprocessing data to change the results based on a newly edited method.

The Use Method In Result File option is not available if you start reprocessing with peak detection because peak detection uses the raw data file rather than the result file. Starting reprocessing with peak detection always uses the method file from disk and creates a new result file (or overwrites the original, if you select that option).

If you start reprocessing with report generation (or later), select Use Method in Result File to regenerate the original report or replot. This ensures that the result file does not change.

16. Choose OK to close the dialog box and have TotalChrom enter the files in the reprocessing queue. Network processing will start automatically.

17. For local reprocessing, choose the Start button to begin.
Reprocessing Sequentially Named Data Files

You can reprocess sequential data files by using the Base File Name command. Before you begin this procedure, however, you need to know the exact base file name for the files you want to reprocess and the path where the files can be found.

This mechanism for specifying files cannot be used with files that have automatically appended time/date stamps.

To reprocess sequentially named data files:

1. Choose Base File Name from the File menu.
2. In the Base Name text box, enter the base file name (or root name) for this group of files.
3. Enter or select the Data Path where these files are located.
4. Enter the Starting Cycle number. This is the same as the injection (or run) number. The name of the first file reprocessed is generated by appending this three-digit number to the Base Name. For example, if the Base Name is “TEST” and the starting cycle number is 2, the first file name will be “TEST002.”
5. Enter the Ending Cycle number.
6. In the Start Analysis list, select a starting point for this reprocessing operation.

For descriptions of the analysis functions, refer to “Reprocessing a Sequence or Index File” on page 15-4.
7. In the End Analysis list, select an ending point for this reprocessing operation.
   To reprocess these files with a different method, enter or select the new method file name.

8. To process files locally, select Local from the Batch Execution list.
   To process files on a server, select the server name in the Batch Execution list.

9. To change your printer from one that is specified in your sequence file, select one from the Batch Printer list.
   See your application manager for information about default and other printers.

10. To use a plotter, select one from the Batch Plotter list, if available.

11. To suppress a printer that has been specified in the method, deselect the Enable Optional Reports In Method check box.

12. To retain the new information under the existing file name, select the Overwrite Existing Result Files check box.
    TotalChrom generates a duplicate file name if you do not select the overwrite option.

13. If you want the reprocessing to use the parameter values originally used to create the result file, select the Use Method In Result File check box.
    The Use Method In Result File option is not available if you start reprocessing with the peak detection phase because peak detection uses the raw data file rather than the result file. Starting reprocessing with peak detection always uses the method file from disk and creates a new result file (or overwrites the original, if you select that option).
    If you start reprocessing with report generation (or later), select Use Method In Result File to regenerate the original report or re-plot. This will ensure that the result file does not change.

14. Choose OK to close the dialog box and start reprocessing the files.
Reprocessing an Individual Data File

The third alternative for reprocessing data is to reprocess one file at a time using the Data File command on the File menu.

➢ To reprocess a single data file:

1. Choose Data File from the File menu.

2. Enter or select the name of the data file you want to reprocess.

To reprocess these files with a different method, enter or select the new method file name.

To reprocess these files with a different report format, enter or select the new Report format file name.

3. In the Start Analysis list, select a starting point for this reprocessing operation.

For descriptions of the analysis functions, refer to “Reprocessing a Sequence or Index File” on page 15-4.

4. In the End Analysis list, select an ending point for this reprocessing operation.

5. To process files locally, select Local from the Batch Execution list.

To process files on a server, select the server name in the Batch Execution list.

6. To change your printer from one that is specified in your sequence file, select one from the Batch Printer list.
Reprocessing an Individual Data File

See your application manager for information about default and other printers.

7. To use a plotter, select one from the Batch Plotter list, if available.

8. To suppress a printer that has been specified in the method, deselect the Enable Optional Reports In Method check box.

9. To save the new file under the current file name and overwrite the old file, select the Overwrite Existing Result Files check box.

10. If you want the reprocessing to use the parameter values originally used to create the result file, select the Use Method In Result File check box.

   The Use Method In Result File option is not available if you start reprocessing with the peak detection phase because peak detection uses the raw data file rather than the result file. Starting reprocessing with peak detection always uses the method file from disk and creates a new result file (or overwrites the original, if you select that option).

   If you start reprocessing with report generation (or later), select Use Method In Result File to regenerate the original report or re-plot. This will ensure that the result file does not change.

11. Choose OK to close the dialog box and start reprocessing the data file.
Using the Control Buttons During Interactive Reprocessing

Use the Move, Delete, Clear, Cancel, Pause, and Resume buttons on the Batch Reprocessing toolbar to manipulate files during interactive reprocessing operation. These buttons function as follows:

- Move, Delete, and Clear affect files waiting in the queue.
- Cancel affects only the file being processed.
- Pause halts the local processing operation. The Pause button becomes active when TotalChrom starts processing the data.
- Resume restarts the processing operation. This button becomes active after you select Pause.

To pause and restart local processing in order to add files to the queue:
1. In the Batch Reprocessing window, choose Pause while TotalChrom is processing the data.
   The program responds when it reaches the first possible stopping point. The Cancel, Clear, and Resume buttons become active when you choose Pause.
2. Select the additional files you want to reprocess.
3. Choose Resume to start processing again.

To move a file in the queue:
1. Choose Pause to halt processing.
   The Resume, Cancel, and Clear buttons become available.
2. In the list of files to be reprocessed, select the entries for the files that you want to move. The Move and Delete buttons become available.
3. Choose Move to open the Move Queued Entry dialog box. It shows the file by its place in the queue.
4. Select an option to move the selected file(s) where you want. You can also move a file by entering a row number in the New Position text box.
5. Choose OK. The dialog box closes and the files are rearranged.
6. Choose Resume to start processing again.

To delete a file from the queue:
1. Choose Pause to halt processing.
2. Select the file in the queue that you want to delete.
   The selected file is highlighted and the Move and Delete buttons become active.
3. Choose Delete.
   The selected file is deleted from the processing queue.

4. Choose Resume to start processing again.

➢ **To clear all files from the queue:**
   1. Choose Pause to halt processing.
   2. Choose Clear.
      All files are removed from the processing queue. The file that is currently
      being processed is not affected.
   3. Choose Resume to start processing again.

➢ **To cancel the current run:**
   1. Choose Pause to halt processing.

   2. Choose Cancel to cancel the current run. Reprocessing resumes
      immediately, starting with the first file in the queue. Whenever you choose
      Cancel, a message similar to the following is sent to the printer:

      Errors and Warnings From Data Processing
      Data File: d:\tcws\ver6.3.0\files\halo002.raw
      Date/Time: 12/15/04 11:45 AM
      ANALYZE: run stopped during peak detection

   3. Choose Resume to start processing again.
Chapter 16
Summarizing Component Data

This chapter describes how to use the Summary application in TotalChrom to generate reports based on result file data and how to edit summary report formats.

<table>
<thead>
<tr>
<th>To learn about:</th>
<th>Go to page:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using Summary</td>
<td>16-2</td>
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<td>Using the Summary Report Format Editor</td>
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<td>Automating Summary Reports</td>
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</tbody>
</table>
Using Summary

Each time TotalChrom analyzes a raw data file, it stores the results in a result file. Summary lets you combine the result files from several analyses into a single summary report. You can customize the report by including the specific component information that is of interest to you, and you can calculate average and/or standard deviation values for numeric quantities.

To generate a summary report, you must perform three tasks:

- Select the result files you want to summarize
- Create a list of the components whose results will be summarized
- Define the report format you want to use for the summary

There are two parts to the Summary application: the Summary function itself and the summary report format editor function. You use the Summary function to select the data to be summarized and to print the report. You use the summary report format editor function when you want to create a new report format or edit an existing report format. The menu bar changes depending on which function you are using. You can switch back and forth between the two functions as necessary.

This chapter is organized as follows:

- The “Summarizing Results” section describes how you select result files, component data, and print a summary report.
- The “Using the Summary Report Format Editor” section describes how to create and edit report formats.
- The last section of this chapter, “Automating Summary Reports,” describes how you can set up a sequence file to automatically print a summary report.
Summarizing Results

Use the Summary functions to select summary data and print a report.

➢ To open the Summary window:

- In the Navigator, choose Summary from the Reprocess menu or click the Summary button.

The status bar contains the only information that appears in the Summary window. This information shows how many result files you have selected to summarize and how many components are in the component list.

The Summary window contains the following menus:

File Menu

Lets you open and edit summary report format files; print summary reports; and, exit Summary to return to the Navigator. The Edit Format command starts the summary report format editor function, which contains another set of menus and commands. Refer to “Using the Summary Report Format Editor” on page 16-12.

Results Menu

Lets you develop a list of result files by using a base file name or a sequence (or index) file. You can also develop a list by individually selecting result files or edit the list created from a base name or sequence.
Summarizing Results

Components Menu

Lets you build a component list by identifying which result or method file you want to use as the source of component information. You can also merge the components from either of these sources. Edit Component List lets you revise the component list manually.

The rest of this section describes how to use the Summary functions to select result files, select component data, and print summary reports.

Developing the Result File List

Use the commands in the Results menu to select the result files you want to summarize:

From Base Name — Lets you select result files by base name, make a basic components list, select a summary report format, and print the report as one operation.

From Sequence — Lets you select the sequence or index file that contains the methods with the desired result files, select a summary report format, and print the report as one operation.

Edit File List — Lets you individually select the result files you want to summarize.

In most cases, you will want to use the From Base Name or From Sequence functions because they let you perform all three summary report tasks as one operation. You can create the result file list manually if you wish; however, in order to print a summary report you must then create the component list, identify the desired report format file, and output the report as separate operations.

Creating a List of Result Files by Base File Name

The From Base Name command in the Results menu lets you perform all summary operations at once: you select the files you want to summarize, select the appropriate summary report format file, load the component list from the first result file, and print the summary report. Alternatively, you can use this command to create the result file list, then create the component list and generate the summary report as separate operations.
To create a list of result files by base file name:

1. Choose From Base Name from the Results menu to open the Result Files From Base Name dialog box.

2. In the Base Name text box, enter the base file name you want to use.

3. In the Result Files Path text box, enter or select the path where your data files are stored.

4. Enter a value in the Starting Cycle Number text box.
   This is the first file you want to include in the list. For example, you might have files named DATA001.RST through DATA012.RST. To begin with DATA006.RST, you would enter 6 in the text box.

5. Enter a value in the Ending Cycle Number text box to indicate the last file you want to include in the list.

6. Enter or select the name of the summary report format file you want to use.

7. Decide whether or not you want to load a component list.
   - If Load Component List Automatically From Result File is checked, Summary creates a component list from the first result file it locates that matches the selection criteria specified in this dialog box. TotalChrom loads all components from the selected result file, including named and timed groups.
   - If Load Component List Automatically From Result File is not checked, then a component list is not created and you must build one yourself. Also, unless you have already built a component list, the Print button becomes disabled. Refer to “Creating/Editing a Component List Manually” on page 16-9 for more information on how to build a component list.

8. Decide whether or not you want to print a summary report based on the information in this dialog box.
• Choose Print to generate a printed summary report using the specified results file(s), a basic components list, and the specified summary report format file.

OR

• Choose OK to load the results file list and a basic components list (if specified in step 6) without generating a report. This closes the dialog box and updates the summary information in the status bar.

**Loading Result Files From a Sequence or Index File**

When you load a result file from a sequence or index file, you can perform all summary operations at once: you select the files you want to summarize, select the appropriate summary report format file, load the component list from the first result file, and print the summary report. Alternatively, you can use this command to create the result file list and then create the component list and generate the summary report as separate operations.

➢ To load a result file from a sequence or index file:

1. Choose From Sequence from the Results menu to open the Results From Sequence File dialog box.

![Results From Sequence File dialog box]

2. In the Sequence File text box, enter or select the name of the sequence or index file you want to use as the source of result files.

---

*Once you specify a file, TotalChrom automatically sets the starting and ending rows to the number of rows in the sequence. You may override these by entering row numbers.*

3. In the Result Files Path text box, enter or select the path where your data files are stored.
An entry here is necessary only when the path is not defined in the sequence and if the files are not in the same path as the sequence file.

4. Enter a Starting Row number to indicate the first row you want included in the report.

5. Enter an Ending Row number to indicate the last row you want included in the report.

6. Select Load Files From Channel A and/or Channel B.
   
   If you select both Channel A and Channel B, the Separate Channel A & B Files and Mix Channel A & B Files options become active.

7. To load Channel A files first, then Channel B files, select Separate Channel A & B Files.
   
   The results from the two channels will be reported separately.

8. To load Channel A and B files together for each row, select Mix Channel A & B Files.
   
   The results from the two channels will be included in a single summary report.

9. Enter or select the name of the summary report format file you want to use.

10. Decide whether or not you want to load a component list.

   - If Load Component List From Method is checked, Summary creates a component list from the method in the first result file it locates which matches the selection criteria specified. The component list will contain all components, including named and/or timed groups, and the single peak components that make up the groups.

   - If Load Component List From Method is not checked, then a component list is not created, and you must build one yourself. Also, unless you have already built a component list, the Print button becomes disabled. Refer to “Creating/Editing a Component List Manually” on page 16-9 for more information on how to build a component list.
11. Decide whether or not you want to print a summary report based on the information in this dialog box.

- Choose Print to generate a printed summary report using the specified results file(s), a default components list, and the specified summary report format file.

- Choose OK to load the results file list and a default components list (if specified in step 9) without generating a report. This closes the dialog box and updates the summary information in the status bar.

**Creating/Editing a List of Result Files Manually**

You can use the Edit File List command from the Results menu to select the individual result files that you want to summarize. However, in order to generate a summary report, you must then create the component list, identify the desired summary report format file, and print the report as separate operations. See “Creating/Editing a Component List” on page 16-9, “Selecting a Summary Report Format” on page 16-11, and “Printing a Summary Report” on page 16-11 for more information on how to perform each of these tasks.

➢ To create or edit a list of result files manually:

1. Choose Edit File List from the Results menu.

2. To add a file to the Files To Summarize list, choose Add and select the desired file.

   The Replace, Delete, and Clear buttons become available after you add one or more files.
3. Use the buttons in this dialog box to modify the Files To Summarize list as desired.

**Insert** - Inserts a new file in a specific place in the list. Select the file above which you want to add another file, select the file you want to add and choose Insert.

**Replace** - Replaces one file with another. Select the file you want to replace, choose Change, and select the replacement file.

**Delete** - Removes a single file. Select the file to remove and choose Delete.

**Clear** - Removes all files.

**Reset** - Resets the list to what it was before you changed it.

4. Choose OK to save your choices and close the dialog box.

**Creating/Editing a Component List Manually**

To include data about only some of the components in your summary report, create or edit the component list manually rather than use the component list from the result file(s).

Using the commands in the Components menu, you can enter or merge components from result or method files, and edit the component list. The components list includes single-peak components as well as named and/or timed groups.

When you load components from a result or method file, TotalChrom overwrites and deletes all components already in the component list. When you merge components from a result or method file, TotalChrom adds the new components to the existing list.

Once you have loaded and/or merged the components you want to use from the result and method files, you can further revise the component list.

➢ **To load components:**

1. Choose the component source.
   - To load components from a result file, select Load From Result File from the Components menu.
   - To load components from a method, select Load From Method from the Components menu.

   A file selection dialog box appears and shows the list of result or method files in the current directory.

2. Select the name of the result or method file you want to use.

3. Choose Open.
The status bar displays the number of components that were loaded.

➢ **To merge components:**

1. Choose the component source.
   - To merge components from a result file, select Merge From Result File from the Components menu.
   - To merge from a method, select Merge From Method from the Components menu.

A file selection dialog box appears and shows the list of result or method files in the current directory.

2. Select the name of the result or method file you want to use.

3. Choose Open.

The components from the selected file are added to the component list. Notice that the number of components listed in the status bar increases to reflect the merge.

➢ **To edit the component list:**

1. Choose Edit Component List from the Components menu.

The Component List dialog box appears.

2. Select the Sort Criteria option you want to use.
   - Retention sorts components by retention time.

3. To delete a component, select it in the list and choose Delete.
   - You can select and delete more than one component at a time.

4. To restore the component list to what it was when you opened the dialog box, choose Reset.

5. Choose OK to save your changes and close the dialog box.
Selecting a Summary Report Format

To identify a summary report format to use for a report, use the Open Format command from the File menu. To edit an existing report format or create a new report format, refer to “Using the Summary Report Format Editor” on page 16-12.

➢ To select a summary report format file:

1. Choose Open Format from the File menu to open the file selection dialog box.
   A list of summary report format files available is displayed.
2. Select a file and choose Open.
   The Summary window remains blank, but TotalChrom will use the summary report format you selected when you print summary reports.

Printing a Summary Report

The Print command in the File menu of the Summary window lets you print a copy of the current report. The command becomes available only after you define the result files you want to summarize and create a component list.
Using the Summary Report Format Editor

TotalChrom provides a default summary report format file that you can use as is or edit using the summary report format editor. You can also create multiple summary report formats and modify them as necessary to use for different summary reports.

Although summary and analysis report formats use different file extensions (SUM as opposed to RPT), contain different data columns, and use different column layouts, the summary report format editor and Report Format Editor functions themselves are identical.

Summary Report Data

A summary report consists of a series of component tables, and each component table includes data from all the result files for a given component. Each row contains the results of one analysis (from one result file), and each column contains one type of data. The following is an example of a summary report.

```
*********** SUMMARY REPORT ***********

<table>
<thead>
<tr>
<th>File</th>
<th>Sample Name</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST01.</td>
<td>First run</td>
<td>0.23</td>
<td>1.701</td>
<td>0.66</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST02.</td>
<td>Second run</td>
<td>0.23</td>
<td>1.779</td>
<td>0.66</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST03.</td>
<td>Third run</td>
<td>0.25</td>
<td>1.779</td>
<td>0.66</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST04.</td>
<td>Fourth run</td>
<td>0.26</td>
<td>1.779</td>
<td>0.69</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST05.</td>
<td>Fifth run</td>
<td>0.23</td>
<td>1.779</td>
<td>0.66</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST06.</td>
<td>Sixth run</td>
<td>0.23</td>
<td>1.742</td>
<td>0.66</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST07.</td>
<td>Seventh run</td>
<td>0.25</td>
<td>1.742</td>
<td>0.68</td>
<td>1.319</td>
</tr>
<tr>
<td>TEST08.</td>
<td>Eighth run</td>
<td>0.24</td>
<td>1.779</td>
<td>0.67</td>
<td>1.318</td>
</tr>
<tr>
<td>TEST09.</td>
<td>Ninth run</td>
<td>0.23</td>
<td>1.742</td>
<td>0.66</td>
<td>1.284</td>
</tr>
<tr>
<td>TEST10.</td>
<td>Tenth run</td>
<td>0.27</td>
<td>1.760</td>
<td>0.70</td>
<td>2.318</td>
</tr>
</tbody>
</table>

Averages

<table>
<thead>
<tr>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>1.77</td>
<td>0.71</td>
<td>1.65</td>
</tr>
<tr>
<td>1.319</td>
<td>1.319</td>
<td>1.01</td>
<td>1.45</td>
</tr>
<tr>
<td>1.652</td>
<td>1.652</td>
<td>1.45</td>
<td>2.012</td>
</tr>
<tr>
<td>1.45</td>
<td>2.021</td>
<td>1.47</td>
<td>2.017</td>
</tr>
<tr>
<td>1.46</td>
<td>2.022</td>
<td>1.45</td>
<td>2.021</td>
</tr>
<tr>
<td>1.45</td>
<td>2.021</td>
<td>1.48</td>
<td>2.007</td>
</tr>
</tbody>
</table>

Sample deviation

<table>
<thead>
<tr>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>0.10</td>
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<td>0.09</td>
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<td>0.09</td>
</tr>
</tbody>
</table>

The summary report format editor lets you specify the types of component data you want to include in a report. The component tables contain all of the same data columns. Therefore, when you make a change to the data in one component table, you affect the data in all component tables. You can also include information about the sample in a summary report. Because the sample information is the same for each component in a method file, it appears only once for all the component tables that are printed across a report page.
Menus and Commands

The summary report format editor includes the following menus and commands:

File Menu

Allows you to create, edit, save, and print summary report format files; display an on-screen print preview of a summary report format file; and enter and review documentation about a summary report format file.

User Notes Menu

Lets you enter header, footer, and title text for a summary report format file.

Sample Data Menu

Provides access to the column information dialog boxes relating to sample data.

Component Data Menu

Displays the types of component columns that are available for summary reports, including blank columns and those containing custom expressions.

Edit Menu

Lets you move and delete columns from the summary report format file.

Options Command

Opens the Report Options dialog box, which lets you select and deselect options such as entering an area reject value; printing column averages; and automatically arranging components.

Return Command

Closes the summary report format editor and returns you to the main Summary function.

General Procedure for Creating a Summary Report Format

The following is the general procedure for creating a summary report format. Specific procedures for the tasks referenced in this procedure are provided later in this chapter.

You can also create a new report format by editing an existing file and renaming it with the Save As command.
To create a new summary report format file:

1. If the main Summary window is open, choose Edit Format.

2. In the summary report format function, choose New from the File menu. The name <untitled> appears in the title bar, and TotalChrom displays the default format. Your defaults may look different from those shown if the default file has been modified.

3. If you wish, change the default title and create a report header and footer. Refer to “Creating Title, Header and Footer Text” on page 16-15.

4. Select the component and sample data columns you want to use for this report. Refer to “Editing Report Columns” on page 16-16 and “Creating a Custom Expression” on page 16-19.

5. When you are finished developing the report format, choose Save from the File menu.

The Documentation dialog box opens.

6. Complete the Description tab in the Documentation dialog box and choose OK.

For a discussion about entering descriptive information about a file, refer to Chapter 2, “TotalChrom Basics.”

The TotalChrom File Save As dialog box opens.
6. Enter a name for the report format in the File Name text box and choose Save.

The dialog box closes, and the new report format name appears in the title bar.

7. When you are finished working in the summary report format editor, choose Return from the menu bar to return to the main Summary function. You can then print a summary report or return to the Navigator.

If a report format is loaded in the summary report format editor when you return to the Summary function, that same report format will be used for printing summary reports. If necessary, you can change the report format by using the Open Format command from the File menu.

Creating Title, Header and Footer Text

The commands in the User Notes menu enable you to create report title, header and footer text in a report format.

➢ To create a report title:

1. Choose Title from the User Notes menu to open the Title dialog box.

2. Enter the title you want to use for this report and choose OK.

When you choose OK, the dialog box closes and the title for this report format file appears at the top of the report.

After you have created a report title, you can click the title area to reopen the Title dialog box.

The steps to create header and footer text are nearly identical, and they have been combined into a single procedure.
To create header or footer text:

1. Choose Header from the User Notes menu to open the Header dialog box.

   OR

   Choose Footer from the User Notes menu to open the Footer dialog box.

The following shows the Header dialog box. However, except for the title, the two dialog boxes look and function the same way.

2. Enter the text you want to appear as the report header or footer in the text area of the dialog box and choose OK.

   When you come to the end of a line, press Ctrl+M to start a new line. You can enter up to 512 characters. To delete all existing text, choose Delete.

   When you choose OK, the report header or footer appears in the report window.

   After you have created a report header or footer, you can click the header or footer area to open the corresponding dialog box.

Editing Report Columns

The Sample Data and Component Data menus each display the sample and component data columns you can include in a summary report. The Sample data types are self-explanatory. For an explanation of component data types, refer to the Glossary at the back of this manual.

The Component Data menu also includes the Custom Expression command. Custom Expression is a special type of data column that allows you to include mathematical operations within a column. Refer to “Creating a Custom Expression” on page 16-19.

Choosing any of the data types from the Sample Data and Component Data menus, or clicking on the column name in the report format itself, opens the column information dialog box, which is described in the procedures that follow. You can edit the column information, including the name, and place the columns wherever you want them to appear in the report.
You can also create blank columns in a report by choosing the Blank Columns command in the Component Data menu. The Blank Column dialog box is the same as the Column Information dialog box, except that it inserts a blank space between columns or adds space in the margin if you make it the first or last column on the page.

**Adding and Deleting Columns**

The default summary report format file shipped with TotalChrom includes the following columns:

<table>
<thead>
<tr>
<th>File Name</th>
<th>Sample Name</th>
<th>Time (min)</th>
<th>Area (μm²)</th>
<th>Adjusted Area (μm²)</th>
<th>Time (min)</th>
<th>Area (μm²)</th>
<th>Adjusted Area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>xxxxxxxx</td>
<td>xxxxxxxxxxx</td>
<td>0000.00</td>
<td>000000.00</td>
<td>0000.00</td>
<td>0000.00</td>
<td>000000.00</td>
<td>0000.00</td>
</tr>
</tbody>
</table>

You can add and delete component and/or sample data columns as desired.

1. **To add a report column:**

   1. Choose Sample Data or Component Data to display the available data types.
   2. Choose one of the data types listed (except for Custom Expression in the Component Data menu).

   The column information dialog box appears. The name of the data type is shown in the dialog box title. The word “<none>” appears next to Current Column until you add this column to the report. Once the data column is added, click on the column name to open this dialog box rather than choose the name from the Sample Data or Component Data menu.

   3. Enter a column number in the Column Number text box.

   This establishes the location of this column relative to other columns on the page. For a column type that is not currently in the report, the default value is the next available column number. For a column type that already appears in the report, the default is the existing column number.
If you assign the new column the same number as an existing column, TotalChrom automatically shifts the columns to insert it.

4. Enter a number to specify the Column Width in characters.

5. If Digits is available, type the number of digits you want to display after the decimal point.

6. Enter text for the top line of the column name in the Top Label text box.

A column name can consist of one or two lines of alphanumeric characters. The Top Label text box is the upper line.

If the column label exceeds the number of characters in the Column Width text box, the column will expand to the label width.

7. Enter text for the column name bottom line in the Bottom Label text box.

8. Choose Insert/Add.

The new column appears in the report format.

Choose Reset to change all entries back to their original settings. The dialog box remains open and you may continue editing the parameters.

There are two ways to delete a column from a report format: from the column information dialog box or by using the Delete Column command in the Edit menu.

To delete a column:

- Click on the column you want to delete and choose Delete in the column information dialog box.

OR

- Choose Delete Column from the Edit menu to open the Delete Column dialog box and enter the number of the column that you want to delete and choose OK.

Replacing/Editing Column Information

Use the Replace command to modify the information that appears for any column in a summary report format file.

To edit an existing column:

1. Click on the column you want to change to open the column information dialog box.

2. Edit the information as necessary, but do not change the column number or you will move the column.

3. Choose Replace.
**Moving Columns**

You can change the position of columns in a report format file in two ways: by choosing the Move Column command in the Edit menu or by choosing the Move button in the column information dialog box.

偺 To move a column:

1. Click on the column you want to move to open the column information dialog box.
2. In the Column Number text box, enter the new column number.
3. Choose Move to close the dialog box and reposition the columns.

OR

1. Choose Move Column from the Edit menu to open the Move Column dialog box.
2. Enter the number of the column you want to move in the Which Column To Move text box and enter the number where you want the column to appear in the New Column Position text box.
3. Choose OK to close the dialog box and move the column to the new location.

**Creating a Custom Expression**

Custom expressions let you include information that is the result of a calculation that is performed when the report is generated.

For example, you must use custom expressions to calculate scaled percentage values. To obtain scaled values, the largest peak is assigned a value of 100% (or some other percentage), and percentage values for other peaks are scaled proportionately. You can use the following expressions:

- Scaled area: \(#PA/#MA*100\)
- Scaled height: \(#PH/#MH*100\)
- Scaled amount: \(#AA/#MC*100\)
In these expressions, #PA, #PH, and #AA represent peak area, peak height, and adjusted amount, respectively; #MA, #MH, and #MC represent the maximum peak area, maximum peak height, and maximum adjusted amount, respectively.

Creating a custom expression is much like building a mathematical expression, except that you use the data values and operators provided in the Custom Expression Editor:

- Data values can be numbers or predefined values such as Area Percent and Sample Volume.
- Operators can be arithmetic, exponential or logarithmic.
- Calculations can be binary (performed with two values) or multi-level.

Use US conventions for all number formats in the Custom Expression Editor. For example, although a Windows system may be set up to use German number formats, such as using a comma to designate a decimal point, the Custom Expression Editor only accepts a period as a decimal point.

When a calculation is invalid (for example, division by zero), a dashed line appears in the report instead of a numeric value.

After you build the calculation for an expression, you specify column parameters in the Custom Expression column information dialog box. This dialog box is identical to the column information dialog box that is used for all other report columns. Once you create a custom expression, it can be edited, moved or deleted just like any other report column.

**Predefined Custom Expression Data Values**

When you develop a custom expression, you select from a list of predefined data values.

Most of the items refer to individual peak values (such as #PA for Peak Area); others refer to the entire run (such as #TA for Total Area).

Predefined values for the items with the phrase (from Suit) are available only if Suitability has been run on the data before this report is generated:

Values for #SA Lambda Max; #SP Peak Purity Index; #SR Absorbance Ratio are available only if they are present in the result file. Additionally, for #SP the LC235 must have been set to AUTO mode for spectra.
To create a custom expression:

1. Choose Custom Expression from the Component Data menu to open the Custom Expression Editor dialog box.

The list box on the left shows the types of the data that are available for calculations.

2. Enter the desired expression in the Custom Expression text box.

You can enter the expression either by choosing items from the Available Column Values and Available Operations lists, or by typing the values and operations directly into the Custom Expression text box. To use constants as values, type the desired constants directly into the Custom Expression text box.

Most terms in the Available Column Values list are defined in the Glossary, which is available in this manual and in Help.

3. Choose OK when the expression is complete.

The Custom Expression Editor dialog box closes and the Custom Expression dialog box appears.

4. Enter the column number where you want the expression to appear.

5. Enter the other parameters for this column.

6. Choose Insert/Add.

The label you assigned to the custom expression appears at the top of the column. When TotalChrom generates a summary report based on this format file, the result of that expression will appear in the column.

When you click on this column in the window, the Custom Expression Editor dialog box appears, followed by the Custom Expression dialog box.
**Creating Additional Custom Expressions**

Data from an existing custom expression column is always used as a basis (or template) for creating additional custom expressions. This enables you to derive new custom expressions from existing custom expressions quickly without having to re-enter data.

When there is only one custom expression in the report, its data are always used when creating a template for another custom expression. When there is more than one custom expression, you must select the specific custom expression whose data you want to use. You can either:

- Click on the desired custom expression

  OR

- Select Custom Expression from the Component Data menu to open the Duplicate Columns dialog box. Choose the custom expression column whose data you want to use and choose OK.

Whichever method you choose, you get to the Custom Expression Editor dialog box, which is already filled in with expression data. Use the procedure described in “Creating a Custom Expression” to build the custom expression.
**Editing Summary Report Options**

The Options command in the summary report format editor function opens a dialog box that contains a variety of options that relate to the information you want to include in the report.

➢ **To edit summary report options:**

1. Choose Options to display the Summary Report Options dialog box.

2. Enter a value of zero or greater in the Area Reject text box.

This option allows you to specify a minimum area value for peaks in the summary report. Peaks with smaller areas will not be included in the calculation of values that may appear in the summary report. For example, area percent and normalized area percent are two values that would be affected. If any component area is below the area reject value, that component will be treated as if it were not found.

3. Select Include Unidentified Peaks In Calculations if you want to include this feature.

This option includes data associated with unidentified peaks in the calculation of values that may appear in the summary report. For example, area percent and normalized area percent are two values that would be affected. To exclude unidentified peaks from calculations, deselect this option.

4. Select Print Column Averages if you want to report the average of the values in each numeric column.

5. Select Print Column Standard Deviations if you want to report the %RSD (relative standard deviation) for each numeric column.
6. To substitute a zero for the dash that appears in reports for components that are not found, select Use Zero For Components Not Found.

7. Decide how you want to arrange the report components.
   - Select Automatically Arrange Components if you want to automatically place the maximum number of components that will fit in one row in the summary report. The number of component tables that can fit in one row depends on the number of results reported in the tables, and the width of the paper on which the report is printed. To manually set the number of components in a row, deselect this option.
   - If you do not select Automatically Arrange Components, enter a value in the Number of Components/Row text box. Each row can have up to six components.

8. To create an ASCII text file of the summary report, select Store Report In An ASCII Formatted File.

   The .CSV, .PRN, and File Name options become available. Use the .CSV File option for a comma-separated format (suitable for transferring results to Microsoft Excel), and the .PRN File option to transfer results to Lotus 1-2-3. After you have selected .CSV or .PRN, enter a name for the file in the File Name text box.

   If you select this option, the ASCII file is saved to disk at the same time the report is printed. A one-line message appears at the bottom of the printed report which identifies the file and where it was saved.

9. Choose OK to implement the current options and close the dialog box.

**Printing Summary Report Format Information**

The Print command in the File menu lets you print a copy of the current report format file, optionally with its audit trail information. This output lists the contents of the report format file; it does not print an example of the report format like the one you see in the window.
Automating Summary Reports

To print a summary report at the end of a sequence, you can set up Summary as a user program in the Sequence Editor. You will need to use the following information to fill in the Program Information dialog box:

**Program Name:**

Enter or select SUMMARY.EXE

**Command Line:**

\$
\text{SEQ} \ [\text{(Row Range)}] \ [\text{.SUM file name}] \ [\text{A}] \ /\text{prn}<\text{printer}\_\text{name}>
\$
\text{IDX}

$\text{IDX}$

- The variable $\text{SEQ}$ passes the name of the sequence file and $\text{IDX}$ passes the name of the index file.
- $\text{(Row Range)}$ enables you to specify a subset of files from either SEQ or IDX files. The subset specification must be enclosed within parentheses. You can specify individual row numbers, or by using a hyphen, you can specify a range of row numbers. Commas are used as separators. For example, the subset definition $(1, 3-6, 8, 11-13)$ would cause cycles 1, 3, 4, 5, 6, 8, 11, 12, and 13 to be analyzed.
- $\text{.SUM file name}$ is the name of the summary file to be used. This file name and complete path must be in double quotes. For example,

  ```
  "c:\penexe\tcws\data\name.sum"
  ```
- Option $\text{A}$ means that only Channel A result files are used.
- Option $\text{B}$ means only Channel B result files are used.
- Option $\text{A B}$ tells Summary to print separate reports for Channels A and B result files.
- Option $\text{A B M}$ tells Summary to generate a single report incorporating both Channel A and B result files.
- $\text{/prn}<\text{printer}\_\text{name}>$ specifies which printer to use. The $\text{printer}\_\text{name}$ should be its UNC name as it is displayed in System Configuration and it should also be in double quotes. Depending on the printer configuration in your system, the switch may not be required, but using it will ensure that the specified printer will print.

Refer to Chapter 11, “Building a Sequence,” for instructions on how to include a user program in a sequence.
This chapter explains how to use the Fit Analysis function in TotalChrom to plot calibration curves for components identified in the calibration section of the method.

*Changes made to calibration information using the Fit Analysis function have no effect on the calibration data stored in the calibration section of the method.*

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What is Fit Analysis?

Fit Analysis allows you to plot the calibration curve for any component that has amount and response data in a method file. It lets you vary the data and the curve parameters to see the effects on the fitted curve.

Although Fit Analysis is intended primarily for viewing calibration curves, you can enter data points directly to view regression curves for any type of data.

If you are not familiar with how TotalChrom performs calibrations, refer to Chapter 8, “Setting Calibration Parameters in the Method.”
The Fit Analysis Window

Like other functions in TotalChrom, Fit Analysis runs in its own window and includes a separate set of menus and commands. Fit Analysis also has a spreadsheet function that enables you to examine data using commands which are similar to those in the Sequence Editor.

You run the Fit Analysis program from the Method Editor.

You could also open the Fit Analysis program by making a shortcut, or by associating Fit Analysis with MTH files so that double-clicking on a method file opens the Fit Analysis program. Refer to your Windows documentation for more information on how to make shortcuts and file associations.

To open the Fit Analysis program:

1. From the Navigator, choose Method from the Build menu or click the Method button to open the Method Editor window.

2. Choose an option in the Startup dialog or choose Open from the File menu to select the method for which you want to perform a fit analysis.

3. Choose Fit Analysis from the Other menu.

If you have changed the currently selected method file, the Save As dialog box appears. You can save the changes to the current file or create a new method file.

The Fit Analysis window opens along with the Component List for the method you selected.

If the method has no components, an error message appears. Choose OK. The Fit Analysis window opens without a method file. Select Open from the File menu to open another method.

4. Select a component in the Component List dialog box and choose OK.
The Fit Analysis Window

The calibration curve for that component is displayed in the Fit Analysis window. If there is insufficient calibration information to construct a curve, an error message will appear.

![Fit Analysis Window](image)

5. You can change components in two ways:

- Choose Component List from the Data menu to display the Component List dialog box. Select the component whose calibration curve and data you want to display and choose OK. Note that you can scroll quickly through the component list by typing the first letter of the desired component.

  OR

- Step through all of the method calibration curves one at a time by pressing PgDn (Next Component from the Data Menu) or PgUp (Previous Component from the Data menu).

**Fit Analysis Status Bar and Menus**

The Fit Analysis status bar at the bottom of the window shows (starting from the left) the current component, which component number this represents in the method file, and curve information. The Fit Analysis window has the following menus:

**File Menu**

Lets you open the method file to view component data; print the calibration curve and calibration information; change the printer or plotter and printing options; copy the calibration curve to the Clipboard; and close the Fit Analysis window.
Data Menu

Lets you select the calibration data you want to plot from a list of components and display each component's calibration curve in turn. The Data List command, which opens a spreadsheet window, lets you change the calibration information by editing the data points in the curve (plot). Refer to “Using the Data List Spreadsheet” on page 17-12.

Display Menu

Lets you define the limits for horizontal and vertical plot-scaling manually, automatically and customize plot labels, and select to display the origin.

Fit Type Menu

Lets you change X-axis scaling, curve type, and regression weighting; set the plot origin; and, display information about the calibration curve.

Solve Menu

Calculates the response for a given amount or the amount for a given response.

Viewing the Calibration Curve

The calibration curve that appears in the Fit Analysis window for each component represents the response produced by each replicate for a component at each calibration level plotted against the replicate amount. In other words, each point on a calibration curve has an amount and response coordinate that corresponds to a different calibration replicate. Likewise, the calibration replicate corresponds to a specific component amount used in a standard sample.

During calibration, TotalChrom stores data (peak areas and heights) from the standard run in the calibration section of the method file. Once the program determines what the component's response is for each replicate, these response values (and amounts) are used to compute the calibration curve. This lets the program quantify unknown component amounts.

The amount is the X-coordinate and the response is the Y-coordinate. The more calibration replicates a component has, the more points will be plotted and used in fitting a calibration curve. You can remove or modify one or more of these points using the Data List command to see the effect on the curve.

Throughout this chapter, the term amount refers primarily to quantity values, which are plotted against response values in calibration curves. However, the way in which these amount values are derived depends on the specific method that is being used. If the method uses an external standard, the amount value plotted in the calibration curve represents the level amount multiplied by the sample volume. For any given level, the amount value will be either the global sample volume (if no replicates exist), or the sample volume from each replicate. The calibration curve displayed in
the Fit Analysis window and the data list spreadsheet use the label “Volume Adjusted Amount” for the amount value.

If the method uses an internal standard, the amount value plotted is the ratio of the component level amount, divided by the internal standard amount from the method or from replicates. The value for sample volume, in this case, cancels out.

Likewise, the response value plotted in the calibration curve is the absolute response for a method using an external standard and the response ratio for a method using an internal standard. The response will be either area or height as determined by the method. The calibration curve displayed in the Fit Analysis window and the data list spreadsheet use the label “Area” for the response value.

For more information on calibration, refer to Chapter 8, “Developing Calibration Parameters in the Method.”

**Changing the Calibration Curve Display**

Once you display the calibration curve, you can change how the plot appears by changing the scale to which it is drawn. You can also change the labels that appear on each axis. Make these changes using the Display menu commands.

**Setting Limits for the Calibration Curve**

The Limits command lets you choose between letting TotalChrom calculate plot scale limits or entering the plot scale manually. Allowing TotalChrom to calculate limits optimizes the plot display. However, you might want to override the TotalChrom-generated values to perform other functions such as viewing the relationship of the curve to the origin.

The limits entered in the Limits dialog box will NOT take effect if Origin is checked in the Display menu. Checking Origin sets the X and Y axis minimums to 0 and the maximums are set to the same values calculated when the Automatically calculate limits option is checked in the Limits dialog. Checking Origin actually sets the parameters in the Limits dialog to those values the next time you open it.

To manually define the scaling limits for a calibration curve:

1. Choose Limits from the Display menu to open the Limits dialog box.

   Automatically Calculate Limits is selected. The other options are unavailable because TotalChrom calculates the scaling limits automatically.
2. To set the plot scale parameters manually, deselect Automatically Calculate Limits.

3. In the Minimum and Maximum text boxes for each axis, enter the values you want to use. These values will be used for all components.

4. Choose OK.

**Changing Calibration Curve Labels**

TotalChrom automatically displays plot labels, including the title (component name), the X-axis (volume adjusted amount), and the Y-axis (area). You can change these labels using the Labels command. The labels you enter will be used for all component plots, unlike the automatically assigned labels, which use the individual component name and appropriate amount and response labels.

The Labels command is most useful when you are plotting manually entered data that is unrelated to component calibration.

➢ To create or change the labels on a calibration curve:

1. Choose Labels from the Display menu to open the Labels dialog box.

   ![Labels dialog box]

   The Automatically Assign Default Labels check box is selected, and the label text boxes are unavailable.

2. To change or modify the labels, deselect Automatically Assign Default Labels.

3. Enter a new title in the Plot Title text box.
   
   You can create a label up to 30 characters long.
4. In the X-Axis Label text box, enter the information you want to appear as the X-axis label.

5. In the Y-Axis Label text box, enter the information you want to appear as the Y-axis label.

6. Choose OK.

**Changing Fit Parameters**

Fit parameters determine exactly how the calibration curve is fitted to the data. The commands in the Fit Type menu let you change the scaling factor used for the X-axis, change the curve fit type and regression weighting, and set the plot origin.

**Changing the Scaling Factor**

The data TotalChrom uses to create a calibration curve depend on the type of calibration you specify in the calibration section of the method: internal standard or external standard. If you use an external standard in the method, the curve will show the relationship between amount and response values. Response may be either the peak area or the peak height: whichever you indicate in the method. If you use an internal standard in the method, the calibration curve will show the relationship between the amount ratio and response ratio.

TotalChrom offers several alternatives to simply plotting response as a function of amount, or response ratio as a function of amount ratio. For example, you can plot response as a function of the log of the component amount.

- **To change the scaling factor for the X-axis:**
  1. Choose Change Scaling from the Fit Type menu to open the Change Scaling Dialog box.

   ![Change Scaling Dialog Box]

   2. Select a new scaling factor from the following choices:
      
      1. **(no scaling)** — The default, it does not add a scaling factor.
      2. **1/x** — Shows response as a function of the reciprocal of the amount.
      3. **1/(x*x)** — Shows response as a function of the reciprocal of the square of the amount.
log(x) — Shows response as a function of the base-10 log of the amount.

1/log(x) — Shows response as a function of the reciprocal of the log of the amount.

These scaling alternatives rule out the use of some values for amounts. For example, you cannot use 0.0 with l/x or l/(x*x). Also, you cannot use an amount of less than 1.0 with a logarithmic option.

3. Choose OK.

**Fitting and Weighting the Calibration Curve**

To compute a calibration curve, TotalChrom performs a regression calculation using all replicates for the component at all available calibration levels. TotalChrom performs this regression analysis by computing a set of orthogonal polynomials and using them to compute the best least-squares approximation. For a mathematical description of how TotalChrom performs this analysis, refer to Chapter 18, “Discussion of Data Analysis.”

You can change the way TotalChrom fits the calibration curve to the data points by selecting a new fit type.

You can also weight the calibration curve by selecting one of several weighting options when you create the calibration section of the method. Your selection determines the type of weighting factor TotalChrom will apply to the points of the calibration curve. The larger the weighting factor is for a point (based on the selected weighting expression), the more important that point becomes in the curve-fitting calculation.

◆ **To select a different fit type for the calibration curve:**

1. Choose New Fit from the Fit Type menu to open the New Fit Parameters dialog box.

2. Select the Curve Fit Type option you want to use.

   **Point to Point** — Averages all replicate amount and response data at each calibration level to derive a point. (Each pair of points is connected by a
Changing Fit Parameters

straight line segment.) You can use this curve type with one or more calibration levels.

1st Order Polynomial — Calculates a first-order polynomial (linear) fit using the coefficients of the curve (intercept and slope). A component must have at least two calibration levels to use this type of fit.

2nd Order Polynomial — Calculates a second-order polynomial (quadratic) fit using the curve coefficients. A component must have at least three calibration levels to use this type of fit.

3rd Order Polynomial — Calculates a third-order polynomial (cubic) fit using the curve coefficients. A component must have at least four calibration levels to use this type of fit.

3. Under Origin, select Include or Force if required.

   If you force the origin, the intercept term is always zero in the calculation. If you include the origin, the point (0,0) is added to the calculation but does not appear in the calibration level list.

4. Select a Regression Weighting option.

   1 (no weighting) — This is the default setting and has no effect.

   1/x — Applies the reciprocal of a point's amount value as the weighting factor.

   1/(x*x) — Applies the reciprocal of the square of a point's amount value as the weighting factor.

   1/y — Applies the reciprocal of a point's response value as the weighting factor.

   1/(y*y) — Applies the reciprocal of the square of a point's response value as the weight factor.

5. Choose Replace, Add, or Cancel.

   Replace — Replaces the current calibration curve with the curve specified by the parameters in the dialog box.

   Add — Adds a new curve to the display derived from the fit parameters specified in the dialog box. You can view up to four curves at the same time.

   Cancel — Closes the dialog box without saving the changes.
Displaying Curve Information

The Curve Info command in the Fit Type menu displays the type of curve fit, the weighting factor used, the origin option in effect, and the calculated coefficients for the polynomial curve (if applicable).

➢ To display information on the current calibration curve:

1. Choose Curve Info from the Fit Type menu to open the Curve Information dialog box.

2. If the Next button is enabled, click on it to display information on the next calibration curve for that component (if any).

3. Choose Close when you have finished viewing calibration curve information.

Solving For New Amounts and Responses

Solving for new amount and response values allows you to calculate an expected response from an amount value or the amount corresponding to an observed response from a sample.

➢ To calculate an amount value:

1. Choose For Amount from the Solve menu to open the Solve For Amount dialog box.

2. In the Enter A Response text box, enter a new value for which you want to solve an amount.

3. Choose Calculate.

   The amount value appears following the Amount = label and marker lines appear on the plot. You can change the response value and recalculate the result.

4. Choose Close.
To calculate a response value:

1. Choose For Response from the Solve menu to open the Solve For Response dialog box.

2. In the Enter An Amount text box, enter a new value for which you want to solve a response.

3. Choose Calculate.

   The response value is displayed following the Response = label, and marker lines appear on the plot. You can change the amount value and recalculate the result.

4. Choose Close.

Using the Data List Spreadsheet

The Data List command in the Fit Analysis window opens a spreadsheet that lists the data from which the plot is derived. By manipulating information in the Data List spreadsheet, you can test various scenarios without disturbing the calibration settings in the method file. For example, you can delete a data point to see how it affects the curve fit.

To open the Data List spreadsheet:

- Choose Data List from the Data menu.

The Data List opens and shows the Amount and Response values that created the calibration curve together with the associated level names. Excluded replicates are shown in red in the Data List dialog and are printed in bold type.
The Data List has the following elements:

**Pop-up menu** — Lets you insert, append, copy, move, and delete rows and insert a repeating value in a column.

**Column labels** — Indicates the type of data that appear in the cells for that column.

**Row numbers** — Identifies spreadsheet rows. These cannot be changed.

**Work area** — Shows the area in which you can enter information. The Data List spreadsheet includes three working columns:

- **Amount Ratio** — The X-axis value of the data point.
- **Response Ratio** — The Y-axis value of the data point.
- **Level Name** — The name of a calibration level (if any).
Using the Data List Spreadsheet

**Editing Calibration Data in the Data List**

- **To edit calibration data:**
  1. Click the mouse inside the cell you want to edit.
  2. Enter the new value (or calibration level).
  3. Press Enter.

**Editing Rows in the Data List**

Use commands from the pop-up menu to edit entire rows.

- **To insert a new row:**
  1. Click in a row before which you want to insert the new row.
  2. Choose Insert from the pop-up menu.
     
     TotalChrom copies all the data in the selected row to the new row directly beneath it.

- **To add a row to the end of the spreadsheet:**
  - Choose Append from the pop-up menu.
    
    TotalChrom appends a new row to the bottom of the spreadsheet. The new row will contain the same data as the row above it.

- **To copy spreadsheet rows:**
  1. Click on the row number of the row(s) you want to copy.
  2. Choose Copy from the pop-up menu.
  3. Click anywhere in a row above which you want to paste the copied row(s).
  4. Choose Paste from the pop-up menu.
     
     This creates extra points (duplicates) in the spreadsheet.

- **To delete a spreadsheet row:**
  1. Click on the row number of the row or rows you want to delete.
  2. Choose Delete from the pop-up menu.
**Entering Repeating Values**

The Fill Down command provides a quick way to fill a column, from a designated cell downward, with the same value. You may perform this operation for any column in the spreadsheet, but you must begin by selecting an individual cell.

➢ To insert a repeating value in an entire column:

1. Select a cell whose value you want to copy to all cells below it in the current column.
2. Choose Fill Down from the pop-up menu.

The information from the selected cell now appears in every cell in the column, from that row down to the bottom of the spreadsheet. The rows above the selected cell are not affected.

**Closing the Data List Dialog**

When you close the Data List dialog, TotalChrom recalculates the plot information based on the changes you have made.
Printing and Plotting Calibration Data

The Print command in the File menu of the Fit Analysis window allows you to print a copy of the calibration curve as well as a summary of calibration information for each component in the selected method file. The Print Setup command lets you change the current default printer or plotter and printing options. The Print Preview command shows an on-screen version of how your calibration data will look when printed.

To print the calibration plot displayed in the Fit Analysis window and/or a summary of calibration information:

1. In the Fit Analysis window, choose Print from the File menu.

The Print dialog box appears. The default setting is to print the Calibration Data and the Calibration Plot.

2. Deselect Calibration Data or Calibration Plot to exclude either one.
3. Choose OK to open the Print Setup dialog box.
4. Choose OK to print the selected data for the current component.
Saving Calibration Data to the Clipboard

If you want to insert a copy of the calibration plot into another application, you may save the information to the Clipboard and paste it into another file. You can use either black and white or color. Since a single color bitmap requires much more memory, use black and white unless you want to use the bitmap in a color report.

➢ To copy calibration data to the Clipboard:

1. Choose Copy To Clipboard from the File menu.

   A cascading menu appears.

2. Choose either BW or Color.

   TotalChrom copies the information to the Clipboard. You can paste the information into another file as desired.
Automating Fit Analysis Functions

To print the calibration information as part of your data analysis, set up Fit Analysis to run as a user program in the processing section of the method. You will need to use the following information. Make sure Synchronize With Data Analysis is unselected.

**Program Name:**
Enter or select CalPlot.EXE

**Command Line:**

