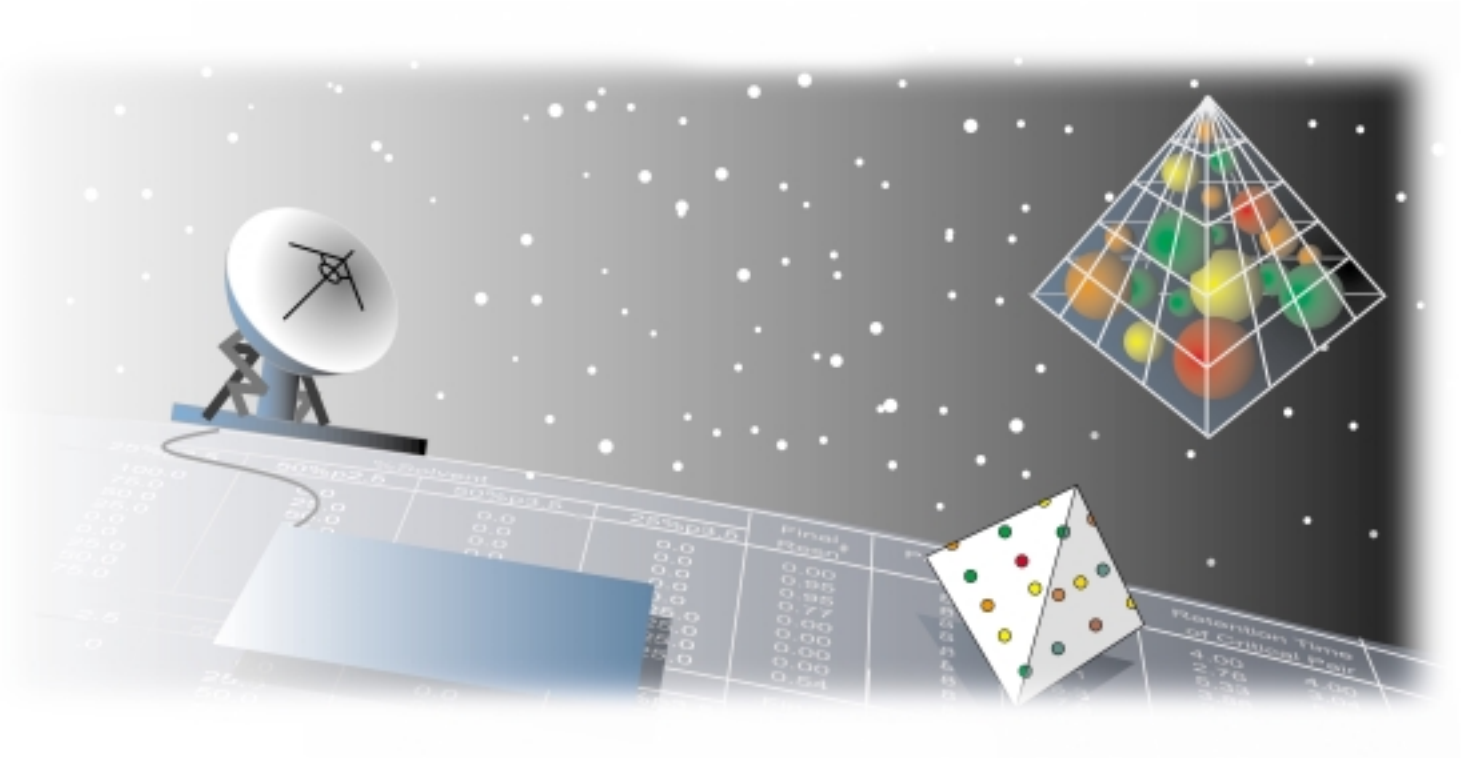
Turbo Method Development System



automated optimization tools

for developing HPLC methods.

PerkinElmer™
instruments.