```
[Method name] [Component name] [PLOT] /prn<printer_name>
[ALL] [DATA] [BOTH] [NONE]
```

- **[method name]** is the name of the method to be used. This file name and complete path must be in double quotation marks (for example, “c:\penexe\tcws\method name”).

- **[Component name]** is the name of a specific component. This name must be enclosed in double quotation marks.

---

*The component name must appear exactly as in the method. The commands must be uppercase (for example, ALL, PLOT, etc.).*

---

- **[PLOT]** prints a plot for the designated component(s).
- **[DATA]** prints the data for the component(s).
- **[BOTH]** prints a plot and data.
- **[NONE]** displays the information on the screen.
- **/prn<printer_name>** specifies which printer to use. The printer_name should be its UNC name as it is displayed in System Configuration and it should also be in double quotes. Depending on the printer configuration in your system, the switch may not be required, but using it will ensure that the specified printer will print.

For information on how to set up and run user programs in a method, refer to Chapter 7.
Chapter 18

Discussion of Data Analysis

This chapter explains how TotalChrom uses the parameters defined in the method to produce an analysis of the raw data.

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What is Data Analysis?

Data analysis is the process by which TotalChrom interprets the data obtained from an instrument and stored in a raw data file. The outcome of this interpretation is stored as a result file.

TotalChrom analyzes data at three different points:

- Immediately after an interface collects the data from an instrument and stores it in a raw data file. This is the original data analysis.
- When you reprocess a series of raw data files using the Batch Reprocessing function.
- When you reprocess an individual raw data file using the Graphic Method Editor or Results Reprocessing function. These processes use the same routines as Analyze but on a smaller scale and independent of Analyze.

Data Analysis Parameters

TotalChrom acquires and analyzes data based on the values (parameters) you enter in a method. A method consists of three sections — instrument, processing, and calibration — each of which has its own set of parameters that guide their respective parts of the analysis.

The instrument and processing sections are essential to all methods. Calibration parameters are essential to identifying peaks and calculating component amounts. A separate but related file, the report format, defines the contents and layout of the printed report containing the results of the analysis.

How the Method Controls Data Analysis

For every injection, you define the method parameters you want to use to analyze the data. For a series of injections, this is done through the sequence file, where you can use different methods to define the instrument, processing, and calibration parameters. This sequence file, which you create using the Sequence Editor, defines how you want TotalChrom to acquire and analyze the data from a particular series of injections.

When you initialize and set up an interface to acquire data from these injections, you specify the sequence name. TotalChrom then acquires and analyzes the data based on the information in this sequence.

For more information on how to create and modify a sequence file, please refer to Chapter 11, “Building a Sequence.”
Overview of the Steps in Data Analysis

There are nine distinct steps in the data analysis process. Some of the steps in data analysis are optional — their occurrence depends on the information you provide in the method and sequence. For example, you can omit from the method instructions for adding component names to peaks, generating a report and plot, and running a user program during analysis.

Also, the calibration step is relevant only when TotalChrom analyzes the data from a standard sample.

When you reprocess data, you can choose which steps you want to perform as part of the reprocessing operation. For example, you can start with component identification or quantitation and end with report generation. As analysis proceeds from one step to the next, the icon at the bottom of the screen changes to reflect the step currently under way. The following shows the nine icons that can appear during data analysis if local analyze is selected.

The following is a brief description of each step of data analysis. More detailed information about each step (except report generation, replot generation, and post-analysis programs) is given later in this chapter.

**Baseline Subtraction** — Subtracts the points in a baseline file, obtained by running a sample blank, from those in the current raw data file in order to correct the baseline. This subtraction process is carried out only if the name a baseline file is given in the sequence cycle that controls a chromatography run. The results of this are stored in a modified raw data file.

**Peak Detection** — Scans the data points in a raw data file (or modified raw data file) to find peaks. This step produces a preliminary result file containing a peak list that consists solely of peak starting and ending points. To edit peak detection parameters, refer to Chapter 7, “Developing Processing Parameters in the Method.”

**Integration** — Groups peaks into clusters, assigning baselines and summing the areas under the peaks. This step adds final values to the result file for peak starting and ending points, retention times, areas and heights. To edit peak integration parameters, refer to Chapter 7, “Developing Processing Parameters in the Method.”
Overview of the Steps in Data Analysis

**Component Identification (optional)** — Determines the peak identities in the result file by comparing their retention times with a pre-defined list of expected components and retention times specified in the calibration section of the method. This step adds component names to peaks in the result file. Refer to Chapter 8, “Developing Calibration Parameters in the Method.”

**Calibration** — Updates amount and response values for components during the analysis of calibration standards. TotalChrom stores calibration data, including the results of the analysis of standard samples, in the calibration section of the method. The calibration step in data analysis also produces a report containing the current calibration data for each component. Refer to Chapter 8, “Developing Calibration Parameters in the Method.”

**Quantitation** — Calculates the amounts of components represented by peaks in the result file. These calculations are based on the peak areas or heights and on the calibration data for the corresponding components. This step adds component amounts to the result file.

**Report Generation (optional)** — Produces one or more reports containing the results of the analysis. You specify the primary report in the sequence file controlling the analysis. You can specify additional reports in the processing section of the method. Refer to Chapter 7, “Developing Processing Parameters in the Method” and Chapter 9, “Building Report Formats.”

**Replot Generation (optional)** — Produces a fully annotated plot of the chromatographic data. The annotations can include peak retention times, peak starting and ending points, and component names. Refer to Chapter 7, “Developing Processing Parameters in the Method” and Chapter 9, “Building Report Formats” for more information.

**Post-Analysis Programs (optional)** — Includes any user programs that TotalChrom runs after data analysis. You designate which programs you want to run, and when, in the processing section of the method. Refer to Chapter 7, “Developing Processing Parameters in the Method” for more information.
Baseline Subtraction

If you enter a name of a baseline file when you are creating or editing a sequence cycle (in the Sequence Editor), TotalChrom subtracts the data points in the baseline file from those in the raw data file and produces a modified data file. The following describes how baseline subtraction is done.

How TotalChrom Performs Baseline Subtraction

If the data in the baseline and raw data files were collected at the same sampling rate, and the same run time and delay time were used, the files will contain an equal number of data points and subtraction will be done on a point-by-point basis.

If the baseline file contains more points than the raw data file, TotalChrom computes averaged values from points in the baseline file such that the number of averages equals the number of points in the raw data file. These averaged points are then subtracted from the points in the raw data file.

If the baseline file contains fewer points than the raw data file, TotalChrom uses points in the baseline file more than once to make up for the difference.

TotalChrom does not use an automatic offset, so if the difference between two files is less than one count, the program will make the offset equal to one count. This can cause the bottom of the chromatogram to appear truncated.
Peak Detection

During the peak detection step in data analysis, TotalChrom scans the data points in a raw data file (or modified raw data file) to find peaks.

TotalChrom saves the point data and point index data it collects in a result file. A point index is a number indicating the position of a data point in the series of data points within the raw data file.

The following sections describe peak detection in detail.

Peak Detection Parameters

The peak detection parameters include bunching factor, noise threshold, and area threshold.

Bunching Factor

The bunching factor specifies how many sequential data points in a raw data file are grouped in a bunch. During peak detection, TotalChrom counts bunches and then averages the voltage values of the points in each bunch. The resulting averages are assigned to bunched points.

The following diagram shows how bunching smoothes out raw data, which helps prevent TotalChrom from identifying baseline noise as peaks. Small dots represent the raw data, and squares represent bunched points. In the following figure, one bunched point is shown for every five raw points; thus, the bunching factor is 5. Each bunched point is located at the same time position as the last raw point in a bunch.

A bunching factor also compensates for over-sampling: that is, collecting more points than are necessary in a peak. Ideally, peaks will have about 20 points from start to end. This provides the best balance between acceptable processing time and correct peak detection and integration.

If the sampling rate remains constant throughout a run, you might not be able to avoid over-sampling because peak widths can vary, perhaps broadening later in the run. The broader a peak, the more points it is likely to have; thus, it can be over-sampled.

In this situation, where both narrow and broad peaks occur in the same run, you cannot lower the sampling rate. However, you can increase the bunching factor one
or more times by using a timed event. You specify the event (increasing the bunching factor) and the time in the run when the event will take place.

Setting the bunching factor too high can lead to small, unresolved peaks being smoothed out completely, so they are undetected. The goal in setting a bunching factor by timed event is to maintain the number of points within the peaks reasonably close to the 20-point optimum.

Too few bunched data points (□) can smooth small peaks, making them invisible to peak detection.

Because bunched points are positioned at the time of the latest point in the bunch, a peak plotted from bunched data tends to shift to the right (refer to the above figure). This shift has no adverse effect on peak detection and integration because the bunched data are used only to identify the potential start, top, and end of a peak. After finding the potential peak values, the software reverts back to the bunched data points to pinpoint the actual peak start, top, and end. Therefore, there is no loss of resolution in calculating baseline positioning or peak integration.
**Noise Threshold**

Expressed in units of microvolts, the noise threshold (NT) is the parameter that enables TotalChrom to discriminate between baseline noise and peaks. If the vertical difference between two consecutive bunched data points is greater than the noise threshold, TotalChrom recognizes the potential start of a peak. For example, in the following figure, point 4 would be detected as a potential peak start because the distance (b) between points 4 and 5 exceeds the noise threshold. Point 2 would not be detected as a peak start because distance (a) is too small.

The lower the noise threshold, the more sensitive peak detection will be. Conversely, raising the noise threshold decreases sensitivity. If the threshold is too high, however, TotalChrom will not be able to detect wanted peaks.

The following figure shows how increasing the noise threshold affects peak detection. The lower the noise threshold, the earlier the peak start is detected, and the later the peak end. The data values at the end taper off to a point that is below the noise threshold.

A higher noise threshold requires a more abrupt rise between data values before a peak start can be detected. By the same token, the peak end is found sooner as differences between consecutive data values quickly reach the threshold.

*When the noise threshold is higher, peaks start and end more abruptly.*
Area Threshold

Area threshold is used to discriminate between noise spikes and peaks. Expressed in microvolts, this parameter is used after the noise threshold to confirm the potential start of peaks that pass the noise threshold test.

After passing the noise threshold test, pairs of bunched data points must continue to exceed the noise threshold, and the cumulative sum of the bunched data points on the leading edge must eventually exceed the area threshold for the peak to be confirmed.

For example, the following figure shows a noise spike whose first two points pass the noise threshold test, but subsequent pairs of consecutive bunched points fail the area threshold test. Consequently, the spike does not sustain a leading edge that accumulates enough area under it to exceed the area threshold. The spike is not detected as a peak.
The following figure illustrates a peak that passes the area threshold test. The sum of the bunched area slices on the leading edge exceeds the area threshold, so the peak is detected. There is no direct relationship between area threshold and the smallest peak that can appear on a report. To exclude all peaks smaller than 100 μV-sec, use the Area Reject setting in the Report Format Options dialog box.

*A peak is confirmed when it passes the area threshold test.*

Higher area threshold values make it harder to confirm a peak, and TotalChrom might not detect smaller peaks at all, especially those that appear as shoulders on the leading or trailing edges of larger peaks.

A good policy when starting out is to use a low noise threshold and a high area threshold. This maintains a high degree of sensitivity in detecting peak starting and ending points, but still screens out noise spikes. You can visually optimize noise and area threshold settings/values for your data in the Graphic Method Editor. Refer to Chapter 10, “Editing Methods and Results Graphically.”

In addition to confirming a peak start, area threshold also helps determine a peak top. Refer to the next section, “The Stages in Peak Detection.”
The Stages in Peak Detection

In order to use timed events and peak detection and integration parameters correctly, you must be familiar with the stages in the peak detection process. A simplified discussion of each stage in peak detection follows.

Finding the Potential Peak Start

To find the potential start of a peak, TotalChrom examines the difference in value between each bunched data point and the preceding one. If the difference exceeds the current noise threshold value, then a potential peak start point has been found.

Confirming the Peak Start

After a potential peak start is found, TotalChrom begins to sum the differences between each bunched point and the last baseline point. Because each bunched point represents an area slice, the sum is the accumulated area for the potential peak, as shown in the figure that follows. The differences between bunched points must also continue to exceed the noise threshold, or the peak start will be canceled.

A peak is confirmed when it passes the area threshold test.

If the accumulated area exceeds the area threshold before a bunched point fails the noise threshold test, then the peak is confirmed.

After a peak is confirmed, the peak detection software scans backward from the potential peak start looking for the lowest raw data point. It scans backward through five bunches of raw data to find this minimum, but will stop the search if it reaches the end of the preceding peak. The lowest raw data point found in this process becomes the actual peak start.

The following figure shows how the software finds the actual start point. In this example, the bunching factor is set to 2, so there are only two raw data points for each bunched point. Therefore, the bunched points do not rise much faster than the raw data. The potential peak start was found at a point well up the side of the peak because...
previous consecutive pairs of bunched points failed the noise threshold test. It is evident in this figure that, without the backward search, a high noise threshold would drastically alter the peak start value. The same principle applies to peak end values.

**Finding the Peak Top**

To find the top of the peak, TotalChrom first tries to identify a local maximum bunched point value. When a bunched point is lower than the previous one, the previous point is considered to be the potential peak top. To avoid finding a false peak top because of noise, TotalChrom performs a confirmation test by summing the differences between the potential top and subsequent bunched points. If the sum exceeds two-thirds of the area threshold value, the potential peak top is confirmed. However, if a higher bunched point is found before the area test is passed, a new potential top is identified and the area test is restarted.

*If the shaded area exceeds two-third of the area threshold, the maximum point becomes the peak top.*
Note that the reported retention time for a peak is not simply the time of the point identified as the peak top. The reported retention time is determined from a quadratic fit based on the five highest bunched data points.

Because of this top-of-peak test, the choice of an area threshold value affects both peak confirmation and how shoulders are detected on the leading edge of a larger peak. Shoulders on the leading edge of a larger peak are not detected as peaks unless they have a discernible maximum point and a crest area that is greater than two-thirds of the area threshold.

The shoulder in the first figure could be detected as a peak if the area threshold were lower. However, the shoulder in the second figure could not be detected automatically regardless of the peak detection parameter values that are used because it lacks a maximum. To separate this shoulder as a distinct peak, you must use the timed event S (split peak).

**Finding the Peak End**

There are two indicators of a peak end:

- Two consecutive bunched point differences are less than half the noise threshold.

  or

- The start of another peak is detected.
Peak Separation Criteria

Width Ratio and Valley-To-Peak Ratio are two peak separation criteria that are considered overlapped or separated. This determination will affect how the baseline is drawn beneath the peaks. In peak detection, a peak is defined as overlapped with its neighbor on the right if the pair meet two criteria: the valley-to-peak ratio is greater than 0.01, and the separation is less than 0.2w, where w is the width of the first peak in the pair. (You can change these values in TotalChrom.) Peaks defined as overlapped are assigned an overlap flag of 1; those not defined as overlapped receive an overlap flag of 0. A set of overlapped peaks is called a cluster and, by default, shares a common baseline. Peaks that are separated each have an individual segment of the baseline.

The Width Ratio is the ratio of the distance(s) between the end of the first peak and the start of the second peak to the width of the second peak at its base (w). If this ratio is greater than the set value, the peaks are considered to be separated. Otherwise, they are marked as overlapped.

Criteria for overlapping peaks

The Valley-to-Peak Ratio is the ratio of the height of the valley between peaks (v) to the height of the smaller peak (p). If this ratio is less than or equal to the set value, the peaks are considered to be separated.
How Timed Events Affect Peak Detection

The following is a description of how each timed event, or pair of timed events, affects the way TotalChrom detects peaks.

The Bunching Factor, Noise Threshold, and Area Threshold Events

Using the bunching factor (BF), noise threshold (NT), and area threshold (AT) timed events allows you to override previously set values. These events take effect at the time they are scheduled: they do not work retroactively. For example, in the first figure that follows, the current area threshold is low, which enables TotalChrom to confirm the peak before the AT event occurs. The new area threshold value does not affect the confirmation of the peak.

In the second figure, the AT event is scheduled during peak confirmation, so TotalChrom uses the new, larger area threshold to determine where the peak actually starts.

For best results, schedule the BF, NT, or AT timed events on the baseline as far away from peaks as possible. If you schedule these events at times too close to a transition from one peak detection stage to another, detection might differ significantly from chromatogram to chromatogram because of small shifts in retention time or random noise. For example, in the previous figure, a slight shift by the peak to an earlier retention time would move the AT timed event past the Confirm stage.

Bunching Factor Event

The BF event helps compensate for changes in peak width over the course of a run. For example, in packed-column, isothermal gas chromatography, peaks tend to broaden as the run progresses. In these cases, scheduling this event at a higher value at least once can help maintain the ideal 20 bunched points per peak.


**Peak Detection**

**Noise Threshold Event**

The NT event helps compensate for changes in baseline noise. You can increase the noise threshold to decrease peak detection sensitivity or decrease the noise threshold to increase sensitivity. For example, suppose the beginning of a chromatogram is noisy because of fast-eluting contaminants in the solvent, and this noise diminishes later in the run. To optimize peak detection, you would set the default noise threshold high enough to screen out the initial noise and schedule the NT event to decrease the noise threshold at the time when the noise diminishes.

**Area Threshold Event**

In addition to the NT event, the AT event also affects peak detection sensitivity. If you are scheduling an NT event to change the noise threshold, schedule an AT event at the same time to change the area threshold. For example, if you are lowering the noise threshold to gain sensitivity, lower the area threshold as well. Otherwise, TotalChrom might not confirm the smaller potential peaks that the lower noise threshold allows it to detect. A general guideline is to set the area threshold to five times the value of the noise threshold.

**Disable/Enable Peak Detection (−P/+P)**

The pair of timed events −P and +P turn peak detection off and on, respectively, allowing you to disable and re-enable peak detection during a run. For example, if valve switching causes noise spikes in the middle of a chromatogram, you can schedule a −P event before the affected region and a +P event after it to avoid the detection of false peaks.

If TotalChrom is at the point where it is searching for a peak ending point (for example, after the peak crest) when the −P event occurs, the peak will end at exactly that point and all peak detection will stop. If TotalChrom is at any other point in peak detection, the process will stop and the current peak will not be detected.

The +P event has no effect unless peak detection has been disabled by a −P event. Likewise, a −P event has no effect if peak detection is currently disabled. The following figures show the effect of the −P and +P timed events.
Without –P and +P timed events, insignificant peaks are detected.

With –P and +P timed events, peak detection is disabled in this region.
Enable/Disable Negative Peak Detection
(+/–N)

The pair of timed events +N and –N turn negative peak detection on and off, thereby enabling and disabling the detection of negative peaks during a run. However, positive peak detection (+P) must be in effect before you can set the negative peak detection (+N). By default, TotalChrom detects only positive signals, so you must add a +N event to have it detect negative peaks. You should also position the +N as close to the negative peaks as possible to get accurate results. This event does not affect the detection of positive peaks.

TotalChrom detects a potential negative peak if a bunched data point value decreases from the previous bunched point value by more than the noise threshold, and if both points lie below the “theoretical” baseline used to separate positive and negative peaks. It also applies the area threshold to negative peaks.

The –N event takes effect immediately if peak detection is in the Begin or Confirm stages. Otherwise, it is delayed until the end of the current peak is found.

If you schedule a +N event to occur when negative peak detection is already enabled, the event has no effect. Likewise, nothing happens if you schedule a –N event to occur when negative peak detection is disabled.

The following figures show the effect of the +N and –N timed events.

Without the +N and –N events, the negative peak is not detected, and a false positive peak is found.
With the +N and –N events, the negative peak is detected, and then negative peak detection is disabled.

To detect a negative peak that follows immediately after a positive peak, TotalChrom must determine the baseline point at which the positive peak ends and the negative peak begins. The same is true for detecting a positive peak that follows a negative peak.
This theoretical baseline is set at the voltage level of the bunched point within whose data range the +N event occurs. The theoretical baseline is not likely to coincide with actual raw data point values when it intersects peaks on the chromatogram, so TotalChrom uses the closest actual data. Positive peaks are forced to start and end on raw data points above this baseline, and negative peaks are forced to start and end on raw data points below this baseline. As a result, the areas between the theoretical baseline and peak baselines are excluded from the peak areas. The following figure, deliberately exaggerated for clarity, illustrates this.

This is how TotalChrom finds the dividing line between a positive and a negative peak.

**Inhibit/Allow End-of-Peak Detection (+I/–I)**

The pair of timed events +I and –I inhibit and allow the stage in peak detection when the peak end is detected. When +I is in effect, TotalChrom does not attempt to determine the ending point of a peak. The –I event re-establishes peak detection for ending points.

It is appropriate to use these events when isomers of a compound elute as shoulders on the trailing side of the main peak, and you want all isomers to be treated as part of the main peak. In this case, you can schedule the +I event to prevent TotalChrom from finding the end of the main peak, and schedule the –I event to allow peak end detection again after the last isomer. The following figures show the effect of using +I and –I events.
Without the +I and –I events, the peak at 31.58 minutes ends naturally, but the shoulder on its trailing edge goes undetected.

With the +I and –I events, the shoulder is included in the peak.

The +I event forces TotalChrom to remain in the Find End stage. The result is that it will neither find a peak end nor detect the start of the next peak until the event is switched off. All data from the time of a +I event to the time of a –I event is perceived as part of the same peak.

The +I event takes effect immediately, but has no impact on processing until peak detection naturally enters the Find End stage. The –I event takes effect immediately, and allows TotalChrom to find the first peak end it encounters, based on the current noise threshold.

You should never use +I and -I events to group together distinct resolved peaks whose area you want to report as a single area. This is because the baseline that would drawn under such a composite peak may be penetrated in places by the
Peak Detection

chromatographic signal. If TotalChrom detects that this has occurred between the start of a peak and the crest (or the crest and the end point), it will adjust the start (or end) point to eliminate the penetration. (See "Baseline Penetration at the Start or End of a Cluster" on page 18-26.) If this process occurs during a composite peak, you will get results you did not intend.

Instead of +I/-I events, use the timed group component capability of TotalChrom to group distinct resolved peaks into a single component for quantitation and reporting.

**Locate Maximum Event**

The LM event locates the peak retention time at the maximum data point that falls within the peak rather than attempting to fit a quadratic to the peak crest.

**Retention Time and User Forced Peak Events**

The RT event forces the retention time of the current peak to be the event time. If it is used within a user forced area with daughter peaks, the optional peak number \(n\) specifies to which peak the RT event is applied.

The RT event can be used in combination with the User Forced Peak event (UFn).

The UFn event allows a baseline to be forced at any arbitrary point within a data file. The events generally occur in pairs — for example, UF1 and -UF1 start a peak at the UF1 time and end the peak at the -UF1 time. If the computer is within a peak and has found a peak crest when the UFn event occurs, an end of peak will be forced so the user peak start can occur. If the crest has not yet been found, then the potential peak will be discarded.

Daughter peaks can be forced within a user peak. For example, the timed events UF1...UF2...UF3...UF4...-UF1 would define three connected daughter peaks (#2, 3, and 4) within the mother peak (#1). In addition to the peak number, you can specify a signal level.

The following figure shows both the Retention Time and the User Forced Peak events.
**Smooth Peak Ends Event**

The SM event smoothes the beginning and ending of all peaks that start or end on a baseline. The following figure shows a peak before the SM event is run.

![Figure showing a peak before the SM event](image1)

This event applies a Savitsky-Golay smoothing algorithm to a group of n data points at both ends of a peak, where n can be any odd value between 5 and 25, inclusive. The algorithm adjusts the start and end data levels used for the baseline calculation to average baseline values (start and end times are not affected. The start and end point for each peak are put in the “center” of the noise band. The following figure shows the same peak after the SM event has been run.

![Figure showing the same peak after the SM event](image2)

The SM event is useful when a high noise level, regardless of the signal strength, or a poor signal-to-noise ratio makes baseline resolution a problem. It is especially useful where accurate, reproducible integration is critical.
Integration

This step of data analysis groups peaks into clusters, assigns baselines, and sums the areas under the peaks. This step adds final values to the result file for peak starting and ending points, retention times, areas, and heights. The following section describes integration.

Baselines Within Clusters

A peak cluster is a single resolved (non-overlapped) peak or a group of contiguous peaks that are found to be overlapped during peak detection.

Identifying peak clusters enables the software to establish preliminary baselines during peak detection according to a simple rule: All peaks in a cluster share a common baseline and resolved peaks have a unique baseline.

Adjustment of Preliminary Baselines

During integration, the software reviews the preliminary baselines to find sections of the chromatogram that cross the baseline. These are called penetrations. If a baseline is forced horizontally by a +HF, HR, or +M timed event, the software ignores any penetrations. In other cases, however, the software makes adjustments to eliminate baseline penetrations. Refer to “How Timed Events Affect Integration” on page 18-15.

Baseline Penetration by Chromatogram Valleys

If a cluster consists of more than one peak, the software checks the valley point between each pair of peaks to ensure that its height is above the height of the preliminary cluster baseline. The valley point is the lowest point between the two peaks as determined during peak detection.

If the valley point goes below (penetrates) the cluster baseline, the software ends the cluster at the valley point. The overlap flag for the first peak in the pair is reset to 0. A new cluster baseline is calculated, and the software continues testing for penetration. The following figure shows an example of this type of baseline adjustment.
During integration, the software corrects baseline penetration by chromatogram valleys.
**Baseline Penetration at the Start or End of a Cluster**

In addition to checking each valley point, the software examines all the points of each cluster for baseline penetration.

If any point penetrates the baseline, the start point of the first peak in the cluster is set to the point following the penetrator. If any of the final points penetrates the baseline, the end point of the last peak in the cluster is set to the point preceding the penetrator. A new cluster baseline is calculated, and the software continues testing for penetration. The following figure shows an example of baseline adjustment at the start of a cluster.

![Baseline Adjustment](image)

*The software corrects baseline penetration at the start of a cluster.*

**Peak Areas**

After establishing final baselines, the software determines peak areas. In performing this task, the software works with area slices. An area slice is defined as the area beginning at raw data point \( n \) and extending horizontally backward to point \( (n - 1) \). The start point of the peak does not contribute to the peak’s area.

To determine a peak’s area, the software first sums the area slices from the peak start to the peak end. Initially, these slices extend vertically from the level of point \( n \) to the zero-microvolt level.

Next, the software corrects this sum for the height of the cluster baseline by subtracting the baseline area, which is the area of the trapezoid between the cluster baseline and the zero-microvolt level. The result is the final peak area if the peak is resolved (refer to the following figure).
Peak areas are determined by summing area slices and then subtracting the baseline area.

Dropline integration means that the boundaries of overlapped peaks are defined by droplines: vertical lines dropped from a peak's start and end points to the peak baseline such that they are perpendicular to the time axis (see the next figure). The outside vertical edge of the first and last area slices of a peak define the positions of the droplines.

Peak boundaries defined by droplines (the outside edge of the first and last area slices).

**Area Adjustment**

For some peaks, additional processing beyond dropline integration is required to obtain a suitable peak area. The system offers two methods to adjust peak areas: exponential skimming and tangential skimming. The necessity for an exponential skim is determined automatically by the software; however, you can also impose an exponential skim by means of a timed event. A tangential skim is never implemented automatically; it can only be imposed by a timed event.
**Exponential Skims**

An exponential skim is a curve drawn by using an exponential equation to approximate the trailing edge of a parent peak. The skim passes under one or more peaks that follow the parent. These are called child peaks. The area underneath the skim is subtracted from the child peaks and given to the parent peak. A small area above the skim is subtracted from the parent peak and given to the first child peak. All droplines, beginning at the end of the first child, are adjusted to drop only to the skim. The following figure illustrates an exponential skim.

![Exponential Skim Diagram](image)

**An exponential skim**

**Exponential Skim Criteria**

Peak Height Ratio, Adjusted Height Ratio, and Valley Height Ratio are exponential skim criteria that determine whether an exponential skim line will be used to calculate the area of a child peak eluting on the trailing edge of a parent peak. TotalChrom will not use these parameters if a +X timed event (which always forces an exponential skim) or a −X timed event (which prevents an exponential skim) is in effect.

Peak Height Ratio is the ratio of the baseline-corrected height of the parent peak (Hm) to the baseline corrected height of the child peak (Hd). This ratio must be greater than the set value for the child peak to be skimmed. To disable exponential skimming throughout a run, you can set this parameter to its maximum value (1.0e+06).
**Integration**

$H_m$ divided by $H_d$ must exceed the set value for peak height ratio.

Adjusted Height Ratio is the ratio of the height of the parent above its start point ($L_m$) to the height of the child above the same point ($L_d$). This ratio must be greater than the set value for the child peak to be skimmed.

$L_m$ divided by $L_d$ must exceed the set value for adjusted height ratio.
Valley Height Ratio is the ratio of the baseline corrected height of the child peak (Hd) to the height of the valley between the parent and child peaks above the baseline (Hv). This ratio must be less than the set value for the child peak to be skimmed.

\[ \frac{H_d}{H_v} \text{ divided by } H_v \text{ must be less than the set value for valley height ratio.} \]
**Calculation of Exponential Skims**

Following is the equation you use to calculate an exponential skim:

\[ Y = H_b + H_e - G^{(t-t_0)} \]

where

- \( Y \) is the height of the exponential skim at time \( t \)
- \( H \) is the height (above the cluster baseline) of the start of the exponential skim
- \( H_b \) is the height of the cluster baseline at the end of the exponential skim
- \( G \) is the decay factor of the exponential
- \( t_0 \) is the time corresponding to the start of the exponential skim

The following figure illustrates this calculation.

*Values used to calculate an exponential skim*
How Timed Events Affect Integration

The following is a description of how each timed event or pair of timed events affect the way TotalChrom integrates peaks.

Force/Discontinue Common Baseline
(+CB/–CB)

The +CB event causes the software to assign an overlap flag of 1 to all peaks that occur while the event is in effect. As a result, during integration, TotalChrom treats all peaks as though they were clustered even if they do not meet the criteria for being overlapped. TotalChrom draws a common baseline for these peaks as shown below.

Without the +CB event, baseline placement is determined by the peak separation criteria.

With the +CB event, all peaks share a common baseline.
Even when the $+CB$ event is in effect, TotalChrom checks the artificial cluster for valleys that penetrate the forced baseline. If such valleys are present, it redefines the baseline, where necessary, to eliminate penetration.

The $+CB$ event takes effect at the time it is scheduled and applies to the current peak — the peak on which the event is located, if any. It also applies to subsequent peaks.

The $-CB$ event discontinues the $+CB$ event. TotalChrom continues to evaluate each peak against the overlap criteria and assigns overlap flags of 0 or 1, as warranted. This event takes effect immediately and applies to the current peak and subsequent peaks.

**Force/Discontinue Valley-to-Valley Baselines ($+V/-V$)**

The $+V$ event assigns an overlap flag of 0 to all peaks that occur while the event is in effect. As a result, during integration, all peaks are treated as though they are resolved, even if they meet the criteria for being overlapped. TotalChrom draws an individual baseline for each peak extending from valley to valley. No peaks are clustered. The following figures illustrate this concept.

*Without the $+V$ event, some peaks are clustered, having a common baseline.*
With the +V event, each peak has an individual baseline regardless of possible overlapping.

The +V event takes effect at the time it is scheduled and applies to the current peak. It also applies to subsequent peaks.

The −V event discontinues the +V event. TotalChrom resumes evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1 as warranted. This event takes effect immediately and applies to the current peak and subsequent peaks.

**Force Baseline to Point (BL)**

The BL event forces the baseline to the start of the current peak. (The current peak is the one on which the event occurs, or the one that follows the event if it occurs between peaks.)

BL causes TotalChrom to assign an overlap flag of 0 to the peak preceding the current peak. As a result, the preceding peak is treated as though it is resolved from the current peak. TotalChrom terminates the peak cluster at the end of the preceding peak by ending the cluster baseline there. Because the baseline is forced into this position, the baseline for the current peak is also forced to start at or near the peak start.

If this event occurs when peak detection is in the Begin or Confirm stages, the baseline of the current peak is forced to start at the bunched point representing the data bunch at the event's time. If the event occurs when peak detection is in the Find Top or Find End stages, the baseline is forced to start at the starting point of the current peak.

In the following figure, no BL event has been scheduled. A common baseline has been drawn for the two peaks because they meet the overlap criteria. The lower figure shows the effect of the BL event when it occurs during the Find Top or Find End stages. The preceding peak is assigned a 0 overlap flag and thus has a valley-to-valley
baseline. As a result, the baseline of the current peak is forced to begin at the peak starting point.

**Without the BL event, the two peaks are clustered and integrated by using drop lines.**

**With the BL event, a baseline is forced to begin at the peak start.**

You can use the BL event to reposition the theoretical baseline used for negative peak detection when a negative peak and a positive peak are contiguous. If the BL event is placed within the negative or positive peak, the theoretical baseline is moved to the level of the peak start. If the BL event is outside either peak, the theoretical baseline is set at the level of the point within whose range the event is scheduled.
**Force/Discontinue Horizontal Baseline Forward (+HF/−HF)**

The +HF event projects a horizontal baseline from the time of the event to the end of the chromatogram or until the event is turned off by a −HF event. When the +HF event is not in use, TotalChrom adjusts the baseline to avoid penetration by valleys (as shown between 43 and 46 minutes in the following figure). Conversely, the baseline established by the +HF event always remains horizontal (as shown next).

*Without the +HF event, baseline placement is determined by peak separation criteria.*

*Peaks within the +HF event are treated as a single cluster and share a common, horizontal baseline. The −HF event restores normal baseline treatment.*

The +HF event takes effect at the time it is scheduled. The baseline is set at the voltage level and time value of the raw data point nearest the event placement.
In sections of the chromatogram where the +HF event is in effect, peaks cannot have starting or ending points below the baseline. If the baseline intersects a peak, it is not likely to intersect exactly at a raw data point, so the peak starting and ending points usually lie some distance above the projected baseline. As a result, the area between the projected baseline and peak starting and ending points are excluded from the peak area. The following figure, which has been exaggerated for clarity, shows this.

The peak starting and ending points usually lie above the horizontally projected baseline.
You must be careful where you place the +HF event. If the projected baseline is set too high, as in the figure shown on the next page, large areas of peaks will be submerged below the baseline and, therefore, will not be integrated. Some peaks might be submerged entirely and go undetected. To avoid this problem, do not schedule a +HF event on a peak: place it on the baseline just before the start of a peak.

This shows the possible effect of scheduling the +HF event at a point too high on the chromatogram.

The −HF event discontinues the +HF event. TotalChrom then resumes evaluating each peak against the overlap criteria and assigns overlap flags of 0 or 1 as warranted.

If TotalChrom is searching for a peak ending point when either event is encountered, the current peak will end at the event. Otherwise, the current peak (if any) will be discarded.

You can schedule any number of +HF/−HF event pairs in a chromatogram, but they must not be overlapped. If two +HF events are scheduled in a row, without an intervening −HF event, the second event will be ignored. The lack of an intervening −HF event might even prevent TotalChrom from drawing a baseline.

**Force Horizontal Baseline Backward (HR)**

The HR timed event projects a horizontal baseline backward from the time the event is scheduled to the start of the chromatogram. The baseline event is set at the voltage level and time value of the raw data point nearest the event placement. TotalChrom does not adjust the baseline to prevent penetration by valleys; thus the baseline remains horizontal.

Where the HR event is in effect, peaks cannot have starting or ending points below the baseline. If the baseline intersects a peak, the intersection is not likely to occur exactly at a raw data point. Therefore, the peak starting and ending points will usually
lie some distance above the projected baseline. As a result, the area between the projected baseline and the peak starting and ending points will be excluded from the peak area. This result is because of the algorithm that requires peak boundaries to occur at raw data points, not between them.

Without an HR event, peaks can have separate baselines.

With the HR event, a common horizontal baseline projects backward to the start of the chromatogram.

To achieve the best result, do not schedule the HR event on a peak, but place it on the baseline between peaks or after any peak. This helps avoid baselines that are too high or too low.
End Peak Now (E)

The E event establishes a peak end at a selected point. You can use this event in conjunction with the +I and −I events to ensure that a peak ends at the desired point.

If TotalChrom is in the Begin or Confirm stages at the time the E event is scheduled, the event will have no effect. Otherwise, the last point of the current bunch becomes the ending point. After this event, TotalChrom re-enters the Begin stage. The following figure shows the effect of the E event.

Without the E event, the peak end is found automatically.

With the E event, the peak end is set manually.
**Start Peak (S)**

The S event starts a new peak when the event occurs, regardless of whether a peak would normally be detected and confirmed at this position. Typically, you use this event to force a split between a shoulder of a parent peak.

*Without the S event, the shoulder on the peak at 31.58 minutes is not detected as a separate peak.*

*With the S event, the shoulder is detected.*

If TotalChrom is in the Begin or Confirm stage at the time the S event occurs, the last raw data point of the current bunch (located at the time the event is scheduled) becomes the peak starting point. Also, TotalChrom enters into the Find Top stage.
If TotalChrom is at the Find Top stage when the S event is scheduled, it forces the present peak to end on the last point of the current bunch, and a new peak is forced to begin.

**Start Manual Integration/End Manual Integration (+M/–M)**

The timed events +M and −M allow you to manually integrate peaks. You place the +M event where you want a peak's baseline to start and the −M event where you want it to end. If necessary TotalChrom will interpolate within a data point to start and/or end manual integration at exactly the time you specify.

**Force Exponential Skim (+X)**

To ensure that TotalChrom creates an exponential skim, schedule the +X event anywhere within the parent peak on which you want the skim to start. The +X will have no effect unless you place it between the starting and ending points of a peak: it will have no effect if you place it on the baseline outside a peak.

If you schedule more than one +X event on the same peak, only one event will be recognized. If you place +X events on two adjacent peaks in the same cluster, TotalChrom will ignore the second one because it cannot decipher multiple skims when they converge.

If you place +X events on non-adjacent peaks in the same cluster, TotalChrom will draw exponential skims. However, what is drawn from the first peak will not extend past the start of the next peak with a +X event.

**Prevent Exponential Skim (–X)**

Scheduling a −X event anywhere on a peak ensures that TotalChrom will not create an exponential skim that originates on that peak. This event does not prevent the software from drawing a skim beneath the peak if the skim originated on a preceding peak.

The −X event will have no effect unless placed between a peak's starting and ending points: it will have no effect if you place it on the baseline outside a peak.

If you schedule more than one −X event on the same peak, only one event will be recognized.

**Tangential Skim (T)**

To create a tangential skim, TotalChrom draws a straight baseline from the starting point to the ending point of each child peak of a designated parent peak, as shown in the following figure. Such baselines are drawn for each child peak that occurs before the end of the cluster. TotalChrom then checks to see that the new baselines are not penetrated by the child peaks.
Finally, new areas for the child peaks are calculated, and the difference between the old and new areas (the area between the tangent baselines and the cluster baseline) is added to the parent peak.

Peaks before and after a tangential skim.

Unlike an exponential skim, TotalChrom never creates a tangential skim automatically. If you want to adjust peak areas by using a tangential skim, you must use a T-timed event. The T event must be placed between a peak's starting and ending points; it will have no effect if you place it on the baseline outside a peak.

If you schedule more than one T event on the same peak, the effect will be the same as having one T event.

Avoid scheduling T events on more than one peak in a cluster. If you place T events on two adjacent peaks in the same cluster, the second one will be ignored.
Calculation of Peak Height and Retention Times

The final step in integration is to determine the retention time and height of each peak. Although peak detection determines a top point, this point is a bunched (averaged) data point and might not be as high as the highest raw data point. Furthermore, even raw data points represent discrete samples of the analog input signal from a chromatograph and might not include the highest possible value in the continuous function. To remedy these shortcomings, the software

- Finds the highest raw data point lying between the peak start and end points. (If integrating a negative peak, it finds the lowest raw data point.)

- Calculates a quadratic regression based on the highest point and the first two points before and after it. The derivative of the result is found and solved to obtain the maximum of the curve (or minimum for a negative peak). This value is considered to be the absolute peak height, and the time of the maximum is the peak retention time. The actual peak height is the absolute peak height minus the height of the peak baseline at the retention time.
Component Identification

The component identification step identifies and assigns component names to the peaks in the result file. The following discusses how TotalChrom handles overlapping search windows and how you can optimize how TotalChrom performs component identification.

Overlapping Search Windows

Overlapping search windows are a source of potential misidentification of peaks. If windows overlap, a peak can be situated in more than one window at a time. The peak detection software must then determine which component the peak represents.

In such cases, the software might initially identify the peak as being two different components. Then it makes a further determination as to which component will be permanently assigned to the peak and which will be assigned to a different peak.

Another situation arises when one of the overlapping windows is that of a reference component. If a peak in the overlap region meets the selected criterion for the reference component (such as being the tallest peak in the reference component's window or the closest peak to the expected retention time of the reference component), it will first be identified as the reference component.

If this misidentified reference component lies within another component search window — even if it is closer to the adjusted retention time of the other component than the expected retention time of the reference component — the peak will retain its identity as the reference component. The software never re-identifies a peak that has already been identified as a reference component.

Optimizing Component Identification

You can optimize component identification by taking the following actions:

- Use at least one reference component to compensate for large shifts in retention times. The reference component should not elute close to other components of interest. Ideally, it will not be a component that you want to quantify; thus you can use a large concentration of it ("spike") in the sample to ensure a large reference peak.

- Make the search windows for reference components as wide as possible but not wide enough to overlap with the windows of other components. A reference peak will be easy to distinguish from the surrounding peaks if it is made larger.

- Make the search window for non-reference components narrow enough to avoid overlapping with the windows of other components. If you eliminate all overlapping, some peaks might not be identified and some components might not be assigned to a peak, but all identified peaks will be correctly identified unless a drastic shift causes the reference component to be misidentified.
Calibration

TotalChrom detects, integrates, and identifies peaks in the raw data produced by a standard sample, just as it does in analyzing any other sample. After these processes are complete, the treatment of a calibration run diverges from that for ordinary analyses. During calibration, TotalChrom performs the following tasks:

- Enters a calibration replicate into the calibration section of the method for each component calibrated. A replicate is the result data from a calibration injection.
- Averages the replicates for each component and uses these averages to update the response data (area and height values) in the current level.
- Updates component retention time replicate data for the current level.
- Computes or recomputes the calibration curve.
- Produces a report of the calibration.

The first task performed by the software in calibration is to store new calibration data in the method. This task consists primarily of building a new replicate structure. The replicate structure contains a component volume adjusted amount, a component response, internal standard amount ratio and internal standard response ratio (if the internal standard method of calibration is specified), and other data obtained from the current calibration run.

Note that it is a volume adjusted amount that is stored in the replicate structure. This is the product of the level amount and the sample volume values set in the method. For External Standard analyses, this allows you to correct for differences in injection volume between standards and samples, or to make concentration-to-amount conversions. In building the replicate data structure the software must also correct for possible differences in the expected component amounts (those currently stored with the calibration level data in the method) and the actual component amounts in the standard sample (as specified in the sequence). Similarly, the software must correct for differences between the expected internal standard amounts and actual internal standard amounts.

Calibration Levels

An unknown component amount can be determined by comparing the response (peak area or height) it produces with the response obtained from a known amount of the same component in a single standard sample. For better accuracy, more than one standard sample is normally used where standard sample contains different amounts (or levels) of the same component. Thus, a calibration level corresponds to a specific component amount used in a standard sample.
**Calibration Curves**

The response produced by the component at each calibration level plotted against the level amount defines the points of the component's calibration curve (see the next figure).

Each point on a calibration curve has amount and response coordinates corresponding to different calibration levels.

Each calibration level for a component is assigned a different name. For example, consider the two standard samples, Standard 1 and Standard 2, as follows:

### Standard 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Level</th>
<th>Amount</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Alpha</td>
<td>10</td>
<td>100000</td>
<td>50000</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Alpha</td>
<td>15</td>
<td>250000</td>
<td>17000</td>
</tr>
<tr>
<td>Propanol</td>
<td>Alpha</td>
<td>5</td>
<td>120000</td>
<td>10000</td>
</tr>
</tbody>
</table>

### Standard 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Level</th>
<th>Amount</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Beta</td>
<td>20</td>
<td>180000</td>
<td>90000</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Beta</td>
<td>30</td>
<td>370000</td>
<td>35000</td>
</tr>
<tr>
<td>Propanol</td>
<td>Beta</td>
<td>10</td>
<td>290000</td>
<td>25000</td>
</tr>
</tbody>
</table>
In Standard 1, the calibration level of each component is named “alpha,” and, in Standard 2, the calibration levels are named “beta.” Note that you can assign any name to a level. Level names need not connote a series such as “alpha” and “beta.” For example, “Red” and “Blue” are equally acceptable names.

Calibration level names are assigned individually to each component. A component can have up to 20 calibration levels, but the level names for all components in a standard sample must be the same. For example, it is a good practice to combine an alpha level of methanol in the same standard sample with alpha levels of ethanol and propanol, not beta levels.

The reason for this requirement lies in the nature of a calibration; one calibration (the analysis of one standard sample) provides data at only one calibration level. This level is specified in the sequence cycle guiding the calibration run. During analysis of the data produced by the run, the system stores the data (such as peak areas and heights) in the calibration method specified in the sequence cycle. The data enter fields reserved for the specified calibration level and no other.

Every component analyzed in a particular type of sample need not be present in each standard. That is, all components need not be represented at every calibration level. For example, if a sample contains ethanol, propanol, and methanol, the following standards could be used for its calibration:

**Standard 3**

<table>
<thead>
<tr>
<th>Component</th>
<th>Level</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Gamma</td>
<td>40</td>
</tr>
<tr>
<td>Propanol</td>
<td>Gamma</td>
<td>12</td>
</tr>
</tbody>
</table>

**Standard 4**

<table>
<thead>
<tr>
<th>Component</th>
<th>Level</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Zeta</td>
<td>30</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Zeta</td>
<td>50</td>
</tr>
</tbody>
</table>
**External Standard Versus Internal Standard Calibration**

TotalChrom offers a choice between the external standard and the internal standard method of calibration.

**External Standard Method**

When the external standard method is selected, the system relates component amounts with response values to compute the component's calibration curve. The amount and response values at each calibration level contribute a data point to a component's calibration curve. The amount is the $x$ coordinate (the independent variable), and the response is the $y$ coordinate (the dependent variable). Thus, the more calibration levels a component has, the more points will be plotted and used in fitting a calibration curve. For example, suppose a component has the following calibration data:

<table>
<thead>
<tr>
<th>Amount</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10,000</td>
</tr>
<tr>
<td>20</td>
<td>40,000</td>
</tr>
<tr>
<td>30</td>
<td>90,000</td>
</tr>
</tbody>
</table>

Using the external standard method and a quadratic curve fit, the system would generate the data contained in the calibration curve in the following figure.

*External standard calibration curve: amount versus response*
**Internal Standard Method**

Internal standards are compounds introduced in known amounts into chromatography samples — both samples to be analyzed and standard samples. One or more internal standards can be added to a sample.

When the internal standard method is selected, the system relates amount ratios with response ratios to compute the calibration curve. The amount ratio is the amount of component in a standard sample divided by the amount of an internal standard component in the same sample. The response ratio is the area or height of the component divided by the response of an internal standard in the same sample.

The amount and response ratios at each calibration level contribute a data point to a component's calibration curve. The amount ratio is the \(x\) coordinate (the independent variable), and the response ratio is the \(y\) coordinate (the dependent variable). For example, suppose a component has the following calibration data:

<table>
<thead>
<tr>
<th>Component</th>
<th>Internal Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td>Response</td>
</tr>
<tr>
<td>10</td>
<td>10,000</td>
</tr>
<tr>
<td>20</td>
<td>40,000</td>
</tr>
<tr>
<td>30</td>
<td>90,000</td>
</tr>
</tbody>
</table>
Using a quadratic curve fit, the system would generate the calibration curve shown in the following figure.

*Internal standard calibration curve: response ratio versus amount ratio*
Internal Standard Amounts and Calibration Levels

TotalChrom treats internal standards much like other sample components. That is, when you create a calibration method for samples that contain internal standards, you enter component information for the internal standards and for the components to be quantified. This information includes calibration level data. Like other components, internal standards can have multiple calibration levels.

<table>
<thead>
<tr>
<th>Component</th>
<th>Internal Standard</th>
<th>Calibration Level</th>
<th>Amount Average</th>
<th>Area Average</th>
<th>Height Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Ethanol</td>
<td>Alpha</td>
<td>10</td>
<td>100000</td>
<td>50000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta</td>
<td>20</td>
<td>180000</td>
<td>90000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeta</td>
<td>30</td>
<td>200000</td>
<td>100000</td>
</tr>
<tr>
<td>Propanol</td>
<td>Butanol</td>
<td>Alpha</td>
<td>5</td>
<td>120000</td>
<td>10000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta</td>
<td>10</td>
<td>290000</td>
<td>25000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeta</td>
<td>12</td>
<td>320000</td>
<td>30000</td>
</tr>
<tr>
<td>Ethanol (Internal standard)</td>
<td>—</td>
<td>Alpha</td>
<td>15</td>
<td>250000</td>
<td>17000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta</td>
<td>30</td>
<td>370000</td>
<td>35000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gamma</td>
<td>40</td>
<td>420000</td>
<td>40000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeta</td>
<td>50</td>
<td>460000</td>
<td>50000</td>
</tr>
<tr>
<td>Butanol (Internal standard)</td>
<td>—</td>
<td>Alpha</td>
<td>100</td>
<td>300000</td>
<td>100000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta</td>
<td>150</td>
<td>430000</td>
<td>150000</td>
</tr>
</tbody>
</table>

Internal standards, like other sample components, have one or more calibration levels.

When multiple calibration levels for an internal standard exist, and the system refers to this method to obtain the expected internal standard amount $I_{\text{exp}}$, from which calibration level does it take that amount?

When a method contains only one internal standard, the system performs the following actions:

- If the internal standard has a calibration level name matching the level in the sequence cycle controlling the calibration, the system takes $I_{\text{exp}}$ from that level.
- If the internal standard does not have a calibration level name matching the level in the sequence cycle, the system takes $I_{\text{exp}}$ from the first calibration level.

For example, suppose a method contains only methanol and ethanol at the calibration levels depicted in the previous figure, and ethanol is the internal standard. If a sequence cycle specifies the zeta calibration level, the system will use 50 for $I_{\text{exp}}$ — the zeta amount of ethanol.
However, if a method contains only propanol and butanol at the levels shown in the previous figure (butanol being the internal standard), and the sequence cycle specifies the zeta level, the system will use \(100\) — the alpha amount of butanol. The alpha level is used because butanol has no zeta level, and alpha is the first level.

When a method contains more than one internal standard, the system takes all \(I_{exp}\) values from the first calibration level. For example, suppose a method contains all the components shown in the previous figure, and the sequence cycle specifies the zeta calibration level. The system will use the alpha amounts of the internal standards ethanol and butanol, 15 and 100, respectively.

The system makes no attempt to obtain \(I_{exp}\) values from the level specified in the sequence cycle; otherwise, it would be mandatory for internal standards to have calibration data at every level represented by the related components. Non-calibration runs (ordinary analyses) are treated in the same manner by necessity. In an ordinary analysis, the sequence cycle does not specify a calibration level; thus, internal standard amounts are arbitrarily taken from the first calibration level in the calibration method.

If a component amount in a standard sample differs from the previously established expected level amount in the calibration method, TotalChrom can correct this discrepancy. Refer to the next section for more information.

**Component Amount Corrections**

The method contains *expected* component amounts for each component at each calibration level. The sequence contains *actual* sample amounts equal to the sum of the actual component amounts in the standard sample (excluding internal standard components).

If the expected component amounts at the appropriate level differ from the actual component amounts in the standard sample and, therefore, do not add up to the sample amount specified in the sequence cycle, the following correction might be made for each component:

\[
A_{adj} = A_{exp} \times \frac{S}{\sum A_{exp}}
\]

where

- \(A_{adj}\) is the adjusted component amount
- \(A_{exp}\) is the expected component amount in the method
- \(S\) is the sample amount specified in the sequence cycle
- \(\sum A_{exp}\) is the sum of the component amounts at the appropriate level in the method (not including internal standard component amounts)
There is a setting in the method that determines whether sample amount is used for correcting calibration standards as described above. If that option is selected, all component amounts in the method are adjusted by the same factor. You cannot correct the amount of one component without correcting the amounts of all the others. If the sample amount $S$ in the sequence cycle equals the sum of the component amounts $A_{exp}$ in the method, the expected component amounts need no correction. They are used as is in the new replicate structure and in computing the calibration curve.

**Internal Standard Amount Corrections**

The method contains *expected* amounts for each internal standard component (possibly at various calibration levels). In a sequence cycle, you enter an *actual* internal standard (ISTD) amount equal to the total amount of internal standard added to a sample. If more than one internal standard is added, the ISTD amount in the sequence cycle will match the sum of the *actual* amounts of the individual internal standard components.

If more than one internal standard is used in the standard sample, and their expected amounts do not add up to the ISTD amount in the sequence cycle, the software makes a correction. An adjusted internal standard amount $I_{adj}$ is calculated for each internal standard component:

$$I_{adj} = I_{exp} \times \frac{I_{seq}}{\Sigma I_{exp}}$$

where

- $I_{exp}$ is the expected internal standard component amount in the method
- $I_{seq}$ is the total ISTD amount specified in the sequence cycle
- $\Sigma I_{exp}$ is the sum of the internal standard component amounts at the appropriate level in the method

If only one internal standard component is added to the standard sample, $I_{exp}$ is equal to $\Sigma I_{exp}$; thus, $I_{seq}$ becomes equal to $I_{adj}$, as shown in the following equation:

$$I_{adj} = I_{exp} \times \frac{I_{seq}}{I_{exp}}$$

If $I_{seq}$ has a value of 1.0, the software interprets this value to mean that no internal standard amount corrections are needed, and it will not perform corrections. The expected internal standard amount(s) in the component list of the method are used without adjustment in the new replicate structure and in computing the calibration curve.
Replace Versus Average

For an auto-calibration, the sequence row type parameter determines what happens when a new replicate is stored: whether new calibration data replace previously stored data or are averaged with previously stored data.

If this parameter specifies “Cal:Replace,” or is part of the opening set of a bracketed calibration, all previously stored replicates for a component at the same calibration level are deleted, and only the new replicate remains. When the component's calibration curve is computed, only the new replicate provides amount and response data at that calibration level. Also, the level average values (area and height) entered into the calibration level section of the method equal the data in the latest replicate.

If the sequence row type is “Cal:Average,” or part of the closing set of a bracketed calibration, the new replicate is added to the list of previously stored replicates for the component at the same calibration level. When the component's calibration curve is computed, the software uses data from all the replicates in the list. Likewise, the level average values entered into the calibration level section of the method reflect contributions from all replicates present.

These options also affect how the updating of retention time data is handled as described in the next section.

Retention Time Data

You can also use calibration samples to update the expected retention time data for components. In the sequence cycle, you specify whether or not you want retention time data updated, and if so, how you want TotalChrom to perform this operation. The Update Retention Times option can be set to one of the following:

- **N (No)** — The retention time data from the run are not updated in the method.
- **Y (Yes)** — The retention time data from the run are updated in the method. If the calibration is an average, then a retention time replicate is added for the current level. If the calibration is a replace, then the retention time data from the run replace all existing retention time replicates for the current level.
- **R (Reset)** — All existing retention time replicates for all levels are deleted and replaced by the retention time data from this run.
Computation of the Calibration Curve

After a calibration replicate for a component is added to the method, the software computes or recomputes the component's calibration curve. There are four cases in which a curve is not derived:

- When a user-supplied constant calibration factor has been entered in the method,
- When an average calibration factor has been specified,
- When calibration by reference has been specified, or
- When a point-to-point curve fit has been specified.

In the first case, the software uses the calibration factor instead of a calibration curve to quantify unknown component amounts.

In the second case, the ratio of response to amount is calculated for each replicate in all of the levels and averaged together to give the average calibration factor (ACF).

Calibration by reference uses the calibration from the reference component to calculate amounts for the other component.

In the case of a point-to-point fit, the software simply draws straight lines between each point plotted from the replicate averages. For all other calibration curve fit types, the software computes a calibration curve by performing a linear regression.

The data used to create a calibration curve depend upon the calibration method specified: internal standard or external standard.

If the external standard method has been specified, the curve shows the relation between volume adjusted amount and response values. Response can be either the peak area or the peak height, whichever is specified for a given component in the method.

If the internal standard method has been specified, the curve shows the relation between amount ratios and response ratios. In this case, the sample volume cancels out because the same volume applies to both component and internal standards.
**Alternative Amount Scaling**

TotalChrom offers alternatives to simply plotting response as a function of amount or response ratio as a function of amount ratio. For example, you can choose to plot response as a function of the log of the component amount. Or, in the case of internal standard calibration, you could plot response ratio as a function of the ratio

\[
\log \frac{x_{\text{comp}}}{x_{\text{istd}}}
\]

where

- \( x_{\text{comp}} \) is the component amount, and
- \( x_{\text{istd}} \) is the internal standard amount.

The following is a list of the alternatives for amount scaling:

- \( 1/x \) Response is a function of the reciprocal of the amount.
- \( 1/x^2 \) Response is a function of the reciprocal of the square of the amount.
- \( \log(x) \) Response is a function of the base-10 log of the amount.
- \( 1/\log(x) \) Response is a function of the reciprocal of the log of the amount.

Use of these scaling alternatives rules out some values for amount that otherwise would be allowed. Specifically, the amount 0.0 cannot be used with \( 1/x \) or \( 1/x^2 \) scaling. Also, amounts less than 1.0 cannot be used with a logarithmic option; otherwise, an invalid operation will result.
**Regression Calculation**

To compute a calibration curve, TotalChrom performs a regression calculation using all replicates for the component at all available calibration levels. If the Include Origin option has been selected in the method, a (0,0) point (amount=0, response=0) is added to the regression data set. The regression is performed by computing a set of orthogonal polynomials and using them to compute the best least-squares approximation with the following equation:

$$ p_n^*(x) = \sum_{j=0}^{n} < y, p_j > p_j(x) $$

where

- $p_n^*(x)$ is the best least-squares approximation to the data,
- $p_j(x)$ are the solutions to the orthogonal polynomials at $x$ (the amount)
- $p_j$ are the orthogonal polynomials
- $<y, p_j>$ are the generalized Fourier coefficients at $y$ (the response)
- $n$ is the fit order, which represents the number of polynomials

The notation $<f, g>$ is defined as:

$$ < f, g > = \sum_{j=0}^{m} w_j f(x_j) g(x_j) $$

where

- $f$ and $g$ are two functions of $x$
- $w$ is the weighting factor for each data point
- $m$ is the number of data points in the regression


The best least-squares approximation obtained from the regression is a polynomial of the following form:

$$ p_n^* = c_0 + c_1 x + c_2 x^2 + c_3 x^3 $$
The curve coefficients, $c_0$ through $c_3$, are stored in the method for each component. They define the calibration curve and constitute all the information necessary to plot calibration curves and quantify unknown amounts of the component.

The equation above represents a cubic fit. A quadratic fit will not have the $c_3$ term, and a linear fit will have neither the $c_2$ nor the $c_3$ terms. If the curve is forced through the origin, the $c_0$ term will not appear in the equation.

**Calibration By Average Calibration Factor**

Performing calibrations by using an average calibration factor (ACF) is a technique that is commonly used in environmental analyses as a shortcut for performing a linear calibration. Rather than doing a linear regression on the data, this method takes an average of the slope at each calibration point on the curve and uses that average as a single calibration factor. This implies that the intercept of the curve must pass through the origin.

To calculate average calibration factors, TotalChrom determines either the ratio of response-to-amount for external standard calibration, or response-ratio to amount-ratio for internal standard calibration, for each replicate. It then sums these ratios and takes the simple average for the average calibration factor, as follows:

\[
ACF = \frac{\sum_{i=1}^{n} \frac{R_{sp_i}}{A_{mt_i}}}{n}
\]

where

- $ACF$ is the average calibration factor
- $R_{sp_i}$ is the response for the $i$th replicate of the standard
- $A_{mt_i}$ is the amount for the $i$th replicate of the standard
- $n$ is the number of replicates for the standard

For external standard calibrations, the unknown amount is calculated as:

\[
A_{mt_c} = \frac{R_{sp_c}}{ACF}
\]

where

- $A_{mt_c}$ is the amount for the component,
- $R_{sp_c}$ is the response for the component, and
- $ACF$ is the average calibration factor for the component.
For internal standard calibrations, the unknown concentration is calculated as:

\[ Amt_c = \frac{Rsp_c \times Amt_{is}}{Rsp_{is} \times ACF} \]

where

- \( Amt_c \) is the amount for the component
- \( Rsp_c \) is the response for the component
- \( Rsp_{is} \) is the response for the internal standard
- \( Amt_{is} \) is the amount of the internal standard
- \( ACF \) is the average calibration factor for the component
Auto-Calibration Report

TotalChrom prints a calibration report after it performs an auto-calibration, either after data acquisition or during batch reprocessing. However, it does not print a report after calibrations that are performed through the Graphic Method Editor or the Method Editor.

An example of a calibration report follows. This shows the full, or “long” auto-calibration report. It contains three sections: header information, a table of the current calibration level averages and confidence limits test results, and a table of curve coefficients and calibration status. A description of the contents of each section follows. The ACR (Auto-Calibration Report) column in the sequence allows you to specify for each calibration cycle whether you want the long report (L), a shorter report (S), or no report (N). The shorter report does not include the table of calibration averages or the confidence limits test results.

Software Version: 6.0
Date: 1/27/97 01:05 PM
Sample Name: Cal Level High
Data File: C:\TC\TCCS\VER6.0.0\DATA\CALS003.RAW Date: 1/17/97 09:27 AM
Sequence File: C:\TC\TCCS\VER6.0.0\DATA\AUTOCAL.SEQ Cycle: 3 Channel: A
Instrument: 970-0 Rack/Vial: 0/0 Operator: MEA
Sample Amount: 1.0000 Dilution Factor: 1.00

AUTO-CALIBRATION REPORT

Updating method: C:\TC\TCCS\VER6.0.0\DATA\970METH4.mth
Calibration performed at level: High Level
Values will be averaged with previous runs in the method
Retention times in the method will be updated
Reported response values are the method averages.

<table>
<thead>
<tr>
<th>Component</th>
<th>Retn</th>
<th>Vol Adj</th>
<th>Adjusted Response</th>
<th>Calibration Factor</th>
<th>%RSD</th>
<th>#</th>
<th>%RSD</th>
<th>#</th>
<th>CLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.140</td>
<td>54.00000</td>
<td>1376443.066667</td>
<td>25489.686420</td>
<td>0.84</td>
<td>9</td>
<td>0.00</td>
<td>1.34</td>
<td>3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.397</td>
<td>67.50000</td>
<td>1774326.000000</td>
<td>26286.311111</td>
<td>0.68</td>
<td>9</td>
<td>0.00</td>
<td>1.34</td>
<td>3</td>
</tr>
<tr>
<td>iso-Propanol</td>
<td>0.592</td>
<td>27.00000</td>
<td>809886.470131</td>
<td>29995.795190</td>
<td>0.71</td>
<td>9</td>
<td>0.00</td>
<td>1.48</td>
<td>3</td>
</tr>
<tr>
<td>iso-alcohols</td>
<td>0.856</td>
<td>94.50000</td>
<td>2362881.114020</td>
<td>25004.032953</td>
<td>----</td>
<td>1</td>
<td>0.00</td>
<td>1.39</td>
<td>3</td>
</tr>
<tr>
<td>iso-Butanol</td>
<td>0.864</td>
<td>67.50000</td>
<td>1552994.643889</td>
<td>23007.328058</td>
<td>0.73</td>
<td>9</td>
<td>0.00</td>
<td>1.35</td>
<td>3</td>
</tr>
<tr>
<td>n-butanol</td>
<td>1.140</td>
<td>8.500000</td>
<td>196378.000000</td>
<td>23103.294118</td>
<td>0.74</td>
<td>9</td>
<td>0.00</td>
<td>1.34</td>
<td>3</td>
</tr>
</tbody>
</table>

Confidence Limits Test (CLT) Result Explanations:
4 = Probably not outlier (failed at 95% 1st pass, passed at 99.9% 1st pass)

Calibration Status:

<table>
<thead>
<tr>
<th>Component</th>
<th>C0</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>r^2</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>17380.714120</td>
<td>32701.340388</td>
<td>-161.847049</td>
<td>----------</td>
<td>0.997189</td>
<td>9</td>
</tr>
<tr>
<td>Ethanol</td>
<td>22240.655996</td>
<td>33714.007085</td>
<td>-116.690546</td>
<td>----------</td>
<td>0.997186</td>
<td>9</td>
</tr>
<tr>
<td>iso-Propanol</td>
<td>10227.749794</td>
<td>38467.097191</td>
<td>-333.286366</td>
<td>----------</td>
<td>0.997048</td>
<td>9</td>
</tr>
<tr>
<td>iso-alcohols</td>
<td>-42918.523345</td>
<td>39421.568689</td>
<td>-146.557065</td>
<td>----------</td>
<td>0.993545</td>
<td>9</td>
</tr>
<tr>
<td>iso-Butanol</td>
<td>19430.123976</td>
<td>29525.488478</td>
<td>-102.502191</td>
<td>----------</td>
<td>0.997246</td>
<td>9</td>
</tr>
<tr>
<td>impurity2</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>n-butanol</td>
<td>2751.355457</td>
<td>31307.527708</td>
<td>-1015.978678</td>
<td>----------</td>
<td>0.997066</td>
<td>9</td>
</tr>
</tbody>
</table>

Calibration Status Explanations:
6 = Component not calibrated: Uses calibration reference
9 = Component missing from named groups
**Header Information**

The header information for an auto-calibration report consists of three parts: (1) a report header, which contains information about the standard sample; (2) the title, “Auto-Calibration Report,” and (3) a calibration header, which gives the calibration level that has been updated and tells whether the Average or Replace option was in effect for the calibration.

**Current Calibration Level Averages**

Next in the report is a table of the current calibration level averages for each component in the method being calibrated. These values are the averages of replicate data in the file after the addition of new replicates as a result of the calibration.

The following information is in this table:

- **Component name** — The name of the component from the method
- **Retn Time** — Average expected retention time
- **Vol Adj Amount** — Average volume adjusted amount or amount ratio for the calibration level
- **Adjusted Response** — Average response (area, height, or response ratio)
- **Calibration Factor** — Average response/volume adjusted amount ratio (using the raw Amount from the method)
- **Purity Adjusted Calibration Factor** — Average response/volume adjusted amount ratio (using the Amount from the method after correction for the Purity of the component)
- **%RSD** — Relative standard deviation of retention time, amount, and response values in the new replicates
- **# Reps** — Number of replicates averaged to obtain the current calibration level data (separate counts for RT and calibration replicates)
- **CLT code** — The result of a confidence limits test on the newest replicate

The calibration level table contains data for all the components in the method even if some components were not calibrated during the current calibration run. This table contains no indication of which components were and which were not calibrated from this result file, but the calibration status table which follows does provide that information.

Because the calibration level table contains level averages, the retention time, amount, and response values might or might not match the corresponding values in the subsequent analysis report for the calibration. The values will not match if the Average option was in effect for the calibration; they will differ because the values in the analysis report are not averages but simply results produced by the current
calibration. The response and retention time values will match if the Replace option was in effect because only the current calibration results contribute to the level averages. The amount values will only match if, in addition, the sample volume was 1.

**Relative Standard Deviation Values**

The calibration level table contains three types of relative standard deviation (RSD) values: component retention time, amount, and response. For a given component, these values reflect the standard deviation of the level average expressed as a percentage of the average. At least three replicates are required for %RSD values to be calculated.

**Confidence Limits Test**

The CLT column in the calibration level table reports the results of a confidence limits test on the calibration data. This test will only be performed when three or more replicates exist for the current level. The results of the confidence limits test do not affect the calibration information stored in the method. They provide an assessment of the data, which you can choose to act upon or to ignore.

The Confidence Limits Test (CLT) was derived as follows:

For any normal distribution, the confidence limits of the distribution can be expressed as

\[ \mu = \bar{x} \pm t \times \left( \frac{s}{\sqrt{n}} \right) \]

where

- **\( \mu \)** is the true mean
- **x-bar** is the average of the replicates
- **t** is the coefficient for the confidence interval at (n-1) degrees of freedom
- **s** is the standard deviation of the replicates
- **n** is the number of replicates

To find if a given value is within the confidence interval, the right side of the equation is calculated compared to the value of interest. TotalChrom tests the hypothesis that

\[ |x_i - \bar{x}| \leq t \times \left( \frac{s}{\sqrt{n}} \right) \]

**or rearranged**
The first pass calculates the limits for the distribution without the current replicate and compares them for the 95% and the 99.9%. The second pass calculates the limits for the distribution including the current replicate at the same points.


The CLT codes reported in the full auto-calibration report have the following meanings:

0 = Insufficient data for outlier test (<3 replicates)
1 = Significant outlier (failed at 99.9% 2nd pass)
2 = Probable outlier (failed at 95% 1st pass, failed at 95% 2nd pass)
3 = Probable outlier (failed at 95% 1st pass, failed at 99.9% 1st pass)
4 = Probably not outlier (failed at 95% 1st pass, passed at 99.9% 1st pass)
5 = Not outlier (passed at 95% 1st pass)

**Calibration Curve Coefficients and Status**

The last section of a calibration report consists of a table of calibration curve coefficients ($c_0$ to $c_3$), an $r^2$ value, and a status for each component. If a component's calibration curve does not include a coefficient, the table contains a dashed line in its place.

The $r^2$ value is a correlation coefficient that gives a qualitative indication of how good the fit is — a measure of how close the calibration curve comes to the data points. The correlation coefficient is calculated by the following equation:

$$r^2 = 1 - \frac{SSE}{SST}$$

$$SSE = \sum w_i y_i^2 - \left( c_0 \sum w_i y_i + c_1 \sum w_i y_i x_i + c_2 \sum w_i y_i x_i^2 + c_3 \sum w_i y_i x_i^3 \right)$$

$$SST = \sum w_i y_i^2 - \left( \frac{\sum w_i y_i}{\sum w_i} \right)^2$$

where

$w_i$ represents the weighting factors for each data point
To report the results of the auto-calibration, a Status column appears in the Calibration Status table, as shown above. Values in the column will range from 1 to 9, representing the possible calibration outcomes. The following explanations appear beneath the Calibration Status table:

1 = Component not calibrated: Rejected based on user criteria  
2 = Component not calibrated: Was not found in peak/group list  
3 = Component not calibrated: No ISTD specified in method  
4 = Component not calibrated: ISTD was not found in peak list  
5 = Component not calibrated: Uses constant calibration factor  
6 = Component not calibrated: Uses calibration reference  
7 = Component not calibrated: No calibration at this level  
8 = Component not calibrated: Incomplete named group  
9 = Component missing from named group  
10 = Component not calibrated: signal level out-of-range in peak  
11 = Component calibrated successfully

The first status indicates that the component has been excluded from the calibration because it failed the user-selected outlier test.

To activate this test, select the Reject Outliers During Calibration in the Global Information dialog in the Components window of the Method Editor, and enter the percentage error you want to apply in the Allowed Deviation text box.

---

This outlier test is distinct from the confidence limits test (CLT) described in the previous section. The outlier test described here is used to reject calibration replicates that exceed a user-specified deviation from current calibration data. A calculated calibration factor is used as the basis of the comparison (as described following this note). The confidence limits test provides an indication of the consistency of the replicate data for that level, but it does not reject any data.

The outlier test is performed according to the following rules:

- The test is applied both in manual calibration and auto calibration
- No test occurs for a Replace operation or an empty calibration
- For an Average operation, the test is applied either to the level named in the sequence for normal curves or to the average calibration factor, if this option has been selected.
In each case, it works out that:

$$\text{Abs} \left( \frac{CF_{\text{Method}} - CF_i}{CF_{\text{Method}}} \right) \times 100 < \%\text{Entered}$$

where

$$CF_{\text{Method}} = \text{Average} \left( \frac{R_i \times Amt_{\text{STD}}}{Amt_i \times R_{\text{STD}}} \right)$$

- $R_i$ is the response of the individual replicate
- $Amt_{\text{STD}}$ is the amount of the internal standard at this level
- $Amt_i$ is the amount at this level
- $R_{\text{STD}}$ is the response of the internal standard at this level

This applies to all of the replicates at the named level or for all levels of an average calibration factor. For external standard calibrations, the internal standard correction does not apply, but all amounts are volume-adjusted amounts (for example, level amount $\times$ sample volume).

The $CF_i$ is calculated as:

$$CF_i = \left( \frac{R_i \times Amt_{\text{STD}}}{Amt_{\text{corr}} \times R_{\text{STD}}} \right)$$

where

- $R_i$ is the response of the current replicate
- $Amt_{\text{STD}}$ is the amount of the internal standard in this run. This is taken from the sequence. If there are multiple internal standards and the actual ISTD amount is other than one, then:

$$Amt_{\text{STD}} = Amt_{\text{STD actual}} \times \frac{Amt_{\text{This ISTD}}}{\sum Amt_{\text{Each ISTD}}}$$

where

- $Amt_{\text{STD actual}}$ is the ISTD amount from the sequence
- $Amt_{\text{This ISTD}}$ is the ISTD amount for this internal standard at this level from the method
- $\sum Amt_{\text{Each ISTD}}$ is the sum of the internal standard amounts at this level from the method
- $Amt_{\text{corr}}$ is the amount at this level corrected for the amount ratio, if the option is selected. If not, it is the level amount from the method
Calibration

\[ Amt_{corr} = Amt_i \times \frac{Amt_{Seq\ File}}{\sum Amt_{Method}} \]

where

- \( Amt_i \) is the standard amount for this level from the method
- \( Amt_{Seq\ File} \) is the sample amount from the sequence
- \( \sum Amt_{Method} \) is the sum of the standard amounts at this level from the method

If TotalChrom either rejects or cannot find a component during a manual calibration, you will see a message box after trying to perform a calibration update (from the Manual Calibration dialog box in the Method Editor). This message box asks if you want to view a calibration log. If you choose OK, Windows Notepad opens automatically and displays a log file showing what happened.
Quantitation

The quantitation step involves the following tasks:

- Correction of internal standard amounts

- Determination of initial component amount values for each component by solving the calibration curve for the component, by applying a user-supplied constant calibration factor, by applying an average calibration factor, or by solving the calibration curve of another component specified for this purpose (for example, reference calibration).

- Reversal of the scaling of initial amount values if they have been derived by the application of a scaling alternative (for example, if they are expressed as the log or the reciprocal of the component amount).

- Conversion of amount ratios to amounts or volume adjusted amounts to amounts. If internal standard calibration is specified in the method, initial amounts will be in the form of amount ratios — the ratio of the component amount to the internal standard amount. If external standard calibration is specified in the method, initial amounts are in the form of volume adjusted amount and are divided by the actual sample volume listed in the sequence.

- Computation of adjusted amounts by applying the dilution factor, multiplier, divisor, and addend to raw amount values. Raw amounts are those that, if necessary, have had scaling reversed and/or have been converted from amount ratios to amounts.

- Quantitation of unidentified peaks.

Note that component amounts can go through three stages in quantitation: the initial stage when they are first determined from the calibration curve; the raw stage after scaling reversal and/or conversion from amount ratios or volume adjusted amounts to amounts; and, the adjusted stage after the dilution factor, multiplier, divisor, and addend have been applied. The initial stage is synonymous with the raw stage if an initial amount has not been scaled and is not in the form of an amount ratio. A raw amount will be equal to the adjusted amount if the dilution factor, multiplier, and divisor have values of 1.0 and the addend has a value of 0.0.

The following sections explain the quantitation steps in greater detail.
Correction of Internal Standard Amounts

If the calibration associated with a sample was done by the internal standard method, the software must correct any discrepancy between the total amount of internal standard actually added to the sample and the amount(s) of internal standard component(s) specified in the method.

When the method specifies an internal standard calibration, internal standard components are added to all samples — those being analyzed and standard samples used in calibration runs. The internal standard components are identified in the method, and the amounts expected to be added to samples are listed in the calibration level section of the file. Amounts can be listed for one or more calibration levels.

The total actual amount of internal standards added to a sample is specified in the sequence cycle controlling the run. If this actual ISTD amount differs from the sum of the internal standard amounts specified for the first calibration level in the method, the software makes a correction to obtain an adjusted internal standard amount \( I_{adj} \) for each internal standard component.

In quantitation, the software looks only at internal standard amounts at the first calibration level. Other levels are not considered because quantitation, unlike calibration, is not associated with a particular calibration level.

\( I_{adj} \) is calculated as follows:

\[
I_{adj} = I_{exp} \times \frac{I_{seq}}{\sum I_{exp}}
\]

where

- \( I_{exp} \) is the expected amount of an internal standard component at the first calibration level in the method
- \( I_{seq} \) is the total ISTD amount specified in the sequence cycle
- \( \sum I_{exp} \) is the sum of all the internal standard component amounts at the first calibration level in the method

If only one internal standard component is added to the sample, \( I_{exp} \) is equal to \( \sum I_{exp} \); thus, \( I_{seq} \) becomes equal to \( I_{adj} \) and \( I_{seq} \) is used as the actual internal standard amount in subsequent calculations.

If \( I_{seq} \) has a value of 1.0, the software interprets this value to mean that no internal standard amount corrections are needed, and it will not perform corrections. The expected internal standard amount(s) in the method are used without adjustment in subsequent calculations.
Calculation of Initial Component Amounts

Initial component amounts are calculated in one of three ways: by solving the component's calibration curve (the normal method), by applying a calibration factor (user-supplied or calculated average), or by using the calibration curve of another component (the calibration reference).

Calibration Curves

To solve a calibration curve for amount, the software uses response in the same form as that used to build the curve. That is, if an external standard calibration was performed, the response used is the peak area or height (whichever has been specified in the method). If an internal standard calibration was performed, the response used is the area ratio or height ratio (component to internal standard).

The initial result of solving a calibration curve is an amount in the same form as that used in building the curve. That is, if an internal standard calibration was performed, the initial amount is an amount ratio. If an alternative amount scaling option was selected, the initial amount is the log of the amount or another variation.

The following sections tell how the software solves the various calibration curve types.

Point-to-Point Fit

In a point-to-point fit, each pair of points (calibration levels) is connected by a straight line segment. When the response of the component is examined with respect to the calibration levels, four outcomes are possible:

The response lies below the first calibration level, and the curve has been forced through the origin; or, there is only one calibration level, and the response lies below it. In either case, a straight line is drawn to connect the first point (or the only point) with the origin. The initial amount is calculated by solving the equation of this line.
The response lies between two calibration levels. In this case, the equation of the straight line drawn between these levels is used to calculate the initial amount.

The response lies above the last calibration level. If there is more than one calibration level, a straight line is drawn to connect the last two levels and extended beyond the last level. If there is only one calibration level, a straight line is drawn to connect the origin with the level and is then extended beyond the last level. The initial amount is calculated by solving the equation of this line.
In each case, the software determines the slope and the $y$ intercept of the line and then solves the following equation to obtain the initial amount $A_{ini}$:

$$A_{ini} = \frac{(R - b)}{m}$$

where

- $R$ is the response
- $b$ is the intercept of the line at the $y$ axis
- $m$ is the slope of the line

Note that a slope of 0.0 (a horizontal line) prevents an amount from being calculated; thus, two adjacent calibration levels must not have the same response. A slope of infinity (a vertical line) also prevents an amount from being calculated; thus, two adjacent calibration levels must not have the same amount.

**First Order Fit**

A first order (linear) curve is specified by the equation

$$y = c_0 + c_1x$$

where

- $c_0$ is the $y$ intercept
- $c_1$ is the slope of the line

The software calculates the initial amount by using the same equation as used for point-to-point fits. If the slope of the linear curve is 0.0, the equation cannot be solved.

**Second Order Fit**

A second order (quadratic) curve is specified by the equation

$$y = c_0 + c_1x + c_2x^2$$

This equation can be solved to yield two amount ($x$) values, as follows:

$$x = \frac{-c_1 + \sqrt{c_1^2 - 4c_2(c_0 - y)}}{2c_2}$$

$$x = \frac{-c_1 - \sqrt{c_1^2 - 4c_2(c_0 - y)}}{2c_2}$$
An attempt to solve the equation will have one of four possible results:

- The curve cannot be solved because the square root term is negative. A quadratic curve has a single minimum or maximum. The inability to solve the curve for this reason means that the response \((y)\) is either below the minimum or above the maximum. The software will produce an error message signaling that the response is outside the domain of the calibration.

- The curve can be solved, and only one of the two versions of the quadratic equation yields a positive result. This is the optimal case. The positive result is taken as the initial amount, and the negative solution is ignored.

- The curve can be solved, and both solutions yield a positive result. The software chooses the amount value closer to the range of the calibration data.

- The curve can be solved, and both solutions yield a negative result. The software always chooses the larger amount value (the lower absolute value). See the discussion on negative amounts on page 18-74.

**Third Order Fit**

A third order (cubic) equation has the form

\[
y = c_0 + c_1x + c_2x^2 + c_3x^3
\]

Unlike linear and quadratic equations, cubic equations cannot be conveniently solved directly for \(x\). Instead, TotalChrom uses an iterative process based on Newton's method of approximating the root of an equation.

The software first computes the solution for a point-to-point fit as a preliminary estimate. Then, by refining that estimate, it tries to converge on the correct solution.

This method of solving cubic equations has some limitations. Most notable is the inability to continue converging to a solution if an intermediate estimate lies at a minimum or maximum on the curve. Another problem occurs when successive estimates oscillate around the correct value without converging. In both cases, the software issues a warning message after the analysis report, notifying you of the inability to converge on a solution to the curve.
**Negative Amounts**

TotalChrom cannot use negative amounts when performing calculations, therefore it interprets negative numbers as zero (0). Occasionally, solving a calibration curve can result in a negative amount value. Although there is certainly no such thing as a negative amount of a component in a sample, negative results are caused by a positive y intercept value \(c_0\) when the slope of the curve at the intercept is positive. Any positive response less than the intercept value will correspond with a negative amount.

Physically, a positive intercept can be interpreted to mean that an injection of zero amount of the component causes a positive response. This effect can be caused by instrumental interferences that produce a positive offset (such as stray light in a UV detector). Alternatively, it can arise from calibration data which shows excessive scatter (the positive intercept being merely a consequence of the regression). You can avoid the positive intercept by forcing the curve through the origin. If an amount of zero truly yields a positive response \(R\), then, mathematically, any response less than \(R\) (even if positive) must correspond with a negative amount.

A more probable calibration curve has a negative \(y\) intercept. This indicates that at a point below a certain positive amount value, there is no response from the chromatograph detector. This amount is the detection limit for the equipment.
Constant Calibration Factor

Component amounts are normally determined by solving a calibration curve as described above. However, you have the option to enter into the method a constant calibration factor to be used to calculate a component's amount. If you choose this alternative, the software does not create a calibration curve. Instead, it divides the component's response $R$ by the calibration factor $F$ to obtain an initial amount $A_{ini}$:

$$A_{ini} = \frac{R}{F}$$

Reference Calibration

Another alternative to using a component's own calibration curve to determine an unknown amount of it in a sample is to use the curve of another designated component. You choose this alternative by selecting the option Calibrate by Reference when you enter the component information. At that time, you also designate the appropriate reference component. The software calculates a raw amount for the component as though it were the reference component, except that the response of the component and not that of the reference component is used in the calculation.

Reversal of Scaling Options

The method can specify alternative scaling for the amount or amount ratio scale of a component's calibration curve. For example, the response can be plotted versus the log of the amount, rather than the sample amount. In such cases, the initial amount value obtained by solving the calibration curve is in scaled form, and the scaling must be reversed for quantitation.

The operation of transforming scaled amount values is the inverse of that used to scale amount values during calibration:

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<tr>
<td>log$(x)$</td>
<td>$10^x$</td>
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<tr>
<td>$1/log(x)$</td>
<td>$1/(10)^x$</td>
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A negative initial amount ($x$) will prevent $1/x^2$ scaling from being reversed because it is impossible to calculate the square root of a negative number. An initial amount of 0.0 is considered to indicate an error during the solution of the calibration curve. The software will leave it at 0.0 and not attempt to reverse the scaling. You cannot reverse scaling if $x$ is exactly one or much less than one. The software displays a message if it cannot reverse the scaling.
Conversion of Amount Ratios to Raw Amounts

If a component's calibration curve is based on the internal standard method of calibration, the initial amount value obtained by solving the curve and reversing scaling, if necessary, is actually the amount ratio (component amount to internal standard amount).

Amount ratios are converted to raw amounts $A_{\text{raw}}$ as follows:

$$A_{\text{raw}} = A_{\text{ini}} \times I_{\text{adj}}$$

where

- $A_{\text{ini}}$ is the initial component amount ratio
- $I_{\text{adj}}$ is the internal standard amount as corrected during quantitation

Conversion of Volume Adjusted Amounts

If a component's calibration is based on the external method of calibration, the initial amount obtained by solving the curve and reversing scaling, if necessary, yields a volume adjusted amount. Volume adjusted amount is converted to raw amounts:

$$A_{\text{raw}} = \frac{A_{\text{ini}}}{V_{\text{samp}}}$$

where

- $A_{\text{raw}}$ is the initial component amount
- $V_{\text{samp}}$ is the sample volume from the sequence cycle
Computation of Adjusted Amounts

The final step in computing a component amount is to calculate an adjusted amount $A_{adj}$. This is done by applying the dilution factor, multiplier, divisor, and addend (found in the sequence cycle) to raw amounts. The software uses the following equation:

$$A_{adj} = \frac{A_{raw} \times f \times m}{S \times d} + a$$

where

- $A_{raw}$ is the raw component amount
- $f$ is the dilution factor
- $m$ is the multiplier
- $d$ is the divisor
- $a$ is the addend
- $S$ is the sample amount

The sample amount ($S$) is used only when the method indicates that unknown samples should be converted to concentration units.
Quantitation of Unidentified Peaks

The method specifies how the software quantifies unidentified peaks. Three methods are available: using a constant calibration factor, using the calibration of the nearest component, or using the calibration of the nearest reference component.

Constant Calibration Factor

In creating a method, you can enter a constant calibration factor for possible quantitation of unidentified peaks. If the method specifies that this factor is to be used (rather than the calibration of the nearest component or reference component), the software divides a peak's area by the constant calibration factor to yield a raw amount. Then the adjusted amount is calculated as described under “Computation of Adjusted Amounts” on page 18-77.

Calibration of the Nearest Component or Reference Component

Unidentified peaks can be quantified by using the calibration curve of a neighboring peak — the nearest identified component or the nearest reference component, whichever is selected in the method. The software finds the appropriate neighboring peak and calculates a raw amount for the unidentified peak as if it were the neighbor (except the response of the unidentified peak is used in the calculation). Then the software calculates an adjusted amount as described under “Computation of Adjusted Amounts” on page 18-77.
Chapter 19

Converting File Formats

The Convert application lets you work with TotalChrom files in ASCII format. It also converts files between different formats.

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Converting TotalChrom Files to ASCII Text

All of the files used by the various TotalChrom functions, including methods, sequences, raw data, and result files, are stored in binary format. This means that you cannot examine the contents by simply viewing or printing the files directly. Most functions, such as the Method Editor and Reprocess Results windows, provide sufficient information about the files.

However, for some purposes, such as data system validation, you may need to examine these files in more detail. To do so, use the TCtoASCII menu to convert the file information to ASCII text.

The following procedure explains how to convert a TotalChrom source file into ASCII data for viewing, copying for use in another program such as Microsoft Excel, printing, or saving to disk.

➢ To convert TotalChrom source files to ASCII data:

1. Choose Convert from the Navigator Apps menu to open the Convert window.

2. Choose Create ASCII File in the TCtoASCII menu if you want to create ASCII file output as each file is converted.

   Convert changes the last letter of the source filename extension into an X. For example, the raw data file HALO001.RAW will be HALO001.RAX. The exception is index (.IDX) files, which are given the extension .IDN.
3. Choose a source file type from the TCtoASCII menu.
   A multiple file select dialog opens.
4. Select one or more files to convert and choose Open.
   ASCII data for the first converted file appears in the bottom portion of the Convert window. If you converted multiple files, you can select another file in the File Name list box.
5. View, copy (right click), print, or save the ASCII data as needed.

The ASCII files produced are accurate records of the contents of the TotalChrom files but, because of the nature of the data, they are not formatted for readability. The different sections of each file are marked by a header line, which indicates the structure, but no other internal documentation of the information is included. To identify each data item, you will need to refer to the help file TCSTRUCTURE.HLP (located in the Examples/TcApi directory in the TotalChrom path), which defines the structure of each TotalChrom file.

Many parameter values, including those you enter (such as sample amount) and those that are calculated (such as average calibration factor or calibration curve coefficients) are stored in double-precision floating-point format. TotalChrom always uses the full precision of these parameters internally but reduces the precision for readability when displaying or printing the information. For example, the coefficients reported from a first-order curve fit in an auto-calibration report or method printout might be

- Intercept = 0.058894
- Slope = 0.212087

but the full double-precision values stored in the method might be

- Intercept = 0.0588939316319134
- Slope = 0.212087362115202

While the precision reported by TotalChrom is sufficient for all normal analytical purposes, you will need to know the full internal precision if you need to duplicate the calculations that TotalChrom performs.
Converting Files to Other Formats

The formats into which you can convert TotalChrom files include PEN Metafiles from ACCESS*CHROM, Galactic multi-CGM files from the optional LC spectral analysis application, and a special data file in the standard format defined by the Analytical Data Exchange Protocol for Chromatographic Data (ASTM E 1947-98) to transfer chromatography data between data systems. TotalChrom conforms to Category 1 (raw data) and Category 2 (final results) of this specification.

There are significant differences in how A*C and TotalChrom store configuration data. When converting A*C methods to TC methods, the instrument information contained in the converted TC method is the TC default information and not the values that were in the original A*C method.

If you have been using the 2600 Chromatography Software, you can convert the raw data files to make them compatible with TotalChrom. You must start with a 2600 or PC Integrator .PTS file and the corresponding .HDR file to create a TotalChrom raw data file (.RAW file). You can also convert 2600 or PC Integrator .MET files to TotalChrom method files.

When converting 2600 data and methods to TotalChrom, you must review your new methods in the Method Editor to ensure that all of the options are set correctly. TotalChrom methods contain more information than 2600 methods. Due to minor differences in the way the 2600 software detects and integrates peaks, you may see slight differences in results when you use TotalChrom.

You can also use Convert as a user program by putting a file name on the command line. For example, if you put a .RAW file name on the command line, you can automatically create an .LC file during data analysis. Similarly, if you put an .RST file name on the command line, you can automatically create a .CDF file.
To convert files into other formats:

1. Choose Convert from the Navigator Apps menu to open the Convert window.

2. Choose the type of conversion you want from the Convert menu.

   The following conversions are available:

   - AIA to TC = .CDF files
   - TC to AIA = .RST files
   - TC to LC = .RAW files
   - PEN to TC = .PEN files
   - TC to PEN = .RST files
   - 2600 to TC Method = .MET files
   - 2600 to TC Data = .HDR files
   - ICP-MS to TC = .NC files
   - A*C to TC Data = .ACR files
   - A*C to TC Method = .ACM files

   A multiple file select dialog opens.

3. Select one or more files to convert and choose OK.

   TotalChrom displays messages about the progress of the conversion in the Conversion Status box. All messages are also written to a conversion file (CONVERT.LOG), which is stored in the LOG directory in the TotalChrom path.
4. To see a list of files that were converted in a single operation, click in the file name text box.

If an error occurred during the conversion of a file, then that file is shown in red.
The Review and Approve application opens when you select the Review button in the TotalChrom Navigator screen.

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What is Review and Approve?

The Review and Approve process requires one or more people, other than the analyst generating the data, to review and sign the work done by that analyst. Once data have been reviewed and the reviewer’s signature applied, that data is then available for one or more levels of approval signatures. After final approval has been applied to the reporting of a particular set of data, the status and security of that data must be changed to prevent any further modification.

21 CFR Part 11 is the set of United States federal regulations that governs how the use of electronic signatures can be applied to electronic records in a similar fashion. These regulations establish, among other things, the conditions under which the FDA will accept a computer-generated “signature”, such as a unique UserID and Password, as equivalent to a handwritten signature. The application of handwritten signatures to laboratory documents is guided by the Good Laboratory Practices (GLP) Regulations.

The Review and Approve environment consists of three main windows:

- **File/Sample List** window contains a list of sample information,
- **Chromatogram/Plot** window contains a chromatogram plot, or plots of the currently selected sample, or samples, from the file list, and
- **Report Window** contains a report that displays results from the samples.

These separate windows are docking windows, so that you are able to adjust the layout and relative sizes of the windows.
The display of any window may be toggled on or off using menu commands or toolbar buttons. Each window may also be closed using the close button on the docked window itself.

When a single file is selected in the file list, the plot window displays the chromatogram from that file and the report window displays results from that run (if available). You can annotate the chromatogram with sample information, peak names and/or other peak information, baselines and labels. The result data can be displayed in the form of a simple peak table and/or in a specific TotalChrom report format as defined by a .rpt file.

When more than one file is selected in the file list, the plot window displays all the chromatograms, in either a stacked or overlay display format and the report window displays a table containing a summary of results from all the files. There is no arbitrary limit to the number of chromatograms that can be displayed.

**About the Review and Approve Environment**

Data review and approval can only be performed by a person using TotalChrom with the proper permission configured through TotalChrom system configuration. The application does *NOT* provide any mechanism either to modify the data being reviewed (other than the application of signatures) or to allow that data to be reprocessed. All data reports and chromatographic images are rendered simply by displaying previously generated results, including peak baselines, and no report can be signed until it has been displayed on the screen.

**General Permissions**

The Review and Approve environment is incorporated into the standard TotalChrom Function Permissions Editor scheme (details are in the TotalChrom Application Manager's Guide). This means that an administrator is able to selectively disable any menu command (and associated toolbar button) or dialog control for a specific Job Type. However, the independent Review and Approve permissions will have the prevailing influence on commands relating to the review and approve process. For example, the administrator will not need to use the FPE to disable menu commands related to Review and Approve for Job Types that do not have the explicit approve permissions set.

**Selecting Data**

The Review and Approve environment is excellent for viewing different types of data files. Within the Review and Approve environment you will be able to load data for examination by selecting one or more files of any of the following file types:

- TotalChrom Report (TCR) --- This is the default data type
- Index (IDX)
- Sequence (SEQ)
- Result (RST)
TCR files are a new TotalChrom file type, specifically created for the Review and Approve process. It is a composite file consisting of an associated set of TotalChrom raw (.RAW), result (.RST) and report format files (.RPT).

If your intent is to approve reports then TCR files must be used. You can casually review the data of other file types but none of the signature Review and Approve functions will be available. Initially the Review and Approve looks for TCR files (with the same file name as the RST files found in the IDX/SEQ). If TCR files are not found it next looks for RST files. If no TCR or RST files are found, it then looks for RAW files.

**From SEQ or IDX files**

The selected IDX or SEQ files are read to obtain the result file names referenced within. These files are then sought using the standard TotalChrom search procedure (i.e. specified directory, then same directory as the primary file). Each result file found will produce an entry in the file list and the following columns will be populated with data from the result file.

**From RST files**

The rules relating to how the Raw File column is filled are as follows:

1. For TCR files - the RAW file is contained within.
2. For RST files - If there is no Modified file OR the "Load RAW file in preference to modified file" flag is set. The RAW file referenced in the seqdesc of the result file will be sought. If it is found then its name will appear in the Raw File column. If it is not found then there will be no line in the file list for that sequence row.
3. For RST files - If there is a Modified file AND the "Load RAW file in preference to modified file" flag is NOT set. The modified file referenced in the seqdesc of the result file will be sought. If it is found then its name will appear in the Raw File column. If it is not found then the RAW file will be sought instead. If this is found then its name will appear in the Raw File column of the file list, otherwise will be no line in the file list for that sequence row.

The Acquisition Time is always read from the file whose name appears in the Raw File column. This is basically the same procedure as before, that is, starting from the point at which RST files referenced in the IDX or SEQ file have been located.

**From RAW files**

When you select a RAW file in the File Open dialog, that file will be opened directly and no results will be available.

**Missing Files**

Any files sought during the process of loading the file list will be recorded in an error log. For each file not found a line will be added to the log in the format:
You may view the Error Log in the Property List dialog for file list. The log displays Channel A files followed by Channel B files. If no Channel B files are found then they will be entirely eliminated from the log, since this most likely indicates only single channel data were collected.

To view the Error Log:

1. Right click on a file in the file list and select Properties. The File List Properties dialog appears.
2. Select the Error Log tab to view the error log. See File List Window Properties Dialog.

The Review and Approve Process

The United States FDA regulations for the review and approval of electronic records are described in the general GLP and GMP regulations, the “Predicate Rules”, upon which 21 CFR Part 11 is based. These regulations require that any relevant records or data, in paper form or electronic, are reviewed by one or more authorized individuals other than the one who was responsible for the generation of those records, and that the reviewer(s) sign and date that record with an indication of the meaning of that signature (for example, Reviewed By). Once data have been reviewed, it can be submitted for final review by another individual, or submitted for approval and/or final approval, by a similar process of signings including a date and meaning of the signature. Such review and approval e-signatures are applied to TCR (TotalChrom Report) files.

The function of Review and Approve is associated with the review and approval of the reporting of data, rather than the data itself. This consists of a combination of information from the existing TotalChrom file types listed below:

- The Raw data for the sample (necessary to redraw plot images required for the report).
- The Result data used for the report.
- The Method used to generate that result file (as embedded in the TotalChrom RST file).
- The Report Format files used for all reports generated during processing (as specified in the Sequence, as Optional Reports in the Method, or as selected during processing in Batch or Graphic Editing).

In addition to the above, the TCR contains a Signatures Table associated with each report (effectively the same as each RPT file) defined within the TCR file. The signatures are applied to each report independently within the TCR file.
What is Review and Approve?

Assigning Job Type Permissions for Review and Approve

Based upon the configuration of the Review and Approve levels, the Job Type settings available for user access will be adjusted accordingly.

The permissions set for the Job Type indicate whether a member of that Job Type is basically authorized to sign for that level. Whether a user can sign a specific report at a particular level depends on whether he or she has already signed that report at some level and whether the system administrator has defined that no user can sign the same reports at two levels, for example:

<table>
<thead>
<tr>
<th>User A permissions</th>
<th>Existing signatures</th>
<th>Can a User sign two levels?</th>
<th>Next level</th>
<th>Can User A sign this level?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Review, Final Review</td>
<td>Reviewed – User A</td>
<td>Yes</td>
<td>Final Review</td>
<td>Yes</td>
</tr>
<tr>
<td>Final Review, Final Approve</td>
<td>Reviewed – User X Reviewed Final – User A</td>
<td>Yes</td>
<td>Approve</td>
<td>No</td>
</tr>
<tr>
<td>Final Review, Final Approve</td>
<td>Reviewed – User X Reviewed Final – User A Approved – User Z</td>
<td>Yes</td>
<td>Final Approve</td>
<td>Yes</td>
</tr>
<tr>
<td>Review, Final Review</td>
<td>Reviewed – User A</td>
<td>No</td>
<td>Final Review</td>
<td>No</td>
</tr>
<tr>
<td>Final Review, Final Approve</td>
<td>Reviewed – User X Reviewed – User Y</td>
<td>No</td>
<td>Final Review</td>
<td>Yes</td>
</tr>
<tr>
<td>Final Review, Final Approve</td>
<td>Reviewed – User X Reviewed Final – User A Approved – User Z</td>
<td>No</td>
<td>Final Approve</td>
<td>No</td>
</tr>
</tbody>
</table>

Report Status

Each report within a TCR file will indicate one of seven states. The status of a report can only be changed via the Review and Approve process and only following entry of a valid electronic signature by an authorized and valid (in the sense of not having signed it before, where applicable) user. The possible states a given report may take depend on the signature levels defined for the system.

- **Initial**: The state of each report within a new TCR file produced by TotalChrom will be Initial.
- **Reviewed**: A Review signature has been applied to the report but further Review signatures remain to be applied. (That is, the Final Review setting is enabled.)
- **Reviewed – Final**: The final Review signature has been applied but approval levels remain to be applied.
• **Reviewed – Complete:** The final Review signature has been applied and no approval levels are enabled.

• **Approved:** An Approve signature has been applied to the report but further Approve signatures remain to be applied. (That is, the Final Approve setting is enabled.)

• **Approved – Complete:** The final Approve signature has been applied.

• **Hold:** A Hold signature has been applied.

The signature levels that can be applied to a report depend upon its current status and the signature levels set in System Configuration.

<table>
<thead>
<tr>
<th>Current status</th>
<th>Next level enabled</th>
<th>Signatures that can be applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Review</td>
<td>Review, Hold</td>
</tr>
<tr>
<td></td>
<td>Final Review</td>
<td>Final Review, Hold</td>
</tr>
<tr>
<td></td>
<td>Approve</td>
<td>Approve, Hold</td>
</tr>
<tr>
<td></td>
<td>Final Approve</td>
<td>Final Approve, Hold</td>
</tr>
<tr>
<td>Reviewed</td>
<td>Final Review</td>
<td>Review, Final Review, Hold</td>
</tr>
<tr>
<td>Reviewed – Final</td>
<td>Approve</td>
<td>Approve, Hold</td>
</tr>
<tr>
<td></td>
<td>Final Approve</td>
<td>Final Approve</td>
</tr>
<tr>
<td>Reviewed – Complete</td>
<td>n/a</td>
<td>None</td>
</tr>
<tr>
<td>Approved</td>
<td>Final Approve</td>
<td>Approve, Final Approve, Hold</td>
</tr>
<tr>
<td>Approved – Complete</td>
<td>n/a</td>
<td>None</td>
</tr>
<tr>
<td>Hold</td>
<td>n/a</td>
<td>None</td>
</tr>
</tbody>
</table>

*A report with a status of Reviewed – Complete, Approved – Complete, or Hold cannot be changed.*

**General Requirements for Status Changes**

- The signature status of a report cannot be changed until it has been displayed on the screen.
- The reviewer and approver signatures can only be applied in that order.
- The reviewer and approver signatures cannot be from the same user if the option to require unique signatures is enabled in System Configuration.
- A status field in the .TCR file will be modified to reflect the state of each report.
- The review and approval signatures will be written to a “Signatures Table” appended to the printed report.
What is Review and Approve?

Initial

All TotalChrom .TCR files, when first created, will have the status of Initial applied to each report contain within. This status indicates that the data have not yet been reviewed or approved.

Reviewed

The status of a report will be changed to Reviewed when a Review signature has been applied to it but a Final Review is still required. Additional Review signatures may still be applied; these will create additional entries in the signature table but the status of the report will remain as Reviewed.

A file with a Reviewed status can still be set to Hold.

Reviewed - Final

This status indicates that the final Review signature has been applied but that at least one level of Approval is also required. This status may be reached following a single Review signature, when Final Review is not enabled, or following the Final Review signature.

A file with a Reviewed – Final status can still be set to Hold.

Reviewed – Complete

This status indicates that the final Review signature has been applied and no Approve signatures are required. This status may be reached following a single Review signature, when Final Review is not enabled, or following the Final Review signature.

A file with a Reviewed – Complete status cannot accept further signatures nor can its status be changed.

Approved

The status of a report will be changed to Approved when an Approve signature has been applied to it but a Final Approve is still required.

As with Review signatures, additional Approve signatures may still be applied; these will create additional entries in the signature table but the status of the report will remain as Approved.

A file with an Approved status can still be set to Hold.

Approved – Complete

This status indicates that the final Approve signature has been applied. This status may be reached following a single Approve signature, when Final Approve is not enabled, or following the Final Approve signature.
A file with an **Approved – Complete** status cannot accept further signatures nor can its status be changed.

**Hold**

The status applied by a reviewer or approver at any step of the process (except when the report is **Complete**) when a sample report is considered unacceptable for approval.

A file with a **Hold** status cannot accept further signatures nor can its status be changed.

**Review and Approve Levels**

In order to provide the greatest flexibility for different implementations, the configuration of review and approve levels required are accommodated through the TotalChrom System Configuration (SysConfig) and Job Type settings to provide single as well as multiple levels of signing capability.

The levels defined are:

- Review
- Final Review
- Approve
- Final Approve
- Hold

The system administrator will be able to enable any combination of these levels (including none). If any is enabled then the system will be considered to have **Review and Approve** functionality enabled.
The general requirements for signature levels are as follows:

- Any number of Review signatures can be added until a Final Review is applied. When the Final Review signature (there can only be one of these) has been added the report’s status changes to ‘Reviewed – Final’ (assuming Approve levels also exist).

- Once a report’s status has been changed to Reviewed – Final, no further Review signatures can be applied.

- Any number of Approve signatures can be added until a Final Approve is applied. When the Final Approve signature (there can only be one of these) has been added the report’s status changes to ‘Approved – Complete’

- Once a report’s status has been changed to Approved – Complete, its status can no longer be changed.

- Once a report’s status has been changed to Hold, its status can no longer be changed.

- Signature levels must be applied in order. For example, (assuming Review, Final Review, Approve and Final Approve are all enabled) the Final Review signature cannot be applied until at least one Review signature has been applied, nor can an Approve signature be applied until the report’s status is Reviewed – Final.

- The use of review and approve signatures can be completely disabled.

- The system administrator will be able to specify whether or not a user will be allowed to sign at more than one level for each report. Note that setting this option will NOT prevent a user being given permission to sign at more than one level, only that he or she will only be able to apply a signature at one of the permitted level for any given report.

**Software Behavior Based on Levels**

The combination of settings for either the Review or the Approval levels will control whether a single signature is required for each, or if multiple levels of signatures will be allowed/required. The following describes how the actions taken for each level depend on what other levels are enabled.

➢ **Review**

When both Review and Final Review are enabled, multiple Review signatures can be applied to a report prior to the Final Review signature. At least one Review signature will be required in this case.

Enabling the Review level without Final Review, will cause the system to allow only a single reviewer’s signature to be applied to a report. This will
act in effectively the same way as a configuration in which Final Review is enabled but not Review, although there will be differences in the user interface.

- **Final Review**
  
  When both Review and Final Review are enabled, at least one Review signature will be required before the Final Review signature can be applied. Enabling the Final Review level without Review will cause the system to allow only a single reviewer’s signature to be applied to a report.

- **Approve**
  
  When both Approve and Final Approve are enabled, multiple Approve signatures can be applied to a report prior to the Final Approve signature. At least one Approve signature will be required in this case. Enabling the Approve level without Final Approve, will cause the system to allow only a single approver’s signature to be applied to a report. This will act in effectively the same way as a configuration in which Final Approve is enabled but not Approve, although there will be differences in the user interface.

- **Final Approve**
  
  When both Approve and Final Approve are enabled, at least one Approve signature will be required before the Final Approve signature can be applied. Enabling the Final Approve level without Approve will cause the system to allow only a single approver’s signature to be applied to a report. Following the Approve signature the status of the report will always be set to ‘Approved – Complete’.
What is Review and Approve?

**Printed Reports**

This example shows a typical TotalChrom report generated from a result file (.RST) in conjunction with a report format file (.RPT) and the raw data file (.RAW) for the chromatogram data).

There is also a Report with Signature Table.
The components of such a report depend upon the definition in the .RPT file but they can include:

- System header — small, medium or large and optionally including instrument conditions
- Chromatogram (optional)
- User Title — printed below the System Header (or the chromatogram if one is included)
- User Header — printed immediately above the peak table (not included in this example)
- Peak Table — can include identified and/or unidentified peaks
- Group Tables — lists of components or peaks in each named or time group (not included in this example)
- Missing Component Table — list of components not found in the sample (not included in this example)
- Timed Event Table — lists timed events from the method (not included in this example)
- User Footer — printed below the last table (not included in this example)
What is Review and Approve?

Report with Signature Table

The following example shows how the same report would appear when printed from a TCR file. The signature table is appended to the very bottom of the report (it follows the footer).
Main View

The Review and Approve environment displays up to three docking windows in the main view. They are:

- The File List
- The Plot Window
- The Report Window

File List

When a single file is selected in the file list, the plot window displays the chromatogram from that file and the report window displays results from that run (if available).

When multiple files are selected the plot window displays all of the selected chromatograms (in stacked or overlay format depending on the current mode) and the report window displays a summary table of the results (if available).
Main View

**Plot Window**

When a single file is selected, selection of a peak in the chromatogram causes that peak to be selected in the peak table of the report window — if or when that tab is displayed.

When multiple files are selected, selection of a peak in any chromatogram causes the summary table to be scrolled so that the data relating to that peak is visible.

**Report Window**

When a single file is selected, selection of a peak in the peak table causes that peak to be selected (highlighted) in the chromatogram (unselecting any other object as necessary). If the chromatogram is scaled such that the peak is not currently visible, the chromatogram will be rescaled so that the peak is displayed.

Selection of a group component on the group tab of the peak list will cause the member peaks currently visible in the chromatogram to become selected (highlighted), unselecting any other object as necessary. If the chromatogram is scaled such that none of the member peaks is visible, the chromatogram will be rescaled so that at least the first member of the group identified in the plot is visible.

When multiple files are selected, selecting any cell containing peak data in the summary table causes that peak to be selected (highlighted) in the active chromatogram (unselecting any other object as necessary). If the chromatogram is scaled such that the peak is not currently visible, the chromatogram(s) will be rescaled so that the peak is displayed.
**Main Toolbar**

The Main Toolbar displays in the Main View when you select **Main Toolbar** from the View menu. The toolbar contains the following:

<table>
<thead>
<tr>
<th>Icon</th>
<th>Associated menu command</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="icon" alt="File/Open" /></td>
<td>File/Open</td>
</tr>
<tr>
<td><img src="icon" alt="File/Previous Data File" /></td>
<td>File/Previous Data File</td>
</tr>
<tr>
<td><img src="icon" alt="File/Next Data File" /></td>
<td>File/Next Data File</td>
</tr>
<tr>
<td><img src="icon" alt="File/Print" /></td>
<td>File/Print</td>
</tr>
<tr>
<td><img src="icon" alt="File/Print Preview" /></td>
<td>File/Print Preview</td>
</tr>
<tr>
<td><img src="icon" alt="View/File List" /></td>
<td>View/File List</td>
</tr>
<tr>
<td><img src="icon" alt="View/Chromatogram" /></td>
<td>View/Chromatogram</td>
</tr>
<tr>
<td><img src="icon" alt="View/Report" /></td>
<td>View/Report</td>
</tr>
<tr>
<td><img src="icon" alt="Plot/Overlay" /></td>
<td>Plot/Overlay</td>
</tr>
<tr>
<td><img src="icon" alt="Plot/Stacked" /></td>
<td>Plot/Stacked</td>
</tr>
<tr>
<td><img src="icon" alt="Plot/Default Scaling" /></td>
<td>Plot/Default Scaling</td>
</tr>
<tr>
<td><img src="icon" alt="Plot/Rescale" /></td>
<td>Plot/Rescale</td>
</tr>
<tr>
<td><img src="icon" alt="Plot/Select Active" /></td>
<td>Plot/Select Active</td>
</tr>
<tr>
<td><img src="icon" alt="Help/Contents" /></td>
<td>Help/Contents</td>
</tr>
<tr>
<td><img src="icon" alt="File/Exit" /></td>
<td>File/Exit</td>
</tr>
</tbody>
</table>

**Menu Bar**

The menu bar items are described in the following table.

The symbol ✔ in front of an item indicates that this is an on/off toggle command. The check mark appears in front of the item when the function is active or selected. The default for these commands is switched on. This applies to all menus (including context menus) described in this document.
<table>
<thead>
<tr>
<th>Menu</th>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>File</td>
<td>Open...</td>
<td>Displays the TotalChrom File Open dialog, enabling you to select data for review.</td>
</tr>
<tr>
<td></td>
<td>Ctrl+O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Previous Data File</td>
<td>Displays the data for the previous file in the list. If a single file is currently being displayed then the new data replaces the existing data. If multiple files are currently displayed (stacked or overlay) then the new file is added to the display set.</td>
</tr>
<tr>
<td></td>
<td>Next Data File</td>
<td>Displays the data for the next file in the list. If a single file is currently being displayed then the new data replaces the existing data. If multiple files are currently displayed (stacked or overlay) then the new file is added to the display set.</td>
</tr>
<tr>
<td>Print...</td>
<td>Ctrl+P</td>
<td>Displays the Print Options dialog, enabling you to select the type of data to be printed.</td>
</tr>
<tr>
<td>Print Preview...</td>
<td>Displays the Print Options dialog, enabling you to select the type of data to be displayed in the subsequent Preview window.</td>
<td></td>
</tr>
<tr>
<td>Print All Reports</td>
<td>Displays the Print All Reports dialog, enabling you to print reports from all files in the file list.</td>
<td></td>
</tr>
<tr>
<td>Save Plot Information</td>
<td>Saves the current properties and user labels of the displayed plot to a file associated with the raw data file. This file is automatically accessed the next time (in this session or another) that data file is loaded to recreate previous display. If multiple files are selected in the list then multiple plot information files will be created.</td>
<td></td>
</tr>
<tr>
<td>Action</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Save Plot As Metafile</td>
<td>Saves the current plot as an enhanced Window metafile (.emf)</td>
<td></td>
</tr>
<tr>
<td>Exit</td>
<td>Closes the Review and Approve window. If there are currently reports in the file list which have been marked with signature levels but have not been signed then, before the window closes, you will be warned that these exist. A ‘No’ response will cause the signature level markings to be discarded and no change will be made to the TCR files.</td>
<td></td>
</tr>
</tbody>
</table>
| Edit                   | Delete
Ctrl+Del
Copy Plot to Clipboard > B/W
Color

Enabled whenever a plot is displayed. Copies the selected plot to the Windows clipboard as a black and white bitmap. The background of the plot is set to white and all other items are set to black. Copies the selected plot to the Windows clipboard as a color bitmap. |
| View                   | ✔ File List
✔ Chromatograms
✔ Report

Toggles display of the file list window on and off.
Toggles display of the chromatogram/plot window on and off.
Toggles display of the report window on and off. |
| ➡ Main Toolbar
✔ Sign Toolbar
✔ Status Bar

Toggles display of the main toolbar on and off.
Toggles display of the sign toolbar on and off.
Toggles display of the status bar on and off. |
<p>| Properties             | Displays the Properties dialog for the active window (view).                |</p>
<table>
<thead>
<tr>
<th>Plot</th>
<th>Default Scaling</th>
<th>Restores the selected plot to default scaling. If the ‘Expand All Plots’ option is set then all displayed plots are restored to default scaling.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rescale...</td>
<td>Displays the Rescale dialog.</td>
<td></td>
</tr>
<tr>
<td>✓ Expand All Plots</td>
<td>Toggles the Expand All Plots function on and off.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| ✓ Overlay                | Sets Overlay mode for display of multiple plots                                 | Sets Overlay mode for display of multiple plots.  
|                          | Overlay and Stacked are mutually exclusive.                                    | Overlay and Stacked are mutually exclusive.                                                                                     |
| ✓ Stacked                | Sets Stacked mode for display of multiple plots                                 | Sets Stacked mode for display of multiple plots.  
|                          | Overlay and Stacked are mutually exclusive.                                    | Overlay and Stacked are mutually exclusive.                                                                                     |
|                          |                                                                                  |                                                                                                                                 |
| Title                    | Displays the Title Properties dialog.                                           |                                                                                                                                 |
| Add Label…               | Displays the Label Properties dialog and adds the label at the center of the active chromatogram. |                                                                                                                                 |
|                          |                                                                                  |                                                                                                                                 |
| Add Static Overlay…      | Displays the TotalChrom File Open dialog, enabling you to select a data file to act as a static overlay reference. Changes to ‘Remove Static Overlay’ when one exists. | Displays the TotalChrom File Open dialog, enabling you to select a data file to act as a static overlay reference. Changes to ‘Remove Static Overlay’ when one exists. |
| Replace Static Overlay… | Enabled when static overlay exists                                               |                                                                                                                                 |
| Add Plot Above Stack…    | Changes to ‘Remove Plot Above Stack’ when one exists.                           | Changes to ‘Remove Plot Above Stack’ when one exists.                                                                             |
| Replace Plot Above Stack…| Enabled when plot exists above stack                                            | Enabled when plot exists above stack                                                                                             |
| Properties               | Displays the Plot Properties dialog for the selected plot.                     | Displays the Plot Properties dialog for the selected plot.                                                                         |
This duplicates the action of View/Properties (when the Plot windows is selected) but its importance warrants this duplication

<table>
<thead>
<tr>
<th>Sign</th>
<th>Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Displays the Electronic Signature dialog. If the signature is validated then the review signature is added to the report. The report status changes appropriately (see Software Behavior Based on Level Settings) Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is in a state to accept a Review signature. Disabled if the TCR file is open for reading only.</td>
<td></td>
</tr>
</tbody>
</table>

| Final Review |
| Displays the Electronic Signature dialog. If the signature is validated then the final review signature is added to the report. The report status changes appropriately (see Software Behavior Based on Level Settings) Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is in a state to accept a Final Review signature. Disabled if the TCR file is open for reading only. |

<p>| Approve |
| Displays the Electronic Signature dialog. If the signature is validated then the approve signature is added to the report. The report status changes appropriately (see Software Behavior Based on Level Settings) Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is in a state to accept a Approve signature. Disabled if the TCR file is open for reading only. |</p>
<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
</table>
| Final Approve | Displays the Electronic Signature dialog. If the signature is validated then the final approve signature is added to the report. The report status changes appropriately (see Software Behavior Based on Level Settings)  
Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is in a state to accept a Final Approve signature.  
Disabled if the TCR file is open for reading only. |
| Hold     | Displays the ‘Reason for the Hold Report’ dialog if so configured. Then displays the Electronic Signature dialog. If the signature is validated then the Hold signature is added to the report. The report status changes to ‘Hold’)  
Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is in a state to accept a Hold signature.  
Disabled if the TCR file is open for reading only. |
| Comment  | Displays the ‘reason for a Hold’ associated with an unapproved report.  
Disabled unless a single TCR file is selected in the file list and the report currently displayed in the Report Window (if any) is ‘Hold’ (signed or unsigned).  
Disabled if the TCR file is open for reading only. |
| All Reports | Displays the Report Review Window.  
**Disabled unless the file list contains only TCR files.** |
| Tools    | Align >  
See Align commands for a full |
<table>
<thead>
<tr>
<th>Action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Ref (Align)</td>
<td>‘Ref’ if no ref plot set yet, else ‘Align’. Disabled if 2 time markers exist.</td>
</tr>
<tr>
<td>Plot Time Marker</td>
<td>‘Ref’ if no ref plot set yet, else ‘Align’.</td>
</tr>
<tr>
<td>Add Ref (Align)</td>
<td>Disabled if 2 voltage markers exist.</td>
</tr>
<tr>
<td>Plot Voltage</td>
<td>Enabled when markers exist.</td>
</tr>
<tr>
<td>Marker</td>
<td>Enabled when 1 or more time markers + 1 or more reference time markers.</td>
</tr>
<tr>
<td>Clear Markers</td>
<td>Enabled when 1 or more voltage markers + 1 or more reference voltage markers.</td>
</tr>
<tr>
<td>Shift Time</td>
<td>Enabled when 1 or more time markers + 1 or more reference time markers.</td>
</tr>
<tr>
<td>Shift Voltage</td>
<td>Enabled when 1 or more voltage markers + 1 or more reference voltage markers.</td>
</tr>
<tr>
<td>Scale Time From Origin</td>
<td>Enabled when 2 time markers + 2 reference time markers.</td>
</tr>
<tr>
<td>Scale Voltage From Origin</td>
<td>Enabled when 2 voltage markers + 2 reference voltage markers.</td>
</tr>
<tr>
<td>Scale Time</td>
<td>Enabled when 2 time markers + 2 reference time markers.</td>
</tr>
<tr>
<td>Scale Voltage</td>
<td>Enabled when 2 voltage markers + 2 reference voltage markers.</td>
</tr>
</tbody>
</table>

### Options
- **Options**
  - Displays the Review and Approve environment Options dialog.
  - Enabled at all times.

### Save Preferences
- **Save Preferences**
  - Saves the properties of the active plot as the default properties for initial display of plots (for the current user). Also saves current settings for docking window display/position/size, and columns/font settings for the file list and report windows.
  - Enabled whenever a plot is displayed.

### Help
- **Help Topics**
  - Displays the table of contents for the Help associated with the Review and Approve environment.
  - Although this command is not current Windows standard terminology it is used here for consistency with the rest of TotalChrom.
  - Enabled at all times.

### About Review and Approve
- **About Review and Approve**
  - Displays the About box containing version and copyright information.
**Status Bar**

The status bar (located along the bottom of the window) is used to display various miscellaneous information about the displayed data. It is divided into four segments (from the left-hand side of the window):

- The fully qualified file name of the active plot. If the file list contains .TCR files then this will be the TCR file name. If an alternative file type was used to access the data, then if result data are available the result file name will be displayed, otherwise the raw file name will be displayed.

- The coordinates (in time and mV units) of the current position of the mouse pointer. If the mouse pointer is not over the plot area then there will be a static display of the last pair of coordinates observed.

The status bar segments are dynamically sized as the main window is sized so that as much information as possible is displayed.
**About the Properties Inspector (Dialog)**

This is an overview of common components used throughout the application. The Properties Inspector dialog is a key dialog, used throughout the Data Review environment for displaying and editing the properties of various objects (such as labels). The Properties dialog remains open when a new object is clicked and its contents are updated to show the properties of the newly selected object. Since the contents of the dialog depend on the currently selected object, the dialog displays the generic title of Properties.

This dialog is always the same size and has the same basic layout. It will generally contain at least two pages (tabs) but for complex objects (such as components) more pages will appear.

The below example shows two pages from the Properties dialog for a user label. The **General tab** displays information that is characteristic of the setting type and **specific** to that particular setting. In this example the General tab contains just the number of the label within the set and the name of the raw data file for the plot the label appears on.

![Properties Dialog Example](image)

The second example tab, showing properties applied to text, is common to several of the graphic objects. When in a dialog, clicking F1 will display information specific to the displayed tab.
### Control Description

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
</table>
| ![Dialog mode button.](image) | Dialog mode button.  
Up position — dialog will close when a new object is selected.  
Down position — dialog remains open in the front-most window when a new object selected and displays its properties. |
| ![Selects the previous object of the same type.](image) | Selects the previous object of the same type.  
The dialog displays the new objects properties, regardless of the state of the dialog mode button.  
This button will appear when the selected object is a peak, baseline timed event, instrument timed event or user label.  
This button will not appear when the selected object is the active plot or plot title. |
| ![Selects the next object of the same type.](image) | Selects the next object of the same type.  
The dialog displays the new objects properties, regardless of the state of the dialog mode button.  
This button will appear when the selected object is a peak, baseline timed event, instrument timed event or user label.  
This button will not appear when the selected object is the active plot or plot title. |
| ![Displays Help window relating to the current display.](image) | Displays Help window relating to the current display. |
| ![Status bar, for display of tool tips relating to selected control.](image) | Status bar, for display of tool tips relating to selected control.  
For example, when you are selecting a font the following displays:  