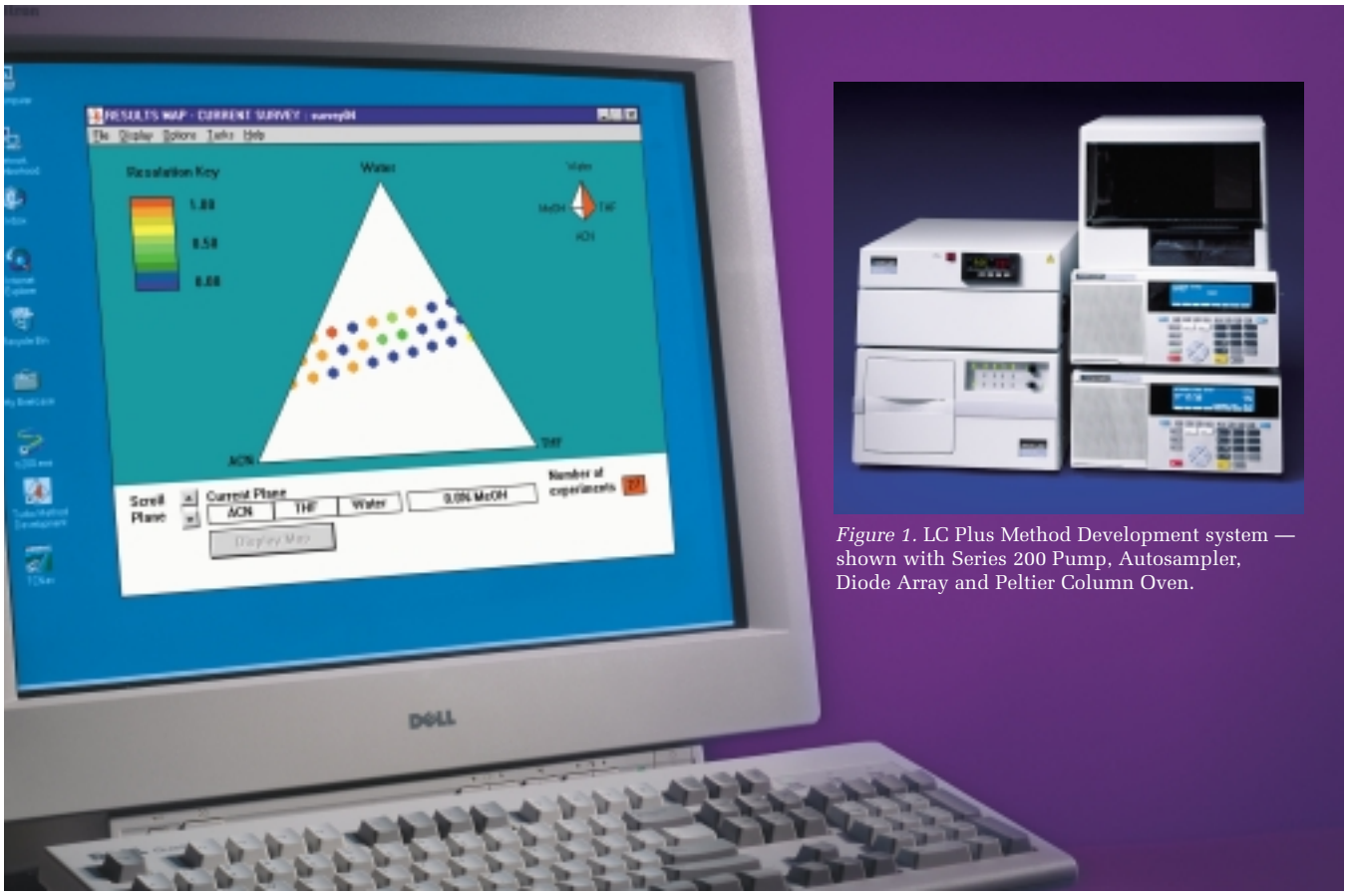


Figure 1. LC Plus Method Development system — shown with Series 200 Pump, Autosampler, Diode Array and Peltier Column Oven.

Method development and validation **MADE EASY**...

PerkinElmer's **UNIQUE** TMD system

PerkinElmer is *the* pioneer in automated method development — and has continued to build on that reputation and expertise. Today, by combining the proven performance of our HPLC instruments, the comprehensive and award-winning power of our TotalChrom chromatography data system AND our intuitive Turbo Method Development (TMD) software, only PerkinElmer is fully prepared to meet all your method development needs with a unified HPLC system solution! While others may claim to offer tools that "validate" methods statistically — no other method validation solution actually automates the *entire* process of generating methods, collecting data and assessing method effectiveness all with a minimum of user intervention!

Everyone develops methods...

All chromatographers develop, optimize or validate methods. Whether it's refining a method transferred between labs, adapting a working method to a different sample matrix, or the extreme case of developing of an entirely new method to separate a new sample, there are usually similar chromatographic goals and requirements.

Those working in a regulated environment — in order to comply with ICH or similar guidelines — must perform all of these activities. These experiments have traditionally been performed one-by-one, manually and were therefore very labor intensive and time consuming.

Today, with the ever increasing need to shorten development cycles and minimize labor costs — all while ensuring regulatory compliance — this type of solvent optimization and method validation can be done faster, easier and under greater process control with a total, automated solution from PerkinElmer.

FAST FACTS

Method Development Checklist

→ Develop an HPLC method to perform the separation in the minimum analysis time while retaining specificity, precision and accuracy.

→ Confirm the specificity of the method; purity and identification of component peaks, and eliminate co-elution concerns via spectral techniques.

→ Ensure that the method is fully optimized and that it's robust enough to resist the effects of small changes in mobile phase composition.

→ Evaluate how variations in the brand, type, lot and age of the HPLC column can effect the performance of the method.

→ Ensure that all methods and data are developed and stored in compliance with all applicable regulatory requirements.