```
Select the font for display of the text.
``` |
### About the Options Dialog

The Options dialog displays when you choose **Options** from the Tools menu.

![Options Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plot columns</td>
<td>An edit box defining the number of columns in the plot window.</td>
</tr>
<tr>
<td>Number of plot rows</td>
<td>An edit box defining number of rows visible in the plot window (additional rows may scroll into the window).</td>
</tr>
<tr>
<td>Display x axes on plots</td>
<td>A check box indicating whether the X-axis should be displayed on each plot. If the X-axis is not displayed there will be no vertical grid lines.</td>
</tr>
<tr>
<td>Display y axes on plots</td>
<td>A check box indicating whether the Y-axis should be displayed on each plot. If the X-axis is not displayed there will be no horizontal grid lines.</td>
</tr>
</tbody>
</table>
Options Dialog — Initial Scaling Tab

Control | Description
---|---
Time | The scaling mode (Autoscale and Absolute) applied to the time scale for chromatograms the first time they are displayed (assuming the file does not have an associated graphics properties file (.tcg)).
  - Autoscale: When this option is selected the ‘Start time’ and ‘End time’ fields will be disabled.
  - Absolute: When this option is selected the ‘Start time’ and ‘End time’ fields will be enabled.

Start time (min) | The start time for the plot when the Time scaling setting is Absolute.

End time (min) | The end time for the plot when the Time scaling setting is Absolute.

Response | The scaling mode (Autoscale, Relative, A/Z offset and Absolute) applied to the response scale for chromatograms the first time they are displayed (assuming the file does not have an associated graphics properties file (.tcg)).
  - Autoscale: When this option is selected the ‘Offset’ and ‘Full scale’ fields will be disabled.
Main View

- **A/Z offset**: When this option is selected the ‘Offset’ field will be disabled and the ‘Full scale’ field enabled.
- **Absolute**: When this option is selected the ‘Offset’ and ‘Full scale’ fields will be enabled.
- **Relative**: When this option is selected the ‘Offset’ field will be disabled and the ‘Full scale’ field will be replaced by the ‘Scale factor’ field.

<table>
<thead>
<tr>
<th>Offset</th>
<th>The offset in mV for the plot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full scale (mV)</td>
<td>The full scale range for the plot in mV.</td>
</tr>
<tr>
<td>Scale factor</td>
<td>The factor by which to expand the largest peak from full scale.</td>
</tr>
</tbody>
</table>
Options Dialog — Peaks/Components Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active peak</td>
<td>A color selector control for setting the color an integrated peak will be filled with when the mouse pointer moves over it.</td>
</tr>
<tr>
<td>Selected peak</td>
<td>A color selector control for setting the color an integrated peak will be filled with when it is selected.</td>
</tr>
<tr>
<td>Time reference</td>
<td>A drop-down list (of Regular, <strong>Bold</strong>, <em>Italic</em>, <strong>Bold Italic</strong>) enabling you to choose a text attribute to be applied to the names of peaks that are time reference peaks.</td>
</tr>
<tr>
<td>Internal standard</td>
<td>A drop-down list (of Regular, <strong>Bold</strong>, <em>Italic</em>, <strong>Bold Italic</strong>) enabling you to choose a text attribute to be applied to the names of peaks that are internal standards</td>
</tr>
</tbody>
</table>
**Report Print Options Dialog**

This dialog displays when you choose **Print** from the **File** menu.

![Print Options Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report</td>
<td>A check box that selects printing of the report using the currently assigned report format file (.rpt).</td>
</tr>
<tr>
<td>Optional reports</td>
<td>A check box that selects printing of the optional reports defined within the TCR file. It is only enabled when a TCR file that contains optional reports is selected.</td>
</tr>
<tr>
<td>Annotated replot</td>
<td>A check box that selects printing of the chromatogram as defined in the processing method.</td>
</tr>
<tr>
<td>Current view</td>
<td>A check box that selects printing of the current contents of the plot window.</td>
</tr>
<tr>
<td>Portrait</td>
<td>A radio button that selects printing of the replot in portrait orientation. It is only enabled when Current view is checked.</td>
</tr>
<tr>
<td>Landscape</td>
<td>A radio button that selects printing of the replot in landscape orientation. Only enabled when Current view is checked.</td>
</tr>
</tbody>
</table>
| OK                    | - From Print… Displays the standard Windows Print dialog for printer selection.  
                        | - From Print Preview… Displays the Preview window. |
| Cancel                | Closes the Options dialog with no action. |
Busy TCR File Warning Dialog

This dialog displays when you attempt to open a TCR file that is in use elsewhere on the TotalChrom system.

The dialog displays the name of the file that has been determined is busy and cannot be opened for editing. Your choices are:

- **Yes**: Opens the indicated file in read–only mode, which means it cannot be signed. Choosing this option will cause the warning dialog to be displayed again if any other TCR file selected (directly or indirectly via an IDX file) for opening are found to be busy.

- **Yes to All**: Opens the indicated file in read–only mode, and also any other TCR file selected (directly or indirectly via an IDX file) for opening that are found to be busy.

- **Cancel**: Cancels the File Open process.
**Print All Reports**

This dialog is displayed when you choose the Print All Reports command from the File menu.

![Print All Reports Dialog]

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>A drop-down list to select the printer to be used for output of the reports.</td>
</tr>
<tr>
<td>Properties</td>
<td>A command button that enables you to access the standard Windows Properties dialog for the selected printer.</td>
</tr>
<tr>
<td>Type</td>
<td>A static text field displaying the printer type (for example, the model name).</td>
</tr>
<tr>
<td>Where</td>
<td>A static text field displaying the port the printer is attached to.</td>
</tr>
<tr>
<td>Include optional reports from method</td>
<td>A check box indicating whether just the primary report or all reports will be printed.</td>
</tr>
<tr>
<td>Print only signed reports</td>
<td>A check box indicating whether only reports with associated signatures will be printed.</td>
</tr>
</tbody>
</table>
Selecting Data for Review

This section describes how to select TotalChrom data for review.

TotalChrom File Open Dialog

This is an enhanced version of the standard TotalChrom File Open dialog. Most of the elements of the File Open dialog are the standard Windows File Open controls. Only those elements unique to TotalChrom, or customized for this application will be described in the table below.

If you select one or more TCR files that are already being reviewed by another person elsewhere on the system, a warning message is displayed. You are given the option of opening the file in a read–only mode (i.e. Review without Approve) or quitting the file open process.

The initial directory in File Open dialog will be your default RST path, then last path accessed.
Selecting Data for Review

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Files of type</td>
<td>A drop-down list containing descriptions and extension of the types of file that may be selected. For example, Report Files (<em>.tcr), Result Files (</em>.rst), Raw Files (<em>.raw), Index Files (</em>.idx), Sequence Files (*.seq).</td>
</tr>
<tr>
<td>Quick paths</td>
<td>A drop-down list from which a new path can be selected. This changes the currently displayed directory to the selected path.</td>
</tr>
<tr>
<td><strong>Header &lt;&lt;</strong></td>
<td>A command button that reduces the size of the dialog, hiding the File Header Information box.</td>
</tr>
<tr>
<td>Clear existing file list</td>
<td>This check box determines whether the selected files will be added to the Review and Approve file list or they will replace the existing contents of the file list.</td>
</tr>
<tr>
<td>File Header Information</td>
<td>A scrolling memo field that displays the TotalChrom file header information from the currently selected file. The field is blank when more than one file is selected.</td>
</tr>
</tbody>
</table>

**File List Window**

When a single file is selected in the file list, the plot window displays the chromatogram from that file and the report window displays results from that run (if available).

When multiple files are selected the plot window displays all of the selected chromatograms (in stacked or overlay format depending on the current mode) and the report window displays a summary table of the results (if available). Each column header is described in the below table.
### Column in Table (abbreviation shown)

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Name</td>
</tr>
<tr>
<td>The name associated with the sample, taken from the sequence entry for this file.</td>
</tr>
<tr>
<td>Sample Number (Sample #)</td>
</tr>
<tr>
<td>The secondary identification associated with the sample, taken from the sequence entry for this file.</td>
</tr>
<tr>
<td>Type</td>
</tr>
<tr>
<td>The type of sample row, taken from the sequence entry for this file.</td>
</tr>
<tr>
<td>Channel (Chan)</td>
</tr>
<tr>
<td>The data channel the raw data was acquired on (A or B).</td>
</tr>
<tr>
<td>Acquisition Date/Time (Acq Date/Time)</td>
</tr>
<tr>
<td>The date and time of data acquisition. This is always taken from the raw data file (if it exists).</td>
</tr>
<tr>
<td>Raw File Name (Raw File)</td>
</tr>
<tr>
<td>The fully qualified name of the raw data file.</td>
</tr>
<tr>
<td>Result File Name (Result File)</td>
</tr>
<tr>
<td>The fully qualified name of the result file.</td>
</tr>
<tr>
<td>Instrument Method Name (Instrument Method)</td>
</tr>
<tr>
<td>The fully qualified file name of the instrument method referenced in the raw data file.</td>
</tr>
<tr>
<td>Processing Method Name (Process Method)</td>
</tr>
<tr>
<td>The fully qualified file name of the processing method referenced in the result file.</td>
</tr>
<tr>
<td>Calibration Method Name (Calibration Method)</td>
</tr>
<tr>
<td>The fully qualified file name of the calibration method referenced in the result file.</td>
</tr>
<tr>
<td>Baseline File Name (Baseline File)</td>
</tr>
<tr>
<td>The fully qualified name of the baseline raw data file.</td>
</tr>
<tr>
<td>Signature Status</td>
</tr>
<tr>
<td>The lowest common denominator status of the reports associated with the data file.</td>
</tr>
</tbody>
</table>

- If all files in the list use a single name for raw and result files, then only a single column (Data) appears.
Selecting Data for Review

- If all files in the list use only a single method then only a single column (Method) appears.

- The Channel column only appears if the list contains a mixture of files acquired on channels A and B. If all files in the list were acquired on the same channel (whether it was A or B) this column is not displayed (or available).

- The Baseline column only appears if a baseline subtraction was performed for at least one of the data files loaded in the file list.

- Signature Status is only available when the list contains TCR files.

- The lowest common denominator Signature Status is set to the lowest status existing for any report associated with the data file, where the status order is as follows: Hold, Review, Reviewed – Final, Approved, Approved – Final, Reviewed – Closed, Approved – Closed. When a mixed status is shown, it is displayed in blue.

The list columns are updated as necessary as new files are added. Ideally the column set would be updated (if necessary) when files are deleted, but this is not a requirement.

Missing data files will be indicated in the list by displaying their file names in a user-selected color. This would occur, for example, if a raw file referenced by a result file was not found or if a method referenced in a data file was not found.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Age (y)</th>
<th>Channel</th>
<th>Raw File</th>
<th>Result File</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/6/018</td>
<td>1</td>
<td>10/27/01 51 26AM</td>
<td>A</td>
<td>D:\Sample\sc_vuln_001.raw</td>
<td>D:\Sample\sc_vuln_001.txt</td>
<td>D:\Sample\sc_vuln_001.txt</td>
</tr>
<tr>
<td>18/6/018</td>
<td>3</td>
<td>05/01/01 08:30 15AM</td>
<td>B</td>
<td>D:\Sample\sc_wuln_001.raw</td>
<td>D:\Sample\sc_wuln_001.txt</td>
<td>D:\Sample\sc_wuln_001.txt</td>
</tr>
<tr>
<td>EUCO1</td>
<td>1</td>
<td>12/27/01 18:18AM</td>
<td>A</td>
<td>D:\Sample\sc_vuln_001.raw</td>
<td>D:\Sample\sc_vuln_001.txt</td>
<td>D:\Sample\sc_vuln_001.txt</td>
</tr>
<tr>
<td>EUCO1</td>
<td>2</td>
<td>07/01/01 20:01AM</td>
<td>B</td>
<td>D:\Sample\sc_wuln_001.raw</td>
<td>D:\Sample\sc_wuln_001.txt</td>
<td>D:\Sample\sc_wuln_001.txt</td>
</tr>
</tbody>
</table>

If there is a single Data column and either the raw or result file cannot be found then the file name will be displayed in a different user-selected color.

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column width</td>
<td>You can change column width by dragging the header boundary, in the standard way for list views. The boundary at the right-hand side of a column will size that column.</td>
</tr>
<tr>
<td>Columns shown</td>
<td>You may hide any column in the list. Columns to be displayed and columns to be hidden are defined in the Show/Hide Columns dialog.</td>
</tr>
<tr>
<td>Column order</td>
<td>Columns may be ordered by drag-and-drop. If the mouse button is held down while a column header is selected, that header may</td>
</tr>
</tbody>
</table>
Selecting Data for Review

- List sorting: The file list may be sorted based upon the data in any column. Clicking on the column header will cause the list to be sorted in ascending order of those values. Clicking the same column again will cause the list to be sorted in descending order. This follows standard Windows list view behavior. The file list may also be ordered manually by dragging an item to a new location. The default will be to move the dropped item to the line above the item it is dropped on, so that moving an item to the top of the list can be readily accomplished.

- Selection: A single row or multiple rows may be selected in the list. The standard Windows mechanisms for multiple selection (i.e. Shift+Click and Ctrl+Click) will be utilized. Whenever a row is selected the process of searching for the referenced files will be repeated. The appropriate column entries, and their colors, will be updated accordingly.

---

**File List Window Show/Hide Dialog**

- You may customize the File List window to display only the columns of interest.

  1. To show or hide the columns in the File List window, right click in the File List window and a context menu appears:
2. Clicking on the Show/Hide Columns... displays the following dialog:

![Show/Hide Columns dialog]

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns shown</td>
<td>A multiple–select list box containing the names of columns that are currently visible in the file.</td>
</tr>
<tr>
<td>Columns hidden</td>
<td>A multiple–select list box containing the names of columns that are available to be displayed but are hidden.</td>
</tr>
<tr>
<td>▶️</td>
<td>A command button that moves the selected column(s) to the <strong>Columns hidden</strong> list.</td>
</tr>
<tr>
<td>▶▶</td>
<td>A command button that moves all items in the shown list to the <strong>Columns hidden</strong> list.</td>
</tr>
<tr>
<td>◀️</td>
<td>A command button that moves the selected column(s) to the <strong>Columns shown</strong> list.</td>
</tr>
<tr>
<td>◀▶</td>
<td>A command button that moves all items in the hidden list to the <strong>Columns shown</strong> list.</td>
</tr>
</tbody>
</table>

*If you Hide all columns in the file list and the file list area becomes blank, you can redisplay (Show) columns in that area, by moving your cursor to the top of the blank area and right-click. Choose Show/Hide columns from the displayed menu then select the hidden columns that you wish to redisplay (Show).*
**File List Window Properties Dialog**

You can customize the colors and text in the File List window.

- To set the colors in the File List window, right click in the window and select Properties from the context menu. The following appears:

![File List Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missing files</td>
<td>A color selector for choosing the color indicating a missing file in the file list.</td>
</tr>
<tr>
<td>Raw or result file missing in Data column</td>
<td>A color selector for choosing the color indicating a missing raw or result file when the Data column appears in the file list.</td>
</tr>
<tr>
<td>Font</td>
<td>A drop–down list for choosing the font for display of text in the file list.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop–down list for choosing the size of the font used for file list display.</td>
</tr>
<tr>
<td>&lt;font sample&gt;</td>
<td>A read-only display of the word ‘Sample’ in the selected font at the selected size.</td>
</tr>
</tbody>
</table>
**Error Log**

The File List Error Log maintains a list of files that cannot be found as shown in the following example.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source File Name</td>
<td>The file that references the file which cannot be found.</td>
</tr>
<tr>
<td>Row #</td>
<td>The row of the sequence or index file which references the missing file (if applicable).</td>
</tr>
<tr>
<td>Chan</td>
<td>The channel of the sequence or index file which references the missing file (if applicable)</td>
</tr>
<tr>
<td>Missing File Name</td>
<td>The name of the missing file.</td>
</tr>
</tbody>
</table>
**Plot Window**

The plot window in the Data Review environment contains either a single plot layout or a number of stacked plots (that is, distinct plot layouts of equal size, one above the other).

- A single plot layout includes the case where several plots are overlaid on one another.
- In the case of stacked plots, you can adjust the number of plots in the stack is an environment parameter that can be (from 2 to 10, default 3).

**Graphic Objects**

These descriptions define the meaning of the following terms as they are used in this application.

- **Plot** - The display of a chromatogram, or several overlaid chromatograms, annotated with any or all of the following: baselines, timed events, peak names, retention times, user labels, etc.

- **Chromatogram** - The curve drawn as visual representation of the raw data collected from a chromatographic detector.

- **Annotation** - Additional information displayed superimposed on the chromatogram or plot area. This can be in the form of lines (as in baselines), text, numbers or pictures.
• **Labels** - Annotations consisting of text (or in the future pictures). These may be system labels or user labels.

• **System labels** - Labels whose content is supplied by the system rather than by the user. System labels are generally locked to the data (as for peak names) but certain types may be at a fixed position in the window (as for header information).

• **User labels** - Labels whose content is supplied by the user. User labels may be locked to the data or at a fixed position in the window.

• **Zoom box** - A rectangular object drawn on the plot by the user by performing a drag operation with the mouse. A zoom box is really a selection of a portion of the plot. Zooming is only one of the actions that can be performed on the selection.

### Sign Toolbar

The Sign Toolbar is displayed along with the Main Toolbar when Sign Toolbar in the View menu is checked.

<table>
<thead>
<tr>
<th>Icon</th>
<th>Associated menu command</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Icon]</td>
<td>Sign/Review</td>
</tr>
<tr>
<td>![Icon]</td>
<td>Sign/Final Review</td>
</tr>
<tr>
<td>![Icon]</td>
<td>Sign/Approve</td>
</tr>
<tr>
<td>![Icon]</td>
<td>Sign/Final Approve</td>
</tr>
<tr>
<td>![Icon]</td>
<td>Sign/Hold</td>
</tr>
<tr>
<td>![Icon]</td>
<td>Sign/Comment</td>
</tr>
</tbody>
</table>

*The enabled/disabled state of the Sign buttons does **not** depend on the job type of the current user or what permissions they have. It is solely determined by the signature level the currently displayed report can accept.*
Report Window

The Report window displays data from the row(s) currently selected in the File List.

<table>
<thead>
<tr>
<th></th>
<th>Component</th>
<th>RT</th>
<th>Area</th>
<th>BL</th>
<th>Adj Amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>0.057</td>
<td>184799.86</td>
<td>BV</td>
<td>0.0000</td>
</tr>
<tr>
<td>2</td>
<td>MEK</td>
<td>0.182</td>
<td>5934038.44</td>
<td>VE</td>
<td>2109.7097</td>
</tr>
<tr>
<td>3</td>
<td>iso-Butanol</td>
<td>0.353</td>
<td>133204.73</td>
<td>EV</td>
<td>0.1332</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl Acetate</td>
<td>0.411</td>
<td>7452255.49</td>
<td>VE</td>
<td>2553.3860</td>
</tr>
<tr>
<td>5</td>
<td>Cyclo-C6</td>
<td>0.549</td>
<td>397701.80</td>
<td>VV</td>
<td>173.1826</td>
</tr>
<tr>
<td>6</td>
<td>MIBK</td>
<td>0.620</td>
<td>7438945.89</td>
<td>VV</td>
<td>2578.6918</td>
</tr>
<tr>
<td>7</td>
<td>Toluene</td>
<td>0.828</td>
<td>2118558.53</td>
<td>W</td>
<td>794.8801</td>
</tr>
<tr>
<td>8</td>
<td>Butyl Cellosolve</td>
<td>0.959</td>
<td>2596701.96</td>
<td>W</td>
<td>2.5967</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl Benzene</td>
<td>1.105</td>
<td>1724250.31</td>
<td>VE</td>
<td>552.1854</td>
</tr>
<tr>
<td>10</td>
<td>o-Xylene</td>
<td>1.361</td>
<td>100776.43</td>
<td>*EV</td>
<td>0.1008</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When a single result file is selected you can elect to display a report created by applying a format file to the result file, an ad hoc report based on data from the result file, or both. The formatted report will appear on a tab labeled Report while the ad hoc may produce up to three tabs (Peak Table, Group Table and a specific detail tab for a selected group). A tab labeled Header, displaying the standard TotalChrom report header, will always appear.

When multiple files are selected in the file list, the contents of the report window will display a summary of the component results for all selected files. The list of components will be obtained from the first file in the selection. The summary will be displayed in a tabular (grid) format, with a row for each selected file.
Viewing Chromatograms

You view chromatogram through the plot window. This next section describes the plot window and how it works in Review and Approve.

About the Plot Window

The plot window in the Data Review environment contains either a single plot layout or a number of stacked plots (that is, distinct plot layouts of equal size, one above the other).

- A single plot layout includes the case where several plots are overlaid on one another.
- In the case of stacked plots, you can adjust the number of plots in the stack is an environment parameter that can be (from 2 to 10, default 3).

Graphic Objects

These descriptions define the meaning of the following terms as they are used in this application.

- **Plot** - The display of a chromatogram, or several overlaid chromatograms, annotated with any or all of the following: baselines, timed events, peak names, retention times, user labels, etc.
• **Chromatogram** - The curve drawn as visual representation of the raw data collected from a chromatographic detector.

• **Annotation** - Additional information displayed superimposed on the chromatogram or plot area. This can be in the form of lines (as in baselines), text, numbers or pictures.

• **Labels** - Annotations consisting of text (or in the future pictures). These may be system labels or user labels.

• **System labels** - Labels whose content is supplied by the system rather than by the user. System labels are generally locked to the data (as for peak names) but certain types may be at a fixed position in the window (as for header information).

• **User labels** - Labels whose content is supplied by the user. User labels may be locked to the data or at a fixed position in the window.

• **Zoom box** - A rectangular object drawn on the plot by the user by performing a drag operation with the mouse. A zoom box is really a selection of a portion of the plot. Zooming is only one of the actions that can be performed on the selection.

### About the Plot

The fundamental layout of a plot is shown below:

Each instance of such a layout is also referred to as a container. The Plot Area is the central subdivision of the whole plot in which the actual chromatograms are plotted.
All elements of the plot except for the plot area are in fact optional. You may choose to show or hide the axes and also choose whether component names and peak labels are shown in the optional zones or within the plot area itself (or not shown at all).

The X–Axis represents time and the Y–Axis represents signal level (detector response). One or more chromatograms may be displayed within a single plot container (not all host environments will support this feature.). When the Overlay display mode is selected multiple chromatograms are displayed within the same container, although each may be displayed at a different scaling. When up to four overlaid chromatograms are shown additional Y axes will appear on the right–hand side of the plot, as indicated above. The labels on these axes will use the same color as the associated curve (grayed if necessary). The Y–axis displayed on the left–hand side will be that of the currently active chromatogram. When more than four chromatograms are overlaid, no additional Y–axes will be displayed (only that of the active curve).

- **The size of the axis zones will be optimized based on their contents.** For example:

  1. If no Y–axes are displayed on the right hand side then this zone will simply not exist and take up no space.

  2. If the Y–axis label (e.g. “Response (mV)” as shown below) is not displayed then the size of the zone will be reduced.

All axis numeric labels will use horizontal text. The plot window can also consist of multiple containers. When the mouse pointer is over the plot window its form changes from the default arrow to a cross hair cursor: 

### Viewing Chromatograms - Plot Window

In its simplest form, the chromatogram is a plot of the data acquired from a chromatographic detector. Chromatograms may be selected (generally by picking from a list, but direct selection may be used if it proves practicable), but they will not generally be directly manipulated (dragged).

Provided that associated results data exist, the chromatogram may be annotated with peak names, retention times, baselines and baseline timed events. If results data exist, a peak may be selected by clicking on it (on the plot or in the area enclosed below the signal curve). When you click on a peak it fills with color. You can also click on peak information in the peak table to select the peak.
You have many options for changing the scaling of the chromatogram, to enable examining various portions of the plot in greater detail.

The normal form of plotting the chromatogram data is as a line (as shown above). In this style each data point is simply joined to the next by a straight line. (The initial line segment is drawn horizontally from the Y-axis to the first data point at a time given by the data interval.)

**Timed Event**

A timed event will be indicated by its standard letter code (see table below) displayed rotated by 90° (i.e., perpendicular to the X-axis). The exact time of the event will be indicated by a short tick mark, intersecting the chromatogram, preceding the event name. Depending on the event type the abbreviation may be preceded by a plus sign (+) or minus sign (−), indicating On or Off and/or followed by a number representing an associated value. Examples of both formats are shown below. A timed event label will generally be located a fixed distance above the signal level at the time at which it occurs.

Timed event properties include the time of the event, its type and its values (where applicable). The color and font attributes (including text color) of the annotation could be global settings.

**Integration Timed Event**

Integration timed events are also known as baseline timed events. The former term is preferred since they include events that affect aspects of integration other than baseline placement.
When you place the mouse over an event (after a brief or usual delay), a tool tip displays the full description of the event type.

Integration timed event information will always be obtained from the results. In Data Review the event is always shown at the actual time at which it was executed. That is, at the adjusted time calculated by Analyze from the method time of the event and the method and actual times of the nearest reference peak. This adjusted time is saved in the result file. The event type is provided in the results as a numeric value.

The available timed events used in the chromatographic display are listed below. The function or application of these events to chromatographic processing will not be performed through any action of the Data Review environment. That will remain the function of the TotalChrom Analysis software. The Plot Window is only responsible for placement of these labels appropriately on the chromatogram display.

<table>
<thead>
<tr>
<th>Event</th>
<th>Value</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunching factor</td>
<td>1 – 99</td>
<td>BF</td>
</tr>
<tr>
<td>Noise threshold</td>
<td>1 – 999999</td>
<td>NT</td>
</tr>
<tr>
<td>Area threshold</td>
<td>5 – 10E15</td>
<td>AT</td>
</tr>
<tr>
<td>Enable peak detection</td>
<td>+P</td>
<td></td>
</tr>
<tr>
<td>Disable peak detection</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Enable negative peak detection</td>
<td>+N</td>
<td></td>
</tr>
<tr>
<td>Disable negative peak detection</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Inhibit end of peak detection</td>
<td>+I</td>
<td></td>
</tr>
<tr>
<td>Allow end of peak detection</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Start non-forced common baseline</td>
<td>+CB</td>
<td></td>
</tr>
<tr>
<td>End non-forced common baseline</td>
<td>CB</td>
<td></td>
</tr>
<tr>
<td>Force the end of current peak</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Force start of new peak</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Force baseline to current point</td>
<td>BL</td>
<td></td>
</tr>
<tr>
<td>Force horizontal forward baseline</td>
<td>+HF</td>
<td></td>
</tr>
<tr>
<td>Stop horizontal forward baseline</td>
<td>HF</td>
<td></td>
</tr>
<tr>
<td>Force horizontal backward baseline</td>
<td>HR</td>
<td></td>
</tr>
<tr>
<td>Start manual integration window</td>
<td>+M</td>
<td></td>
</tr>
<tr>
<td>End manual integration window</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Start valley-to-valley baselines</td>
<td>+V</td>
<td></td>
</tr>
<tr>
<td>End valley-to-valley baselines</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Force an exponential skim</td>
<td>+X</td>
<td></td>
</tr>
<tr>
<td>Prevent an exponential skim</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Force a tangential skim</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Smooth Peak Ends On</td>
<td>+SM</td>
<td></td>
</tr>
<tr>
<td>Smooth Peak Ends Off</td>
<td>-SM</td>
<td></td>
</tr>
<tr>
<td>Force retention time of current peak to be the event time</td>
<td>RT</td>
<td></td>
</tr>
<tr>
<td>Locate peak retention time at the maximum data point</td>
<td>LM</td>
<td></td>
</tr>
<tr>
<td>Force a baseline at any point in data file</td>
<td>UF</td>
<td></td>
</tr>
</tbody>
</table>

**Instrument Timed Event**

In most respects these are handled in an identical manner to integration time events except for one important difference: The data defining the instrument events will always be obtained from the Method rather than from the results. This is because instrument event times are never adjusted.

**User Label**

The user label consists of free form text that may be positioned anywhere on the plot. You have complete control over the content as well as all other properties. The properties include most font attributes (including text color); rotation, border (yes/no); background color; and check boxes to indicate whether the label is linked to the chromatogram (as for peak names positioned at the peak apex), a data axis location (as for peak names displayed below the plot) or a fixed position in the window (as for header information).
It will be possible to save user labels (and type and location of system labels) associated with a raw data set, so that when that raw data set is displayed in the future the previous view of the data can be restored.

**Multiple Chromatogram Layouts**

There are two fundamental display modes available for displaying multiple chromatograms:

- **Stacked** - Each chromatogram is displayed within its own container. (The containers are of equal size)

- **Overlay** - All chromatograms are display within the same container

  There are also two variant formats — static stacked and static overlay — that are, respectively, a variant of the stacked form and a hybrid of stacked and overlay.

**Stacked**

The basic form of the Stacked display is the most flexible layout and allow you to define how many containers appear in the plot window by setting values for Number of Rows (from 2 to 10, initial default 3) and Number of Columns (from 1 to 10, initial default 1).

The individual containers will not be sizable, but whenever the plot window is resized the displayed containers will be resized appropriately. The following diagrams illustrate some of the possible layouts within the plot window:

These examples show some of the rules for creating the plot grid.

- Each column is filled with a plot before additional rows are created
- All containers are the same size; blank containers exist where necessary

The following examples illustrate these rules using real chromatograms. First, a plot window configuration of Columns = 2 and Rows = 3.
Overlay

When Overlay is selected, all selected chromatograms are displayed in a single container. The displayed X–axis and primary Y–axis (the one on the left–hand side) scales will be those of the active chromatogram.

- **The display of secondary Y–axis scales is determined by the following rules:**
  1. If all plots are at the same Y scale, then only the primary Y–axis scale appears (since it is shared by all chromatograms)
  2. If the plots do not share the same y scale then secondary Y–axis scales will appear for each additional chromatogram, up to a maximum of three (four including the primary scale)
  3. If there are more than four overlaid chromatograms, then only the scale of the active chromatogram (the primary Y–axis scale) is shown.

The currently active chromatogram can be selected through a drop–list control (containing the file names) in the toolbar, or by clicking on that curve in the plot window. The baselines, component names, peak labels displayed on an overlay plot are also those of the active chromatogram. User labels however, are a special case.
**Static Stacked Plot**

To compare several chromatograms against a standard chromatogram (or conversely, several standards against an unknown sample) there is a command (Add Plot Above Stack…) which inserts a specified chromatogram above the current stack. This command is not available (not enabled in the main menu and not present in the context menu) if the number of columns in the plot window is greater than one.

Upon selecting the command, you are prompted to select a raw or result file, via the standard TotalChrom File Open dialog. The standard is not taken from the file list (although it could appear there) and it will not be added to the file list once chosen. The space occupied by the plot window will not change, nor will the total number of plot containers visible (including the blank plot containers), nor the size of each container. The chosen chromatogram will now occupy the top container in the plot window and the previously stacked chromatograms (one fewer of these than before is initially visible in the window) will scroll in the area beneath the static standard.

*Clicking the scroll bar down button (this moves the contents of the window up) will cause the ‘static’ plot to remain where it is and just the remaining plots in the stack will scroll beneath it.*

**Static Overlay Plot**
This feature is similar in purpose to the static stacked plot. In this case the standard chromatogram is overlaid on one of the stacked plots, but it says in that relative position such that when the stack is scrolled the standard appears overlaid on a different plot. This command is not available (not enabled in the main menu and not present in the context menu) if the number of columns in the plot window is greater than one.

When the command (Add Static Overlay) is issued you will be prompted to select a raw or result file, via the standard TotalChrom File Open dialog. The standard is not taken from the file list (although it could appear there) and it will not be added to the file list once chosen. The space occupied by the plot window will not change, nor will the total number of plot containers visible (including the blank plot containers), nor the size of each container. The stack appears essentially unchanged, except that the standard is overlaid at the selected position.

**Scaling the Chromatogram via a Zoom Box**

To draw a zoom box, you can drag the mouse across the plot area. It should really be called a selection rectangle, since it selects an area of the plot and is used for other actions in addition to zooming, but zoom box is well understood and much shorter.

The zoom box is sizeable by dragging handles positioned on its corners and edges, and movable by dragging.

If the Expand All Plots menu item is checked then a zoom on any chromatogram will be applied to all other displayed chromatogram (either stacked or overlay mode). By this is meant that the scale for each plot is changed by the same factors as for the zoomed chromatogram, not that each is set to the same X and Y scale values.
To cause the zoom to occur, click within the zoom box, or select Zoom from the context menu displayed when you right-click within the zoom box. To remove the zoom box without zooming click anywhere outside the zoom box.

**Scaling the Chromatogram Using Keystrokes**

It will be possible to perform simple pan and zoom operations on a chromatogram using keystrokes. Supported actions will be as described in the table below.

<table>
<thead>
<tr>
<th>Key</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>→</td>
<td>The visible segment of the chromatogram is shifted later in time by 10% of the current X-axis range. That is, the start time and end time are both increased by 10% of the current X-axis range.</td>
</tr>
<tr>
<td>←</td>
<td>The visible segment of the chromatogram is shifted earlier in time by 10% of the current X-axis range. That is, the start time and end time are both decreased by 10% of the current X-axis range.</td>
</tr>
<tr>
<td>↑</td>
<td>The Offset of the chromatogram is increased by 10% of the Full Scale. The viewed segment of the chromatogram is moved down within the window.</td>
</tr>
<tr>
<td>↓</td>
<td>The Offset of the chromatogram is decreased by 10% of the Full Scale. The viewed segment of the chromatogram is moved up within the window.</td>
</tr>
</tbody>
</table>
**Viewing Chromatograms**

<table>
<thead>
<tr>
<th>Key Sequence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl + →</td>
<td>The chromatogram is zoomed around the center of the X–axis scale. The start time is increased by 5% of the X–axis range and the end time is decreased by an equal amount.</td>
</tr>
<tr>
<td>Ctrl + ←</td>
<td>The chromatogram is un–zoomed around the center of the X–axis scale. The start time is decreased by 5% of the X–axis range and the end time is increased by an equal amount.</td>
</tr>
<tr>
<td>Ctrl + ↑</td>
<td>The chromatogram is zoomed, in both X and Y directions, about the midpoint of the plot. The start time is increased by 5% of the X–axis range and the end time is decreased by an equal amount. The Offset is increased by 10% of the Full Scale and the Full Scale is decreased by 10%.</td>
</tr>
<tr>
<td>Ctrl + ↓</td>
<td>The chromatogram is unzoomed, in both X and Y directions, about the midpoint of the plot. The start time is decreased by 5% of the X–axis range and the end time is increased by an equal amount. The Offset is decreased by 10% of the Full Scale and the Full Scale is increased by 10%.</td>
</tr>
</tbody>
</table>

**Selecting Objects in a Plot**

Since a plot is a variegated collection of discrete objects rather than a continuous stream like a block of text, objects are selected by clicking on them, rather than by dragging over them. In general, a selected object will be indicated by a line (generally dotted but not always) surrounding it.

- Pressing the Esc key when the plot window has focus will deselect any selected object on the active plot (but the active plot remains active).
- A right–click on an object will first select it (deselecting other objects if appropriate) and then display the context menu for that item. This avoids confusion about which object the chosen command will act on. Selecting an object will deselect any other existing selected object.

**Selecting a peak**

If the chromatogram is associated with a result file then when the mouse pointer moves over them the peaks are filled in with color. When the mouse pointer moves off the peak again the fill–in is removed. Clicking on a peak also fills the peak with color. This time it is a different color (to indicate selection) and the color is not removed when the mouse pointer moves off the peak. Clicking again within the same peak removes the selection, or clicking on another peak switches the selection,
removing the color from the first peak. The colors used for this feature are selectable in the Options dialog from the Tools menu (Peaks/Components tab).

Selecting any part of the composite peak object (peak curve, peak label or peak name) serves to select the entire object. For example, clicking on the peak name will cause the peak curve to be filled in with color and the peak label to be shown in the selected state, as well as the peak name.

If the report window is currently displaying the peak table or RPT report, then when a peak is selected in the chromatogram the line corresponding to that peak is highlighted in the report (if it is included). If necessary the report is scrolled so that the line is visible. If a group tab (or the header tab) is showing there is no selection.

When the Peak Properties dialog is open clicking on the Next button will select the peak immediately following the currently selected one in the chromatogram. If the currently selected peak is the last peak in the chromatogram, then the Next command will wrap around and selected the first peak in the chromatogram. If the crest of the newly selected peak is not within the currently displayed segment of the chromatogram, the display is updated to display the segment (maintaining the same time span) that contains the peak. The segment is set such that the newly selected peak is approximately in the center of the window. In this example (the peak selection highlight has been eliminated here for clarity:

![Chromatogram Diagram](image)

The initial time span is from 10.5 to 12.5 minutes (i.e. 2 minutes). The Next command selects the peak at 18.838 minutes.
The 2 minute time span has been maintained and the new X–axis scale is 17.85 to 19.85 minutes.

The Previous button in the Peak Properties dialog acts in a similar manner, selecting the preceding peak in the chromatogram, rescaling if necessary, and wrapping to the last peak in the run when required.

**Selecting a Timed Event**

When the mouse pointer is moved over a timed event (instrument or baseline) it responds by displaying the event code with a border around it (if the event properties already include a border then the border will change color).

Left or right clicking on an event will select it. When an event is selected, the code is displayed with a dotted border around it. Right clicking on an event will display its context menu.

**Selecting a User Label**

When the mouse pointer is moved over a user label it will respond by displaying the label with a border around it (if the label properties already include a border then the border will change color).

Left or right clicking on a user label will select it. When a user label is selected, it will be displayed with a dotted border around it. Right clicking on a user label will display its context menu.
Viewing Chromatograms

Plot Window Properties Dialog

You can set the Plot Window Properties using the Properties dialog. You can access it by right clicking in the Plot Window and select Properties from the context menu.

Plot Properties Dialog – General Tab

The General tab displays the information that is characteristic of the object type and specific to the particular object.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Indicates the type of object displayed (a TotalChrom raw file in this example) and the following information about the raw file:</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full raw file path name of the active plot.</td>
</tr>
<tr>
<td>Acquisition date and time</td>
<td></td>
</tr>
<tr>
<td>Name of user who set up the acquisition</td>
<td></td>
</tr>
<tr>
<td>Actual run time</td>
<td></td>
</tr>
<tr>
<td>Delay time (if any)</td>
<td></td>
</tr>
<tr>
<td>Sampling rate</td>
<td></td>
</tr>
<tr>
<td>Number of raw data points in the file</td>
<td></td>
</tr>
</tbody>
</table>
### Plot Properties Dialog – Active Curve Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>A drop-down list used to set the type of plot display. <strong>Line, Raw Points, Raw/Bunch Pts</strong></td>
</tr>
<tr>
<td>Style</td>
<td>A drop-down list used to set the style of the plot line. <strong>Solid, Dot, Dashed, Dot–Dashed</strong></td>
</tr>
<tr>
<td>Width</td>
<td>A drop-down list used to set the width of the plot line. <strong>Default, 1 point, 2 points, 3 points, 4 points, 5 points</strong></td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control used to set the color of the plot line.</td>
</tr>
</tbody>
</table>
## Plot Properties Dialog – Options Tab

![Plot Properties Dialog – Options Tab](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color (Baselines)</td>
<td>A color selector control used to set the color of the baselines.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop-down list used to set the style of the baselines.</td>
</tr>
<tr>
<td>Color (Background)</td>
<td>A color selector control used to set the color of the plot background.</td>
</tr>
<tr>
<td>Grid (check box)</td>
<td>A check box that determines whether or not vertical and horizontal grid lines are displayed on the plot.</td>
</tr>
<tr>
<td>Grid (drop-down list)</td>
<td>A drop-down list that sets the style of the grid lines.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list that determines the scope of the parameter change (This plot, All plots).</td>
</tr>
</tbody>
</table>
## Plot Properties Dialog – Annotations Tab

![Plot Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Show baselines</td>
<td>A check box that determines whether or not peak baselines are displayed on the plot.</td>
</tr>
<tr>
<td>Show component names</td>
<td>A check box that determines whether or not peak names are displayed on the plot.</td>
</tr>
<tr>
<td>Show peak labels</td>
<td>A check box that determines whether or not peak labels are displayed on the plot.</td>
</tr>
<tr>
<td>Show instrument events</td>
<td>A check box that determines whether or not instrument timed events are displayed on the plot.</td>
</tr>
<tr>
<td>Show baseline events</td>
<td>A check box that determines whether or not baseline timed events are displayed on the plot.</td>
</tr>
<tr>
<td>Show user labels</td>
<td>A check box that determines whether or not user labels are displayed on the plot.</td>
</tr>
<tr>
<td>Component names</td>
<td>Radio buttons that determine the position of peak names when these are displayed.</td>
</tr>
<tr>
<td>Peak labels</td>
<td>Radio buttons that determine the position of peak labels when these are displayed.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list (This plot, All plots) that determines the scope of the parameter change.</td>
</tr>
</tbody>
</table>
**Plot Properties Dialog – Axes Tab**

![Plot Properties Dialog](image_url)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start (min)</td>
<td>An edit box which sets the start time of the plot display (i.e. the minimum X-axis value).</td>
</tr>
<tr>
<td>End (min)</td>
<td>An edit box which sets the end time of the plot display (i.e. the maximum X-axis value).</td>
</tr>
<tr>
<td>Offset</td>
<td>An edit box which sets the minimum of the Y-axis.</td>
</tr>
<tr>
<td>Full scale</td>
<td>The full range displayed on the Y-axis (i.e. Ymax – Offset)</td>
</tr>
<tr>
<td>Line color</td>
<td>A color selector control used to set the color of the axes.</td>
</tr>
<tr>
<td>Labels color</td>
<td>A color selector control used to set the color of the axes labels.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop–down list that determines the scope of the parameter change (This plot, All plots).</td>
</tr>
</tbody>
</table>
**Plot Properties Dialog – Title Tab**

![Plot Properties Dialog – Title Tab](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left (Upper row)</td>
<td>A drop–down list that determines the data item to be displayed on the left–hand side of the top row of the plot title.</td>
</tr>
<tr>
<td>Center (Upper row)</td>
<td>A drop–down list that determines the data item to be displayed in the center of the top row of the plot title.</td>
</tr>
<tr>
<td>Right (Upper row)</td>
<td>A drop–down list that determines the data item to be displayed on the right–hand side of the top row of the plot title.</td>
</tr>
<tr>
<td>Left (Lower row)</td>
<td>A drop–down list that determines the data item to be displayed on the left–hand side of the second row of the plot title.</td>
</tr>
<tr>
<td>Center (Lower row)</td>
<td>A drop–down list that determines the data item to be displayed in the center of the second row of the plot title.</td>
</tr>
<tr>
<td>Right (Lower row)</td>
<td>A drop–down list that determines the data item to be displayed on the right–hand side of the second row of the plot title.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop–down list that determines the scope of the parameter change.</td>
</tr>
</tbody>
</table>
**Plot Window - Plot Title Properties Dialog**

Information about the chromatogram is displayed above the plot. Through these dialogs you can customize the plot title text properties (such as the type, color, and size of the font) and position of the plot title.

*Plot Title Dialog — General Tab*

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Plot Title</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full raw file path name of the displayed plots(s).</td>
</tr>
</tbody>
</table>
### Plot Title Dialog — Text Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop-down list enabling you to select a font for the baseline event label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop-down list enabling you to select the font size for the title.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop-down list enabling you to select the text style for the title.</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the title.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal</td>
</tr>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list that determines the scope of the parameter changes (<em>This plot</em>, <em>All plots</em>).</td>
</tr>
</tbody>
</table>
**Plot Title Dialog — Plot Title Tab**

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left (Upper row)</td>
<td>A drop-down list that determines the data item to be displayed on the left-hand side of the top row of the plot title.</td>
</tr>
<tr>
<td>Center (Upper row)</td>
<td>A drop-down list that determines the data item to be displayed in the center of the top row of the plot title.</td>
</tr>
<tr>
<td>Right (Upper row)</td>
<td>A drop-down list that determines the data item to be displayed on the right-hand side of the top row of the plot title.</td>
</tr>
<tr>
<td>Left (Lower row)</td>
<td>A drop-down list that determines the data item to be displayed on the left-hand side of the second row of the plot title.</td>
</tr>
<tr>
<td>Center (Lower row)</td>
<td>A drop-down list that determines the data item to be displayed in the center of the second row of the plot title.</td>
</tr>
<tr>
<td>Right (Lower row)</td>
<td>A drop-down list that determines the data item to be displayed on the right-hand side of the second row of the plot title.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list that determines the scope of the parameter change.</td>
</tr>
</tbody>
</table>
Plot Window - Baseline Events Dialog

Using the Baseline Events dialog, you can customize how you want your report to display. This dialog contains two tabs — General and Text properties. Details of these two tabs are shown below.

Baseline Event Dialog — General Tab

The General tab displays the information that is characteristic of the object type and specific to the particular object.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Indicates which baseline event is selected of the total number on the plot.</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full result file path name from which the event data were taken.</td>
</tr>
</tbody>
</table>
Baseline Event Dialog — Baseline Timed Event Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>A read–only edit box displaying the actual time at which the event took effect (Adjusted for actual RT of nearest reference peak where applicable). This value is taken from the result file.</td>
</tr>
<tr>
<td>Event</td>
<td>A read–only drop–down list, displaying the event type.</td>
</tr>
<tr>
<td>Value</td>
<td>A read–only edit box displaying the value associated with the event (where applicable).</td>
</tr>
<tr>
<td>Level</td>
<td>A read–only field displaying the signal level associated with a User Forced peak start or end. This control does not appear unless the event is a User Forced peak start or end.</td>
</tr>
</tbody>
</table>
## Baseline Event Dialog — Label Tab

![Baseline Event Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop-down list enabling you to select a font for the baseline event label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop-down list enabling you to select the font size for the title.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop-down list enabling you to select the text style for the title. Styles supported by the selected font (of Regular, Bold, Italic, Bold Italic)</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the title.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal</td>
</tr>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list (of This event, All baseline events - this plot, All baseline events - all plots) that determines the scope of the parameter changes.</td>
</tr>
</tbody>
</table>
**Plot Window - User Label Properties Dialog**

You have the ability to create and position a label anywhere on the plot. Through these dialogs you can create the content and properties for the label, such as the type, color, and size of the font.

**User Label Properties Dialog — General Tab**

![User Label Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Indicates which label is selected of the total number on the plot.</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full raw file path name of the displayed plots(s).</td>
</tr>
</tbody>
</table>
### User Label Properties Dialog — Text Tab

![User Label Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchor label to X data</td>
<td>Determines whether the label is displayed at a specific time on the plot (On) or at the same horizontal position in the window regardless of scaling (Off).</td>
</tr>
<tr>
<td>Anchor label to Y data</td>
<td>Determines whether the label is displayed at a specific response level on the plot (On) or at the same vertical position in the window regardless of scaling (Off).</td>
</tr>
<tr>
<td>Text</td>
<td>The text for the label.</td>
</tr>
<tr>
<td>Execute shell command</td>
<td>A check box to run the shell command defined in the <code>&lt;entry field&gt;</code>.</td>
</tr>
<tr>
<td>Test button</td>
<td>Click to test that the shell command runs correctly.</td>
</tr>
<tr>
<td><code>&lt;entry field&gt;</code></td>
<td>Enter the shell command (for example, file name, program name, URL, etc) to be associated with this label. You can use the shell command to link to a document that provides more information. For example, c:\document.htm. To execute a shell command</td>
</tr>
</tbody>
</table>
### User Label Properties Dialog — Label Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop–down list enabling you to select a font for the label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop–down list enabling you to select the font size for the label.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop–down list enabling you to select the text style for the label.</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the label.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal</td>
</tr>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop–down list that determines the scope of the parameter changes.</td>
</tr>
</tbody>
</table>
**Plot Window - Instrument Event Properties Dialog**

The displayed timed events display read-only information obtained from the TotalChrom analysis.

**Instrument Event Dialog — General Tab**

![Instrument Event Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Indicates which instrument event is selected of the total number on the plot.</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full result file path name from which the event data were taken.</td>
</tr>
</tbody>
</table>
**Instrument Event Dialog — Instrument Timed Event Tab**

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>A read–only edit box displaying the time at which the event was executed.</td>
</tr>
<tr>
<td>Event</td>
<td>A read–only drop–down list, displaying the event type.</td>
</tr>
<tr>
<td>Value</td>
<td>A read–only edit box displaying the value associated with the event (where applicable).</td>
</tr>
</tbody>
</table>
**Instrument Event Dialog — Label Tab**

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop–down list enabling you to select a font for the baseline event label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop–down list enabling you to select the font size for the title.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop–down list enabling you to select the text style (of <em>Regular</em>, <em>Bold</em>, <em>Italic</em>, <em>Bold Italic</em>) for the title.</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the title.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal.</td>
</tr>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop–down list (of <em>This event</em>, <em>All instrument events - this plot</em>, <em>All instrument events - all plots</em>) that determines the scope of the parameter changes.</td>
</tr>
</tbody>
</table>
Viewing Peak/Component Information

The Peak Component text is taken from the result file (or the method depending on the environment) and is not editable. You may choose to have the basic location of the peak names below the plot or at the top of the peaks. Properties of peak names that will be editable (apart from position) include most font attributes (including text color), rotation, border (yes/no) and background color.

➢ To edit the label, right click on it and select Properties from the context menu.

The following example is the Plot Window with a Selected Peak Shown.

Only one peak can be selected at a time.
Plot Window: Viewing Peak/Components - Properties Dialog

Through the Peak or Component Properties dialog, you can customize how you want your peak or component to display. This dialog contains eight tabs. Details of these tabs are described below.

Peak/Component Properties Dialog — General Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object Description</td>
<td>Indicates the type of object displayed (a peak in this case) and the following information about the peak:</td>
</tr>
<tr>
<td></td>
<td>• The peak number (and the total number of peaks)</td>
</tr>
<tr>
<td></td>
<td>• The retention time of the peak (in minutes)</td>
</tr>
<tr>
<td></td>
<td>• The times of the start and end of the peak baseline</td>
</tr>
<tr>
<td></td>
<td>• The peak name (if it is matched to a component)</td>
</tr>
<tr>
<td>Date File/s:</td>
<td>The full path name of the result file from which the data are taken.</td>
</tr>
</tbody>
</table>
### Peak/Component Properties Dialog — Peak Tab

![Properties dialog]

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak information</td>
<td>Detailed information about the peak:</td>
</tr>
<tr>
<td></td>
<td>• Peak # (and total number of peaks in the plot)</td>
</tr>
<tr>
<td></td>
<td>• Type (Normal Peak, Expo Parent or Expo Child)</td>
</tr>
<tr>
<td></td>
<td>• Retention time (minutes)</td>
</tr>
<tr>
<td></td>
<td>• RT found using (Quad Fit at Crest, Assigned Crest, Maximum Crest, First Moment)</td>
</tr>
<tr>
<td></td>
<td>• Peak Area (µV*s)</td>
</tr>
<tr>
<td></td>
<td>• Peak Height (µV)</td>
</tr>
<tr>
<td></td>
<td>Baseline start time, level and type (Baseline resolved, Valley fit, Forced begin, Forced end, Forced valley’, Pos to neg, etc.)</td>
</tr>
<tr>
<td>Label type</td>
<td>A drop-down list containing the data items that can be selected to label the peak. The data items are: &lt;None&gt;, Adjusted Amount, Area, Area %, Delta RT Percent, Height, Raw Amount, Relative Retention, Retention Time.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list that determines the scope of the parameter change. Your choices are: This peak, All peaks – this plot, All peaks – all plots</td>
</tr>
</tbody>
</table>
Peak/Component Properties Dialog — Label Tab

This tab contains parameters identical to the Name tab.

*The Rotation slider and entry field are disabled if both the Peak Label and the Peak Name are displayed above the peak. That is, when the two labels are concatenated they cannot be rotated.*

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop–down list enabling you to select a font for the label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop–down list enabling you to select the font size for the label.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop–down list enabling you to select the text style for the label.</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the label.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal</td>
</tr>
</tbody>
</table>
## View Peak/Component Information

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop-down list (of <strong>This peak</strong>, <strong>All peaks – this plot</strong>, <strong>All peaks – all plots</strong>) that determines the scope of the parameter changes.</td>
</tr>
</tbody>
</table>

### Peak/Component Properties Dialog — Component Tab

![Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>A read-only combo box displaying the component name (if the peak is matched to a component). This field is blank if the peak is unidentified.</td>
</tr>
<tr>
<td>Reference</td>
<td>A read-only drop-down list displaying the name of the time reference component used in the identification process for this component. This field is blank if the peak is unidentified or if a time reference peak was not used.</td>
</tr>
<tr>
<td>ISTD</td>
<td>A read-only drop-down list displaying the name of the internal standard assigned to this component (if any). This field is blank if the peak is unidentified or if an internal standard was not assigned.</td>
</tr>
</tbody>
</table>
Use Component as Retn reference

A read–only check box that indicates whether the current component is a time reference peak. This is unchecked if the peak is unidentified or if the component was not a time reference peak.

Use Component as ISTD

A read–only check box that indicates whether the current component is an internal standard. This is unchecked if the peak is unidentified or if the component was not an internal standard.

---

Peak/Component Properties Dialog — ID Tab

Control | Description
---|---
Expected | A read–only edit box that displays the expected retention time for the component (i.e. the time set in the method). This field is blank is the peak is unidentified.
Absolute window | A read–only edit box that displays the absolute search window value. This field is blank is the peak is unidentified.
Relative window | A read–only edit box that displays the relative search window value. This field is blank is the peak is unidentified.
Find tallest peak in window | A read–only check box that indicates whether the tallest peak in the search window was identified as the component (checked) or the peak nearest the center of the search window (unchecked).
## Peak/Component Properties Dialog — Calibration Tab

![Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
</table>
| Calibration type   | A read–only drop–down list indicating the type of calibration process used for the component. For an unidentified peak, this list will show the process used for unidentified peaks:  
  - Use calibration factor  
  - Use nearest component  
  - Use nearest reference |
| Curve fit type     | A read–only drop–down list indicating the type of curve fit used for calibration of the component. This control is blank if the calibration type is not Use curve. |
| Scaling            | A read–only drop–down list indicating the Amount scaling function used. This control is blank if the peak is unidentified. |
| Weighting          | A read–only drop–down list indicating the calibration data weighting function used. This control is blank if the peak is unidentified. |
| Response           | Read–only radio buttons indicating the response type used for quantitation. |
### Viewing Peak/Component Information

<table>
<thead>
<tr>
<th>&gt; Area</th>
<th>When selected, area was used for component quantitation. When selected, height was used for component quantitation. For an unidentified peak this list will show the default response type set for unidentified peaks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; Height</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin Treatment Include</th>
<th>A read-only check box indicating whether or not the calibration curve included the origin in the curve fit process. This is unchecked if the peak is unidentified or if the curve did not include the origin in the curve fit process.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin Treatment Force</td>
<td>A read-only check box indicating whether or not the calibration curve was forced to pass through the origin. This is unchecked if the peak is unidentified or if the curve was not forced to pass through the origin.</td>
</tr>
</tbody>
</table>
**Peak/Component Properties Dialog — Levels Tab**

![Properties Dialog](image)

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity (%)</td>
<td>A read–only edit box displaying the standard purity for the component. The edit box is blank if the peak is unidentified.</td>
</tr>
<tr>
<td>&lt;grid&gt;</td>
<td>A read–only grid displaying the calibration level information for the component. The grid is blank if the peak is unidentified or if the component has no calibration level data.</td>
</tr>
</tbody>
</table>
Peak/Component Properties Dialog — Name Tab

This tab is identical to that for the User Label Properties dialog, with the exception of the contents of the Apply to drop-down list (see below).

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop–down list enabling you to select a font for the label.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop–down list enabling you to select the font size for the label.</td>
</tr>
<tr>
<td>Color</td>
<td>A color selector control enabling you to select the color of the text.</td>
</tr>
<tr>
<td>Style</td>
<td>A drop–down list enabling you to select the text style for the label.</td>
</tr>
<tr>
<td>Background</td>
<td>A color selector control enabling you to select the background color for the label.</td>
</tr>
<tr>
<td>Border</td>
<td>A check box that determines whether a border (in the same color as the text) will be drawn around the label.</td>
</tr>
<tr>
<td>Rotation (slider)</td>
<td>A slider that determines the rotation of the label counterclockwise from horizontal</td>
</tr>
<tr>
<td>Rotation (edit box)</td>
<td>An edit box in which you can directly type the rotation.</td>
</tr>
<tr>
<td>Apply to</td>
<td>A drop–down list (of This peak, All peaks – this plot, All peaks – all plots) that determines the scope of the parameter changes.</td>
</tr>
</tbody>
</table>
Report Window Overview

Interactions Between Windows

File List Selection

If a **single row** is selected in the file list then the report window displays results from that file (if a result file is found) or just file header information (if only the raw file is found). The plot window displays the chromatogram from that file (if available).

If **multiple rows** are selected then the report window displays a summary of results from those rows for which result files were found. The plot window shows the chromatograms in stacked or overlay format, depending upon the option setting.

Peak/Group List Selection

You may select a row in the peak list or group list table. If the selected item is a peak, this will cause the associated peak to be selected in the plot (i.e. filled in with the single peak TotalChrom color), unselecting any other object as necessary. If the selected peak is not currently visible (because the plot is expanded) the displayed start and end times will be adjusted (by the same amount) so that the selected peak is displayed approximately in the middle of the window. If the software determines that the peak width (start to end) is greater than the current X-axis scale, then the scale will be increased such that the peak start and end points are included in the displayed time range. Note that a peak cannot be selected on the group detail tab (since this would conflict with being in 'group mode' - of critical importance in the editing environments).

If the selected item is a group then that group will be shown selected (unselecting any other object as necessary). That is, all peaks that belong to the group will be filled in with the TotalChrom group peak color, and the name of the group will be displayed (also in the group peak color) at the approximate mid-point of the group. If the current time scale of the X-axis is such that not all peaks belonging to the group are visible, then the scale will be increased such that all peaks in the group are included.

Peak Selection in Plot Window

When a peak is selected in a chromatogram in the plot window, and the Peak List tab is displayed in the Report Window, then that peak will be selected in the Peak List table.
**Report Window Show/Hide Dialog**

This same basic dialog may be displayed from the Peak Table, Group Table and Group Detail tabs, and also the Summary view. These versions differ only in the contents of the two list boxes; Columns shown and Columns hidden.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns shown</td>
<td>A multiple–select list box containing the names of columns that are currently visible in the selected table.</td>
</tr>
<tr>
<td>Columns hidden</td>
<td>A multiple–select list box containing the names of columns that are available for the selected table but are hidden.</td>
</tr>
<tr>
<td>[&gt;]</td>
<td>A command button that moves the selected column(s) to the hidden list.</td>
</tr>
<tr>
<td>[&gt;&gt;]</td>
<td>A command button that moves all items in the shown list to the hidden list.</td>
</tr>
<tr>
<td>[&lt;]</td>
<td>A command button that moves the selected column(s) to the shown list.</td>
</tr>
<tr>
<td>[&lt;&lt;]</td>
<td>A command button that moves all items in the shown list to the shown list.</td>
</tr>
</tbody>
</table>
Report Properties Dialog

Using the Report Properties dialog, you can customize how you want your report to display. This dialog contains two tabs — General and Text properties. Details of these two tabs are shown below.

Report Properties Dialog — General Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Display peak table and group table</td>
<td>Displays the peak information and (if there is any) the peak group information on separate tabs.</td>
</tr>
<tr>
<td>Display results based on a report format file</td>
<td>Displays a TotalChrom report on the Report tab.</td>
</tr>
<tr>
<td>Use report format referenced in result file</td>
<td>Display report in format specified by report format file named in result file.</td>
</tr>
<tr>
<td>Use specified report format</td>
<td>Use the report format file specified in the entry field to display the results.</td>
</tr>
<tr>
<td>&lt;entry field&gt;</td>
<td>An edit box in which you can type the full path name of a report format file.</td>
</tr>
<tr>
<td>&lt;folder button&gt;</td>
<td>A command button that displays a TotalChrom File Open dialog, enabling you to select a report format file (.rpt). This button is only enabled when the ‘Use specified report format’ option is selected.</td>
</tr>
</tbody>
</table>
Report Properties Dialog — Text Properties Tab

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop-down list enabling you to select a font for the report window display.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop-down list enabling you to select a font size (not greater than 20 pt) for the report window display.</td>
</tr>
<tr>
<td>&lt;sample box&gt;</td>
<td>Displays the word Sample in the select font at the selected size.</td>
</tr>
</tbody>
</table>

Viewing Reports and Results from a Single TotalChrom Report (TCR) File

When a TCR file is selected in the file list, rather than a result file (or raw file), the main differences are:

1. The Report Window will contain tabs for every report associated with the TCR file. This includes the main report (specified via the RPT file in the sequence description) as well as any optional reports (from the Method and/or specified from Batch or Graph Editing).

2. One or more of the Sign menu commands and toolbar buttons may be enabled (depending on the status of the TCR file)
3. If the file list contains more than one TCR file but no other file types then the All Reports command in the Sign menu will be enabled.

Example of Report Window for TCR containing three reports (Report, Opt 1 and Opt 2).
Viewing Reports and Results from a Single Result File

When a single result file is selected you can elect to display a report created by applying a format file to the result file, an ad hoc report based on data from the result file, or both. The formatted report will appear on a tab labeled Report while the ad hoc may produce up to three tabs (Peak Table, Group Table and a specific detail tab for a selected group). A tab labeled Header, displaying the standard TotalChrom report header, will always appear (it is always the rightmost tab).

Report Window — Peak Table Tab

The Peak Table is a list view that displays data from the TotalChrom result file (or the result file embedded in a TCR file). This tab will be displayed when an ad hoc report format is being used. The result data will be displayed in a list view with a row for each peak detected.

The data columns displayed will be determined by selections you made in the Show/Hide Columns dialog (see the Report Window).
The complete set of data columns available is described below:

<table>
<thead>
<tr>
<th>Column in Table (abbreviation shown)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted Amount (Adj Amount)</td>
<td>The final calculated amount obtained in quantitation from the raw amount through the use of the dilution factor and the conversion factors.</td>
</tr>
<tr>
<td>Peak Area (Area)</td>
<td>The measured area of the peak (in microvolt–seconds) in the chromatogram, after the baseline has been drawn and integration performed.</td>
</tr>
<tr>
<td>Area Percent (Area %)</td>
<td>The ratio of the peak’s area to the sum of all peak areas listed in the main report. Expressed as a percentage, the sum in this calculation includes only the areas of peaks listed in the main report. It does not include peak areas of unidentified peaks if these peaks are not reported. Thus, the percentages in an area percent column will always add up to 100.</td>
</tr>
<tr>
<td>Metric/Expression</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Area/Raw Amount (Area/Amt)</td>
<td>The ratio of the peak’s area to the raw amount it represents. For a component with a linear response, this is equivalent to the calibration factor for the peak.</td>
</tr>
<tr>
<td>Area/Height</td>
<td>The ratio of the peak’s area to its height.</td>
</tr>
<tr>
<td>Baseline Code (BL)</td>
<td>A two-letter code that may be printed as part of the analysis report, indicating how the peak’s baseline was drawn. The first letter indicates the baseline treatment at the peak start, and the second letter indicates the baseline treatment at the peak end.</td>
</tr>
<tr>
<td>Calibration Range Flag (Cal Range)</td>
<td>A symbol that indicates whether the peak response is within the calibrated range for the component or not. A minus sign (–) indicates that the peak response lies below the calibrated range. A plus sign (+) indicates that the peak response lies above the calibrated range. A blank represents a response within range. An asterisk (*) indicates one or more levels in the method were uncalibrated for that component.</td>
</tr>
<tr>
<td>Component Name (Component)</td>
<td>The name of the component identified in the method to which the peak was matched.</td>
</tr>
<tr>
<td>Delta RT Percent (Delta RT %)</td>
<td>The difference between the expected retention time of a component (as specified in the calibration section of the method) and the actual retention time of the identified peak. Delta retention time is expressed as a percentage of the expected retention time.</td>
</tr>
<tr>
<td>Peak Height (Height)</td>
<td>The height, above the peak baseline, of the peak crest, measured in microvolts.</td>
</tr>
<tr>
<td>Internal Standard Amount Ratio (ISTD Amt Ratio)</td>
<td>The ratio of the component amount to the related internal standard amount. This data type applies only to peaks that have been identified and quantified based on the internal standard method. The amount ratio is always based on raw amounts, never on adjusted amounts.</td>
</tr>
<tr>
<td>Internal Standard Response Ratio</td>
<td>The ratio of the peak’s response to the response of the related internal standard.</td>
</tr>
</tbody>
</table>
### Viewing Reports and Results from a Single Result File

<table>
<thead>
<tr>
<th>(ISTD Resp Ratio)</th>
<th>component. This data type applies only to peaks that have been identified and quantified based on the internal standard method.</th>
</tr>
</thead>
</table>
| k prime (k’ )     | The capacity ratio of the peak. This expresses how many times longer the compound took to pass through the chromatography column, retarded by the stationary phase, than it would have if it was completely unretained. It is calculated as the ratio of the retention time of the peak (corrected for the void time) to the void time:  

\[
k' = \frac{RT_{\text{peak}} - RT_{\text{void}}}{RT_{\text{void}}}
\]

| Normalized Amount (Norm Amt) | The adjusted amount of the peak expressed as a percentage of the total reported amounts. These percentages add up to the normalization factor specified in the sequence file.  

\[
\text{Normalized Amount} = \frac{Q_i}{\sum_{i=1}^{n} Q_i} F
\]

where \(Q_i\) is the adjusted amount of an individual peak and \(F\) is the normalization factor in the sequence file. |
| Normalized Area Percent (Norm Area) | The peak’s percentage contribution to the total area for all peaks for which results are reported. These percentages add up to the normalization factor specified in the sequence file.  

\[
\text{Normalized Area Percent} = \frac{A_i}{\sum_{i=1}^{n} A_i} F
\]

where \(A_i\) is the area of an individual peak and \(F\) is the normalization factor in the sequence file. The sum in this calculation includes only the areas of peaks listed in the main report. |
| Peak Number (Pk #) | An index assigned to each peak detected in a run. |
### Percent Amount (% Amt)

The adjusted amount for the peak expressed as a percentage of the sum of the adjusted amounts for all the peaks listed in the main report. The values in a percent amount column always add up to 100 because the amounts for peaks excluded from the main report are not added into the sum.

### Raw Amount (Raw Amt)

The amount of a component represented by the peak, as calculated from the calibration curve or by applying a response factor. Unlike adjusted amount, this amount does not take into account the dilution factor, multiplier, divisor, or addend. If necessary, raw amounts have had scaling reversed, have been converted from amount ratios to amounts, and have sample volume adjustments made.

### Relative Retention (Rel RT)

The retention time of the peak compared to that of a specified relative retention reference peak.

\[
RRT = \frac{(RT_{peak} - void \ time)}{(RT_{reference} - void \ time)}
\]

### Retention Time (RT)

In an analysis report, this is the actual elution time of the peak (in minutes), as measured from the start of the run.

### Voltage Range Flag (Volt Range)

A symbol that indicates if the signal to the interface went over or under this range during the elution of the peak. If a 900 Series Interface collected the data analyzed, a plus sign (+) indicates that a peak’s height is higher than the voltage range for which the interface was set during data acquisition. A minus sign (–) indicates that a peak’s height is below the voltage range. If a peak is within the voltage range, its field in this column will be blank. If a LINK Interface was used, a plus sign indicates that a peak’s height exceeds 999990 counts. A minus sign indicates that a peak’s height is less than 10 counts.

The initial default set of columns will be: Pk #, Component, RT, BL, Area, Height, Adj Amt
**Report Window — Group Table Tab**

The Group Table is a list view that displays peak group data from the TotalChrom result file (or the result file embedded in a TCR file). This tab will be displayed whenever an ad hoc report is being used and the result file (or TCR) contains group data.

The selection of data columns displayed for groups will be independent of that set for the peak list (but again, will be the same for all files). The columns displayed will be determined by selections you made in the Show/Hide Columns dialog. The initial default set of columns is: Group #, Type, Component, RT, Area, Height Adj Amt.
The complete set of data columns available is described below:

<table>
<thead>
<tr>
<th>Column in Table (abbreviation shown)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted Amount (Adj Amount)</td>
<td>The final calculated amount obtained in quantitation from the raw amount through the use of the dilution factor and the conversion factors.</td>
</tr>
<tr>
<td>Peak Area (Area)</td>
<td>The total area of the peak group measured as the sum of the areas of all group member peaks (in microvolt–seconds).</td>
</tr>
<tr>
<td>Area Percent (Area %)</td>
<td>The ratio of the total group area to the sum of all peak areas listed in the main report, expressed as a percentage.</td>
</tr>
<tr>
<td>Area/Raw Amount (Area/Amt)</td>
<td>The ratio of the total group area to the raw amount it represents.</td>
</tr>
<tr>
<td>Area/Height</td>
<td>The ratio of the total group area to its ‘height’.</td>
</tr>
<tr>
<td>Component Name (Component)</td>
<td>The name of the group component identified in the method.</td>
</tr>
<tr>
<td><strong>Group Number</strong> (Grp #)</td>
<td>An index assigned to each group in the run.</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td><strong>Peak Height</strong> (Height)</td>
<td>The sum of the heights of all group member peaks, measured in microvolts.</td>
</tr>
</tbody>
</table>
| **Normalized Amount** (Norm Amt) | The adjusted amount of the group area expressed as a percentage of the total reported amounts. These percentages add up to the normalization factor specified in the sequence file.  

\[ N_{\text{norm}} = \left( \frac{\sum Q_i}{\sum Q_i} \right) F \]

where \( Q_i \) is the adjusted amount of an individual peak (or group) and \( F \) is the normalization factor in the sequence file. |
| **Normalized Area Percent** (Norm Area) | The group’s percentage contribution to the total area for all peaks for which results are reported. These percentages add up to the normalization factor specified in the sequence file.  

\[ N_{\text{area}} = \left( \frac{\sum A_i}{A_i} \right) F \]

where \( A_i \) is the area of an individual peak (or group) and \( F \) is the normalization factor in the sequence file. The sum in this calculation includes only the areas of peaks listed in the main report. |
| **Percent Amount** (% Amt) | The adjusted amount for the group expressed as a percentage of the sum of the adjusted amounts for all the peaks and groups listed in the main report. The values in a percent amount column always add up to 100 because the amounts for peaks excluded from the main report are not added into the sum. |
| **Raw Amount** (Raw Amt) | The amount of a component represented by the group, as calculated from the calibration curve or by applying a response factor. Unlike adjusted amount, this amount does not take |
into account the dilution factor, multiplier, divisor, or addend. If necessary, raw amounts have had scaling reversed, have been converted from amount ratios to amounts, and have sample volume adjustments made.

<table>
<thead>
<tr>
<th>Relative Retention (Rel RT)</th>
<th>The retention time of a peak compared to that of a specified relative retention reference peak. ( RRT = \frac{(RT_{\text{min}} - \text{void time})}{(RT_{\text{max}} - \text{void time})} )</th>
</tr>
</thead>
</table>
| Retention Time (RT)        | The ‘retention time’ of the group. This is a manufactured number:  
For timed group — the mid–point of the group  
For named group — an average of the RTs of peaks making up the group |
| Type                       | The type of group — Named or Timed |

When a row in the Group Table list is selected a new tab (the Group Detail tab), labeled with the name of the selected group, will be added to the window, immediately following the Group Table tab. In this example, Aromatics is the new tab.
**Report Window — Group Detail Tab**

A Group Detail tab will only appear when a specific peak group is selected on the Group Table tab; thus only one can be displayed at any time. The Detail tab is labeled with the name of the selected peak group and it displays the data for the peaks making up the group. One row appears in the table for each peak in the group.

The data columns displayed are determined by selections you made in the Show/Hide Columns dialog.
The complete set of data columns available is described below:

<table>
<thead>
<tr>
<th>Column in Table (abbreviation shown)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted Amount (Adj Amount)</td>
<td>The final calculated amount obtained in quantitation from the raw amount through the use of the dilution factor and the conversion factors.</td>
</tr>
<tr>
<td>Peak Area (Area)</td>
<td>The measured area of the peak (in microvolt–seconds) in the chromatogram, after the baseline has been drawn and integration performed.</td>
</tr>
<tr>
<td>Area Percent (Area %)</td>
<td>The ratio of the peak’s area to the sum of all peak areas listed in the main report. Expressed as a percentage, the sum in this calculation includes only the areas of peaks listed in the main report. It does not include peak areas of unidentified peaks if these peaks are not reported. Thus, the percentages in an area percent column will always add up to 100.</td>
</tr>
<tr>
<td>Area/Raw Amount</td>
<td>The ratio of the peak’s area to the raw amount</td>
</tr>
<tr>
<td><strong>Field</strong></td>
<td><strong>Definition</strong></td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
</tr>
<tr>
<td>(Area/Amt)</td>
<td>it represents. For a component with a linear response, this is equivalent to the calibration factor for the peak.</td>
</tr>
<tr>
<td>Area/Height</td>
<td>The ratio of the peak’s area to its height.</td>
</tr>
<tr>
<td>Baseline Code (BL)</td>
<td>A two-letter code that may be printed as part of the analysis report, indicating how the peak’s baseline was drawn. The first letter indicates the baseline treatment at the peak start, and the second letter indicates the baseline treatment at the peak end.</td>
</tr>
<tr>
<td>Component Name (Component)</td>
<td>The name of the component identified in the method to which the peak was matched.</td>
</tr>
<tr>
<td>Peak Height (Height)</td>
<td>The height, above the peak baseline, of the peak crest, measured in microvolts.</td>
</tr>
<tr>
<td>Normalized Amount (Norm Amt)</td>
<td>The adjusted amount of the peak expressed as a percentage of the total reported amounts. These percentages add up to the normalization factor specified in the sequence file.</td>
</tr>
<tr>
<td>[ N_{\text{norm}} = \frac{Q_i}{\sum_{i=1}^{n} Q_i} F ]</td>
<td></td>
</tr>
<tr>
<td>Normalized Area Percent (Norm Area)</td>
<td>The peak’s percentage contribution to the total area for all peaks for which results are reported. These percentages add up to the normalization factor specified in the sequence file.</td>
</tr>
<tr>
<td>[ N_{\text{norm}} = \frac{A_i}{\sum_{i=1}^{n} A_i} F ]</td>
<td></td>
</tr>
<tr>
<td>Peak Number (Pk #)</td>
<td>An index assigned to each peak detected in a run. This is the index of the group member</td>
</tr>
</tbody>
</table>

*Viewing Reports and Results from a Single Result File*
within the entire set of peaks found in the run, not just within the group.

<table>
<thead>
<tr>
<th>Percent Amount (% Amt)</th>
<th>The adjusted amount for the peak expressed as a percentage of the sum of the adjusted amounts for all the peaks listed in the main report. The values in a percent amount column always add up to 100 because the amounts for peaks excluded from the main report are not added into the sum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Amount (Raw Amt)</td>
<td>The amount of a component represented by the peak, as calculated from the calibration curve or by applying a response factor. Unlike adjusted amount, this amount does not take into account the dilution factor, multiplier, divisor, or addend. If necessary, raw amounts have had scaling reversed, have been converted from amount ratios to amounts, and have sample volume adjustments made.</td>
</tr>
<tr>
<td>Retention Time (RT)</td>
<td>In an analysis report, this is the actual elution time of the peak (in minutes), as measured from the start of the run.</td>
</tr>
</tbody>
</table>

The initial default set of columns will be: Pk #, Component, RT, BL, Area, Height, Adj Amt.

The current set of columns shown, as well as their order and their widths are saved as environment defaults (for the current user) when the application window is closed.
Report Window — Report Tab

The Report tab is displayed when a report format file (.RPT) is being used in association with the selected data file. It displays a preview of the TotalChrom format report as it would be printed. This is equivalent to a standard Print Preview but rendered within the tab client area. Vertical and/or horizontal scroll bars will be displayed as necessary.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print …</td>
<td>Displays the standard Windows Print dialog, enabling you to print the currently displayed report.</td>
</tr>
<tr>
<td>Print Preview …</td>
<td>Displays the standard Windows Print Preview window for the currently displayed report. <em>A somewhat pointless exercise maybe, so perhaps this can be removed?</em></td>
</tr>
<tr>
<td>Properties</td>
<td>Displays the Report window Properties dialog.</td>
</tr>
</tbody>
</table>
Report Window — Header Tab

The header tab will always appear (as the rightmost tab in the window) and will be basically the same regardless of what other report tabs are being displayed. The header will be the same format as the standard TotalChrom report header, with small, medium and large versions, which may be selected from a context menu. If a report format file has been associated with the data file and is being displayed, then the report header displayed on the Header tab will be identical to that from the report. If only an ad hoc report is being displayed, then there will be no Area Reject value displayed (since this comes from the RPT file). The initial default header is Medium.

Small Header

![Small Header](image)

Medium Header

![Medium Header](image)
Large Header

![Large Header Image]

Context Menu Commands

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Small</td>
<td>Sets the contents of the Header tab.</td>
</tr>
<tr>
<td>✓ Medium</td>
<td>These items form a set of radio buttons, that is, clicking on an item checks that item and unchecks any other of the group.</td>
</tr>
<tr>
<td>✓ Large</td>
<td></td>
</tr>
<tr>
<td>Print …</td>
<td>Displays the standard Windows Print dialog, enabling you to print the currently displayed report.</td>
</tr>
<tr>
<td>Print Preview …</td>
<td>Displays the standard Windows Print Preview window for the currently displayed report.</td>
</tr>
<tr>
<td>Properties</td>
<td>Displays the Report window Properties dialog.</td>
</tr>
</tbody>
</table>
Report Window — Status Tab

When a single TCR file is selected in the file list the Report Window includes a tab that displays the Signature Status of all reports in the .tcr file. This acts as an expansion of the single Status column in the file list. The reports are listed on the Status tab in the same order as their individual preview tabs are shown in the window.

<table>
<thead>
<tr>
<th>Column in Table</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report</td>
<td>The full path name of the report format file defining the report within the selected TCR file.</td>
</tr>
<tr>
<td>Signature Status</td>
<td>The current status of the report.</td>
</tr>
</tbody>
</table>

You can change the column width by dragging the header boundary and you can sort the list based on the data in any column by clicking on the column header.

Viewing Reports and Results from a Single TotalChrom Report (TCR) File

When a TCR file is selected in the file list, rather than a result file (or raw file), the main differences are:
1. The Report Window contains tabs for every report associated with the TCR file. This includes the main report (specified via the RPT file in the sequence description) as well as any optional reports (from the Method and/or specified from Batch or Graph Editing).

2. One or more of the Sign menu commands and toolbar buttons may be enabled (depending on the status of the TCR file).

3. If the file list contains more than one TCR file but no other file types then the All Reports command in the Sign menu will be enabled.

Example of Report Window for TCR containing three reports (Report, Opt 1 and Opt 2).
**Viewing Multiple Samples Simultaneously**

**Multiple File Selection**

The behavior of the plot window in overlay mode is essentially the same as the single plot case. The active chromatogram can be selected using the plot selector accessed via the toolbar button.

- File List with multiple file selection
- Plot Window with Multiple Chromatograms Overlaid
- Plot Window with Multiple Chromatograms Stacked
- Plot Window Rescale Dialog
- Report Window with Multiple File Selection

An example of the Main View with multiple file selection.
Viewing Multiple Samples Simultaneously

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot Window</td>
<td>Selection of a peak in any chromatogram will cause the summary table to be scrolled so that the data relating to that peak is visible. The active chromatogram can be changed either by clicking on it directly (where possible) or by using the selector control in the toolbar.</td>
</tr>
<tr>
<td>Report Window</td>
<td>Selecting any cell containing peak data in the summary table will cause that peak to be selected (highlighted) in the active chromatogram (unselecting any other object as necessary). If the chromatogram is scaled such that the peak is not currently visible, the chromatogram(s) will be rescaled so that the peak is displayed.</td>
</tr>
</tbody>
</table>

File List with Multiple File Selection

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>File List</td>
<td>The set of files selected can be modified using the standard Shift + Click and Ctrl + Click selection modifiers. Shift + Click will extend or reduce the number of files selected in a contiguous fashion. All files between the current selection boundary will be added to the selection (if they are not currently selected) or removed from the selection (if they are currently selected). Ctrl + Click will add the file clicked on to the selection (if it is not currently selected) or remove it from the selection (if it is currently selected). Following any such a change to the selection the display in all windows will be updated.</td>
</tr>
</tbody>
</table>
**Plot Window with Multiple Chromatograms Overlaid**

The currently active plot is indicated by the dotted line surrounding it. There is always one (and only one) selected plot in stacked mode.
Plot Window with Multiple Chromatograms Stacked

Plot Area Context Menu

This menu appears when you right click in the plot window.
Most of the commands have been described previously for a single plot. The additional commands resulting from the stacked display are described below.

The static overlay is restricted to the top position of the stack.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Static Overlay...</td>
<td>Displays a TotalChrom File Open dialog, enabling you to select a standard file to act as a static overlay.</td>
</tr>
<tr>
<td>Add Plot Above Stack...</td>
<td>Displays a TotalChrom File Open dialog from which you can select a .raw or .rst file. When a file is selected the new data will be plotted in a new container placed at the top of the stack. Does not appear if the plot window is set to Overlay mode, or if the plot window is configured with more than one column. Replaced by ‘Replace Plot Above Stack’ if a static stacked plot has already been set up.</td>
</tr>
<tr>
<td>Replace Plot Above Stack</td>
<td>Displays a TotalChrom File Open dialog for selection of a single file to replace the existing static stacked plot. Does not appear if there is no static stacked plot set up.</td>
</tr>
<tr>
<td>Remove Plot Above Stack</td>
<td>Removes the static stacked plot and restores the plot window to standard stacked format (resetting the first selected file to the top of the stack and displaying as many of the remaining selected files as possible with the current window configuration. Does not appear if there is no static stacked plot set up.</td>
</tr>
<tr>
<td>Add Label ...</td>
<td>Adds a label to the plot.</td>
</tr>
<tr>
<td>Add Ref Plot Time Marker</td>
<td>Displays a vertical alignment cursor on the plot. Disabled if two vertical markers already exist on the plot.</td>
</tr>
<tr>
<td>Add Ref Plot Voltage Marker</td>
<td>Displays a horizontal alignment cursor on the plot.</td>
</tr>
</tbody>
</table>
### Viewing Multiple Samples Simultaneously

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;row #&gt;</td>
<td>A row will be displayed in the grid for each currently selected chromatogram. This column is read–only.</td>
</tr>
<tr>
<td>File Name</td>
<td>The data file name associated with the chromatogram (raw or rst). This column is not editable.</td>
</tr>
<tr>
<td>Start Time (min)</td>
<td>The start time of the plot display (i.e. the minimum X-axis value).</td>
</tr>
<tr>
<td>End Time (min)</td>
<td>The end time of the plot display (i.e. the maximum X-axis value).</td>
</tr>
</tbody>
</table>

### Plot Window Rescale Dialog

One or more rows in the grid may be selected by clicking on the row number, in conjunction with the Shift or Ctrl keys to obtain a contiguous or discontiguous selection of rows.

The complete row does not have to be selected for the AutoScale command to act on it. As long as any cell in the row is part of the current selection (i.e. a block of cells) then AutoScale will apply to that row.

Use default scaling to display chromatogram(s)
<table>
<thead>
<tr>
<th>Offset (mV)</th>
<th>The minimum of the Y-axis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Scale (mV)</td>
<td>The range of the Y-axis in mV.</td>
</tr>
<tr>
<td>AutoScale</td>
<td>Sets the Start Time, End Time, Offset and Full Scale values from the data for all currently selected rows (see Behavior below).</td>
</tr>
</tbody>
</table>

**Report Window with Multiple File Selection**

When multiple files are selected in the file, the contents of the report window will display a summary of the component results for all selected files. The list of components will be obtained from the first file in the selection. The summary will be displayed in a tabular (grid) format, with a row for each selected file.

The summary format will allow a single file identifier column (selectable from file name, sample name, sample number, date/time of acquisition, or any of the ten User values) followed by a set of columns for each component. The data columns displayed are determined by selections you made in the Show/Hide Columns dialog.
The complete set of data columns available will be virtually the same as for the single selection Peak Table but with the following omissions:

- **Peak Number** - This is not applicable since the number might not be the same for the component in every file summarized.
- **Component Name** - This appears as a heading above the group of data columns.
- **Baseline Code** - This is not applicable since the code might not be the same for the component in every file summarized.

There are two rows of column headers (as in the standard summary report): The first will identify the component and the second will indicate the column types. The initial default file identifier column is file name. The initial default set of component columns is: RT, Area, Adjusted Amount. There are no column averages or calculated values (such as Rel. Std. Dev.) displayed with the summary report.

**Summary View Context Menu**

Right clicking in the report window when it contains a summary view displays the following context menu:

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Show/Hide Columns</td>
<td>Displays the Show/Hide Columns dialog</td>
</tr>
<tr>
<td>Properties</td>
<td>Displays the Summary Properties dialog. The alternatives will be:</td>
</tr>
<tr>
<td></td>
<td>• File Name</td>
</tr>
<tr>
<td></td>
<td>• Sample Name</td>
</tr>
<tr>
<td></td>
<td>• Sample Number</td>
</tr>
<tr>
<td></td>
<td>• Date/Time of Acquisition</td>
</tr>
<tr>
<td></td>
<td>• User Values 1 – 10</td>
</tr>
</tbody>
</table>
Report Window with Multiple File Selection

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column width</td>
<td>Columns may be changed in width by dragging the header boundary, in the standard way for list views. The boundary at the right–hand side of a column will size that column. Changing the width of one column changes the width for all columns containing that data type. Hence all ‘Area’ columns (for example) are always the same width.</td>
</tr>
<tr>
<td>Columns shown</td>
<td>Columns to be displayed for each component will be defined in the Show/Hide Columns dialog.</td>
</tr>
<tr>
<td>Selection</td>
<td>Selection of a row in the list will cause that file to become the active plot.</td>
</tr>
</tbody>
</table>

Aligning Chromatograms

The process of aligning two (or more) chromatograms involves the following steps:

1. Marking one or two reference points on the chromatogram to which others will be aligned. This is the ‘reference’ chromatogram.
2. Marking the corresponding of points on the chromatogram(s) to be aligned with the reference chromatogram.
3. Selecting the type of alignment to be performed — Shift (single point align) or Scale (two point align)

Marking the Reference Plot

When the plot window contains a stacked display and there are no existing alignment markers displayed on any plot, a right–click on any plot will display the following context menu:
The Add Ref Plot Time Marker and Add Ref Plot Voltage Marker items are initial commands associated with alignment. Since the first chromatogram to which markers are added is defined to be the reference chromatogram (i.e. the one which will not be rescaled), the menu wording reflects this. Choosing ‘Add Ref Plot Time marker’ will cause a vertical cursor, the full height of the plot area, to be displayed, while choosing ‘Add Ref Plot Voltage Marker’ will cause a horizontal cursor, the full width of the plot area, to be displayed. The cursor will be positioned at the time or response value at the point of the right–click.
Once a reference marker has been added to a plot a subsequent right-click on that plot will display the reference alignment context menu, as shown above. The availability of the alignment commands will depend on what markers exist, as defined in the table below.

*Both Time and Voltage markers may exist on the same plot at the same time.*

### Reference Plot Context Menu

Only the commands associated with alignment are defined in the following table.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Ref Plot Time Marker</td>
<td>Displays a vertical alignment cursor on the plot.  Disabled if two vertical markers already exist on the plot.</td>
</tr>
<tr>
<td>Add Ref Plot Voltage Marker</td>
<td>Displays a horizontal alignment cursor on the plot.  Disabled if two horizontal markers already exist on the plot.</td>
</tr>
<tr>
<td>Clear Markers</td>
<td>Removes all alignment markers from the plot.  Disabled when no markers exist on the plot.</td>
</tr>
</tbody>
</table>

### Marking the Plot to be Aligned

When the plot window contains a stacked display and one of the plots contains one or more reference alignment markers, a right-click on any other plot will display the following context menu:
Choosing ‘Add Align Plot Time marker’ will cause a vertical alignment cursor, while choosing ‘Add Align Plot Voltage Marker’ will cause a horizontal alignment cursor to be displayed. As before, the cursor will be positioned at the time or response value at the point of the right–click.

**Aligned Plot Context Menu**

Only the commands associated with alignment are defined in the following table.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Align Plot Time Marker</td>
<td>Displays a vertical alignment cursor on the plot. Disabled if two vertical markers already exist on the plot.</td>
</tr>
<tr>
<td>Add Align Plot Voltage Marker</td>
<td>Displays a horizontal alignment cursor on the plot. Disabled if two horizontal markers already exist on the plot.</td>
</tr>
<tr>
<td>Clear Markers</td>
<td>Removes all alignment markers from the plot. Disabled when no markers exist on the plot.</td>
</tr>
</tbody>
</table>

Please note that if you clear the markers from the reference plot at any time, then the context menu for the next plot (without existing markers) will contain the Add Ref Plot Time Marker and Add Ref Plot Voltage Marker commands. In other words, any
time a reference plot does not exist the first plot receiving its first marker will be defined as the reference plot.

Of course additional markers could be added to existing align plots when no reference plot exists without affect their status. Clearing markers from an align plot would however make it eligible to be designated as the reference plot.

**Aligning the Plots**

When alignment markers exist on both the reference plot and at least one other plot a right–click on one of the plots containing ‘Align Plot’ Markers will display a context menu similar to the following:
The exact form and behavior of this context menu is defined in the table below.
Choosing one of the align commands will cause the alignment plot(s) to be rescaled such that the alignment marker, or markers, in the reference and aligned plots line up. For example, choosing the Shift/Time command in the above display would yield the following display.

![Graphs showing alignment markers lined up](image-url)
**Align Command Context Menu**

Only the commands associated with alignment are defined in the following table.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Align Plot Time Marker</td>
<td>Displays a vertical alignment cursor on the plot. Disabled if two vertical markers already exist on the plot.</td>
</tr>
<tr>
<td>Add Align Plot Voltage Marker</td>
<td>Displays a horizontal alignment cursor on the plot. Disabled if two horizontal markers already exist on the plot.</td>
</tr>
<tr>
<td>Clear Markers</td>
<td>Removes all alignment markers from the plot. Disabled when no markers exist on the plot.</td>
</tr>
<tr>
<td>Shift Time Voltage</td>
<td>Enabled when a vertical alignment marker exists on the plot. Enabled when a horizontal alignment marker exists on the plot.</td>
</tr>
<tr>
<td>Scale Time from Origin Voltage</td>
<td>Enabled when a vertical alignment marker exists on the plot. Enabled when a horizontal alignment marker exists on the plot.</td>
</tr>
<tr>
<td>Scale Voltage from Origin Time</td>
<td>Enabled when a vertical alignment marker exists on the plot. Enabled when a horizontal alignment marker exists on the plot.</td>
</tr>
<tr>
<td></td>
<td>Enabled when two vertical alignment markers exists on the plot. Enabled when two horizontal alignment markers exists on the plot.</td>
</tr>
</tbody>
</table>

**Moving Alignment Markers**

If necessary, you can move an alignment market to a more accurate position before the shift or scale command is issued. When the mouse pointer (normally a cross–hair cursor).

Is moved over an alignment marker it will change to a vertical or horizontal move cursor (depending on the type of marker selected).
Viewing Multiple Samples Simultaneously

The marker can then be moved by drag–and–drop.

The plot can even be zoomed to perform the movement of the alignment cursor more precisely and then returned to default scale before the shift/scale command is selected, as shown here:

**Viewing a Static Overlay Plot**

When the plot window contains a stacked display and there are no existing alignment markers displayed on any plot, a right–click on any plot will display the following context menu:
Choosing the ‘Add Static Overlay’ command displays a TotalChrom File Open dialog from which you can select a .raw or .rst file. When a file is selected the new data will be plotted in overlay mode on the selected plot (in this example the top one in the stack).
Clicking the scroll bar ‘down’ button (i.e. to move the contents of the window up) will cause the top plot in the stack to be scrolled out of the window and remaining plots will move up one position. However, the overlay plot will remain where it is, so that now it is overlaid on the next plot, thus:
In this example there are only three plots in the stack, so scrolling them up leaves an empty position. A further click of the scroll bar would move the final plot into the top position with the static overlay:

The above examples show the static overlay situated at the top of the stack but it may appear in any position. For example, if the initial right-click and command selection was performed in the middle of a three-row stack the static overlay would appear as follows:
**Static Overlay Context Menu**

Only the commands associated with static overlay are defined in the following table.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Static Overlay…</td>
<td>Displays a TotalChrom File Open dialog for selection of a single file.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if the plot window is set to Overlay mode, or if the plot window is configured with more than one column.</td>
</tr>
<tr>
<td></td>
<td>Replaced by ‘Replace Static Overlay’ if a static overlay has already been set up.</td>
</tr>
<tr>
<td>Replace Static Overlay</td>
<td>Displays a TotalChrom File Open dialog for selection of a single file to replace the existing static overlay.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if there is no static overlay set up.</td>
</tr>
<tr>
<td>Remove Static Overlay</td>
<td>Removes the static overlay and restores the plot window to standard stacked format (resetting the first selected file to the top of the stack and displaying as many of the remaining selected files as possible with the current window configuration.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if there is no static overlay set up.</td>
</tr>
</tbody>
</table>
**Viewing a Static Stacked Plot**

When the plot window contains a stacked display and there are no existing alignment markers displayed on any plot, a right-click on any plot will display the following context menu:

Choosing the Add Plot Above Stack ... command displays a TotalChrom File Open dialog from which you can select a .raw or .rst file. When a file is selected the new data is plotted in a new container placed at the top of the stack.
Viewing Multiple Samples Simultaneously

In the above example the plot ‘Test sample #1’ has been added. Note that ‘Test sample#10’ is now second in the stack.

Clicking the scroll bar ‘down’ button (i.e. to move the contents of the window up) will cause the ‘static’ plot to remain where it is and just the remaining plots in the stack will scroll beneath it. For example, clicking the down arrow twice in the case above produces the display:

![Image of the display with 'Test sample #1' added and the others moved down]

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Note that ‘Test sample #1’ remains at the top of the window, while Test samples #10 & #11 have scrolled out of the window.

Only the commands associated with static stacked plot are defined below.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add Plot Above Stack…</td>
<td>Displays a TotalChrom File Open dialog for selection of a single file.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if the plot window is set to Overlay mode, or if the plot window is configured with more than one column.</td>
</tr>
<tr>
<td></td>
<td>Replaced by ‘Replace Plot Above Stack’ if a static stacked plot has already been set up.</td>
</tr>
<tr>
<td>Replace Plot Above Stack</td>
<td>Displays a TotalChrom File Open dialog for selection of a single file to replace the existing static stacked plot.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if there is no static stacked plot set up.</td>
</tr>
<tr>
<td>Remove Plot Above Stack</td>
<td>Removes the static stacked plot and restores the plot window to standard stacked format (resetting the first selected file to the top of the stack and displaying as many of the remaining selected files as possible with the current window configuration.</td>
</tr>
<tr>
<td></td>
<td>Does not appear if there is no static stacked plot set up.</td>
</tr>
</tbody>
</table>

**Interactions Between Windows**

**File List Selection**

If a single row is selected in the file list then the report window displays results from that file (if a result file is found) or just file header information (if only the raw file is found). The plot window displays the chromatogram from that file (if available).

If multiple rows are selected then the report window displays a summary of results from those rows for which result files were found. The plot window shows the chromatograms in stacked or overlay format, depending upon the option setting.

**Peak/Group List Selection**

You may select a row in the peak list or group list table. If the selected item is a peak, this will cause the associated peak to be selected in the plot (i.e. filled in with the
single peak TotalChrom color), unselecting any other object as necessary. If the selected peak is not currently visible (because the plot is expanded) the displayed start and end times will be adjusted (by the same amount) so that the selected peak is displayed approximately in the middle of the window. If the software determines that the peak width (start to end) is greater than the current X-axis scale, then the scale will be increased such that the peak start and end points are included in the displayed time range. Note that a peak cannot be selected on the group detail tab (since this would conflict with being in 'group mode' - of critical importance in the editing environments).

If the selected item is a group then that group will be shown selected (unselecting any other object as necessary). That is, all peaks that belong to the group will be filled in with the TotalChrom group peak color, and the name of the group will be displayed (also in the group peak color) at the approximate mid-point of the group. If the current time scale of the X-axis is such that not all peaks belonging to the group are visible, then the scale will be increased such that all peaks in the group are included.

**Peak Selection in Plot Window**

When a peak is selected in a chromatogram in the plot window, and the Peak List tab is displayed in the Report Window, then that peak will be selected in the Peak List table.

**Summary Properties**

**Summary Tab**

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>File Identifier</td>
<td>A drop–down list (of File Name, <strong>Sample Name</strong>, Sample Number, Date/Time of</td>
</tr>
</tbody>
</table>
Acquisition, User Values 1 to 10) from which you select the data item used to identify each file in the summary table.

**Text Tab**

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font</td>
<td>A drop-down list for choosing the font for display of text in the summary table.</td>
</tr>
<tr>
<td>Size</td>
<td>A drop-down list for choosing the size of the font used for the summary table.</td>
</tr>
<tr>
<td>&lt;font sample&gt;</td>
<td>A read-only display of the word ‘Sample’ in the selected font at the selected size.</td>
</tr>
</tbody>
</table>
Reviewing and Approving Reports

About Reviewing and Approving Reports

Only TotalChrom Report (TCR) files can be Reviewed and Approved. All Review and Approve commands (the Sign menu and related toolbar buttons) will be disabled if the currently selected file is not a TCR file. These commands are also disabled if more than one file is selected, even if all are TCR files.

While there will be occasions when you only wish to review or approve a single report, the most usual situation will be that a set of reports must be handled.

How to Sign a Single Report

Within the main window only a single report can be signed at a time.

➢ To sign a single report:
   1. Select the associated TCR file in the File Open dialog, so that the sample entry appears in the file list.
   2. Select that item in the file list.
   3. Display the report to be signed, by selecting the Report tab in the Report Window or one of the optional report tabs if there is more than one report associated with the data.
   4. Review the report (and optionally the chromatogram).
   5. Click the button for the signature level to be signed (assuming this is enabled).
   6. Complete the electronic signature dialog.

How to Sign Multiple Reports

Applying a single electronic signature to multiple reports is performed within the Review Reports window, accessed using the Sign/All Reports command.

➢ To sign multiple reports:
   1. Select the associated TCR files in the File Open dialog, so that the file list contains all the necessary data (and no more). If the list contains files other than TCR files (e.g. result files), the All Reports command will be disabled.
   2. Choose the All Reports command from the Sign menu.
   4. Click the button for the signature level to be signed (assuming this is enabled).
   5. Examine the next report in the set – this will be displayed automatically.
6. Repeat steps 4 and 5 for each remaining report in the set (that is all reports from all the TCR files in the file list).

7. Click the Finish button when all reports have been marked

8. Review the Sign Reports dialog, summarizing the reports to be signed. Clear any invalid pending signatures.

9. Click Sign.

10. Complete the Electronic Signature dialog.

**About the Report Review Window**

When the Sign/All Reports command is chosen the Report Review window replaces the contents of the main Review and Approve window (with the exception of the title bar), in the manner of a standard Print Preview window. However, the Report Review window is not merely a display window, you can issue commands from here that affect the status of the TotalChrom Report (TCR) files.

The main characteristics of this window are:

1. The Prev Report and Next Report buttons allow several documents (reports from several TCR files) to be viewed in succession without having to close the window and reopen it.

2. The Sign buttons at the right–hand side of the toolbar flag a potential change to the signature status of the currently displayed report (pending a valid electronic signature).

3. The status bar displays the current status of each report as well the name.
Reviewing and Approving Reports

Report Review Window

This window displays when you choose the All Report command from the Sign menu of the Review and Approve environment.

About the Status Bar

The status bar will be used to identify the displayed report and the displayed page(s). The status bar is divided into two segments:

1. The fully qualified file name of the displayed TotalChrom report (TCR) file, followed by its signature status. This will be the stored status if the report has not been marked for signature or the pending status (followed by ‘– Unsigned’) if it has been marked.

2. The currently displayed page number and the total number of pages in the report, in the form ‘Page n of m’. When two pages are being displayed side–by–side, ideally the page numbers would be shown as ‘Pages n–n+1 of m’ but an acceptable alternative would be to use the single page format, displaying the number of the page on the left as ‘n’.

The page number segment will always occupy the minimum of space required at the right–hand side of the window, regardless of the window size. The other segment will be sized accordingly, with the name shortened in the standard windows manner (with an ellipsis replacing as many characters as necessary in the middle of the name).
The toolbar of the Report Review window enables you to do the following:

<table>
<thead>
<tr>
<th>Button</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print...</td>
<td>Displays the standard Windows Printer dialog, enabling you to print the currently displayed report on the selected printer.</td>
</tr>
<tr>
<td>Prev Page</td>
<td>Displays the page prior to the currently displayed page for the current report.</td>
</tr>
<tr>
<td>Next Page</td>
<td>Displays the page following the currently displayed page for the current report.</td>
</tr>
<tr>
<td>Zoom In</td>
<td>Displays the report at the next larger expansion size. The three sizes available are:</td>
</tr>
<tr>
<td></td>
<td>• Whole Page: Scaled such that the whole page is visible.</td>
</tr>
<tr>
<td></td>
<td>• Mid Size: Intermediate between Whole Page and Actual Size.</td>
</tr>
<tr>
<td></td>
<td>• Actual Size: Displayed at the size it would be on the printed page.</td>
</tr>
<tr>
<td>Zoom Out</td>
<td>Displays the report at the next smaller expansion size.</td>
</tr>
<tr>
<td>Prev Report</td>
<td>Displays the first page of the previous report from the currently selected data set.  This may be another report associated with the same TCR file or, if there are no reports in that TCR file, the last report from the previous TCR file in the file list.</td>
</tr>
<tr>
<td>Next Report</td>
<td>Displays the first page of the next report from the currently selected data set.  This may be the next report associated with the TCR file or, if there are no reports in that TCR file, the first report from the next TCR file in the file list.</td>
</tr>
<tr>
<td>Close</td>
<td>Closes the Report Review window. If (and only if) you marked any reports for signature then a dialog displays asking you want to sign those reports. The message is “You have marked reports for signature. Do you want to sign these now?” Yes and No buttons are provided. If you choose ‘Yes’ then the Sign Reports dialog is displayed. If you choose ‘No’ then you are returned to the main Review and Approve environment. However, the unsigned reports will retain their pending signature marks while the main Review and Approve window remains open. If (and only if) you marked any reports for signature then the Sign Reports dialog will be displayed. Close changes to Finish once the final report in the current data set has been displayed – provided at least one report has been marked for signature.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>(Finish)</td>
<td>Marks the current report as Reviewed and flags it as ‘Unsigned’. Also causes the next report in the data set to be displayed (if there is one).</td>
</tr>
<tr>
<td></td>
<td>Marks the current report as Reviewed – Final and flags it as ‘Unsigned’. Also causes the next report in the data set to be displayed (if there is one).</td>
</tr>
<tr>
<td></td>
<td>Marks the current report as Approved and flags it as ‘Unsigned’. Also causes the next report in the data set to be displayed (if there is one).</td>
</tr>
<tr>
<td></td>
<td>Marks the current report as Approved – Final and flags it as ‘Unsigned’. Also causes the next report in the data set to be displayed (if there is one).</td>
</tr>
<tr>
<td></td>