HPLC instruments you can count on for quality, performance and reliably

During every step in the method development process it's critical to utilize instrumentation that delivers accurate and precise results day after day, week after week. After all, the ultimate effectiveness of the method is predicated on it being built on a solid foundation. Therefore, it's only natural to demand the highest levels of performance from the HPLC system being employed. PerkinElmer's HPLC instruments were designed to meet the *unique* demands of method development as well as meeting the rigors of routine, high throughput usage.

Series 200 Quaternary Gradient Pump

- The only HPLC pump that provides accurate method reproducibility and transferability with results identical to those obtained via premixed (mixed by hand) mobile phases.
- The Series 200 is the only pump available today that automatically and continuously compensates for solvent compressibility — no matter the duration of the run or the complexity of the gradient!
- No other pump is capable of meeting that challenge!

Series 200 Autosampler

- Ensures accuracy and repeatability using a rapid and rugged all gear-driven XYZ sampling mechanism — never any belts to slip or wear out or none of the risk and wasted time inherent with moving sample vials via a robotic arm.
- In addition to its reliability, the Series 200 is the fastest and most flexible HPLC autosampler available — supporting many different vial and microtiter plate formats — including several peltier heated/cooled versions for kinetic studies or temperature labile samples.
- The Series 200 also minimizes carryover and vial-to-vial contamination concerns by washing both the inside and the outside of the sampling needle with a fresh flush solvent after each and every run!

Series 200 Diode Array Detector

- Provides you with true UV/Vis performance and high-resolution spectral data to verify peak homogeneity, track component RT changes vs. solvent composition, and to assist with the identification of unknown components.
- Simultaneous deuterium and tungsten sources ensure performance and high sensitivity across the entire 190 to 700nm wavelength range.
- Peak purity, lambda max, and component ID confirmation values can automatically be calculated and inserted right in to your chromatographic report.

"PerkinElmer is **COMMITTED** to providing you with the highest **QUALITY** instrumentation available anywhere. We're very proud to have been awarded the ***"Readers' Choice 2000"*** Award as the **NUMBER ONE** instrument manufacturer in quality by ***Today's Chemist at Work*** magazine."

TotalChrom Workstation

TotalChrom is the next stage in the evolution of chromatography software — building upon Turbochrom, the industry-standard, most respected name in PC-based chromatography data handling software. A proven solution that's won the *"Readers Choice"* Award from *Scientific Computing & Automation* magazine for the eighth consecutive year!

In addition to aiding in the development, optimization and validation of methods, your laboratory needs a computing strategy to adequately handle the growing volume of chromatographic data quickly and efficiently. A strategy that can easily be implemented and that can grow and adapt to your changing needs. A strategy that satisfies regulatory requirements such as 21 CFR part 11 and the need to archive and recall data — all while maintaining ease of use. TotalChrom is the answer.

TotalChrom uses Microsoft Windows standard interfaces and graphics — allowing you to quickly and easily build instrument control and data handling methods. By combining the power of TotalChrom with PerkinElmer's HPLC instrumentation — several new and valuable capabilities have been added. In addition to control of our isocratic, binary and quaternary pumps, TotalChrom now controls the PerkinElmer Series 200 Micro Pumps — offering for the first time a unified HPLC system capable of scaling from microbore analysis up to traditional analytical flows. In combination with the Series 200 AutoSampler, TotalChrom allows you to quickly and easily perform automated serial dilution routines and derivation studies — freeing you from having to manually dilute samples or prepare several standards of varying concentration. TotalChrom also fully controls the Series 200 UV/Vis variable wavelength detector and the Series 200 Diode Array detector — providing the quantitative and qualitative information you need for method development. This same level of automation and sophistication is available for building lengthy sequences of multiple runs quickly and easily via TotalChrom's template driven Sequence Editor — featuring that spreadsheet look and feel you're familiar with.

Then there's the unique Graphic Method Editor (GME) that builds the best data handling method based on your particular chromatographic results. Identifying the proper peak detection thresholds is critical when evaluating such items as limits of detection and limits of quantitation.

Perhaps most important, once your data is collected, our new TC Publisher raises the bar on data reporting by providing a custom report generator using industry-standard tools. Unique features let you *graphically* format the exact report you want, control the layout and appearance of your chromatograms, and even use "if/then" conditional logic — all without extensive training. TC Publisher even lets you do calculations between samples and do automatic statistics for replicate injections — with full control over the format of the output. In fact, TC Publisher will often replace those special custom applications written for your current system — thus minimizing costs, efforts and validation concerns.