<td>Marks the current report as Hold and flags it as ‘Unsigned’. Displays the ‘Reason for Hold Report’ dialog if so configured. Also causes the next report in the data set to be displayed (if there is one).</td>
</tr>
</tbody>
</table>
The enabled/disabled state of the Sign buttons does not depend on the logged on user’s permissions. The Review and Approve process will support electronic signature by someone other than the logged on user.

If the current TCR file is open it is for reading only.

The following summarizes the combinations of Sign buttons (Review, Final Review, Approve, Final Approve) that can be enabled at the same time. No other combinations are legal.

- Review and Final Review: When both are enabled in SysConfig and the report status is Reviewed
- Approve and Final Approve: When both are enabled in SysConfig and the report status is Approved

Unsigned can exist at the same time in either of the above combinations.
Electronic Signature

Review and Approve does not use the standard TotalChrom Electronic Signature dialog but employs its own custom but similar versions. The reasons for not using the standard TotalChrom electronic signature functionality are:

1. For Review and Approve the signer does not have to be the logged on user.
2. There are two versions of the Electronic Signature dialog within Review and Approve, one for single report signing and one for multiple reports.

Version for single report signing

Version for multiple report signing

The purpose of the additional text in the single report case is to confirm to you what you are signing (i.e. the same function the Sign Report dialog serves in the case of multiple report signing). This will enable you to recognize and cancel an error in the pending signature (for example if he clicked the wrong button in error). Although such an error might be prevented from being finalized by signature permission levels, it also might not be.

Electronic Signature Dialog

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Your signature will apply the following status to the current report</td>
<td>Text to explain the status.</td>
</tr>
<tr>
<td>&lt;Status to be signed&gt;</td>
<td>Indicates the status that you are signing for the current report. For example:</td>
</tr>
<tr>
<td></td>
<td><strong>Reviewed, Reviewed – Final, Reviewed – Complete, Approved, Approved – Complete, Hold</strong></td>
</tr>
</tbody>
</table>
**User name**
A box to enter your logon name to sign the reports. Note that this does not have to be the person logged on to TotalChrom.

**Password**
A box to enter your password to sign the reports.

**OK**
When this command button is clicked, the entered user name and password will be verified with the TotalChrom security system (via TcAccess if necessary). If the combination is not valid then an error message will be displayed:

- User name or password not recognized.
- When the error message is closed the user will be returned to the Electronic Signature dialog.

If the user name/password combination is valid then the software will determine if the user has permission to sign at the marked level (or levels if the set also includes ‘Hold’ reports). If the user does not have permission then an error message will be displayed:

You are not authorized to sign reports as:

<signature level not allowed>

The message could display two levels if neither is allowed, for example:

You are not authorized to sign reports as:

- Reviewed
- Hold

When the error message dialog is closed the Electronic Signature dialog will also close. The reports for which the user did have permission to sign for the marked level will be updated with the new status and signature. The reports for which the user did not have permission will be left unchanged and pending mark will be removed.

If the user does have the necessary permissions then the Electronic Signature dialog will close, the marked reports will have their status updated appropriately and
| Cancel | A command button that closes the Electronic Signature dialog and returns the user to the main Review and Approve environment. The marked reports remain marked but ‘Unsigned’. |
**Reason for Hold Dialog**

This dialog is displayed when you mark a report as Hold and the requirement for entry of a comment for hold reports is set in System Configuration.

The dialog will appear when you click the Hold button (if the comment requirement is set). You cannot cancel out of this dialog (you must enter a comment, even if it is “I just clicked the button to see what would happen”) but you can clear the Hold mark prior to signing, if the button was clicked accidentally.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comment</td>
<td>A memo field for entry of the comment to be applied to this signature event. This will be saved in the signature table for all associated reports.</td>
</tr>
</tbody>
</table>
Comment Review Dialog

This dialog is displayed when the Comment button is clicked.

This dialog is identical to the Reason for Hold dialog except:

- The comment field is read–only in this case
- The button is labeled ‘Close’
- The name of the user who signed the hold report is displayed

The dialog is referred to by a different name here simply to distinguish it from the other dialog, the dialog title is the same in both cases.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comment</td>
<td>A read–only memo field displaying the comment entered when the report was marked as ‘Hold’.</td>
</tr>
<tr>
<td>Signed by:</td>
<td>A read-only display of the full name of the user who signed the hold report</td>
</tr>
</tbody>
</table>

.
Sign Report Dialog

Sign Reports dialog

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reports tree</td>
<td>A tree control listing the reports to be signed and the TotalChrom report file (.TCR) they are associated with.</td>
</tr>
<tr>
<td></td>
<td>• File/Report File Name: Displays the TCR file as the top level nodes and the associated reports as second level nodes.</td>
</tr>
<tr>
<td></td>
<td>• Status to be Signed: The signature level to be signed.</td>
</tr>
<tr>
<td>Sign</td>
<td>A command button that displays the electronic Signature dialog. Note that the Sign Reports dialog will remain displayed (with all buttons disabled), so that you can refer to it if necessary by moving the Electronic Signature dialog aside. The Sign Reports dialog will be closed when the Electronic Signature dialog is closed.</td>
</tr>
<tr>
<td>Cancel</td>
<td>A command button that closes the Sign Reports dialog, leaving the reports marked but unsigned.</td>
</tr>
</tbody>
</table>
### Context menu: Sign Reports dialog

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Pending Signature</td>
<td>Deletes the selected report from the tree. The status of the report remains as it was before it was marked and the ‘Unsigned’ flag is cleared.</td>
</tr>
</tbody>
</table>
Review and Approve Scenario

The following is an example scenario of how the Review and Approve process could be used. This example has been developed to illustrate some key aspects of Review and Approve; however, it is neither the simplest nor the most complex path through the application. While the scenario aims to be accurate in its depiction of Review and Approve, it does not attempt to be completely realistic in all matters.

Set Up Conditions

The configuration of the TotalChrom system for this example contains the following key items:

- **Review and Approve signature levels**: Review, Final Review, Final Approve and Hold
- **Review and Approve options**: The same user cannot sign two levels for a report = True
  You must enter a comment for each Hold report = True

**Job Types**: Manager, Analyst, Technician

**Users**: Susanna (Manager), Michael (Senior Analyst), Vicky (Analyst), and James (Lab Technician)

**Review and Approve permissions**:

- Susanna – Final Approve, Hold
- Michael – Final Review, Hold
- Vicky – Review
- James – no permissions

The sample data set for this example consists of three samples (CK46123, CK46124 and CK46125) each of which has two associated reports (Actives and Impurities). Processing of these samples yielded three TCR files, each containing two report definitions.

Vicky has reviewed the results in the Review and Approve environment and signed each report, such that each now has the status of Reviewed.

**How Review and Approve is used in this Example**

1. Michael logs on to TotalChrom on one of the lab computers and opens the Review and Approve environment. He prefers to be away from his office phone when reviewing reports.

2. He selects the three TCR files representing the CK46123, CK46124 and CK46125 sample reports in the File Open dialog and clicks OK.

   The Status column in the file list shows Reviewed for all three TCR files, as Michael expects (knowing Vicky came in on Sunday to review the samples after James had finished the chromatographic runs). The chromatogram and Actives report for the first sample are displayed in the Plot and Report windows.
3. He zooms in on the chromatogram to examine the integration and baseline for each peak of interest in turn. All look satisfactory. Michael repeats this process for the two remaining TCR files. The chromatography and integration looks fine for all three samples.

4. Now he wants to review the reports more closely prior to signing them so he chooses the Sign/All Reports command from the main menu.

The Report Review window appears with the Actives report for sample CK46123 displayed. Michael notes that the Review, Final Review and Hold buttons are enabled on the Sign toolbar, while the Approve and Final Approve buttons are disabled.

He will not use the Review button since the company SOP only requires two levels of review. He also hopes he won’t be using the Hold button because rejecting batches of product leads to a lot of paperwork and unwelcome attention from upper management. Once he has applied a Final Review signature to these reports they will be ready for Susanna to give Final Approval.

5. Michael clicks the Zoom In button so that the report text is at a size that is comfortable for him to read. He then checks the header information carefully to make sure the correct methods were used. Clicking the scroll bar, he examines the peak table to ensure the reported amount of each active component is within limits and that the suitability parameters are within tolerance.

6. When he is satisfied that all is as it should be Michael clicks the Final Review button.

The Impurities report for CK46123 is then displayed. Michael reviews the header information again and checks the amounts for each impurity, as well as the figure for total impurities. All these values are well within limits and so Michael clicks Final Review once again.

7. Michael repeats his examination on the reports from CK46124. Both of these are also satisfactory, so he marks both using the Final Review button.

8. The Actives report for CK46125 also appears acceptable, so he clicks Final Review once more. This causes the Impurities report for CK46125 to be displayed. Michael notes that the individual impurities are just within limits but the total is too high.

9. He double-checks the addition manually but he knows the TotalChrom custom expression math is flawless. “That process may be getting out of hand he thinks to himself, as he clicks the Hold button.

The Reason for Hold is displayed and, following the SOP, Michael types in an explanation of why he is not approving the report.

10. He clicks OK and the notes that the report name in the status bar of the Review Reports window is now followed by (Hold – Unsigned). Since that was the final
report in the set, the Close button has been replaced by Finish, indicating he has reviewed everything.

11. Michael clicks the Finish button and the Sign reports dialog is displayed. This shows the three TotalChrom Report files he originally selected as top level nodes on the tree control. Each top level node has two second-level nodes under it, representing the individual reports. The Status to be Signed column shows three Reviewed – Final and one Hold:

   C:\data\september\09\CK46123.tc
   C:\data\september\09\actives_r2.rpt          Reviewed – Final
   C:\data\september\09\impurities_r3.rpt       Reviewed – Final
   C:\data\september\09\CK46124.tc
   C:\data\september\09\actives_r2.rpt          Reviewed – Final
   C:\data\september\09\impurities_r3.rpt       Reviewed – Final
   C:\data\september\09\CK46124.tc
   C:\data\september\09\actives_r2.rpt          Reviewed – Final
   C:\data\september\09\impurities_r3.rpt       Hold

12. Michael clicks on the Sign button and the Electronic Signature dialog appears.

13. Michael enters his user name (logon ID) in the first field and his password in the second field.

14. When he clicks the OK button the display returns to the main Review and Approve screen. The file list still contains the three TCR files for samples CK46123, CK46124 and CK46125. The Status column now displays Reviewed – Final for the first five reports and Hold for the final report.

At that moment Susanna walks into the lab. Debbie said that the analyses that had to be run again over the weekend. “Well, I can tell you that Debbie isn't going to be happy because one sample is no good. CK46125 has too high a total level of impurities. We can’t let it go.” “How about the others”, asks Susanna. CK46123 was well within limits and CK46124 was between the two.” “Well I suppose we should be grateful that two out of three are OK.” “Do you want to look these over, since you are here right now?” asks Michael, offering his chair to Susanna. “Sure”, she replies, and sits down at the computer.

15. Susanna chooses the All Reports command from the Sign menu and examines each report in turn in the Review Reports window. The first five reports are all marked with the Reviewed – Final status that Michael signed off.

16. Susanna clicks the Final Approve button for each of these. After the fifth click, the final report is displayed and Susanna notes the Hold designation in the status bar.
17. She clicks the Comment button, which is the only button in the Sign toolbar enabled, since the current unapproved report has a comment attached. A dialog is displayed showing Michael's reason for holding the report.


18. Susanna closes the comment dialog and clicks the Finish button, reviews the names of the five reports she is signing in the Sign Reports dialog, clicks the Sign button and finally enters her logon name and password in the Electronic Signature dialog.

When she accepts that dialog the file list in the main Review and Approve window is updated to show the following statuses for the three sample files:

- C:\data\september\09\CK46123.tcrReviewed – Final
- C:\data\september\09\CK46124.tcrReviewed – Final
- C:\data\september\09\CK46124.tcrHold

At that moment James rushes into the lab. “Morning, James” says Michael. “Hi Mike, I was hoping Susanna would be with you. Debbie is waiting for you in your office, Susanna. She asked me to get you right away.” “Letting me down again, James?” jokes Susanna. “OK, I'll go and break the bad news to her.” “Call on me if you need support”, Michael calls after Susanna as she leaves the lab. “That's what the PerkinElmer salesperson said”, replies Susanna, “but that TotalChrom software is so easy to use I have never had to.”
To configure your system for Review and Approve, follow this procedure:

1. Select System Configuration from the TotalChrom Navigator Admin menu.
2. Select Review and Approve Settings from the System menu to access the Review and Approve Settings dialog.
3. In this dialog define (or a system administrator will define) the required Signature Points (levels) and set other Signature Policy rules. Click OK when done.
If a computer is to be used as an analysis server, and if you want to generate TCR files, then the System Configuration on that server must have at least one box checked in the Review and Approve Settings dialog under Signature Points (for example, Review, Final Review, Approve, Final Approve, or Hold). The analysis server must be configured this way, even if no one will actually at its console and generate result files.

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Review</td>
<td>A check box that indicates at least one Review signature will be required for reports.</td>
</tr>
<tr>
<td>Final review</td>
<td>A check box that indicates a Final Review signature will be required. If the Review check box is also checked then this means at least two signatures will be required (but there could be more Approve signatures).</td>
</tr>
<tr>
<td>Approve</td>
<td>A check box that indicates at least one Approve signature will be required for reports.</td>
</tr>
<tr>
<td>Final Approve</td>
<td>A check box that indicates a Final Approve signature will be required. If the Approve check box is also checked then this means at least two signatures will be required (but there could be more Review signatures).</td>
</tr>
<tr>
<td>Hold</td>
<td>A check box that indicates reports can be marked as hold.</td>
</tr>
<tr>
<td>Same user cannot sign two levels for a report</td>
<td>A check box that indicates a user can only sign a given report at one level.</td>
</tr>
<tr>
<td>User must enter a comment for each Unapproved report</td>
<td>A check box that indicates a user signing to Hold a report must enter a comment.</td>
</tr>
</tbody>
</table>

4. Set (or a system administrator will set) the Review and Approve permissions from the Job Types tab as shown below.
The Review and Approve permissions are described below.

<table>
<thead>
<tr>
<th>Permission</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>User can Review reports</td>
<td>A check box that indicates the user has permission to mark reports as Reviewed.</td>
</tr>
<tr>
<td>User can Final Review reports</td>
<td>A check box that indicates the user has permission to mark reports as Reviewed – Final.</td>
</tr>
<tr>
<td>User can Approve reports</td>
<td>A check box that indicates the user has permission to mark reports as Approved.</td>
</tr>
<tr>
<td>User can Final Approve reports</td>
<td>A check box that indicates the user has permission to mark reports as Approved – Final.</td>
</tr>
<tr>
<td>User can Hold reports</td>
<td>A check box that indicates the user has permission to mark reports as Hold.</td>
</tr>
<tr>
<td>External Programs</td>
<td>A button that provides access to the TcAccess/TCPublisher permissions dialog.</td>
</tr>
</tbody>
</table>
About External Programs

Clicking the External Programs button displays the External Programs dialog. In this dialog the system administrator sets the permissions for members of the Job Type that have to use TcAccess, IRIS and TCPublisher. These are linked together in this dialog because IRIS and TCPublisher make use of TcAccess.
### Abbreviations and Acronyms

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>ES</td>
<td>Enhanced Security (PKI term for s/w designed for 21 CFR Part 11)</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GAMP</td>
<td>Good Automated Manufacturing Practice</td>
</tr>
<tr>
<td>GLP</td>
<td>Good Laboratory Practices</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practices</td>
</tr>
<tr>
<td>GxP</td>
<td>“GLP” plus “GMP”</td>
</tr>
<tr>
<td>IDX</td>
<td>TotalChrom Index file</td>
</tr>
<tr>
<td>MTH</td>
<td>TotalChrom Method file</td>
</tr>
<tr>
<td>RAW</td>
<td>TotalChrom Raw Data file</td>
</tr>
<tr>
<td>RPT</td>
<td>TotalChrom Report Template file</td>
</tr>
<tr>
<td>RST</td>
<td>TotalChrom Result Data file</td>
</tr>
<tr>
<td>SEQ</td>
<td>TotalChrom Sequence file</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>TCR</td>
<td>TotalChrom Report file</td>
</tr>
</tbody>
</table>
Appendix A

How Interfaces Collect Data

This appendix describes how the 600 Series LINK Interface and 900 Series Interface collect data, and how TotalChrom interprets that information as chromatographic data.

<table>
<thead>
<tr>
<th>To learn about:</th>
<th>Go to page:</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Downloading Parameters to the Interface</td>
<td>A-6</td>
</tr>
<tr>
<td>Storing Data in the Interface</td>
<td>A-7</td>
</tr>
</tbody>
</table>
Using Interfaces to Collect Data

During data acquisition, you need to interact with instruments to:

- Set up an instrument for data acquisition
- Start data acquisition
- Pause or stop data acquisition
- Respond to error conditions

You communicate with the instrument through an interface. TotalChrom supports two types of interfaces to acquire data: a 600 Series LINK Interface, commonly referred to as a LINK, and a 900 Series Interface.

How the 900 Series Interface Converts Analog Signals to Digital Values

The principal function of a 900 Series Interface is to convert analog voltage signals to digital values from chromatographic detectors. You can also communicate with instruments in a limited way through these interfaces by synchronizing their operation with TotalChrom, by obtaining rack and vial numbers from autosamplers, and by using the seven relays on the interface to operate valves on other external devices.

Each 900 Series Interface can be connected to one or two chromatographic detectors, usually on a single instrument. The signal from each detector enters the interface through one of two channels in the interface. When you set up a method or sequence to collect data in TotalChrom, you specify Channel A, Channel B, or both channels as being active (950, 960, and 970 only). The sampling rate and voltage range settings are identical for both channels. However, you may not use some sampling rates available for single-channel data acquisition for dual channels.

The 900 Series Interface converts an analog voltage signal to a frequency-modulated pulse train, which is a series of pulses with a frequency that varies in proportion to the signal voltage. The interface then counts the pulses and records a value every 0.01 second. The count accumulated during this interval is called a time slice. The value of each time slice, or the sum of two or more time slices, becomes a data point on the chromatogram.

Because the interface always records a count every 0.01 second, its fundamental sampling rate is 100 points per second. However, you can define a lower sampling rate in the method. The valid range is 100 pts/s to 0.1 pts/s (or 1 point every 10 seconds). If you use a rate that is slower than the fundamental rate, the interface sums the appropriate number of slice values taken at the fundamental rate. This integrated value becomes a data point. The number of time slices that are summed to derive a data point depends on the desired sampling rate. For example, if the method calls for a rate of 10 pts/s, the interface sums 10 time slices taken at the fundamental rate.
Not all sampling rates between 100 and 0.1 are valid because the interface averages only the counts taken at whole 0.01-second intervals, not at fractional intervals. So, if two counts are averaged, the resulting sampling rate would be 50 pts/s. If three counts are averaged, the resulting sampling rate would be 33.33 pts/s. No rates between 50 and 100 or between 33.33 and 50 are possible.

Allowed sampling rates conform with this relationship:

\[
\frac{100}{(\text{sampling rate})(\# \text{ channels used})} = \text{integer}
\]

where the sampling rate is in pts/s, and the number of channels used is either 1 or 2, depending on whether you are performing single- or dual-channel data acquisition. If you enter an impossible sampling rate, TotalChrom will substitute the nearest allowed rate. Certain rates are disallowed on systems with 50Hz power to prevent aliasing effects.

All raw area sums are normalized before storing them in the interface. The normalization equation is as follows:

\[
\text{stored value} = \frac{\text{raw sum} \times 10}{\# \text{ of time slices}}
\]

A value stored in a 900 Series Interface normally falls in the range of 1 to 999999 counts. Values outside this range are adjusted to the appropriate limits. TotalChrom compresses stored data to conserve memory.

**How the 900 Series Interface Interacts with an Instrument**

You can interact with a chromatographic instrument using the 900 Series Interface by:

- Sending a remote Start signal from the autosampler to the interface
- Sending a remote Stop signal from the autosampler to the interface
- Sending a Ready signal from the interface to the autosampler
- Communicating rack and vial numbers from the autosampler to the interface
- Switching chromatographic components by using interface relays

The connections you can make depend on the features of the autosampler. Refer to the *900 Series Intelligent Interface Operator's Manual* from PerkinElmer for additional information.
Using a 600 Series LINK Interface

LINK Interfaces have no analog to digital (A/D) conversion function. Their main purpose is to communicate with chromatographic instruments. You can have up to three gas chromatographs or one liquid chromatography system connected to a LINK. The LINK sends commands to an instrument using the instrument’s own protocol while simultaneously communicating with the Navigator function in TotalChrom. This enables you to operate the instrument from the computer.

The LINK can convey instructions to an instrument in order to control such parameters as sampling rate, run time, injection volume and speed, zone temperatures, detector range and polarity, inlet pressures, solvent conditions and valve settings. The parameters you set depend on which instrument you are using.

You enter instrument control parameters in the Method Editor or by using a Quick Method in the Setup function. These parameters become part of the method file, which is downloaded to the LINK’s memory during Setup.

Instrument Personality Modules

The LINK communicates with an instrument by using software called instrument personality modules (IPMs). These IPMs are copied to your hard disk when you install TotalChrom and installed in a LINK by the Configuration Editor. Each combination of modules that makes up an instrument requires a unique IPM. Each IPM has two parts: a firmware module and a library (LIB) file.

The firmware module controls data acquisition and communication. It enables the LINK to translate downloaded parameters into a form recognized by the instrument. The firmware is downloaded to a battery-backed, non-volatile portion of memory in the LINK, and it remains in memory even in the event of a power failure. When power is restored, a startup file in the LINK automatically reinstalls the IPM.

The LIB file, which resides in the program directory on the host computer, contains information about each of the features that are supported on a given instrument. The Configuration function in TotalChrom consults this file to provide appropriate instrument-configuration options. When you configure the instrument, Configuration generates a CFG file that contains a subset of the information in the LIB file: those features pertaining to the particular instrument that you configured. The CFG file then serves as a source of information for the Method Editor, determining the options available to you when you create a method for the instrument.
How the LINK Communicates with an Instrument

The LINK has four RS232 ports — A, B, C, and D — and can accommodate up to four instrument modules.

If you have more than one similarly configured (using the same combination of instrument and autosampler) gas chromatograph connected to a LINK, you need only one IPM to control them. If you have two configurations on a LINK, you must have an IPM present in the LINK for each one.

A LINK can collect data simultaneously from all attached chromatographs, and this data can be received by one or two channels, depending on the instrument. The LINK sorts and stores the data, which can range in value from 1 to $2^{32}$. TotalChrom does not compress the data because the original data formats may vary widely.

Once you have established the proper communication between your instruments, interfaces, and the computer, you are ready to set up the instruments for data acquisition by defining method parameters, and/or a sequence.
**Downloading Parameters to the Interface**

Downloading prepares the 900 Series Interface or the LINK-controlled instruments to collect data. In downloading, sets of parameters from the instrument section of the controlling method (or methods) are sent to the memory of the interface. For LINK interfaces, these parameters are passed on to the chromatograph as needed.

Downloading is carried out as part of the Setup process. When you set up an instrument using QuickStart or a method, there is only one set of instrument parameters to be downloaded. However, when you set up a sequence, there may be many different instrument methods referenced. Both 900 Series and LINK Interfaces can accommodate up to 21 sets of instrument parameters at once.

The number of sets of parameters actually downloaded depends on the content of the sequence. If all cycles use the same instrument method, then only one set of parameters will be downloaded to the interface. If different instrument methods are used during the course of the sequence, then up to 21 sets of parameters may be downloaded as required. When an instrument method is repeated in a sequence after one or more intervening methods, its parameters are treated as a new set. In the example sequence shown below, five sets of parameters would be downloaded to the interface (in the order SOLV1, SOLV2, SOLV3, SOLV2, SOLV4) even though the sequence contains only four distinct methods.

<table>
<thead>
<tr>
<th>Row</th>
<th>Type</th>
<th>Sample Name</th>
<th>Sample Number</th>
<th>Inst Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample</td>
<td>Solvent 1A</td>
<td>1</td>
<td>SOLV1</td>
</tr>
<tr>
<td>2</td>
<td>Sample</td>
<td>Solvent 1B</td>
<td>2</td>
<td>SOLV1</td>
</tr>
<tr>
<td>3</td>
<td>Sample</td>
<td>Solvent 2A</td>
<td>3</td>
<td>SOLV2</td>
</tr>
<tr>
<td>4</td>
<td>Sample</td>
<td>Solvent 3A</td>
<td>4</td>
<td>SOLV3</td>
</tr>
<tr>
<td>5</td>
<td>Sample</td>
<td>Solvent 3B</td>
<td>5</td>
<td>SOLV3</td>
</tr>
<tr>
<td>6</td>
<td>Sample</td>
<td>Solvent 3C</td>
<td>6</td>
<td>SOLV3</td>
</tr>
<tr>
<td>7</td>
<td>Sample</td>
<td>Solvent 2B</td>
<td>7</td>
<td>SOLV2</td>
</tr>
<tr>
<td>8</td>
<td>Sample</td>
<td>Solvent 2C</td>
<td>8</td>
<td>SOLV2</td>
</tr>
<tr>
<td>9</td>
<td>Sample</td>
<td>Solvent 4A</td>
<td>9</td>
<td>SOLV4</td>
</tr>
<tr>
<td>10</td>
<td>Sample</td>
<td>Solvent 4B</td>
<td>10</td>
<td>SOLV4</td>
</tr>
</tbody>
</table>

The number of sequential cycles that each set of parameters is used for also downloads to the interface. This allows the interface to continue to take data if communication to the Acquire server process is lost (perhaps due to a network failure or power outage).

If more than 21 sets of parameters are needed for the sequence, the Acquire server downloads additional sets when the interface has completed the cycles using the initial sets. In this case, the Acquire server must be running for the sequence to complete.
Storing Data in the Interface

Both 900 Series and LINK Interfaces have internal memory for storing data. They store the data until the Acquire server transfers the data to files on disk. Memory capacity varies for the different interface models. Refer to the operator’s manual for your interface for more information.

Memory Segmentation

At setup time, you specify if you want to store the data from just a single run or from multiple runs in the interface memory. This determines whether or not the interface memory will be segmented. A segmented memory is divided into distinct storage areas or segments. Each segment holds the data from a single run for either single- or dual-channel acquisition.

The maximum number of segments into which the interface memory can be divided is 100. The actual number of segments created depends upon the number of data points generated by each run. When you set up an interface, the Navigator calculates a nominal segment size based on the sampling rate and run time specified for the first run. This calculation is based on an estimate of three bytes per data point in a 900 Series Interface and four bytes per data point in a LINK Interface. However, segmentation is dynamic, and each segment is adjusted to accommodate the actual amount of data acquired during the run.

If the Acquire server is running while the runs are in progress, it transfers the data to raw data files on disk, starting with the first segment filled. If all segments become filled during the course of a sequence, the interface will begin re-using segments from which the Acquire server has read the data. Data points that have not been read by the Acquire server will not be overwritten. A backlog of unread data will accumulate if the Acquire server is not running or if it cannot keep up with the total rate of data collection. If all segments of an interface memory become full before the Acquire server can read the data, the interface stops collecting data.

Unsegmented Memory

When the interface has been set up to store a single run only, the memory is unsegmented and acts like one large segment. In the case where the run produces more data points than can be stored in the interface memory, it is necessary to use unsegmented memory. This allows the interface to “wrap around” once the memory has filled, and begin overwriting those data that were stored first and have already been read by the Acquire server and stored on disk. If the earlier data points have not been read by the Acquire server (for example, because it was not running), then the interface will stop collecting data when the memory is full and the run will end.
**Backlogged Data**

Several conditions determine how long data collection will continue. These are outlined in the table on the following page.

The front panel of an interface indicates whether its memory contains backlogged data. On 900 Series Interfaces, both the Under Range and Over Range indicators light up. On LINK Interfaces, a blinking Ready light for a given port (A-D) indicates a backlog of data from the instrument connected at that port. A blinking Error light, in addition to a blinking Ready light, indicate that the memory is full.

When the Acquire server is started, or when communication is re-established after a network failure, the program automatically begins to read any backlogged data and transfers the data to the host computer.

If communication is disrupted between the interface and the Acquire server, data acquisition will continue in the background until the conditions cited in the following table are met.
### Storing Data in the Interface

**How Interfaces Collect Data**

<table>
<thead>
<tr>
<th>Interface Type</th>
<th>Conditions</th>
<th>Data Collection Continues Until:</th>
</tr>
</thead>
<tbody>
<tr>
<td>900 Series</td>
<td>No autosampler OR Autosampler does not send remote start signals to the interface.</td>
<td>End of current run or interface memory is full.</td>
</tr>
<tr>
<td>900 Series</td>
<td>Autosampler sends remote start signals AND 1 to 21 instrument files in the sequence.*</td>
<td>Interface memory is full.</td>
</tr>
<tr>
<td>900 Series</td>
<td>Autosampler sends remote start signals AND &gt;21 instrument files in the sequence.</td>
<td>End of the last cycle in the sequence that uses the 21st instrument file or interface memory is full.</td>
</tr>
<tr>
<td>LINK</td>
<td>LINK controls an autosampler.</td>
<td>Injections have been made from each vial listed in the sequence or interface memory is full.</td>
</tr>
<tr>
<td>LINK</td>
<td>No autosampler.</td>
<td>End of the current run or interface memory full.</td>
</tr>
<tr>
<td>LINK</td>
<td>Autosampler is not controlled by LINK, but sends remote start signals to chromatograph.</td>
<td>Interface memory is full.</td>
</tr>
</tbody>
</table>

* Sets of parameter from up to 21 instrument files in a sequence can be downloaded to the interface at once. After completing the cycles that utilize these instrument files, Acquire downloads the next 21 sets of parameter or the remaining sets of parameters if less than 21 are left in the sequence.
Data Compression

Data compression is a memory-saving feature of the 900 Series Interface only. It does not store values representing actual points on a chromatogram, except for the first point. Instead, the interface calculates the difference between each two consecutive points and stores these values. Because the difference values are smaller than the actual data points, they occupy less memory. This compression technique is called *delta modulation*.

When the TotalChrom Acquire server transfers data from the interface memory, the interface translates the compressed data to full data point values. These data point values, not the value differences, are stored in the raw data file.
Appendix B
System Suitability Testing

This appendix describes how to use the optional System Suitability program.

<table>
<thead>
<tr>
<th>To learn about:</th>
<th>Go to page:</th>
</tr>
</thead>
<tbody>
<tr>
<td>System Suitability Parameters</td>
<td>B-2</td>
</tr>
<tr>
<td>Program Operation</td>
<td>B-3</td>
</tr>
<tr>
<td>Suitability Reports</td>
<td>B-4</td>
</tr>
<tr>
<td>Using Suitability</td>
<td>B-8</td>
</tr>
<tr>
<td>Creating a Suitability Method</td>
<td>B-9</td>
</tr>
<tr>
<td>Using an Existing Suitability Method</td>
<td>B-11</td>
</tr>
<tr>
<td>Editing the Component List</td>
<td>B-12</td>
</tr>
<tr>
<td>Selecting Suitability Options</td>
<td>B-13</td>
</tr>
<tr>
<td>Selecting Result Files</td>
<td>B-16</td>
</tr>
<tr>
<td>Printing the Suitability Method and Reports</td>
<td>B-18</td>
</tr>
<tr>
<td>Automating Suitability Reports</td>
<td>B-20</td>
</tr>
<tr>
<td>Suitability Calculations</td>
<td>B-22</td>
</tr>
</tbody>
</table>
**What Is System Suitability Testing?**

System suitability testing assesses the performance of a chromatographic system including the column, injector, detector, electronics, mobile phase, operating temperature, and other components. It allows you to verify that the system is operating within the performance requirements of an analytical method and lets you compare the performance of different systems.

For the best results, conduct suitability testing periodically as a routine quality control measure and whenever you replace a system component.

**System Suitability Parameters**

System suitability testing evaluates the system’s separation characteristics and reproducibility. The ASTM (American Society for Testing and Materials), USP (United States Pharmacopeia), and BP (British Pharmacopeia) have defined a set of suitability parameters by which to quantify these characteristics.

To test suitability, you calculate values for these parameters and compare them with acceptable limits. When the parameter values fall within these limits, the system is deemed to be suitable.

The parameters that are evaluated in suitability testing are:

- Theoretical plates
- Tailing factor (asymmetry of peak)
- Relative retention ($\alpha$)
- Capacity factor ($k'$)
- Resolution
- Peak width
- Peak area and height (to test reproducibility of replicate injections)
- Signal-to-noise ratio
**Program Operation**

The Suitability program uses TotalChrom result files and their corresponding raw data files. For each component peak you specify, it calculates values for the suitability parameters you have selected. It compares each value against limits you have defined and reports whether or not the results fall within these limits.

*The date and time of the result files used in System Suitability calculations will be modified.*

Running Suitability is a three-step procedure:

- First, you load a suitability method file. The method specifies (1) the components for which you want to obtain suitability data, (2) the parameters you want calculated, and (3) suitability limits for each parameter.

- Second, you select one or more result files that contain chromatography results for the components listed in the method. Each result file points to the raw data file from which the results were derived.

- Third, you run calculations and print reports.

You can perform all three steps interactively, or have TotalChrom perform them automatically as part of the data analysis process. To do this, you identify Suitability as a user program in the method, and set it up to run during data analysis (at any point after component identification), or after the analysis is complete. Refer to “Automating Suitability Reports” on page B-20.
Suitability Reports

The Suitability program produces two types of reports that contain suitability results:

- System Suitability Reports
- System Suitability Summary Reports

System Suitability Reports

The System Suitability Report contains the results of evaluating the data in a single result file and its companion raw data file (that is, the data produced by a single injection). When no companion raw data file is found for a result file, a message appears that informs you that this is the case.

Parameter values are given for each sample component. The following figure is an example of this type of report.
**SYSTEM SUITABILITY REPORT**

Software Version : 6.0.0  
Date : 2/9/97 04:29 PM  
Instrument : 970 - 0  
Instrument Method : TESTSUIT  
Suitability Method File : C:\TCCS\VER6.0.0\EXAMPLES\USPSUIT.SUI  
Author : BATCH  
Compliance : USP  
Alpha and Resin Calc. : Adjacent SUIT Components  
Tailing Factor Calc. : % Peak Height  
Void Time : 0.100  
S/N Window Start : 0.200 min  
S/N Window End : 0.500 min  
S/N n Sigma : 4  

<table>
<thead>
<tr>
<th>Result File</th>
<th>Sample Name</th>
<th>Acquisition Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>suit003.rst</td>
<td>suit003.rst</td>
<td>1/13/94 03:04 PM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Ret Time</th>
<th>Peak Area</th>
<th>Peak Height</th>
<th>N Tan</th>
<th>N Foley</th>
<th>Tail Fact</th>
<th>k'</th>
<th>Resolution</th>
<th>Alpha</th>
<th>Signal / Noise</th>
<th>Peak Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinyl Chloride</td>
<td>0.655</td>
<td>1232088</td>
<td>204116</td>
<td>288.56</td>
<td>139.71</td>
<td>1.880</td>
<td>5.548</td>
<td>N/A</td>
<td>N/A</td>
<td>828</td>
<td>9.251</td>
</tr>
<tr>
<td>Chloroethane</td>
<td>1.105</td>
<td>2436791</td>
<td>407895</td>
<td>847.63</td>
<td>431.69</td>
<td>1.822</td>
<td>10.049</td>
<td>2.942</td>
<td>1.811</td>
<td>1.65e+03</td>
<td>9.108</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.405</td>
<td>3045509</td>
<td>509543</td>
<td>1367.25</td>
<td>674.89</td>
<td>1.887</td>
<td>13.049</td>
<td>1.975</td>
<td>1.299</td>
<td>2.07e+03</td>
<td>9.119</td>
</tr>
</tbody>
</table>

**Peak Area Criteria**  
718545.00 to 4547371.00  
**Peak Height Criteria**  
119074.00 to 764230.00  
**N Tan Criteria**  
282.00 to 13508.10  
**N Foley Criteria**  
140.00 to 6966.80  
**Tailing Factor Criteria**  
0.05 to 2.00  
**k' Criteria**  
0.00 to 5.90  
**Resolution Criteria**  
0.90 to 8.00  
**Alpha Criteria**  
1.00 to 10.00  
**Signal / Noise Criteria**  
200.00 to 10000.00  
**Peak Width Criteria**  
9.07 to 5.35

- Vinyl Chloride  
  - Tailing Factor : Requirements for suitability were met.  
  - Resolution : Requirements for suitability were not applicable.  
  - Alpha : Requirements for suitability were not applicable.  
  - Signal / Noise : Requirements for suitability were met.  
- Chloroethane  
  - Tailing Factor : Requirements for suitability were met.  
  - Resolution : Requirements for suitability were met.  
  - Alpha : Requirements for suitability were met.  
  - Signal / Noise : Requirements for suitability were met.  
- Chloroform  
  - Tailing Factor : Requirements for suitability were met.  
  - Resolution : Requirements for suitability were met.  
  - Alpha : Requirements for suitability were met.  
  - Signal / Noise : Requirements for suitability were met.

Approved by : _______________________________ Date : _________________
**System Suitability Summary Reports**

The System Suitability Summary Report contains mean parameter values obtained by evaluating the data in a list of result files that you specify.

The program examines the result files to see whether each has the same components. When the number of result files that include the same component (as indicated by “n = x” next to each component) does not equal the number of files summarized, then a message at the end of the report alerts you that there are missing components from the result files.

The summary also contains the relative standard deviation (RSD) for each parameter.
The following figure is an example of a summary report.

**SYSTEM SUITABILITY REPORT**

<table>
<thead>
<tr>
<th>Software Version</th>
<th>6.0.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>1/23/97 04:22 PM</td>
</tr>
<tr>
<td>Instrument</td>
<td>970 - 0</td>
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<tr>
<td>Instrument Method</td>
<td>TESTSUIT</td>
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<tr>
<td>Suitability Method File</td>
<td>C:\TCCS\VER6.0.0\EXAMPLES\USPSUIT.SUI</td>
</tr>
<tr>
<td>Author</td>
<td>MEA</td>
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<tr>
<td>Compliance</td>
<td>USP</td>
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<tr>
<td>Alpha and Resln Calc.</td>
<td>Adjacent SUIT Components</td>
</tr>
<tr>
<td>Tailing Factor Calc.</td>
<td>5% Peak Height</td>
</tr>
<tr>
<td>Void Time</td>
<td>0.100</td>
</tr>
<tr>
<td>S/N Window Start</td>
<td>0.200 min</td>
</tr>
<tr>
<td>S/N Window End</td>
<td>0.500 min</td>
</tr>
<tr>
<td>S/N n Sigma</td>
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</tr>
<tr>
<td># Of Files Summarized</td>
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</tr>
<tr>
<td>Result File</td>
<td>suit001.rst</td>
</tr>
<tr>
<td>Sample Name</td>
<td>1/13/94 02:40 PM</td>
</tr>
<tr>
<td>Acquisition Date</td>
<td>suit002.rst</td>
</tr>
<tr>
<td></td>
<td>1/13/94 02:54 PM</td>
</tr>
<tr>
<td></td>
<td>suit003.rst</td>
</tr>
<tr>
<td></td>
<td>1/13/94 03:08 PM</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Set</th>
<th>Peak</th>
<th>Peak</th>
<th>N Tan</th>
<th>N Foley</th>
<th>Tail</th>
<th>k'</th>
<th>Resln</th>
<th>Alpha</th>
<th>S/N</th>
<th>Base</th>
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<tr>
<td>Vinyl Chloride</td>
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<td></td>
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<tr>
<td>Mean</td>
<td></td>
<td>0.655</td>
<td>7195</td>
<td>1100</td>
<td>282.97</td>
<td>140.45</td>
<td>1.86</td>
<td>5.54</td>
<td>N/A</td>
<td>N/A</td>
<td>9.34</td>
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<tr>
<td>% RSD</td>
<td></td>
<td>0.03</td>
<td>65.33</td>
<td>65.48</td>
<td>1.98</td>
<td>0.93</td>
<td>1.23</td>
<td>0.02</td>
<td>N/A</td>
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<td>1567</td>
<td>26485</td>
<td>835.71</td>
<td>429.38</td>
<td>1.81</td>
<td>10.04</td>
<td>2.91</td>
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<tr>
<td>Mean</td>
<td></td>
<td>0.00</td>
<td>54.06</td>
<td>54.53</td>
<td>1.65</td>
<td>0.56</td>
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<td>0.02</td>
<td>0.83</td>
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<tr>
<td>% RSD</td>
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<td>54.06</td>
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<td>0.63</td>
<td>0.00</td>
<td>0.67</td>
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<td>0.59</td>
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<tr>
<td>Chloroform</td>
<td></td>
<td>1.405</td>
<td>1936</td>
<td>32713</td>
<td>1550.79</td>
<td>675.82</td>
<td>1.88</td>
<td>13.05</td>
<td>1.96</td>
<td>1.29</td>
<td>1.31</td>
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<tr>
<td>Mean</td>
<td></td>
<td>0.04</td>
<td>54.97</td>
<td>55.46</td>
<td>1.17</td>
<td>0.63</td>
<td>0.63</td>
<td>0.00</td>
<td>0.67</td>
<td>0.00</td>
<td>0.59</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.05</td>
<td>50.00</td>
<td>55.46</td>
<td>1.05</td>
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<td>0.63</td>
<td>0.00</td>
<td>0.67</td>
<td>0.00</td>
<td>0.59</td>
</tr>
<tr>
<td>Peak Area Criteria</td>
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<td>1.00 to 1000000.00</td>
<td>Maximum % RSD</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Height Criteria</td>
<td></td>
<td>0.00 to 1000000.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>N Tan Criteria</td>
<td></td>
<td>0.00 to 10000.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N Foley Criteria</td>
<td></td>
<td>0.00 to 10000.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
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<td>Tailing Factor Criteria</td>
<td>0.05 to 1.50</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.05</td>
<td>15.00</td>
<td>15.00</td>
<td>2.00</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Alpha Criteria</td>
<td></td>
<td>0.00 to 10.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signal / Noise Criteria</td>
<td>0.00 to 10.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Width Criteria</td>
<td></td>
<td>0.00 to 10.00</td>
<td>Maximum % RSD</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vinyl Chloride
- Tailing Factor: Requirements for suitability were met.
- Resolution: Requirements for suitability were not applicable.
- Alpha: Requirements for suitability were not applicable.
- Signal / Noise: Requirements for suitability were met.

Chloroethane
- Tailing Factor: Requirements for suitability were met.
- Resolution: Requirements for suitability were met.
- Alpha: Requirements for suitability were met.
- Signal / Noise: Requirements for suitability were met.

Chloroform
- Tailing Factor: Requirements for suitability were met.
- Resolution: Requirements for suitability were met.
- Alpha: Requirements for suitability were met.
- Signal / Noise: Requirements for suitability were met.

Approved by: _______________________________ Date: _________________

---

**System Suitability Testing**

B-7
Using Suitability

The System Suitability application is an optional TotalChrom program. The Suitability command appears on the Apps (Applications) menu in the TotalChrom Navigator.

➢ To open the System Suitability window:
  • In the Navigator, choose Suitability from the Apps menu to open the System Suitability window.

The System Suitability window contains the following menus and commands:

To perform a suitability calculation using the program interactively (as opposed to running it as a user program), you complete the following steps, each of which is described in the following sections:

• Create a new suitability method or load an existing one by using the New or Open command, respectively, in the File menu (refer to “Creating a Suitability Method” on page B-9).

• Select the parameters you want to use and set the value limits for suitability calculations by using the Options command (refer to “Selecting Suitability Options” on page B-13).

• Select the result files that contain the analysis results you want to evaluate by using the Data command in the File menu (refer to “Selecting Result Files” on page B-16).
• Edit the component list as necessary using the Edit List command in the Component menu (refer to “Editing the Component List” on page B-12.

• Initiate calculations by selecting a report to print (refer to “Printing the Suitability Method and Reports” on page B-18).

Creating a Suitability Method

If you do not currently have a suitability method that is appropriate to use, you can create a new one.

➢ To create a new suitability method:

1. In the System Suitability window, choose New from the File menu.
   If you currently have a suitability method loaded, this will clear it. The status bar at the bottom of the window will show zero result files and zero components.

2. To load a component list from an existing TotalChrom method, choose New from the Components menu.
   The File Open dialog box opens.

3. Select the method file from which you want to load the component list.

4. Choose OK to close the dialog box and load the components in the method into the component list. This overwrites any components already in the list.

5. Choose Edit List from the Component menu to edit the component list, as necessary.
   For more information, refer to “Editing the Component List” on page B-12.
Creating a Suitability Method

6. Choose the Options command to open the Suitability Options dialog box.

![Suitability Options dialog box]

For an explanation of each option, refer to “Selecting Suitability Options” on page B-13.

7. Choose Save As from the File menu.

The Documentation dialog box opens.

8. Complete the Description tab in the Documentation dialog box and choose OK.

For a discussion about entering information about a file, refer to Chapter 2, “TotalChrom Basics.”

The TotalChrom File Save As dialog box opens.

9. Enter a name for the new suitability method in the File Name text box and choose Save.

The dialog box closes, and the new file name appears in the title bar.
Using an Existing Suitability Method

You can also perform a suitability analysis based on the component list and suitability options already established in an existing method.

- **To load an existing suitability method:**
  1. In the System Suitability window, choose Open from the File menu.
     
     This opens the file selection dialog box, which lists the suitability method files that are available in the user's default method path.

     *Although both are called methods, TotalChrom method files use the .MTH extension whereas Suitability method files use the .SUI file extension.*

  2. Select the file you want to open and choose OK.
     
     The method is loaded into memory and the name appears in the title bar. The number of components associated with that suitability method appears in the status bar.
     
     If you want to modify the suitability method, you can:

     * Overwrite the existing component list (or create one) by opening either a new method file or one or more result files.

     * Edit the current component list by using the Edit List command in the Components menu.

     * Change the options using the Options command.

  3. Choose Save from the File menu to save the revised suitability method under the same name or choose Save As to save it as a new file.

     For more information on how to modify a suitability method, refer to “Editing the Component List” on page B-12 and “Selecting Suitability Options” on page B-13.
**Editing the Component List**

Suitability parameters are calculated by component. Therefore, you need to identify which components you want to include in the evaluation by developing a component list in each suitability method. When the files in the current result file list contain data for components that are not included in the component list, TotalChrom ignores them.

You can develop a component list by loading the components from the calibration section of a TotalChrom method. Alternatively, you can build a component list from a result file as described in “Selecting Result Files.”

You can edit the component list by deleting components, but you must select a method file again to add or insert components to the list.

➢ **To edit the component list:**

1. In the System Suitability window, choose Edit List from the Components menu.

   The Component List dialog box appears and shows the components in ascending order of retention time.

2. To delete a component from the list, select it and choose Delete.

   The selected component is removed, and the remaining components are renumbered.

3. When you finish changing the component list, choose OK. To reset the component list to what it was when you opened the dialog box, choose Reset.
Selecting Suitability Options

The Options command in the System Suitability window opens a dialog box where you set acceptability criteria and define which suitability parameters will be evaluated. All settings in the Options dialog box are saved as part of the suitability method.

➢ To change or set suitability parameters and values:

1. In the System Suitability window, choose Options to open the Suitability Options dialog box.

2. To enter a specific void time, leave Value selected and enter a time (in minutes) in the adjacent text box or select First Peak instead of Value.

   The Void Time is the time it takes an unretained solute to pass through the column. When you select First Peak, the retention time of the first peak is used as the void time.

3. Select an Efficiency option for reporting column efficiency.

   The default is total theoretical plates. Plates/Meter allows you to compare columns of different lengths. This defines how the results of plate calculations will be expressed in printed reports.

4. If you select Plates/Meter, enter a value (in meters) in the Column Length text box.
5. To calculate a signal-to-noise ratio for each component, enter values in the Start time and End time text boxes. This defines the noise measurement period, which must not contain any peaks. Suitability examines the raw data from this time period and calculates a noise value. First, it determines the linear least-squares fit line through all the raw (not bunched) data points during the specified portion of the run. Then, it calculates the standard deviation of the data point values from this line.

The final noise value used to calculate the signal-to-noise ratio is a multiple of the standard deviation. You set this value using the n Sigma parameter in the Suitability Options dialog box.

6. In the n Sigma text box, enter the value you want to use as the multiple of the standard deviation for noise measurement.

7. To generate a comma-delimited ASCII text file from the suitability analysis, select Generate ASCII-Delimited File.

This will produce a comma-delimited ASCII text file that contains the results of the suitability calculations requested in the current method. This file will be generated whenever you print a System Suitability Report or System Suitability Summary Report. You may use these ASCII files with other software applications.

8. Select either USP or BP as the Compliance option for calculating suitability parameters.

9. Select either Adjacent Peaks or Adjacent Components as the Analyze option for calculating resolution and alpha.

The Analyze option determines how the resolution and alpha calculations are done, either between a named component and the previous adjacent peak (Adjacent Peaks) or between a named component and the previous (adjacent) named component (Adjacent Components).

10. Select either 5% Peak Height or 10% Peak Height as the option for calculating tailing factor.

These two items are not available for testing against your validity criteria.

11. Under Criteria And Limits Selection, select the suitability parameters you want tested against your validity criteria by clicking the appropriate check box.

   - **Area** — Peak area
   - **Height** — Peak height
   - **N Tan** — Theoretical plates calculated by the tangential method
   - **N Foley** — Theoretical plates calculated by the Foley-Dorsey method
Selecting Suitability Options

**Tailing Factor** — Asymmetry of the peak

\( k' \) — Capacity factor

**Resolution** — Separation of a peak from the previous peak

**Alpha** — Relative retention of a peak to the previous peak

**S/N** — Signal-to-noise ratio

**Peak Width** — Peak width at the base

12. For each parameter selected, enter an acceptable Lower Limit and Upper Limit in the adjacent text boxes or accept the default values.

The results of the suitability calculations will be evaluated against these limits. When a result falls within the specified limits, the suitability requirements will be judged to have been met.

13. For each parameter selected, enter an acceptable percent relative standard deviation (% RSD) or leave the default value.

Mean and %RSD values are reported in the System Suitability Summary Report only. The mean values for each parameter are computed based on individual values derived from each file in the result file list. The %RSD for each parameter are calculated. When the calculated value exceeds the %RSD you enter, the requirements for suitability are *not* met.

14. Choose Reset or OK.
Selecting Result Files

To calculate suitability parameters, Suitability requires a result file list that consists of at least one .RST file. The program generates one System Suitability Report for each .RST file on the list or uses all the files to generate a System Suitability Summary Report.

The result files on the list should contain results for the components listed in the current suitability method. If the components do not coincide, you must edit the method or use the first result file as the source of a component list. The latter option is explained in the following procedure.

➢ To build a result file list:

1. In the System Suitability window, choose Data from the File menu to open the Result Files Selection dialog box.

2. Choose Add to display the file selection dialog box.

3. Locate and select the .RST file for which you want to generate a System Suitability Summary Report.

   The selected file name appears in the File List.

4. Repeat steps 2 and 3 for each result file you want to add.

5. If you want to use the components from the first result file in the list rather than from the current suitability method, select Create Component List From Result File.

   If you prefer to derive the component list from the calibration section of a method, leave this option unselected. You can edit the component list regardless of its source. If you create the component list from result files and later choose a method file, the second component list overwrites the first one.
6. Choose OK to close the dialog box and display the number of result files and components in the status bar.

➢ **To insert a file in the result file list:**
   1. Select the file above which you want to insert a file in the File List box.
   2. Choose the Insert button to display the file selection dialog box.
   3. Locate and select the file you want to insert.

➢ **To replace a file in the result file list:**
   1. Select the result file you want to replace in the File List box.
   2. Choose Change to open the file selection dialog box.
   3. Locate and select the replacement file.

➢ **To delete a file from the result file list:**
   1. Select the result file you want to delete in the File List Box.
   2. Choose Delete.

➢ **To clear the result file list:**
   - Choose Clear to delete all files from the File list.
Printing the Suitability Method and Reports

The Print command in the Suitability File menu lets you print method parameters, reports, and plots for the files in the result file list.

To print the method parameters, reports, and plots for the files in the result files:

1. Open the suitability method you want to print.
2. Choose Print from the File menu to open the Print dialog box.
3. Select one of the following options under Print Selection:
   
   **Method** — prints the parameters in the current suitability method. A report similar to the following is printed.