"**YOUR** laboratory needs a **COMPUTING STRATEGY** to handle the growing volume of **CHROMATOGRAPHIC DATA**... **TOTALCHROM** is the **ANSWER.**"

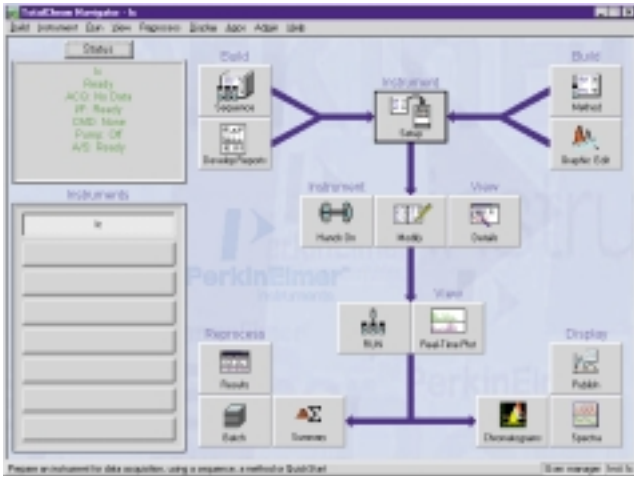


Figure 2. TotalChrom Navigator screen showing intuitive, icon-driven logic path for method development, instrument control and data processing.

Finally, turbo-charge the system with our unrivaled TMD software!

Our truly unique Turbo Method Development software (TMD) is at the heart of our method development solution. TMD allows the chromatographer to perform automated searches of quaternary solvent space (i.e. perform actual experiments blending up to 4 solvents) *and* analyze the resulting data — all with a minimum of time and effort!

Simply enter some background information and then step back and let TMD automatically create all the methods for a series of injections — each using a specific solvent composition — to be performed by the system. You only need to be present for the initial set up of the system and then again to examine the analyzed and compiled final data!

TMD performs all the analyses unattended, and then examines the data and displays the results in an easy to interpret color-coded map so that the best separation can be identified quickly. Graphical tools can be used for viewing the data in greater detail to exploring such effects as peak retention vs. changes in solvent strength.

For further refining the analysis, simulated chromatograms, produced via sophisticated algorithms, can easily be generated to help predict separation results between actual survey points. Once the optimal conditions are found, a robustness and ruggedness test feature is available to help determine the sensitivity of the separation to even the smallest changes in solvent composition.

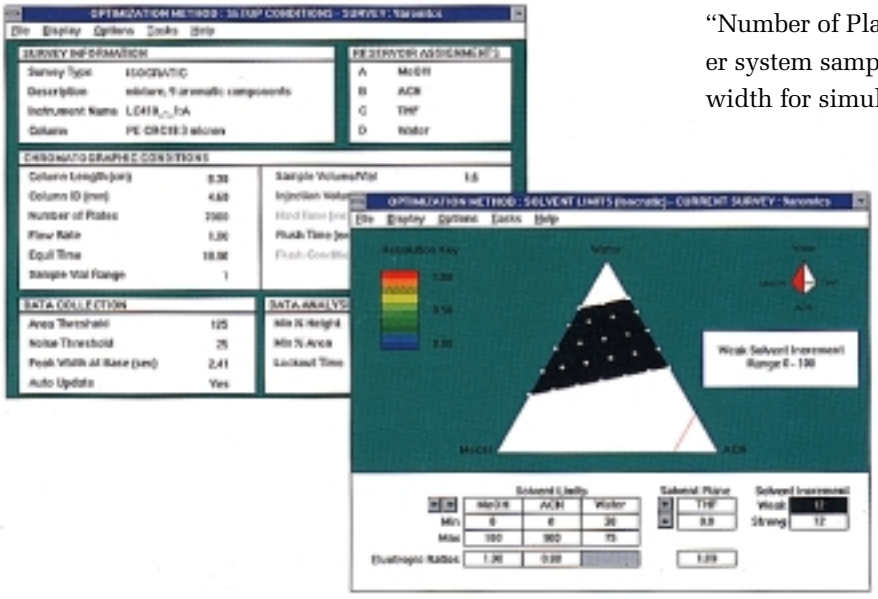


Figure 3. Turbo Method Development setup and graphical survey design screen.

Ternary Solvent Separation Problem

The challenge is to develop an effective and rugged separation for the following mixture of aromatic compounds:

- → 2-phenyl ethanol
- → dimethyl phthalate
- → 3-phenyl 1-propanol
- → diethyl phthalate
- → toluene
- → benzonitrile
- → nitrobenzene
- → anisole
- → 3,4-dinitrotoluene

- Several of these compounds have very similar polarities and while an isocratic separation is possible, it represents a difficult separation problem.
- Previous experiments have shown that binary mixtures of ACN/water and MeOH/water cannot resolve all the chromatographic peaks, so we hope to solve the problems by exploring various ternary mixtures of ACN/MeOH/water.

The Solution:

Intuitive graphical setup to get started quickly and easily. We will use Turbo Method Development to automatically explore the behavior of this separation using a number of strategically chosen binary and ternary solvent combinations of ACN, MeOH and water.

The setup of survey conditions is straightforward in Turbo Method Development (Figure 3). Input parameters include the solvents in each reservoir, the column description and the instrument parameters. This survey was constructed for a diode array detector, but any type of detector can be used with the system. The input "Number of Plates" is used both to help select the proper system sampling rate and to select the correct peak width for simulated chromatograms.

Because Turbo Method Development surveys are constructed graphically and interactively, it is easy to create a series of experiments, which covers the desired solvent combinations. The "space" defined by the four solvents is shown in the tetrahedron at the top right of the Solvent Limits screen, and the selected plane of the survey is defined by the large central triangle (Figure 3). Any solvent plane can be chosen

for an experiment (three solvents are varied, one held constant). You use the solvent limits and increments precisely define range and number of survey experiments to be performed (white points). This survey includes "eluotropic ratios" which correct the solvent mixtures for the relative elution strength of each strong solvent. We want to use them in the case, so that changes in solvent strength do not overcome desired changes in selectivity as we change solvents from MeOH to ACN.

Before the survey is run, a list of experiments can be generated to show the exact solvent composition and run time for each injection (Figure 4). The "calculate usage" function gives information about the total number of experiments, survey duration, and exact solvent usage from each reservoir. The nine-aromatic survey will take 23 hours for 26 experiments. Therefore, you know how much solvent you will need to prepare before the analysis starts.

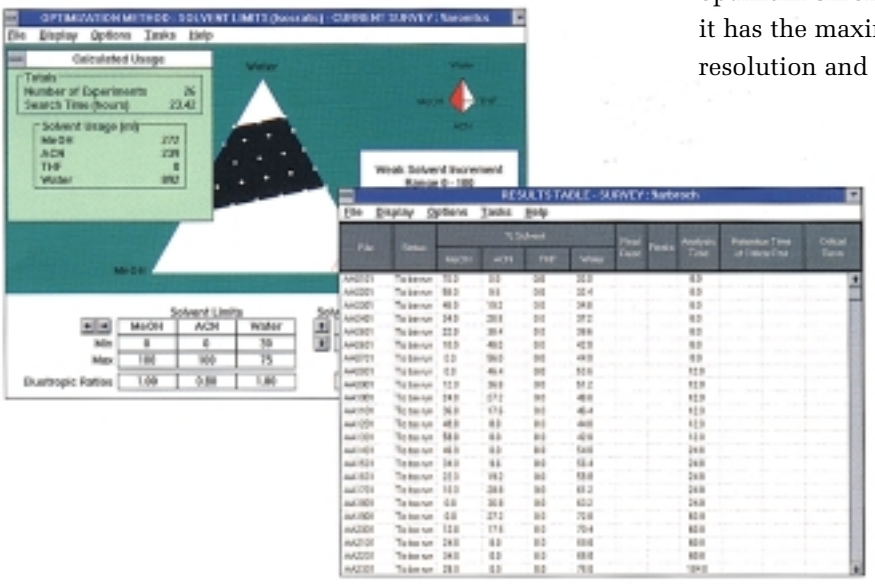


Figure 4. List of survey experiments and "calculated usage" summary highlighting experiment duration and requirement solvent amounts.

Full automation — multitasking system to maximize efficiency. The collection and processing of survey data in Turbo Method Development is completely automatic so that survey injection sequences can be collected overnight or at any time without an operator. Since Turbo Method Development and TotalChrom are fully multitasking, the workstation may be used for a variety of other tasks — even operating other chromatographs on the system or reprocessing TotalChrom data while a survey is in progress.

Intuitive map for rapid interpretation of results. After the run, survey results are displayed as a color resolution map (Figure 5). Each experiment in a survey is evaluated based on the number of detected peaks and the resolution of the two chromatographic peaks, which are least, separated the critical pair. The resolution value can vary between 0 (co-elution) and 1.0 (baseline separation). The "best separation" command in the Turbo Method Development finds the optimum chromatogram in the survey automatically — it has the maximum number of peaks, the best critical resolution and the shortest run time.

In the map shown here, experiments which are colored blue have at least two co-eluting peaks, while those in red or orange contain the maximum number of peaks (present in any chromatogram surveyed), and all peaks are separated at or near baseline level. Because separation optima are located automatically, it is not necessary to use predefined matrices or known mixtures of components. Automatic evaluation of the survey based on peak number and critical resolution can often effectively locate the best separation, even with unknown samples. The resolution map allows you to quickly evaluate the survey results and rapidly locate the most promising

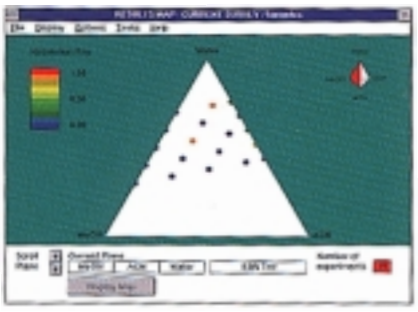


Figure 5. Resolution map quickly summarizes numerous runs in an easy to interpret format

FAST FACTS

Turbo Method Development

- → Intuitive graphical setup to get started quickly and easily.
- → Full automation — multitasking system to maximize efficiency.
- → Intuitive map for rapid interpretation of results.
- → Stacked-plot viewing and peak matching to help visualize chromatographic trends.
- → Chromatographic interpolation to simulate results between survey experiments.
- → Fully automated robustness testing.
- → Spectral confirmation of peak identity and purity.