```
SYSTEM SUITABILITY METHOD FILE

Date                     : 1/28/97 04:05 PM
Suitability Method File  : C:\TCCS\VER6.0.0\EXAMPLES\USPSUIT.SUI
Author                   : MEA
Creation Date            : 1/28/97 04:02 PM

Theoretical plates will be reported in units of Plates
Theoretical Plates and Resolution will be calculated by USP Standards
Alpha and Resolution will be calculated using Adjacent SUIT Components
Tailing Factor will be calculated at 5% of Peak Height

Void Time                : 0.100
S/N Window Start         : 0.200  min
S/N Window End           : 0.500  min
S/N n Sigma              : 4

Limits will be checked on the following results:

<table>
<thead>
<tr>
<th>Result</th>
<th>Min</th>
<th>Max</th>
<th>Max % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Area</td>
<td>718945.00</td>
<td>4547371.00</td>
<td>54.00</td>
</tr>
<tr>
<td>Peak Height</td>
<td>119074.00</td>
<td>764230.00</td>
<td>54.50</td>
</tr>
<tr>
<td>N Tan</td>
<td>282.00</td>
<td>13508.10</td>
<td>.74</td>
</tr>
<tr>
<td>N Foley</td>
<td>140.00</td>
<td>6696.80</td>
<td>.35</td>
</tr>
<tr>
<td>Peak Tailing</td>
<td>0.05</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>k'</td>
<td>0.00</td>
<td>5.90</td>
<td>0.00</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.90</td>
<td>8.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Alpha</td>
<td>1.00</td>
<td>10.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Signal / Noise</td>
<td>2.00</td>
<td>10000.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Peak Width (Base)</td>
<td>9.07</td>
<td>9.35</td>
<td>.37</td>
</tr>
</tbody>
</table>

The following components will be included in the report:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Ret Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinyl Chloride</td>
<td>0.65</td>
</tr>
<tr>
<td>Chloroethane</td>
<td>1.10</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.40</td>
</tr>
<tr>
<td>t-1,2-Dichloroethene</td>
<td>1.90</td>
</tr>
<tr>
<td>Chlorotoluene</td>
<td>2.80</td>
</tr>
</tbody>
</table>
```

**Report** — TotalChrom performs suitability calculations for each file in the result file list and prints a separate report for each file.
Plot — The options in the Plot Style group box are enabled. Select the number of plots you want printed on each page. TotalChrom performs suitability calculations for each file in the result file list, and then prints a plot for each file.

Plot and Report — The options in the Plot Style group box are enabled. Select the number of plots you want printed on each page. TotalChrom performs suitability calculations for each file in the result file list and prints a separate report and plot for each file.

Summary Report — TotalChrom performs suitability calculations for each file in the result list and prints a single report containing the mean parameter and % RSD.

4. Select the number of plots you want printed on each page (1, 2, or 4 Per Page).

5. Choose OK.
Automating Suitability Reports

You can run System Suitability after data analysis or at any point after component identification. When Suitability runs, it will use the result file produced by the data analysis (and the related raw data file) for its calculations.

To run Suitability automatically after each analysis, you can list it as a user program in the processing section of the method. You can execute the program at any point after Component Identification; however, if you want to include the suitability results in a report, you must run suitability before report generation. Refer to Chapter 7 for instructions on how to include a user program in a method.

To run Suitability after a specific analysis, enter it as a user program in a sequence. Make sure the Synchronize With Instrument option is not checked. Refer to Chapter 11 for instructions on how to include a user program in a sequence.

You will need to use the following information. Separate parameters with a space.

Program Name — Enter or select SUIT.EXE.

Command Line —

For a Single File:

$RST [.SUI file name] /R /Q /1 /prn<printer_name>
/P /2
/B /4

- The variable $RST passes the name of the result file.
- [.SUI file name] is the name of the suitability method to be used. This file name and complete path must be in double quotes (for example, "name.sui").
- To automatically print a report, add the /R parameter. To automatically print a plot, add the /P parameter. To automatically print both, add the /B parameter.
- The /Q (quiet) parameter turns off error message display and prints errors to a log file. TotalChrom supplies the /Q parameter when Suitability is run as a user program.
- To print one, two, or four plots per page, add a /1, /2, or /4 parameter.
- If SYNCHRONIZE is set to NO, suitability will not update the RST file. SYNCHRONIZE must be set to YES if you want the RST file and suitability results in the report.
For Multiple Files:

$SEQ[(Row Range)] [.SUI file name] /R /Q /1 /prn<printer_name>

or $IDX

/P /2

/B /4

/R /1

- The variable $SEQ passes the name of the sequence file; and $IDX passes the name of the index file.

- [(Row Range)] enables you to specify a subset of files from either SEQ or IDX files. The subset specification must be enclosed within parentheses. You can specify individual row numbers, or by using a hyphen, you can specify a range of row numbers. Commas are used as separators. For example, the subset definition (1, 3 – 6, 8, 11–13) would cause cycles 1, 3, 4, 5, 6, 8, 11, 12, and 13 to be analyzed.

- [.SUI file name] is the name of the suitability method to be used. This file name and complete path must be in double quotes (for example, “name.sui”).

- To automatically print a report, add the /R parameter. To automatically print a plot, add the /P parameter. To automatically print both, add the /B parameter.

- The /Q (quiet) parameter turns off error message display and prints errors to a log file. TotalChrom supplies the /Q parameter when Suitability is run as a user program.

- To print one, two, or four plots per page, add a /1, /2, or /4 parameter.

- /prn<printer_name> specifies which printer to use. The printer name should be its UNC name as it is displayed in System Configuration and it should also be in double quotes. Depending on the printer configuration in your system, the switch may not be required, but using it will ensure that the specified printer will print.
Suitability Calculations

This section describes how TotalChrom calculates suitability results. For all equations, retention times and peak widths are measured in seconds.

If you have poorly resolved or badly skewed peaks, the System Suitability Report will probably display N/A for these values. Suitability might have been unable to calculate a value because it is unable to properly calculate the tangents needed to determine a peak width. This may produce an insufficient number of points under the peak. The Resolution and N Tan calculations require that there be more than 20 data points under the peak(s) in order to be calculated.

To obtain values, you may have to recollect the sample data using a higher sampling rate (perhaps 50 points per peak or more) to obtain enough data points. You could also use a bunching factor to maintain the optimal 10-20 points across the peaks for peak detection.

Theoretical Plates by the Tangential Method

First, peak tangents are calculated as a linear fit of all the points from 60% to 80% of the peak height on both the leading and trailing sides of the peak. Therefore, each peak has two tangents.

Then, the width at the base of the peak is computed as the time-axis projection between the two points at which the tangents and the baseline intersect. For ASTM and USP compliance, system efficiency $N_{sys}$ is then calculated using this equation:

$$N_{sys} = 16 \left( \frac{T_r}{W} \right)^2$$

where

$T_r$ is the peak retention time

$W$ is the calculated peak width at base

For BP compliance, the width of the peak is measured at half its height, and system efficiency $N_{sys}$ is calculated using the equation:

$$N_{sys} = 5.545 \left( \frac{T_r}{W_{h/2}} \right)^2$$

where

$T_r$ is the peak retention time

$W_{h/2}$ is the peak width at half height
**Theoretical Plates by the Foley-Dorsey Approximation**

The Foley-Dorsey method provides a significantly more accurate calculation of theoretical plates than the tangential method. Foley-Dorsey assumes an exponentially modified Gaussian distribution as the skewed peak model. The equation for system efficiency $N_{sys}$ is:

$$N_{sys} = \frac{41.7 \left( \frac{T_r}{W_{0.1}} \right)^2}{B/A + 1.25}$$

where

- $T_r$ is the peak retention time
- $W_{0.1}$ is the peak width at 10% of peak height
- $B/A$ is an empirical asymmetry ratio

**Tailing Factor**

The tailing factor $T$ is calculated by the equation:

$$T = \frac{W_{0.05}}{2f}$$

where

- $W_{0.05}$ is the peak width at 5% peak height
- $f$ is the width (time) between the peak maximum and the front edge of the peak at 5% of the peak height

**Capacity Factor ($k'$)**

The capacity factor is calculated using the following equation:

$$k' = (T_r / T_v) - 1$$

where

- $T_r$ is the peak retention time
- $T_v$ is the void time
Resolution

The resolution reported for a given peak is the value calculated between itself and the previous named peak. For USP compliance, resolution is calculated by the following equation:

\[ R_{p2} = \frac{2 \left( T_{p2} - T_{p1} \right)}{W_{p2} + W_{p1}} \]

where

- \( R_{p2} \) is the resolution of peak 2
- \( T_{p1} \) is the retention time of peak 1
- \( T_{p2} \) is the retention time of peak 2
- \( W_{p1} \) is the width at the base of peak 1
- \( W_{p2} \) is the width at the base of peak 2

For BP compliance, resolution is calculated by the following equation:

\[ R_{p2} = \frac{1.18 \left( T_{p2} - T_{p1} \right)}{W_{h1/2} + W_{h2/2}} \]

where

- \( R_{p2} \) is the resolution of peak 2
- \( T_{p1} \) is the retention time of peak 1
- \( T_{p2} \) is the retention time of peak 2
- \( W_{h1/2} \) is the width of peak 1 at half height
- \( W_{h2/2} \) is the width of peak 2 at half height

Alpha

The alpha value reported for a given peak is the relative retention of that peak to the previous named peak. This distinguishes it from the relative retention values you can select in the TotalChrom Report Format Editor, which are calculated for each peak relative to a specific named reference component. This latter value is usually designated by the letter “r.”
Alpha (relative retention) is calculated by the following equation:

$$\alpha_{p2p1} = \frac{T_{p2} - T_v}{T_{p1} - T_v}$$

where

- $\alpha_{p2p1}$ is the relative retention of peak 2
- $T_{p1}$ is the retention time of peak 1
- $T_{p2}$ is the retention time of peak 2
- $T_v$ is the void time

**Signal-To-Noise Ratio**

The signal-to-noise ratio is calculated using the following equation:

$$S/N = \frac{\text{Peak Height}}{\text{Noise}}$$

where

- $\text{Noise} = n$ times the standard deviation of $y$ residuals

$$\text{Standard deviation of } y \text{ residuals} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n-2}}$$

$\hat{y}_i = \text{values on the calculated regression line corresponding to the individual x-values}$

"The above equation is clearly similar in form to the equation for the standard deviation of a set of repeated measurements; the former differs in that deviations $(y_i - \bar{y})$ are replaced by residuals $(y_i - \hat{y})$, and the denominator contains the term $(n - 2)$ rather than $(n - 1)$. In a linear regression calculation the number of degrees of freedom is $(n - 2)$. This clearly reflects the obvious consideration that only one straight line can be drawn through two points."

Slope of line through data points given by:

\[
b = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{n \sum x_i^2 - (\sum x_i)^2}
\]

Intercept given by:

\[
c = \frac{\sum x_i^2 \sum y_i - \sum x_i \sum x_i y_i}{n \sum x_i^2 - (\sum x_i)^2}
\]
Appendix C

Interface Validation

This appendix explains how to use the Interface Validation Module software, which is an add-on application to TotalChrom.

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<th>Go to page:</th>
</tr>
</thead>
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<td>C-19</td>
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</tbody>
</table>
Interface Validation Module Software

Interface Validation Module software is designed for use with the Series 500 Interface Validation Module (IVM) to characterize the performance of the 900 Series Interfaces. The IVM provides accurate test patterns for the interfaces to digitize and the software to process. You need to purchase a license and install the license PAK to activate the software from within TotalChrom.

The validation procedure compares the observed data values to the expected values from known patterns. Estimates of performance parameters are made from this comparison. They are printed out as a report and are stored as a text file.

A validation run on any given interface generates three performance parameters:

- Percent Gain Error
- Standard Deviation of Percent Gain Error
- Linearity Error as Percent Full Scale

A comparison to a validation range is made on each parameter. The result passes if the parameter falls inside the validation range; the result fails if the parameter falls outside the validation range. The parameters, results, and ranges with descriptive information about the run are printed out as a report, stored in a result file, and stored in a text report file.

After you connect the IVM to the interface and turn it on, allow 45 minutes for the IVM and interface to stabilize. The validation procedure takes about 10 minutes. The results of the run are printed by the TotalChrom report generator. The result file uses standard TotalChrom naming conventions and replaces the normal result file generated by the Analyze program. The text report file is stored with the same base name as the raw file but with the extension .IVM. Any errors detected by the IVM program will be recorded in the IVM text report file.

A predefined method, report format, and sequence are provided for these tests. They are: IVM.MTH, IVM.RPT, and IVM.SEQ. You may select any instrument in the system that uses a 900 Series Interface as the instrument type in the method. You may also choose to test Channel A or Channel B and the voltage range over which the test will be run. The sequence file is only a template. There are no restrictions on base file names for the data files.

You can use the Summary function to review the data from the result files.
Installing IVM

The IVM application program, IVM.EXE, and the other IVM files were copied to the appropriate directories as part of the TotalChrom installation process. The program may be found with the other TotalChrom programs. The method, report format, sequence, and sample data files were copied to the Examples directory.

The location of example and reference files depends on which version of TotalChrom you have and how your application manager installed the files. For instructional purposes, the default file path is listed in this appendix as c:\penexe\tccs\ver6.0.0\examples, but your actual path may differ.

The software will only run if a license has been installed. Refer to the TotalChrom Application Manager’s Guide for license installation information.
Testing Unipolar and Bipolar Interfaces

There are three versions of the 900 Series Interface. The original 900 Interface (Models 941, 950, and 970) has programmable unipolar voltage ranges with nominal values of 0.1, 1.0, 2.0 and 10.0 Volts. The actual ranges are:

- −0.0005 to +0.0995 Volt
- −0.005 to +0.995 Volt
- −0.01 to +1.99 Volts
- −0.05 to +9.95 Volts

These ranges are considered unipolar since there is only a minimal offset to accommodate slight drift of the signal below zero.

A factory-installed modification to the 900 Interface enables it to operate in an asymmetric bipolar mode with the following nominal ranges:

- −0.1 to +0.1 Volt
- −0.55 to +0.45 Volt
- −0.6 to +1.4 Volts
- −1 to +9 Volts

The latest version of the 900 Series Interface, the 900A (Models 941A, 950A, and 970A), can operate with the same unipolar voltage ranges as the original 900 Interface. However, the 900A Interface can be set to a symmetric bipolar mode simply by setting a switch on the rear panel. See the *Intelligent Interface Operator’s Manual* for further details. The bipolar ranges available are:

- −0.05 to +0.05 Volt
- −0.5 to +0.5 Volt
- −1.0 to +1.0 Volt
- −5.0 to +5.0 Volts

Different Interface Validation Modules are required for testing unipolar and bipolar interfaces. The Model 500 IVM is used for testing unipolar interfaces, and the Model 510 is used for testing bipolar interfaces including A-series interfaces configured for bipolar operation. The Model 510 IVM can be set to operate in a unipolar emulation mode, but this provides less precision than the Model 500 and is not recommended for validation testing.

The procedures for testing unipolar and bipolar interfaces are essentially the same. The key factors are to ensure that the interface is set to the correct mode and that the voltage range set in the data acquisition method matches the range set on the IVM.
Refer to the *IVM 520 Interface Validation Module or 500 Series Interface Validation Module Operator’s Manual* for details on how to set the initial output range. You must also indicate to the IVM program whether you are testing the interface in unipolar or bipolar mode. Complete details for running the interface validation test are provided in the following section.
Validation Procedures

It is important to follow the procedures in this section carefully. The data generated by the IVM must be compatible with the parameters set in the data acquisition method. The data must also match that expected by the IVM application program or accurate validation parameters cannot be calculated.

➢ To connect the IVM to the interface:

1. Plug the black cable coming from the IVM into the appropriate channel on the interface using the attached depluggable connector.

   As supplied, the connector is wired for Channel A. The wires must be moved if you want to test Channel B.

   Do not connect Channels A and B together. The gain test requires that each channel be connected separately. To test both channels, connect one and test it. Then connect the other and repeat the test.

2. Connect the START IN +/- lines of the IVM to Relay 1 of the interface using the relay cable provided.

To conserve bench space, you can place the IVM on top of the interface.

Preparing the Method

The IVM method must be assigned to a 900 Series Interface that is currently configured on the system. It also must be assigned an appropriate data channel.

➢ To prepare an IVM method:

1. In the Navigator, click on the Method button or choose Method from the Build menu to open the Method Editor window.
The Startup dialog appears:

2. Choose “Load method stored on disk” and choose OK.

The TotalChrom File-Open appears:

3. Select the method IVM.MTH from the list.
   This method is typically in the path and: c:\penexe\tcws\ver6.x.x\examples.
4. Choose Open to close the dialog box and display the Instrument Selection dialog box.

![Instrument Selection Dialog Box]

5. Select the required 900 Series-based instrument you want to test from the list of configured instruments and choose OK.

The Data Acquisition dialog appears:

![Data Acquisition Dialog Box]

6. Choose the Data Channels tab and select either Channel A or Channel B in the Data Channels dialog box.
7. Select the Voltage Range of the 900 Series Interface you want to test.
   The voltage range options are the same for all unipolar and bipolar interfaces, and are the nominal overall range. For example, for a unipolar interface to be tested on the −0.005 to +0.995 Volt range, you select 1 Volt. Similarly, for a bipolar interface to be tested on the −5.0 to +5.0 Volts range, you select the 10 Volts option.
   You must use a Model 510 IVM to test the bipolar ranges of a 900A Series Interface or an original 900 Series Interface modified for bipolar operation. Refer to the section “Testing Unipolar and Bipolar Interfaces” on page C-4.

8. Click OK.

9. Choose User Programs from the Process menu.
   IVM.EXE appears in the first Program Name text box.

10. If you are testing an interface in unipolar mode, verify that the program information is as shown here.
    If you are testing an interface in bipolar mode, change the /U command line parameter to /B. The “/F$RST” parameter on the command line (followed by a space) must be left unchanged. This is expanded by TotalChrom to supply the result file name to the IVM application program.
11. If you do not want a replot of the IVM test pattern printed at the end of the run, choose the Replot tab, then deselect the Generate A Separate Replot option.

12. Choose OK to close the Process window.

13. Make no other changes to the method. To do so may invalidate the test.

14. Choose Save As from the File menu.

   The Documentation dialog box opens with either the Description or the Audit Trail tab.

15. Complete the Description or the Audit Trail tab. Refer to Chapter 2, “TotalChrom Basics,” for complete information.

16. Choose OK.

   The Save As dialog box opens.

17. Save the method as IVMA if you selected Channel A or as IVMB if you selected Channel B, and choose OK.

   Save both methods if the IVM sequence is to be used without any changes.

   The following example shows method parameters for testing Channel A of a unipolar interface.
TotalChrom Method File: C:\TCCS\VER6.0.0\EXAMPLES\IVM.MTH
Created by: on: 1/21/97 12:05 PM
Edited by: on: 1/26/97 12:26 PM
Description:
Number of Times Edited: 1
Number of Times Calibrated: 0

Instrument Conditions:
Instrument Control Method:
Instrument name: 970_0

Interface Parameters:
Delay Time: 0.00 min.
Run Time: 10.00 min.
Sampling Rate: 10.0000 pts/s
Interface Type: 900
Analog Voltage Input: 1000 mV
Data will be collected from channel A

Timed Events:
RLY1 set to ON at 0.08 min
RLY1 set to OFF at 0.20 min
RLY1 set to ON at 3.08 min
RLY1 set to OFF at 3.20 min
RLY1 set to ON at 6.08 min
RLY1 set to OFF at 6.20 min

Real Time Plot Parameters:
Channel A -- Pages: 1 Offset: 0.000 mV Scale: 1000.000 mV
Channel B -- Pages: 1 Offset: 0.000 mV Scale: 1000.000 mV

Processing Parameters:
Bunch Factor: 1 points
Noise Threshold: 1 uV
Area Threshold: 100.00 uV

Peak Separation Criteria
Width Ratio: 0.200
Valley-to-Peak Ratio: 0.010

Exponential Skim Criteria
Peak Height Ratio: 5.000
Adjusted Height Ratio: 4.000
Valley Height Ratio: 3.000

Baseline Timed Events:
Event #1 - -P at 0.000

Annotated Replot Parameters:
Offset & Scale determined automatically
Number of Pages: 1
Plot Title: IVM Test Data
X-Axis Label: Time [min]
Y-Axis Label: Response [mV]
Orientation: Landscape
Retention Labels: Top of Plot
Component Labels: Actual Time
Automatically set plot start and end times to data limits

Report Format files:
No report format files given

User Programs:
User Program #1: ivm.exe
Command Line: /F$RST /U
Entry Point: Quantitation
Synchronize: YES

Global Information:
Default Sample Volume: 1.000 u l
Quanti cation Units:
Void Time: 0.000 min
Correct amounts during calibration: YES
Reject outliers during calibration: NO
An External Standard calibration will be used
Unknown peaks will use the response factor of the nearest reference peak

Component Information:
No components present in calibration file
If you are ready to run the IVM test, you can continue with the following procedure.

For Turbo LC Plus systems with an analog detector, you can use IVM to validate a 900 interface used in tandem with a LINK. However, you must create a new IVM method for the specific instrument as follows: (1) open the IVM.MTH file in the Method Editor; (2) select the appropriate instrument in the Instrument Name dialog box; and (3) save the method as a new file (such as IVMLC.MTH). Use this new file for the IVM data acquisition method. This will cause the timed events and other processing parameters specified in IVM.MTH to be used for the validation experiment.

**Setting Up the Sequence**

To run the IVM test, you must set up the IVM sequence (IVM.SEQ) on the appropriate interface. You may want to modify the sequence first to define your own data file names.

```plaintext
TotalChrom Sequence File : C:\TCCS\VER6.0.0\EXAMPLES\IVM.SEQ
Created by :                  on : 1/26/97   12:38 PM
Edited by  :                  on : 1/26/97   12:38 PM
Description :

Number of Times Edited : 0

Sequence File Header Information:

Number of Rows    : 2
Instrument Type   : 760 / 900 Series Intelligent Interface
Injection Type    : SINGLE

Sequence Sample Descriptions - Channel A

<table>
<thead>
<tr>
<th>Row</th>
<th>Type</th>
<th>Sample Name</th>
<th>Sample Number</th>
<th>Amount</th>
<th>Amount</th>
<th>Sample Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample</td>
<td>IVM Test Data</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sample</td>
<td>IVM Test Data</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Sequence Sample Descriptions - Channel B

<table>
<thead>
<tr>
<th>Row</th>
<th>Type</th>
<th>Sample Name</th>
<th>Sample Number</th>
<th>Amount</th>
<th>Amount</th>
<th>Sample Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample</td>
<td>IVM Test Data</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sample</td>
<td>IVM Test Data</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Sequence Process Information - Channel A

<table>
<thead>
<tr>
<th>Row</th>
<th>Site</th>
<th>Rack</th>
<th>Vial</th>
<th>Inst</th>
<th>Process</th>
<th>Calib</th>
<th>Report</th>
<th>Raw</th>
<th>Result</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method</td>
<td>Method</td>
<td>Format</td>
<td>File</td>
<td>Result</td>
<td>File</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>IVMA</td>
<td>IVMA</td>
<td>IVMA</td>
<td>IVM</td>
<td>ivma001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>IVMB</td>
<td>IVMB</td>
<td>IVMB</td>
<td>IVM</td>
<td>nodata</td>
<td>nodata</td>
<td>DEFAULT,</td>
</tr>
</tbody>
</table>