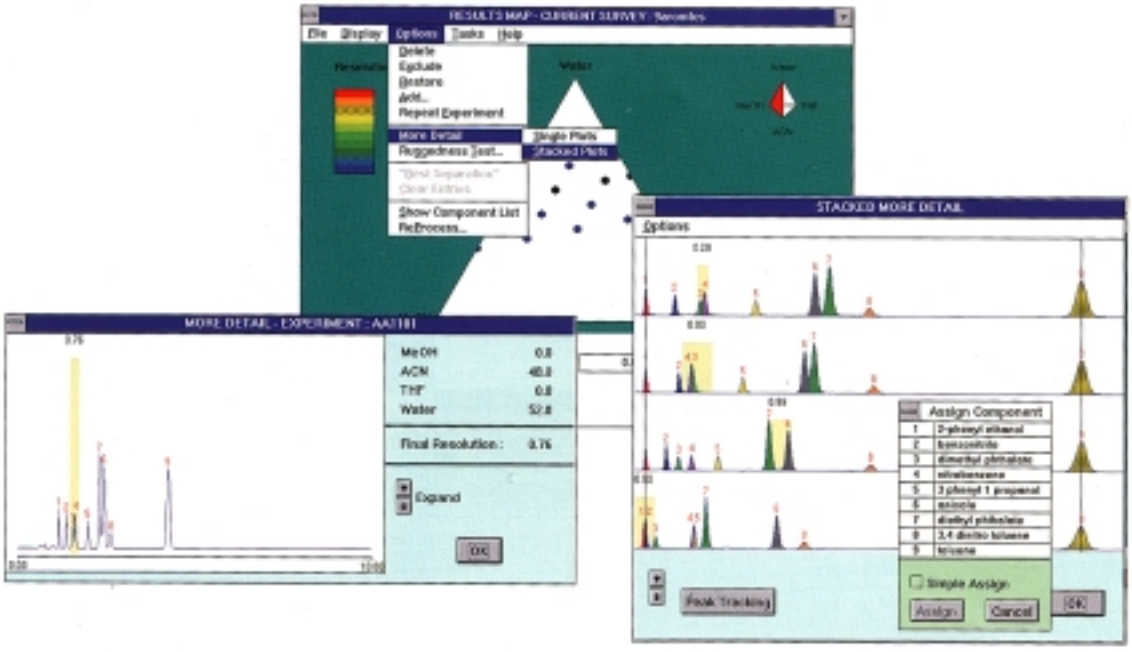


Figure 6. The stacked plot allows you to track trends and identify the critical peak separations vs. changes in solvent.

regions and studied "solvent space." In our case, the map shows some potentially useful regions, but more information would be helpful. Turbo Method Development also provides a convenient way to look at the movement of chromatographic peaks and to help predict chromatographic results in regions not surveyed using computer simulation.

Stacked-plot viewing and peak matching to help visualize chromatographic trends. Any chromatogram, a selected group of chromatograms, or a linear row of experiments in the survey can be viewed via mouse clicks. The chromatograms can be displayed either as a series of plots, or in a stacked-plot format, as shown in Figure 6. In stacked plots integrated peaks are positioned between two vertical markers and are displayed *independent of time*. This provides the best means for visualizing the relative migration of peaks as solvent composition and strength are changed. The stacked plot in Figure 6 shows a row of survey experiments, which have been selected by the mouse and then viewed, as single or stacked chromatographic plots with the critical pair of each chromatogram highlighted. Viewed from the bottom of the screen, the movement of chromatographic peaks relative to one another can easily be observed as the strong solvent is changed from MeOH to ACN. The alignment of chromatographic peaks in a stacked plot can be adjusted for best visual comparison by dragging specific peaks to the left or right of the vertical marker with the mouse.

Peak identities can be rapidly assigned through built-in automatic and visually aided peak matching tools

"**TURBO METHOD DEVELOPMENT** allows the chromatographer to perform **AUTOMATED** searches of quaternary solvent space (i.e. perform actual experiments blending up to 4 solvents) and **ANALYZE** the resulting data — all with a **MINIMUM** of time and effort."

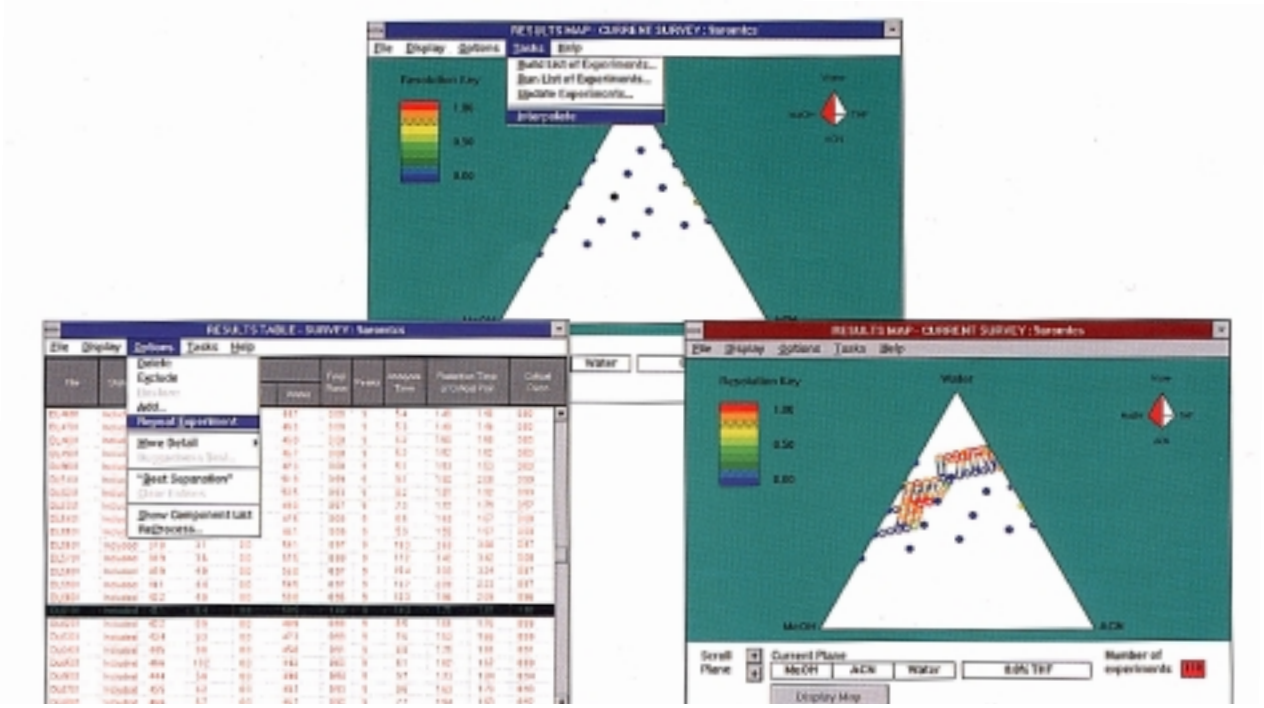


Figure 7. TMD can generate interpolated chromatograms (represented on the map by "hollow" data points) between actual survey points to help predict and uncover better separations quickly and easily.

so that you can track components through various regions of the survey and also so that you can perform chromatographic interpolation experiments. Peaks can be matched visually by using the mouse, or automatically through the "Peak Tracking" button, which assigns numbers to each chromatographic component in a stacked plot based upon relative peak area.

Chromatographic interpolation to simulate results between survey experiments. As shown in Figure 7, you can use the interpolation capabilities of Turbo Method Development to easily generate simulated chromatograms in regions of the survey between actual experiments, helping to uncover new areas where better separations might be possible. The simulated chromatograms for this survey, shown as hollow hexagons, predict that baseline separations are possible in several places not performed in the original survey. All of the simulated chromatograms become a part of the survey and are shown in red in the results table. Simulation is an excellent predictive tool. For instance, there are two areas in this survey which are predicted by interpolation to yield baseline separations — but the simulations at the top of the map (65-70% water) have a 20-25 minute run time while those in the middle of the survey (50% water) have a more practical run time of ten minutes or less. Confirming any simulation can be done automatically through the "Repeat Experiment" command.

Fully automated robustness testing. Now that a viable survey region has been located (50% H_2O, 40% MeOH, 10% ACN), the sensitivity of the separation to small mobile phase changes must be determined. Method robustness testing is required in the pharmaceutical industry and in other regulated HPLC laboratories. In Turbo Method Development it's referred to as a "ruggedness test". The test can be designed around any survey point (actual or simulated) and the range of the test and the number of experiments can be set in accordance with the intended usage of the method. In the nine-aromatic example we have executed a "ruggedness test" around the located survey optimum (Figure 8). Because the optimum was found in a region defined by simulated data, we will both confirm the simulations and test for robustness in the same experiment. "Ruggedness tests" and all additional survey injections become integrated into the survey, and all survey data are reevaluated after the new data have been processed.

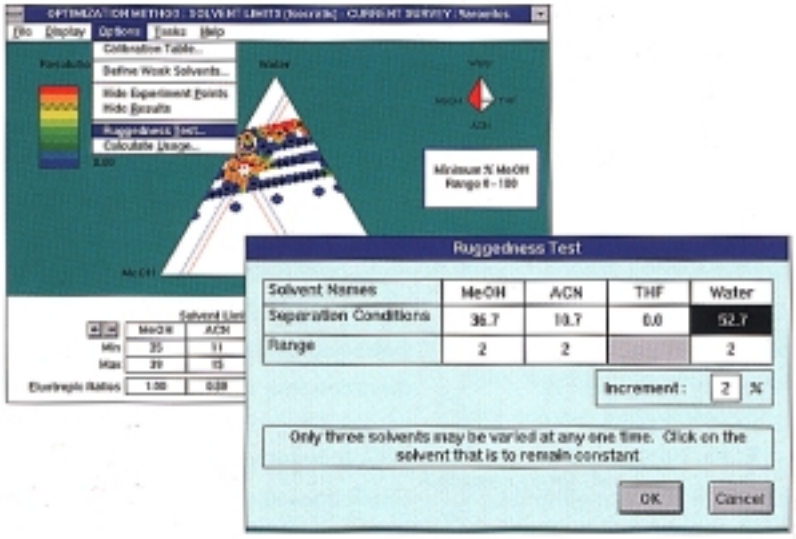


Figure 8. Robustness testing is performed via TMD's "Ruggedness Test", which builds additional experiments to evaluate how small changes impact the separation.