Sequence Process Information - Channel B

<table>
<thead>
<tr>
<th>Row</th>
<th>Site</th>
<th>Rack</th>
<th>Vial</th>
<th>Inst</th>
<th>Process</th>
<th>Calib</th>
<th>Report</th>
<th>Raw</th>
<th>Result</th>
<th>Out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method</td>
<td>Method</td>
<td>Format</td>
<td>File</td>
<td>Result</td>
<td>File</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>IVMA</td>
<td>IVMA</td>
<td>IVMA</td>
<td>IVM</td>
<td>ivma001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>IVMB</td>
<td>IVMB</td>
<td>IVMB</td>
<td>IVM</td>
<td>ivmb001</td>
<td>ivmb001</td>
<td>DEFAULT,</td>
</tr>
</tbody>
</table>
```
To set up the IVM sequence:

1. If the Method Editor window is open, choose Sequence Editor from the Other menu to open the Sequence Editor window.

OR

Click on the Sequence button in the Navigator.

2. Open the IVM.SEQ file and edit either Channel A or Channel B to make any necessary modifications.

The sequence provided assumes you have used the method names suggested above (IVMA.MTH and/or IVMB.MTH). If you used any other name, you will have to enter it in the appropriate spreadsheet.

3. Choose Save from the File menu to save any changes you have made to the sequence file.

4. Choose Set Up from the Actions menu in the Sequence Editor.

The Sequence Editor window closes, the Navigator window opens, and the Setup dialog box appears.

5. Select the instrument to be tested from the list, and enter the directory where you want to store the data files in the Data Path text box.

If you are using a 950 (or 950A) Interface, make sure you select the Single Run data buffering option in the Setup dialog box. This will ensure that this interface will be able to store the complete IVM analysis. DO NOT suppress processing; otherwise, the IVM calculations will not occur. Do not suppress reports/plots if you want a printed IVM report.

The IVM test is now set up and ready to proceed.
Running the Test

Once the data acquisition method and sequence have been customized for the interface to be tested, you are ready to run the test.

➢ To run the test:

1. Make sure that the front panel of the IVM is set to Test 1 and that the Range selected is the same as the one you selected in the Data Channels dialog box of the method.

   It is essential that the initial Range setting on the IVM matches the Voltage Range specified in the method or the test cannot be performed successfully.

2. Start the 900 Series Interface.

   The Ready light will go out on the interface, and the Sampling light will come on. After 4 to 5 seconds, the Range light on the IVM will change from the initial setting to the next lower range and the Data light will start flashing.

   After about 1.5 minutes, the Range light on the IVM will change back to the initial setting and the Data light will continue to flash for another period of about 1.5 minutes. This cycle will repeat three times for a total elapsed time of 10 minutes.

After the run is completed, TotalChrom will run IVM.EXE as a user program. The IVM program stores the calculated values for the validation parameters in the result file and in an ASCII text file. Normal data analysis then continues and the printed IVM report is generated from the modified result file. A replot will also be generated if you specified this in the method.

If you are testing both channels, be sure to change the analog connection from Channel A to Channel B before proceeding. In the example sequence, Channel B is the next run after Channel A.
Example Validation Report

The following figure shows an example of the report generated by the IVM software using the IVM.RPT format file.

---

PerkinElmer Interface Validation Report

<table>
<thead>
<tr>
<th>IVM Test Metric</th>
<th>Test Result</th>
<th>Pass (+)/ Fail (-)</th>
<th>Specification (+/- % Full Scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain Error</td>
<td>-1.0803</td>
<td>+</td>
<td>2.5000</td>
</tr>
<tr>
<td>SD Gain Error</td>
<td>0.0053</td>
<td>+</td>
<td>0.1700</td>
</tr>
<tr>
<td>Linearity Error</td>
<td>0.0496</td>
<td>+</td>
<td>0.1000</td>
</tr>
</tbody>
</table>

---

Approved By: 

Date:
The following figure shows the IVM report format file parameters. This file should not be modified.

---

**Report Title:**

**PerkinElmer Interface Validation Report**

**User Report Header:**

No user header will be printed

**User Report Footer:**

Approved By: 
Date: 

**Report Format Options:**

- **System Header**
  A medium default header will be printed
- **Compressed mode**
  The report body will be in 80 column mode

**Report Body Options**

- Identified components
- Unidentified peaks

**Miscellaneous Options**

Report Area Reject = 0.00

**Report Columns:**

<table>
<thead>
<tr>
<th>Column</th>
<th>Label 1</th>
<th>Label 2</th>
<th>Width</th>
<th>Precision</th>
<th>Total Column</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>7</td>
<td>0</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>'IVM Test Metric'</td>
<td>' '</td>
<td>20</td>
<td>0</td>
<td>NO</td>
<td>Expression</td>
</tr>
<tr>
<td>3</td>
<td>'Test Result'</td>
<td>'(%Full Scale)'</td>
<td>8</td>
<td>4</td>
<td>NO</td>
<td>Expression</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>'Voltage Flag'</td>
<td>'Fail(-)'</td>
<td>1</td>
<td>0</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>'Peak Height'</td>
<td>'Specification'</td>
<td>8</td>
<td>4</td>
<td>NO</td>
<td>Expression</td>
</tr>
</tbody>
</table>
Validation Specifications

The default specifications for 900 Series Interfaces, which your applications manager can modify, are as follows:

- Gain Error: ± 2.50%
- SD Gain Error: ± 0.17%
- Linearity Error: ± 0.10% FS

The Result File

The IVM software modifies the normal TotalChrom result file to incorporate the test parameters for reporting and for review with the Summary program. The IVM information is stored in the following fields:

- Component Names: Test Parameter Names (for example, Gain Error)
- Adjusted Amount: Test Value
- Height: Specified Test Tolerance
- Voltage Over/Under Flag: Pass/Fail Flag

You can format a summary report to include historical IVM data using the above field names. For example, to report test parameter names and values, include the component name and adjusted amount columns in your summary report format.

The IVM Text File

The following figure shows an example of a single validation run text file. The file is stored with the same base name as the raw data file and the extension .IVM (such as TEST001.RAW, TEST001.IVM).

<table>
<thead>
<tr>
<th>IVM ANALYSIS LOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result File: C:\TCCS\VER6.0.0\DATA1\ivma001.rst</td>
</tr>
<tr>
<td>Date          : 1/21/97  04:02 PM</td>
</tr>
<tr>
<td>TotalChrom    : Version 4.0</td>
</tr>
</tbody>
</table>

Instrument : 970 - 0
Serial #     : 2056574541
Operator     :

Gain Error    : -1.0803 * passed * (+/- 2.5000)
SD Gain Error : 0.0053 * passed * (<= 0.1700)
Linearity Error (%FS) : 0.0496 * passed * (<= 0.1000)

In addition to the validation parameters, the file includes the date and time of the run, the TotalChrom revision level under which the run was made, the instrument name, interface serial number, operator name, and other file information.

The file is in ASCII format and, therefore, can be printed or used as input to other programs for subsequent archiving or processing.
Troubleshooting

The IVM software is designed to detect conditions that might prevent the successful completion of a run. The following is a list of potential error conditions.

- Unable to open result file — analysis halted.
- Unable to open raw data file — analysis halted.
- Unable to read information from data file — analysis halted.
- Unable to create modified result file — analysis halted.
- Unable to buffer raw data points — analysis halted.
- Zero determinant in regression — analysis halted.
- Unable to queue analysis request for IVM report.

These error conditions are reported in the IVM text report file. If the validation procedure is not working, this information, as well as any other error reports, can be useful in diagnosing the report.
How Calculations Are Performed

The calculations that the IVM application performs are based on a comparison of observed interface output versus expected interface output.

The IVM divides both the tested voltage ranges into 20 steps. A cycle begins at the bottom of the lower range and holds the voltage fixed for 4 seconds before increasing to the next higher step. A reading is taken of the analog zero before the cycles are triggered by Relay 1. The next reading is of the digital zero. After that, the 20-step pattern begins.

When all 20 steps in the lower range have been traversed, the pattern is repeated across the initially set range as shown in the IVM test plot above. A final window of baseline points at analog zero is taken after the last step. This cycle will be repeated two more times to complete the test. Each cycle is treated as a separate data set.

Zero-adjusted data points are used as the dependent variable set in a linear least-squares (LLS) regression analysis. Five points are taken at the midpoint at each voltage step for both voltage ranges as well as the final return to baseline. This results in 205 data points and 15 baseline points. The analog 0-volt points are fit with an LLS regression to determine the drift, and the data points are adjusted to provide the zero-adjusted point set. The data are also adjusted for the offset between digital and analog zero in the IVM.

The independent variable set is derived from constants specific to PerkinElmer interfaces. For the 0.1-volt range, each step is 4,899.6 counts; for the 1-volt range, each step is 48,996 counts.
If each observed value were identical to the expected value at every voltage step, the LLS regression would produce the equation of a line having a slope of exactly 1, an intercept of exactly 0, and the entire observed point set would fall on that line. Errors, both random and systematic, prevent this exact outcome.

**Gain Error**

The gain error is the deviation of the slope of the LLS line from unity expressed as a percent. It is calculated by the following equation:

\[
Gain\ Error = (Slope - 1) \times 100
\]

**Linearity Error**

From the LLS equation, you can predict what value will be observed at each expected value. That is:

\[
Predicted\ Value = Slope \times Expected\ Value + Intercept
\]

If the Predicted Value is compared to the 5 observed values selected mid-step at a single voltage level, the maximum difference is determined by:

\[
Maximum = \max_{i=1,5} \left| \text{Observed Value}_i - \text{Predicted Value}_i \right|
\]

Since there are 41 voltage levels (21 in the lower range with digital zero and 20 in the initially set range), the previous equation is modified as follows to find the maximum difference over all observed values:

\[
Maximum = \max_{i=1,5} \max_{j=1,41} \left| \text{Observed Value}_{i,j} - \text{Predicted Value}_{i,j} \right|
\]

The linearity error is the Maximum from the previous equation, expressed as a percent of full scale counts:

\[
Linearity = \left[ \frac{Maximum}{Counts\ at\ Full\ Scale} \right] \times 100
\]
**Reported Parameters**

Each IVM run produces three cycles through the next lower range and the initially set range. As a result, three values for both gain error and linearity error are obtained from each IVM run. The parameters reported in the IVM text report file are averages of these values.

**SD Gain Error**

SD Gain Error is the standard deviation of the three values of gain error obtained during each complete run of an IVM test.
Appendix D
Serial Dilution

Serial Dilution is a separate application for use with the Series 200 Autosampler only. This application allows you to perform multiple vial-to-vial transfer and mixing operations.

You can create a Serial Dilution program to transfer any amount of liquid between any two vials in the tray in any order. The Serial Dilution program you create is independent of the series of vials designated as samples by the sequence.

A common use for Serial Dilution is to prepare a series of standards for multilevel calibration. For example, if you automate the dilution of a single concentrated stock solution, you can create a series of increasingly dilute solutions that you can then use to calibrate the chromatograph.

Use the Serial Dilution command in the Apps menu of the Navigator to create Serial Dilution programs. Then specify the Serial Dilution program as a cycle type in the Sequence Editor.
To create a Serial Dilution program:

1. In the Navigator, select Serial Dilution from the Apps menu. A startup dialog appears.
2. Select a startup option and choose OK. The Serial Dilution Editor dialog appears.
3. Select Liquid or Air as the Mixing Mode.
   For the best precision with standard-sized vials, choose air mixing with 2 cycles. If you are using liquid mixing, refer to “Mixing Volume Rules for Liquid Mixing” on page D-4.
4. If you are using air mix mode, select from the Air Mix Volume list the volume of air you want delivered through the flush syringe for each mixing cycle.
5. In the Target Volume text box, enter the volume of liquid in the target vials. This volume is the amount of liquid present before the Autosampler performs any transfers. If the target vials contain different volumes, enter the smallest volume in the series of vials.

6. Select a speed from the Mix Speed Out list. For liquid mixing, this setting controls how fast the syringe dispenses the sample back into the vial for mixing. For air mixing, this setting controls how fast the syringe injects air into the sample vial.

7. Select a speed from the Mix Speed In list. For liquid mixing, this setting controls how fast the syringe draws up a sample from the vial for mixing. For air mixing, this setting controls how fast the syringe draws up air.

8. Since the pump is required to be running when a serial dilution method is being run, set the pump conditions for the serial dilution method.

9. In the Source Vial column, enter the position of the vial from which you want to transfer liquid. To specify the tank as the source vial, enter “Tank” in this field.

10. In the Volume Transferred column, enter the volume of the liquid you want the Autosampler to transfer from the source vial to the target vial.

11. In the Target Vial column, enter the position of the vial to which you want to transfer liquid.

12. In the Number of Mixes column, enter the number of times you want the Autosampler to mix the mixture in the target vials.

13. Repeat Steps 8 through 11 for each transfer you want to perform.

14. Choose Save from the File menu. The Documentation dialog box opens.

15. Complete the Description tab in the Documentation dialog box. For a discussion about entering descriptive information about a file, refer to Chapter 2, “TotalChrom Basics.”

16. In the Save As dialog box, enter a file name for the Serial Dilution program, and choose Save.

17. When you are finished working in the Serial Dilution Editor, choose Exit from the File menu.
Mixing Volume Rules for Liquid Mixing

TotalChrom determines the mixing volume for a vial by the following calculation:

$$(\text{Target Volume} + \text{Transferred Volume}) \times 0.80$$

where Transferred Volume is the sum of all volumes transferred from the source vials to a specific target vial.

Whenever you save a Serial Dilution program, TotalChrom first checks each step to verify that your program conforms to the following rules:

- The amount of liquid you transfer from a vial does not exceed the mixing volume for that vial.
- The sum of the mixing volume and the Air Gap (the separation between multiple samples) does not exceed the transfer line capacity. The transfer line capacity for 2500 mL syringes is 2.4 mL; for all others, the capacity is 1.1 mL.

If any steps in your program violate these rules, TotalChrom displays a dialog box that indicates the specific steps that violate the rule(s). You must correct the values in the steps before you can save the program.
**Glossary**

**600 Series LINK Interface**

Provides instrument control for supported chromatographs and digital data acquisition for chromatographs with digital output capability. A single LINK interface can support a single HPLC system or up to four gas chromatographs (with two channels each).

**900 Series Interface**

Used to acquire data from any one- or two-channel chromatograph that provides analog output. It converts the analog voltage signal from an instrument to digital values, which TotalChrom can then store, analyze, and plot. No instrument control, other than ready/run signals and autosampler rack/vial input, is specifically available with a 900 Series interface, although there are seven general-purpose relays provided.

**Absolute window**

See *Search window*.

**Absorbance ratio**

The ratio of the UV absorbance, at the Channel A and Channel B wavelengths, for each peak detected. The ratio value is obtained at the apex of the chromatographic peak. If the absorbance at either wavelength is too low, the ratio value reported is 0.0.

**Addend**

A user-defined value, which may be positive, negative, or zero, that is added to raw component amounts. The addend, dilution factor, multiplier, and divisor constitute the conversion factors for calculating adjusted component amounts from raw amounts.

**Adjusted amount**

In quantitation, this is the final calculated amount obtained from the raw amount through the use of the dilution factor and the conversion factors.
**Adjusted expected retention time**

If a component has a reference component, the component’s expected retention time is corrected by the percent that the reference peak deviates from its expected retention time. When corrected in this manner, a component’s expected retention time is called the adjusted expected retention time. It is based on the assumption that if the reference peak shifts by a certain percentage of its expected retention time, a peak representing a related component will also shift by the same percentage.

**Aliasing**

An oscillation of the acquired detector signal that may occur when the frequency of the power line is equal to (or even a multiple of) the data sampling rate. This effect will not occur at a line frequency of 60 Hz because the sampling periods available with TotalChrom are all integral multiples of 0.01 second (for example, 100, 50, 33.33, 25, etc. Hz). To prevent aliasing on 50 Hz power lines, certain sampling rates are disabled.

**Amount ratio**

See Internal standard (ISTD) amount ratio and Relative amount ratio.

**Analytical Instrument Association (AIA) metafile**

See Metafile.

**Analyze program**

The data analysis program in TotalChrom that processes all new raw data files produced during data acquisition. It also reprocesses raw data files in the Batch Reprocessing function.

**Annotation**

The labeling of chromatograms, on the screen or on printed output, with baselines, peak retention times and/or peak names.

**Area/Amount ratio**

The ratio of a peak’s area or height to the raw amount it represents. For a component with a linear response, this is equivalent to the calibration factor for the peak.

**Area/Height ratio**

The ratio of a peak’s area to its height. This data item can be included in peak reports or summary reports.
**Area percent**

The ratio of a peak’s area to the sum of all peak areas listed in the main report. Expressed as a percentage, the sum in this calculation includes only the areas of peaks listed in the main report. It does not include peak areas of unidentified peaks if these peaks are not reported. Thus, the percentages in an area percent column will always add up to 100.

**Area reject**

The minimum peak area you want included in the main report and any group reports. Any peak with an area less than this minimum peak area is not included.

**Area slice**

The area accumulated by the analog-to-digital converter of a 900 Series Interface during a sampling period.

**Area threshold**

A parameter in the processing section of the method that discriminates between noise and peaks. After a pending peak has passed the noise threshold test, the cumulative sum of bunched area slices must exceed the area threshold value before the peak crest is detected; otherwise the peak is rejected as noise.

**ASCII text files**

A text file that is used to transfer data between TotalChrom and other applications. You can store peak reports, summary reports and suitability reports in ASCII format and export the data to other programs. Text files can also be used to input sequence information or component data into TotalChrom.

**Auto-calibration**

The automatic updating of method calibration information using data from sequence cycles defined as calibration standards. Auto-calibration can occur either after data acquisition or during batch reprocessing.

**Autoscaling**

The automatic scaling of a chromatogram for display or printing. The X-axis scale is set to the full run time, and the Y-axis scale is set so that the minimum data point value appears at the bottom of the plot window and the crest of the tallest peak appears at the top of the window.
Average calibration factor

In this type of calibration, the ratio of response to amount is calculated for each replicate at all of the calibration levels, and is averaged together to give an average calibration factor. The amount in an unknown sample is calculated by dividing the response by the average calibration factor.

Axis labels

See Labels.

Backlogged data

Data that accumulates in the memory of a 600 Series LINK or 900 Series Interface if the workstation or server goes down while the interface is collecting data. When you restart the workstation or server, TotalChrom automatically begins reading any backlogged data and transfers the data to the host computer for storage on disk.

Base file name

Characters that form the basis of file names created for the data collected during a run or series of runs. TotalChrom appends a three-digit cycle number to the base file name. If you run a sequence more than once, TotalChrom appends a timestamp to file names when necessary to keep them unique.

Baseline adjustment

A correction factor that is applied to each data point on a chromatogram as it is generated from a mathematical operation on two plots in the Chromatograms function. The baseline adjustment ensures that no calculated data point is less than the minimum acceptable value of 1.

Baseline code

See Baseline type.

Baseline file

The data file, usually derived from running a blank sample, that is subtracted from a raw data file to generate a modified raw data file. The name of the baseline file to be subtracted is defined in the sequence. The modified raw data file is then processed, rather than the original data file. See also Baseline subtraction.
Baseline subtraction

The process of subtracting the points in a baseline file from those in a raw data file in order to correct the baseline. The results of this subtraction are stored in a modified raw data file. See also Baseline file.

Baseline timed events

Commands defined in the processing section of the method that affect peak detection and/or integration at specific times during a run.

Baseline type

A two-letter code that may be printed as part of the analysis report, indicating how a peak’s baseline was drawn. The first letter indicates the baseline treatment at the peak start, and the second letter indicates the baseline treatment at the peak end. The baseline codes are:

B — Resolved peak: The peak starts or ends at the baseline.

V — Unresolved peak: The peak is overlapped with the next and/or previous peak. The peak starts or ends at a valley above the baseline, and a dropline is drawn from the valley to the baseline to allow integration.

E — The peak starts or ends with an exponential skim. A parent peak separated from a child peak by an exponential skim has a baseline type of either BE or VE. The child peak’s baseline type is either EB or EV. Successive child peaks do not have the E code because they do not begin at the start of the skim.

T — The peak starts or ends with a tangential skim.

Binary code

See Rack code and Vial code.

Binary coded decimal (BCD) code

See Rack code and Vial code.

Blank column

A blank column of a specified width that is included in an analysis or summary report format to add extra space between data columns.
**Bracket calibration**

A form of calibration where standards are analyzed both before and after a group of samples, and the average of the calibration information is used to quantify the samples.

**Buffering**

The temporary storage of data in a 900 Series or LINK Interface that ensures data will not be lost if the computer malfunctions or if it is performing another task.

**Bunching**

The process during peak detection that averages the voltage values of a number of successive data points. The resulting averages constitute a “bunched point.” Bunching smoothes the raw data so that baseline noise does not cause the system to find too many potential peak starts. Bunching also compensates for over-sampling. See also *Bunching factor*.

**Bunching factor**

A parameter in the processing section of the method that specifies how many sequential data points in the raw data file will be grouped in a bunch. It can range from 1 to 99. Bunching factors should be based on the narrowest peak you want the system to detect. See also *Bunching*.

**Calculation plot**

The new chromatogram resulting from a mathematical operation performed on two raw data files. A calculation plot can be saved as a new raw data file and reprocessed to produce a result file.

**Calibration**

The stage in data analysis that updates amount and response values for components during the analysis of calibration standards. Calibration information is then stored in the method file, and calibration curves are recalculated. There are two methods of calibration:

- **External Standard (EXTD) calibration** — TotalChrom plots component amounts against response values to compute the calibration curve.

- **Internal Standard (ISTD) calibration** — TotalChrom plots component amount ratios against response ratios (with the internal standard) to compute the calibration curve.
**Calibration curve**

Shows the relationship between a component’s responses at different calibration levels and the corresponding amounts. This helps the system to quantify component amounts in unknown samples. See also *Calibration* and *Curve fit type*.

**Calibration curve fit**

See *Curve fit type* and *Fit type*.

**Calibration factor**

A constant value you enter that is used to calculate component amounts. The component’s peak response is divided by the calibration factor value to obtain the raw component amount.

**Calibration level**

Corresponds to a specific component amount used in a standard sample. Each standard sample contains the same component, but at different calibration levels. The response produced by the component at each calibration level is plotted against the level amount and defines the points on which the component’s calibration curve is based. A component can have up to 100 calibration levels, but the level names for all components in a standard sample must be the same.

**Calibration method**

The method named in the Calibration column of a sequence cycle. The calibration information in this method is used to identify components and quantitate samples. The method is updated when the sequence cycle is defined as a calibration sample. A cycle may specify a single method as the source for instrument, processing and calibration parameters, or it may specify up to three different methods.

**Calibration mode**

Specifies the procedure TotalChrom uses when calibrating the method. There are two basic calibration modes:

- **Average** — A calibration replicate is added to each component that has a level defined with the selected name.

- **Replace** — Any existing calibration replicates for the selected level are deleted from the component list, and a single new replicate that corresponds to the peak data is added.
**Calibration range**

An optional report data item that indicates whether the peak response is within the calibrated range for the component or not. A minus sign (\(-\)) indicates that the peak response lies below the calibrated range. A plus sign (\(+\)) indicates that the peak response lies above the calibrated range. A blank represents a response within range. An asterisk (*) indicates one or more levels in the method were uncalibrated for that component.

**Calibration reference**

See Reference component

**Calibration replicates**

A repetitive analysis of the same calibration standard. Replicates allow you to calculate average calibration information. Results from replicates are stored in the method. See also Calibration mode.

**Channel**

See Data channel.

**Channel run counters**

Channel counters count the number of runs made on each channel of an instrument. The current value is displayed for each configured instrument.

**Child peak**

A minor peak skimmed off the major, or parent, peak when exponential or tangential skimming is employed.

**Chromatogram colors**

The colors of items that appear in chromatogram displays in the Graphic Method Editor, Reprocess Results, and Real-Time Plot windows; the background and annotation colors in the Chromatograms window; and the colors in hard copy chromatograms produced by color printers or plotters. The colors you set for the background and plot labels are used by the Chromatograms function. You set colors in the Configuration function.

**Cluster**

A group of unresolved peaks that normally share a common baseline segment.
**Coefficient of multiple determination**

A statistic that gives a qualitative indication of how well the derived curve fits the calibration data.

**Command line**

The text made available to a user program. This is generally used to communicate the name of the TotalChrom result file just collected to an end-of-run program.

**Component**

An identified peak or groups of peaks. There are three types of components:

- **Single peak component** — A component identified as a single peak in the run.

- **Named group** — Two or more single-peak components that are grouped together in order to be calibrated and reported as a single entity. Members of a named group can appear anywhere in the chromatogram; they do not have to be located contiguously. A single peak component can be a member of more than one named group.

- **Timed group** — A group of contiguous peaks whose retention times fall within a defined time window. Each peak is detected and integrated individually; the group area and height are the sum of the individual peak areas and heights. Peaks need not be identified to be included in a timed group.

**Component amount**

The amount of a component present in a calibration standard. Each calibration level defined for a component references a different amount. See also *Calibration level*.

**Component defaults file**

This file (DEFAULT.CMP) contains the default parameters used when new components are added to a method. You specify these values in the calibration section of the method. The location of the active component defaults file is defined by the Component Defaults path setting in the Configuration function.

**Component identification**

The process of determining the identity of peaks in the result file by comparing their retention times with a predefined list of expected components and retention times found in a calibration file. This assigns component names to peaks in the result file.

**Component list**

The list of components defined in a method.
Component name

The name given to a peak, or group of peaks, to be identified in an analysis.

Configuration file

A file that contains all the configuration information for a given instrument configured on the system.

Curve fit type

Determines how the calibration curve is generated from the data points. The curve fit type is the principal parameter in determining the curve fit, and is therefore sometimes simply referred to as fit type. See also Weighting.

Point-to-point — Averages all replicate amount and response data at each calibration level to derive a point. Each pair of points is connected by a straight-line segment. You may use this fit type with one or more calibration levels.

First-order polynomial — Calculates a first-order (linear) polynomial fit using the curve coefficients (intercept and slope). To use this type of fit, a component must have at least two calibration levels (or one plus the origin).

Second-order polynomial — Calculates a second-order (quadratic) polynomial fit using the curve coefficients. To use this type of fit, a component must have at least three calibration levels (or two plus the origin).

Third-order polynomial — Calculates a third-order (cubic) polynomial fit using the curve coefficients. To use this type of fit, a component must have at least four calibration levels (or three plus the origin).

Custom expression

An expression created in the Report Format Editor that calculates a non-standard data value. Such expressions include symbols for mathematical operators and codes representing the data values available in the result file. In a summary report, custom expressions let you include summary information that is the result of a calculation performed automatically when the report is generated.

For example, in the expression

#AA/#WT*100

#AA represents a peak's adjusted amount, #WT represents the sample amount in the sequence, / represents division, and * represents multiplication. This expression calculates a percent amount for the peak, based on the sample amount (in contrast to the percent amount based on the sum of reported peak amounts).
Custom labels

See Labels.

Cycle

A sequence row that defines an analysis. Each cycle contains the parameters TotalChrom needs to acquire and analyze data produced by one (or possibly two) injections. With some LINK-controlled instruments, cycles may contain information controlling two simultaneous injections.

Cycle number

A number appended to the base file name used in a sequence to create a unique file name for the raw and result files created by each cycle.

Data acquisition

The process by which chromatographic detectors obtain data. It is carried out by an interface according to instructions in a method that you create.

Data analysis

The process by which data in a raw data file are interpreted, utilizing parameters from a method, to produce chromatographic results. See also Analyze program.

Data channel

The signal from each detector on the instrument enters the interface through one of two channels in the interface. The parameter set governing data acquisition specifies that Channel A, Channel B, or both channels will take data. The sampling rate and range settings are identical for both channels. However, some sampling rates available for single-channel data collection are not allowed for dual-channel data collection.

Data compression

The process of transforming data point values so that they occupy less memory in an interface, without losing any of the analytical information. See also Delta modulation.

Data path

The directory (defined in the sequence or at setup) where raw data files and result data files created during data acquisition are to be stored. All raw data files containing data collected from an instrument and all result files created as a result of
analyzing these raw data files are placed, by default, in the same directory, unless they are individually given a different path name in the sequence.

**Data points**

See *Raw data.*

**Delay time**

This is the length of time between the start of the run and the start of data analysis.

**Delta modulation**

A data compression feature of 900 Series Interfaces only. These interfaces do not store values representing actual points on a chromatogram, except for the first point. Instead, they calculate the difference between each pair of consecutive points and store the values. Because the difference values are smaller than the actual data points, they occupy less memory.

**Delta retention time**

The difference between the expected retention time of a component (as specified in the calibration section of the method) and the actual retention time of the identified peak. Delta retention time is expressed as a percentage of the expected retention time.

The delta retention time \( T \) (applying to identified peaks only) is calculated as follows:

\[
T = \frac{t_{exp} - t_{act}}{t_{exp}} \times 100
\]

where \( t_{exp} \) is a peak's expected retention time and \( t_{act} \) is the peak's actual retention time.

**Dilution factor**

A value that accounts for the dilution of the sample prior to injection. For example, if a sample is diluted 100:1, the dilution factor would be 100. The software multiplies raw amounts by this factor. If a sample is not diluted, or if you do not want to make this correction, use 1 as the dilution factor. The dilution factor, divisor, multiplier and addend constitute the conversion factors for calculating adjusted component amounts from raw amounts.

**Divisor**

A positive or negative user-defined value, which cannot be zero. TotalChrom divides raw component amounts by this value. The divisor, dilution factor, multiplier, and
addend constitute the conversion factors for calculating adjusted component amounts from raw amounts.

**Downloading**

Preparing an instrument — the interface and, in the case of LINK, the attached chromatograph — to perform a run. When the user completes the Setup dialog box, TotalChrom sends the instrument method parameter sets, as specified in the sequence, to the interface.

**Droplines**

Vertical lines drawn from an overlapped peak’s start point and/or end point to the peak baseline, defining the boundaries of the peak’s area.

**Dual-channel data acquisition**

Simultaneous collection of data from two detectors (or detector channels) on the same instrument.

**Dual-injection sequence**

A sequence in which the parameters for Channel A and Channel B represent injections of two different samples injected into the chromatograph at the same time. Only certain chromatographs support dual simultaneous injections, so this capability is only selectable for certain configurations. See also *Single-injection sequence*.

**Dynamic data exchange (DDE)**

Enables two Windows applications to exchange information dynamically, on a continuing basis. The application that initiates the exchange is referred to as the client. The application that receives the information is referred to as the server.

**Elapsed time**

The length of time, in minutes, for which the current run has been in progress.

**EPROM version number**

Indicates the revision level of the firmware installed in a 900 Series or 600 Series LINK Interface. This is displayed when the interfaces are configured.

**Expected retention time**

The time during a run when the peak corresponding to the component is expected to elute. The component search window is centered on the expected retention time.
**Exponential skim**

A curve drawn by using an exponential equation to approximate the trailing edge of a parent peak. The skim passes under one or more child peaks. The area underneath the skim is subtracted from the child peak(s) and given to the parent peak.

**Exponential skim criteria**

Parameters in the processing section of the method that determine whether or not an exponential skim will be used to determine the area of a peak on the trailing edge of another peak.

**External standard (EXTD) calibration**

See *Calibration*.

**Firmware**

Programs running in an interface (or instrument module) that control its operation. Firmware resides in a type of computer memory that retains its contents even in the event of a power failure.

**First-order polynomial curve**

See *Curve fit type*.

**Fit type**

The way in which TotalChrom fits the calibration curve to the data points. You change the fit type by altering the fit parameters, such as the weighting factor and origin. Because curve fit type is the principal parameter, it is often referred to simply as *fit type*. See also *Curve fit type*.

**Full scale**

The height of the plot window (in mV).

**General-purpose interface bus (GPIB)**

Connects the interfaces to the computer. See also *Ziatech IEEE-488 adapter*.

**Global calibration information**

Parameters that apply to all components in the method (such as ISTD or EXTD calibration) or to all unidentified peaks (such as quantitation type).
**Graphic editing**

The process of modifying parameters and viewing their effects on the data analysis by manipulating the chromatogram itself. This lets you optimize method parameters for a routine analysis (in the Graphic Method Editor), or to optimize the results for a single unique sample (in Reprocess Results).

**Group report**

A tabulation of results for components that are members of groups. A separate (optional) group report is created for each group established in the calibration section of the method. A report for a given group contains information only for peaks that belong to the group. It contains the same data types as the main report and has the same format. Percent and normalized percent calculations are based only on the peaks within the group that are reported.

**Header information**

Information displayed for a selected file when you choose the Header button in the TotalChrom File Open (or Save As) dialog box that helps you locate the file of interest.

**IEEE-488 address**

A unique number assigned to each 900 Series or 600 Series LINK Interface connected to the computer, that enables TotalChrom to identify the interface to which a command is being directed. The address, which can be from 0 to 15 (except 3, which is used by the computer), is set with switches on the back panel of the interface.

**Importing**

The process of integrating parameters from instrument, process or sample files created with TotalChrom Version 3.3 into the current method. You can also import the method parameters saved in a result file into a new method.

**Injection site**

Where the injection takes place on a LINK-controlled gas chromatograph: either the front or the rear injector (inlet). This does not apply to data acquisition by 900 Series Interfaces.
**Injection volume**

The actual volume of the sample to be injected by a LINK-controlled autosampler. This quantity is set in the autosampler dialog box in the instrument section of the method. It should not be confused with the sample volume, defined in a sequence, which is used solely to correct for differences in injection volume between calibration standards and unknown samples.

**Instrument**

A chromatograph or analyzer configured on the TotalChrom system. It can consist of either a single module, or a collection of modules making up a single instrument, controlled through a 600 Series LINK Interface. A 900 Series Interface is also referred to as an instrument, even though TotalChrom has little or no interaction with the chromatograph connected to the interface.

**Instrument file**

Previous versions of TotalChrom stored acquisition and control parameters in a separate file called the instrument file. They are now stored as the instrument section of the method.

**Instrument header**

See **Instrument notes**.

**Instrument method**

The method in a sequence cycle which defines the parameters to be used for instrument control and data acquisition. The cycle may specify a single method as the source for instrument, processing and calibration parameters, or it may specify up to three different methods.

**Instrument name**

The name defined for a particular instrument. The application manager assigns an instrument name which a user may change in the instrument configuration program.

**Instrument notes**

A block of free format text, 511 characters long, that can be printed as part of a report header. There are several templates available for instrument notes that suggest possible contents for this text.
Instrument parameters

Instrument parameters affect how TotalChrom acquires data by controlling settings for the chromatographic instruments configured on your system. These parameters include sampling rate, run time, data channels, instrument timed events, and control parameters for LINK-based instruments. Instrument parameters are defined in the instrument section of the method.

Instrument personality module (IPM)

The LINK interface communicates with an instrument by using an IPM. All IPMs available to you are installed during the installation process. Each combination of modules that make up an instrument require a unique IPM. Each IPM has two parts: a firmware module that is downloaded to the LINK when the instrument is configured, and a library (LIB) file, which defines all possible configuration and control options for that type of instrument.

Instrument timed events

Commands defined in the instrument section of the method that control functions of the instrument during a run. The events available depend on the type of instrument, but they may include the ability to switch relays or valves, or to autozero the detector output.

Integration

See Peak integration.

Interface

A device manufactured by PerkinElmer that connects the instrument and the computer. The two types that may be used are the 900 Series, which digitizes analog detector signals, and the 600 Series LINK, which controls and obtains data from instruments with digital communication capability.

Internal standard (ISTD) amount

The actual total amount of internal standard components in the sample or calibration standard. TotalChrom multiplies each internal standard component amount in the calibration section of the method by this factor to correct differences between the actual amount of internal standards used and the amounts of internal standards specified in the calibration section of the method. If you want to use the internal standard amount(s) specified in the calibration section of the method, leave the ISTD amount in the sequence cycle at 1.0000 (the default). If calibration and quantitation are not based on internal standards, the ISTD amount is not used, but is still available for use in custom expressions in the analysis report.
**Internal standard (ISTD) amount ratio**

The ratio of the component amount to the related internal standard amount. This data type applies only to peaks that have been identified and quantified based on the internal standard method. The amount ratio is always based on raw amounts, never on adjusted amounts.

**Internal standard (ISTD) calibration**

See Calibration.

**Internal standard (ISTD) component**

A known amount of a compound introduced into a chromatographic analysis. This amount is applied to both samples to be quantified and calibration standard samples. One or more internal standards can be added to a sample.

**Internal standard (ISTD) response ratio**

The ratio of a peak’s response to the response of the related internal standard component. This data type applies only to peaks that have been identified and quantified based on the internal standard method.

**k-prime (k')**

The capacity ratio of a peak. This expresses how many times longer the compound took to pass through the chromatography column, retarded by the stationary phase, than it would have if it was completely unretained. It is calculated as the ratio of the retention time of the peak (corrected for the void time) to the void time:

\[ k' = \frac{RT_{obs} - RT_{unretained}}{RT_{unretained}} \]

**Labels**

There are four types of labels than can be added to printed, plotted and/or displayed chromatograms:

- **Axis labels** — The labels that describe the axes and the title of chromatogram replots.
- **Plot labels** — The retention time, component name, and timed events, which can appear on replots and in the Chromatograms, Graphic Method Editor and Reprocess Results windows.
- **Tick labels** — The time values along the X-axis and the mV (response) values along the Y-axis of the chromatogram. The size of the actual tick marks on the axes does
not change. Tick marks appear in the Real-Time Plot and Chromatograms windows and on replots.

**Custom labels** — The labels added by the user to plots in the Chromatograms window.

**Lambda max**

The wavelength at which a peak displays maximum absorbance. This is only available for peaks collected on a system using an LC diode array detector.

**Landscape plot orientation**

See *Plot orientation*.

**Level amount**

The amount of the component in the calibration standard at that level.

**Level name**

The name of the calibration level.

**Library (.LIB) file**

Contains information on all supported features of the given instrument. When you configure an instrument, Configuration generates a .CFG file that contains a subset of the information in the .LIB file, which pertains to the particular instrument configured.

**LINK interface**

See *600 Series LINK Interface*.

**LINK port**

The physical port to which an instrument is connected. This only applies to instruments connected through a 600 Series LINK Interface.

**Maximum adjusted amount**

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the largest adjusted amount value in the data set. For a single report, it is the largest value for all peaks in the report. For a summarized data set, it is the largest value for all components and all report files.
Maximum peak area

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the largest peak area value in the data set. For a single report, it is the largest value for all peaks in the report. For a summarized data set, it is the largest value for all components and all report files.

Maximum peak height

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the largest peak height value in the data set. For a single report, it is the largest value for all peaks in the report. For a summarized data set, it is the largest value for all components and all report files.

Memory segmentation

See Segmented memory.

Metafile

A file used as a mechanism for data interchange between different systems or programs, or for data archival purposes.

Windows metafiles that you have the option of saving in TotalChrom are either WMF or EMF files. The PerkinElmer metafile (.PEN) can be used to exchange data between TotalChrom and Access*Chrom. The AIA metafile (.CDF) can be used to exchange data between data systems from any vendor supporting the standard format defined by the Analytical Data Exchange Protocol for Chromatographic Data (ASTM E 1947-98).

Method

Instructions created by the user in the Method Editor that specify the way in which instrument control, data acquisition, and analysis are carried out by TotalChrom and the attached instruments. A method consists of three sections: instrument, processing, and calibration.

Method setup

See Setup.

Missing component

Components that are listed in the calibration file but are not associated with a peak in the chromatogram.
Modified data file

The data created when a baseline (or blank sample) file is subtracted from a raw data file. The system analyzes the data in this file, rather than that in the original raw data file.

Multiplier

A positive or negative user-defined value (which cannot be zero) entered in a sequence cycle. TotalChrom multiplies raw component amounts by this value. The multiplier, dilution factor, divisor, and addend constitute the conversion factors for calculating adjusted component amounts from raw amounts.

Also, when performing a calculation on two plots in the Chromatograms window to create a resultant plot, a multiplier can be applied to scale the second plot prior to the calculation.

Multiplot colors

The colors, defined in the Configuration function, that are used to display one or more chromatograms in the Chromatograms window. The first multiplot color defined is also used for the reference plot in the Graphic Method Editor and Reprocess Results windows.

Named group

See Component.

Navigator

The graphic display that appears when you start TotalChrom. It provides an easy way to move around in the application and enables you to go directly to the activity you want to perform, such as data acquisition.

Noise threshold

A parameter in the processing section of the method that discriminates between baseline noise and peaks. If the difference between two consecutive bunched data points is greater than the noise threshold, TotalChrom recognizes the potential start of a peak. The lower the noise threshold, the more sensitive and susceptible to noise peak detection will be. Raising the noise threshold decreases sensitivity.
Normalization

The process of expressing a peak area or amount as a percentage of the total area or amount for all peaks. A normalization factor can be used to set the total to a value other than 100%.

Normalization factor

A factor used in calculating normalized area percent and normalized amount percent values. The normalized area and amount percent values for all reported peaks add up to the normalization factor, which is set to 100% by default.

Normalized amount

The adjusted amount of the peak expressed as a percentage of the total reported amounts. These percentages add up to the normalization factor specified in the sequence file. See also Normalization factor.

The normalized amount $N_{\text{amt}}$ for a peak is calculated as follows:

$$N_{\text{amt}} = \frac{\sum_{i=1}^{n} Q_i}{\sum_{i=1}^{n} Q_i} F$$

where $Q_i$ is the adjusted amount of an individual peak and $F$ is the normalization factor in the sequence file. The sum in this calculation includes only the amounts for peaks listed in the main report. Thus, if $F$ is 85, the sum of the normalized amount values will be 85.

Normalized area

One peak’s percentage contribution to the total area for all peaks for which results are reported. These percentages add up to the normalization factor specified in the sequence file. See also Normalization factor.

The normalized area percent $N_{\text{area}}$ is calculated as follows:

$$N_{\text{area}} = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} A_i} F$$

where $A_i$ is the area of an individual peak and $F$ is the normalization factor in the sequence file. The sum in this calculation includes only the areas of peaks listed in the main report. Thus, if $F$ is 85, the sum of the normalized area percent values will be 85.
**Offset**

The amount, in millivolts, by which the Y-axis minimum of a plot is offset from zero. That is, the signal level displayed at the bottom of the plot window. The default is 0.000 mV, and the range is $-10,000$ mV to $+10,000$ mV.

In the calculation plot feature of the Chromatograms window, Offset is a correction applied to each data point of the resultant data to ensure the minimum value is at an acceptable level. It can be used to ensure a subtraction does not generate values less than zero (which would be invalid), or to set the baseline of a division result to the desired level. The offset can be automatically calculated so that no negative data point values are generated or set by the user.

**Orientation**

See *Plot orientation*.

**Outliers**

Responses that, during calibration, fall outside a certain percentage deviation from the average. Such outliers can be excluded from an auto-calibration.

**Output**

The sequence columns, Printer and Plotter, that specify the devices to be used to print reports containing the analysis results and plot annotated chromatograms (replots) at the end of each run. If one device is specified in the sequence, both reports and chromatograms are printed on the same device. If two are specified, the first device will print reports and the second will produce the associated chromatograms.

**Overlap flag**

A marker in the result file that identifies whether a peak is overlapped or resolved from its neighbors. During integration, overlap flags are used to determine how baseline is drawn under the peak or peak cluster. See also *Peak separation criteria*.

**Over-sampling**

Collecting more than the ideal number of points in a peak. You may choose to oversample deliberately if you intend to perform system suitability calculations to obtain sufficient data to accurately measure peak width and other parameters. See also *Bunching*. 
Parameter set

The group of parameters from the instrument section of the method that are downloaded to the interface memory to control the analytical instrument and/or data acquisition. Up to 20 parameter sets can be stored in the interface and used when different methods are specified for different cycles in the sequence.

Parent peak

In exponential or tangential skimming, this is the major peak from whose trailing edge the minor (child) peaks are skimmed.

Peak area

The measured area of a peak (in microvolt-seconds) in the chromatogram, after the baseline has been drawn and integration performed.

Peak cluster

A group of contiguous peaks that are found to be overlapped during peak detection. All peaks in a cluster share a common baseline, whereas all resolved peaks have an individual baseline.

Peak confirmation

The stage in the peak detection process when the accumulated area for an emerging peak is tested against the area threshold value. The peak is confirmed when the accumulated area exceeds the area threshold.

Peak detection

The process of scanning the data points in a raw data file (or modified raw data file) to find peaks. Peak detection parameters provide the criteria for rejecting noise and baseline drift and recognizing the start of true peaks. They also enable TotalChrom to determine the points at which the tops and ends of peaks are located. This produces a preliminary result file containing a peak list of peak start and end points. The principal parameters are the bunching factor, noise threshold, and area threshold.

Peak end

The end of a peak is marked when either of the following occurs: two consecutive bunched points decrease less than the noise threshold, relative to the preceding bunched point; or a bunched point increases over the preceding bunched point by an amount greater than the noise threshold. The latter case indicates the start of another peak.
**Peak height**

The height, above the peak baseline, of the peak crest, measured in microvolts. The exact peak crest is determined from a quadratic curve fit through the raw points that are above 75% of maximum level (with a minimum of five points).

**Peak integration**

Sets the final positions of baselines for clustered peaks and separates these peaks with droplines. It also determines peak areas and adjusts them, if necessary, by exponential or tangential skimming, and calculates peak heights and retention times. This step adds final values for peak start and end points, retention times, areas, and heights to the result file.

**Peak library search**

Identification of all the peaks in a chromatogram by searching a library for a match to the apex spectrum of each peak in the chromatogram. The comparison of the spectra is made using a Euclidean distance algorithm, the numerical result of which is called the Hit Quality.

**Peak number**

An index assigned to each peak detected in a run. This number can be included in the analysis report.

**Peak purity index (PI)**

A number that indicates the purity of a peak. It is derived by comparing the UV spectra collected on the leading and trailing edges of a peak. A purity index of 1.0 indicates that the two spectra are the same. Therefore, there is a good probability that the peak is homogeneous. Peak purity index values may be included in TotalChrom reports from data acquired using an LC diode array detector through a LINK Interface.

**Peak separation criteria**

Tests to determine if two peaks are resolved or part of a cluster. If the distance between the two peaks is less than 0.2 times the width of the second peak, and if the height of the valley (above the baseline) is greater than 0.01 times the height of the smaller of the two peaks (above the baseline), the peaks are considered to be overlapping. If the separation is greater than this, they are not overlapping. See also *Overlap flag*.
**Peak start**

Once a peak is confirmed, TotalChrom scans backward from the potential peak start through five bunches of raw data to find the lowest raw data point. The search stops when it reaches the end of the preceding peak. The lowest raw data point found in this process becomes the actual peak start.

**Peak width**

In the processing section of the method, the user may set the data acquisition (sampling) rate by entering a value for the expected width (at its base) of the narrowest peak in the run. TotalChrom automatically sets the sampling rate so that 20 data points will be collected across the peak.

**Peak width at Base (Suit)**

The width (in seconds) at the base of a peak by doing a linear fit of the points between 60% and 80% of peak height and finding the points of intersection of the baseline.

**Penetration**

When part of the chromatogram (such as a valley point) crosses below the initial drawn baseline. This can occur during the integration process. TotalChrom usually adjusts the drawn baseline to prevent such penetration; however, it does allow penetration for horizontally projected baselines.

**Percent amount**

The adjusted amount for a peak expressed as a percentage of the sum of the adjusted amounts for all the peaks listed in the main report. The values in a percent amount column always add up to 100 because the amounts for peaks excluded from the main report are not added into the sum.

**Plot**

The display of a chromatogram on the screen or output to a printer. The printed version of a chromatogram is frequently referred to as a replot.

**Plot labels**

See Labels.

**Plot orientation**
The way in which plots are arranged on a page. There are two kinds of plot orientation:

**Landscape** — The plots are drawn with the time axis parallel to the long dimension of the page, and the peak tops point toward the right side of the page.

**Portrait** — The plots are drawn with the time axis parallel to the narrow dimension of the page, and the peak tops point toward the top of the page.

**Plot scale**

The time and voltage scales for a displayed chromatogram.

**Plot style**

In graphic editing, you have the choice of three different plot styles. “Normal” chromatograms are plotted as a series of line segments connecting each raw data point. Chromatograms can also be plotted as a series of unconnected raw data points, or they can be plotted as a series of raw data and bunched points. You set the plot style in the Configuration function.

**Point-to-point calibration curve**

See *Curve fit type*.

**Portrait plot orientation**

See *Plot orientation*.

**Post-run processing**

The steps in data analysis that are performed automatically at the end of each run. They consist of peak detection, integration, component identification, calibration, quantitation, report generation, replot generation and the execution of user programs.

**Primary report**

A report based on the format files (for channels A and/or B) you list in the sequence file. Up to six secondary reports can be specified in the processing section of the method.

**Print margins**

The amount of blank space at each edge of reports and replots. These values are set in the Printer Test window of the Configuration function.
**Process file**

Previous versions of TotalChrom stored processing parameters in a separate file called the process file. They are now stored as the processing section of the method.

**Processing method**

The method in a sequence cycle which defines how peaks are detected and integrated, how reports and replots are printed, and which user programs are run. In the case of a sequence setup, the cycle may specify a single method as the source for instrument, processing and calibration parameters, or it may specify up to three different methods.

**Program path**

The drive and directory in which the TotalChrom program and configuration files are located. Configuring the program path stores the path name in the TotalChrom initialization file. If you have installed TotalChrom according to the instructions in the Application Manager’s Guide, you do not need to configure the program path: it will have been configured automatically by the installation program. The TotalChrom program path must also be included in the DOS path list.

**Project Directory**

This command opens a dialog in which the user can type in (or select via a Path Select dialog) a Project Directory setting. When such an entry exists (the default is blank), this path is used by all applications in place of all default path settings for all file types. This includes all editors and Setup.

The specified project directory is saved with other user settings and will continue to be used for all TC sessions of that user until a further change is made.

**Quantitation**

The process of calculating the amounts of the components in chromatography samples. These calculations are based on the peak areas or heights and on the calibration data for corresponding components. This process adds component amounts to the result file.

**Quick Method**

A simple method, defining only essential instrument control, data acquisition, and processing parameters, that is generated as part of a QuickStart setup.
**QuickStart**

A quick way to begin data acquisition that does not require a pre-existing method or sequence. Only essential instrument control, data acquisition and processing parameters need to be specified as a Quick Method. A vial list can be generated if required. See also *Setup*.

**Rack code**

Defines how an autosampler, connected to a 900 Series Interface, communicates the current sample rack number (if any). Numbers in either binary or binary coded decimal (BCD) format can be read.

**Raw amount**

The amount of a component represented by a peak, as calculated from the calibration curve or by applying a response factor. Unlike adjusted amount, this amount does not take into account the dilution factor, multiplier, divisor, or addend. If necessary, raw amounts have had scaling reversed, have been converted from amount ratios to amounts, and have sample volume adjustments made.

**Raw data**

Actual data points acquired during an analysis cycle and stored in a .RAW file. Each raw data point supplied by a TotalChrom interface is a positive whole number. A point collected by a 900 Series Interface can range from 1 to 999999, whereas a point collected by a LINK Interface can range from 1 to $2^{32}$. A point occupies four bytes in the computer’s memory and while stored on disk.

**Real-time plot**

A display of the chromatogram as the data points are acquired from the instrument.

**Reference chromatogram**

A section of the screen, in the Graphic Method Editor and Reprocess Results, which normally displays the whole chromatogram, as opposed to the current expanded working chromatogram.

**Reference component**

There are two kinds of reference components:

- **Retention Reference** — A component whose retention time is used to adjust the expected retention time of other components, and/or to calculate a relative retention value for them.
**Calibration Reference** — A component whose calibration data is used by one or more other components.

**Reference peak**

See *Retention Reference* under *Reference component*.

**Regression calculation**

To compute a calibration curve, TotalChrom performs a regression calculation using all replicates for the component at all available calibration levels. The regression is performed by computing a set of orthogonal polynomials and using them to compute the best least-squares approximation.

**Regression weighting**

See *Weighting*.

**Relative amount ratio**

The ratio of the adjusted amount of a component (in a result file) to the adjusted amount of the same component (in the first result file, in a file list). You can include this data item in a Summary report.

**Relative retention time (RRT)**

The retention time of a peak compared to that of a specified relative retention reference peak.

RRT for a peak is calculated as

$$RRT = \frac{(RT_{obs} - \text{void time})}{(RT_{ref\ peak\ obs} - \text{void time})}$$

**Relative standard deviation (RSD)**

The relative standard deviation of a data set expressed as a percentage of the average value for the data set. In a summary report, RSD values can be calculated for component retention time, amount, and response.

**Relative window**

See *Search window*.
Replicates

See Calibration replicates.

Replot

A printed chromatogram that reflects the analysis of a raw data file. It can be labeled with peak retention times, baselines, component names, and timed event symbols. The X and Y axes are marked with a user-entered label, and header information appears at the top of the page.

Replot generation

The process of producing an annotated plot of the chromatogram. It can be specified to occur after initial data acquisition and analysis, after batch reprocessing, or after graphic editing.

Report format file

Contains some of the report's formatting characteristics and the specifications for the analysis report created through the Report Format Editor. The specifications include the types of results the report will contain and the content of standard sections such as the header, title, footer, and column labels. Report format files always end with a .RPT file extension.

Report generation

The process of producing one or more reports containing the results of the analysis of a raw data file. The reports to be generated are specified in the sequence file governing the analysis and in the processing section of the method. Reports can be generated after initial data acquisition and analysis, after batch reprocessing, or after graphic editing.

Reprocessing

Re-analyzing stored data to reintegrate peaks, re-identify components, recalibrate the method, generate a new report, or to change the report’s format.

Resolved peak

See Baseline type.

Response ratio

See Internal standard (ISTD) response ratio.
Response value

The Y-axis value of a data point. For a peak, the response is its area and/or height.

Result file

After analysis of the data in a raw data file, the results of the analysis are stored in a result file. Each raw data file analyzed has a counterpart result file with the file name extension .RST.

Retention reference component

See Reference component.

Retention time (RT)

In an analysis report, this is the actual elution time of the peak (in minutes), as measured from the start of the run. For a component in the calibration section of the method, this is the expected elution time of the peak.

RMS noise

A measure of average noise, calculated as the root mean square value, in the processed region.

Row

An entry in a sequence. A row can either define an analysis cycle (a single injection of a calibration standard or a sample), or it can specify a program to be run. A row defining an analysis is usually referred to as a cycle. See also Cycle.

Run time

The length of time during which data points are collected.

Sample amount

In a sequence cycle, this is the actual total amount of the sample (greater than 0) in the injection being analyzed. In calibration runs, this is the sum of the actual component amounts in the standard sample (excluding internal standard components).

Based on settings in the method, this value can be used for correcting calibration standards and for converting unknown samples to concentration units.
Sample file

In previous versions of TotalChrom, component and calibration information were stored in a separate file called the sample file. This information is now stored as the calibration section of the method.

Sample ID (LIMS)

An alphanumeric string, originating from a LIMS system, that uniquely identifies the sample defined in a sequence cycle within the LIMS system.

Sample note

A text note associated with the sample defined in the current sequence cycle. This feature applies only to sample and calibration types, not to user programs. You may enter one note (of up to 127 characters) per row, per channel.

Sample type

Defines the nature of each row in a sequence. It can be a calibration standard (Replace, Average, Bracket-Overlapped or Bracket-Non-overlapped), an unknown sample or a program.

Sample volume

The actual amount of calibration standard or sample withdrawn from the vial for injection. In external standard analyses, this value is used to correct peak area or height values if the actual sample volume differs from the default value defined in the calibration section of the method. This parameter is applied to both standard and unknown samples. If no correction is required, the default value of 1.0 can be left unchanged. This parameter should not be confused with the injection volume parameter defined for a LINK-controlled autosampler, which actually controls how much of the sample is injected.

Sampling rate

The number of data points to collect per second. This controls how finely the analog data are resolved into digital values. 900 Series Interfaces allow up to 100 pts/s (50 pts/s dual channel), as well as any even division of this base sampling rate.

Scale

The height (in mV) of the plot window.
**Scale factor**

A factor used to change the default scaling of a replot, which sets the largest peak to full scale. The new full scale value is the default scale divided by the scale factor. Thus, if the scale factor is greater than 1, the plotted peaks increase in size; if the scale factor is less than one, the peaks are plotted smaller.

**Search window**

The time tolerance before and after the expected retention time of a component. A peak whose retention time falls within this tolerance can be identified as that component. This allows components to be identified despite small variations in retention time from run to run. The search window is constructed as the sum of two parts:

- **Absolute window** — A time period, expressed in seconds, before and after the expected retention time of the component. The absolute part of the search window can be used to compensate for shifts in the chromatogram that affect all peaks alike. If you expect the retention time for a component to vary by ±5 seconds, you would assign an absolute window of 5 seconds to the component.

- **Relative window** — The relative part of the search window, expressed as a percentage of the component’s expected retention time, causes the window width to increase as the retention time of the component increases. This helps compensate for the greater uncertainty that frequently occurs for peaks eluting later in the run. If you expect the peaks produced by an analysis to vary by ±5% of the retention time, you would assign a relative window of 5 to the component.

**Secondary report**

A report based on a report format file listed in the processing section of the method. Up to six of these reports may be generated.

**Second-order polynomial curve**

See *Curve fit type*.

**Segmented memory**

The interface memory can be divided into distinct regions (or segments) during data acquisition. Each segment holds the raw data acquired from one injection (or simultaneous dual injections). Segmented memory allows multiple runs to be stored in the interface before the data need be uploaded to the computer. Segmented memory is used when the user selects the Multiple Runs option in the Setup dialog box.
**Segments free**

The number of memory segments available in the interface for storing data. This value is displayed in the Details window.

**Segments used**

The number of memory segments available in the interface containing stored data. This value is displayed in the Details window.

**Selection rectangle**

A highlighted region of the reference chromatogram in the Graphic Method Editor or Reprocess Results windows that indicates which part of the chromatogram is displayed in the working area of the screen.

**Sequence**

Consists of one or more rows that define analysis cycles. The sequence you create controls how data are acquired, analyzed, and/or reprocessed.

**Sequence setup**

See Setup.

**Sequence template**

Lets you define the essential structure of a sequence, which TotalChrom then builds for you. The template can define when calibrations are to be included, and allows for sequential numbering or samples and/or vials.

**Setup**

The Navigator function used to prepare an instrument for data acquisition. There are three Setup options:

- **QuickStart** — Allows you to set up for data acquisition without having an existing method or sequence. You create a Quick Method, entering only the essential instrument control and data collection parameters (or accepting the defaults).

- **Method** — Used when an appropriate method already exists, but you do not wish to create a sequence.

- **Sequence** — Used when a number of similar samples are to be run or when an autosampler is being set up for unattended operation.
**Single-injection sequence**

A sequence for which each analysis cycle represents only a single sample injection. This may involve either single or dual channel data acquisition. See also *Dual-injection sequence*.

**Single-peak component**

See *Component*.

**Spectral library confirmation**

Verification of the named peaks in a sample chromatogram by comparing the apex spectrum of each named peak in the sample to an identically named spectrum in a Spectral Library. The result of the comparison is called the Hit Quality.

**Spectral standard confirmation**

Verification of the identity of named peaks in a sample chromatogram by comparing the apex spectrum of each peak in the sample to the apex spectrum of an identically named peak in a standard chromatogram. The comparison is made using the Absorbance Index test.

**Standard samples**

Samples to be used for the calibration of a method.

**Study**

A name entered for each cycle in a sequence, used in conjunction with the sample name to identify a sample. Generally, “Study” is used to indicate the type of analysis and “Sample Name” is used to distinguish the individual samples.

**Summary report**

A compilation of results from multiple runs, generated by the Summary function. In addition to the individual data for each component, values such as average and relative standard deviation may be calculated and reported. The specific content and format of the report can be customized by the user.

**Summary report format file**

Defines the content and format of a summary report.
**System header**

Summary information about the sample and its processing that precedes the peak table in an analysis report. It is not simply a label, as the user-entered report header is, and the content is not selected, item by item, in the report format file editor. However, you can specify the size of the system header and whether it should include instrument control parameters.

**Tangential skim**

Use of a straight baseline segment, rather than an exponential curve, when skimming minor (child) peaks off the trailing edge of a tailing major (parent) peak. A tangential skim is never created automatically by the system. It must be accomplished by a “T” timed event placed between the peak’s start and end points.

**Task ID (LIMS)**

An alphanumeric string, originating from a LIMS system, that uniquely identifies a test to be run on the sample defined in a sequence cycle.

**Template**

See *Sequence template*.

**Text file**

See *ASCII files*.

**Third-order polynomial curve**

See *Curve fit type*.

**Threshold values**

See *Area threshold* and *Noise threshold*.

**Tick labels**

See *Labels*.

**Timed event**

See *Baseline timed events* and *Instrument timed events*. 
**Timed group**

See *Component*.

**Time-out delay**

A window of time within which an interface must respond to a command from TotalChrom; otherwise, an error state will exist.

**Tokenized file names**

The system manager will be able to define default raw and result file names, for each user, based on the use of "tokens" to represent key data values. The tokens ensure that an appropriate (and even specific, depending on the tokens chosen) file name is provided as a default. For example, `<Inst>` would be replaced by the instrument name (Note: not key), `<User>` by the operator’s name and `<Name>` by the sample name. An option is provided to prevent the user changing these file names.

**Total amount**

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the sum of the adjusted amounts in the data set. For a single report, it is the sum for all peaks in the report. For a summarized data set, it is the sum for all components and all report files.

**TotalChrom Report (TCR)**

TCR files are a specifically created for the Review and Approve process. It is a composite file consisting of an associated set of TotalChrom raw (.RAW), result (.RST) and report format files (.RPT).

**Total area**

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the sum of the peak areas in the data set. For a single report, it is the sum for all peaks in the report. For a summarized data set, it is the sum for all components and all report files.

**Total height**

A variable that can be used in custom expressions in the Report Format Editor and Summary Format Editor. It equates to the sum of the peak heights in the data set. For a single report, it is the sum for all peaks in the report. For a summarized data set, it is the sum for all components and all report files.
**Unidentified peak**

A peak present in the chromatogram, but not matched to a component identified by name in the calibration section of the method.

**Unidentified peak quantitation**

The way in which unidentified peak amounts in the analysis may be calculated. The three ways to calculate this amount are:

- Component amounts are calculated based on the constant calibration factor you enter.
- Each unidentified peak is calculated using the same options as the nearest component, where the amounts are calculated as if the unidentified peak is the same as the component.
- Each unidentified peak is calculated using the same options as the nearest reference peak, where the amounts are calculated as if the unidentified peak is the same as the reference peak.

**Unknown peak**

See *Unidentified peak*.

**Unresolved peak**

See *Baseline type*.

**Unsegmented memory**

When only a single run is to be stored at a time in the interface, the memory is set in unsegmented mode. If the length of the run requires it, the interface wraps around at the end of the buffer memory, overwriting the data that have already been read. If the data have not been read, the interface will stop collecting data when the buffer is full. See also *Segmented memory*.

**Update retention times**

A sequence cycle option to use the actual retention times from a calibration run to update the component retention information in the calibration method.

**User program**

A program you develop or designate to use data from a file generated by TotalChrom. It can be run during or after data analysis.
**User value**

A numeric constant associated with a component in the method. Up to five user values may be defined for each component, for use in custom expressions.

**Valley point**

The lowest point between two unresolved (overlapped) peaks, as determined during peak detection. It can be either the ending point of the first peak, or the starting point of the second peak.

**Vial code**

Defines how an autosampler, connected to a 900 Series interface, communicates the number of the vial sampled for the current run. Numbers in either binary or binary coded decimal (BCD) format can be read. See also Rack code.

**Void time**

The elution time of an unretained peak. This is used in the calculation of k’ or corrected relative retention values.

**Voltage range**

A parameter in the instrument section of a method for a 900 Series interface that defines the range to be used in digitizing the analog data.

You can include a Voltage Range column in an analysis report to indicate if the signal to the interface went over or under this range during the elution of the peak. If a 900 Series Interface collected the data analyzed, a plus sign (+) indicates that a peak’s height is higher than the voltage range for which the interface was set during data acquisition. A minus sign (−) indicates that a peak’s height is below the voltage range. If a peak is within the voltage range, its field in this column will be blank. If a LINK Interface was used, a plus sign indicates that a peak’s height exceeds 999990 counts. A minus sign indicates that a peak’s height is less than 10 counts.

**Volume adjusted amount**

The quantity plotted against response (area or height) to create the calibration curve for an external standard calibration. The volume adjusted amount is the component amount stored with the replicate information multiplied by the sample volume from the same source. The component amount in replicates is obtained from the calibration section of the method when the replicate is run and the sample volume is taken from the sequence cycle information.
**Weighting**

The weighting options in the calibration section of the method allow you to assign different significance (weight) to calibration points during the regression calculation, according to their amount or response values. Your selection determines the form of the weighting factor to be applied to the points of a calibration curve.

The options are:

1. No weighting. (the default)
2. \( \frac{1}{X} \) The reciprocal of a point’s amount value is used.
3. \( \frac{1}{Y} \) The reciprocal of a point’s response value is used.
4. \( \frac{1}{(X*X)} \) The reciprocal of the square of a point’s amount value is used.
5. \( \frac{1}{(Y*Y)} \) The reciprocal of the square of a point’s response value is used.

See also *Curve fit type* and *Fit type*.

**Working chromatogram**

The chromatogram displayed on the screen that you can manipulate in the Graphic Method Editor or Reprocess Results windows. It can include the following annotations from data analysis: retention time labels, baselines, component names, and timed events. See also *Graphic editing* and *Reference chromatogram*.

**Ziatech IEEE-488 adapter**

An add-in board, inserted into an available slot in your computer, that provides the capability to communicate with external devices (the 900 Series or 600 Series LINK Interfaces) that use the IEEE-488 protocol.
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