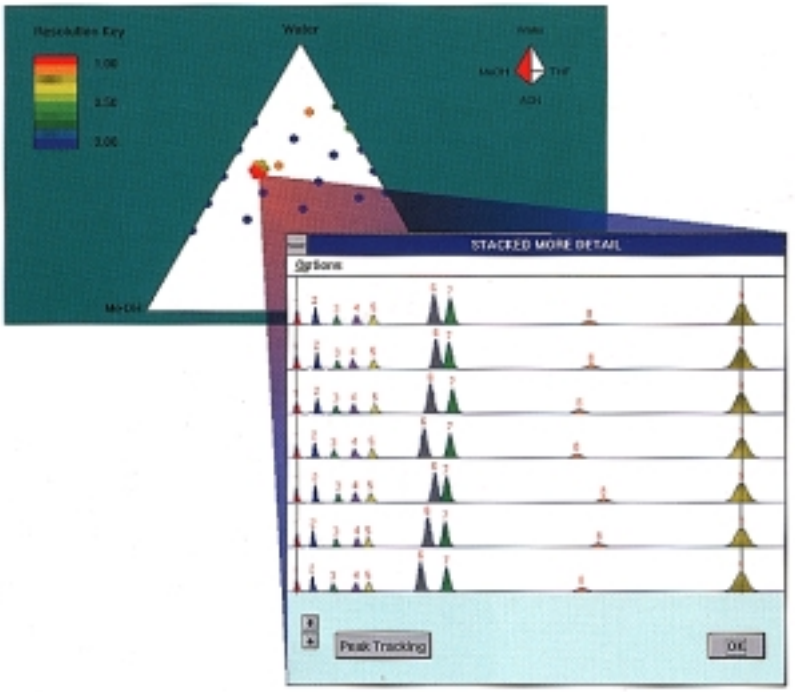


Figure 9. Once performed, the result of the test can be examined in greater detail to confirm whether the separation is indeed stable.

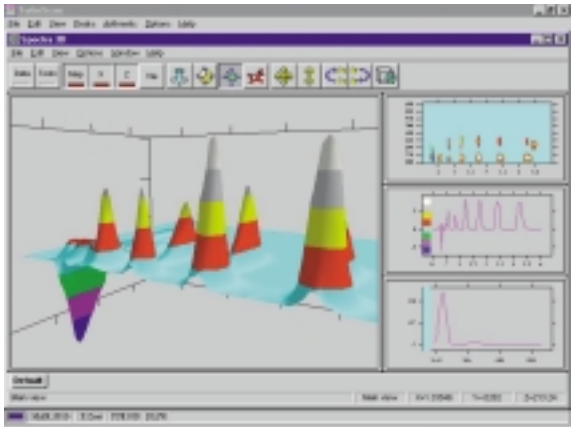


Figure 10. Spectral data eliminates co-elution concerns. The spectral maps generated by TurboScan200 also allows for optimization of wavelength settings.

The results confirm that we can perform the separation in less than 12 minutes in this region of the survey with baseline separation of all components. They also tell us that the separation is more stable to variations in water or strong solvent composition in the portion of the ruggedness test toward MeOH (Figure 9). The stack plot in Figure 9 shows all the chromatograms from the ruggedness test. The recommended conditions according to this ruggedness test are 39% MeOH, 10% ACN, 51% water.

Spectral confirmation of peak identity and purity. If used with the Series 200 Diode Array detector, Turbo Method Development has complete control over both the chromatographic and spectral modes of data acquisition. This means that all chromatographic data collected with Turbo Method Development surveys can be used for analysis of spectral purity and peak identity confirmation.

By using the TurboScan200 software for viewing and processing of the spectral data, you can perform sophisticated 3D plotting and spectral contour mapping. This functionality is ideal for performing detailed studies of the entire "spectral space" of a newly developed separation. You can rotate 3D plots in real time and can reveal subtle spectral features using a unique "side-lighting" effect. The 3D plot in Figure 10 can be rotated in any orientation to reveal minor impurities. Both contour maps and 3D plots are very useful for extracting individual spectra and looking at chromatograms at various detector wavelengths. This can be helpful in finding the optimum analytical wavelength or wavelength program for a newly developed separation. Extracted chromatograms can be integrated and processed within TotalChrom.

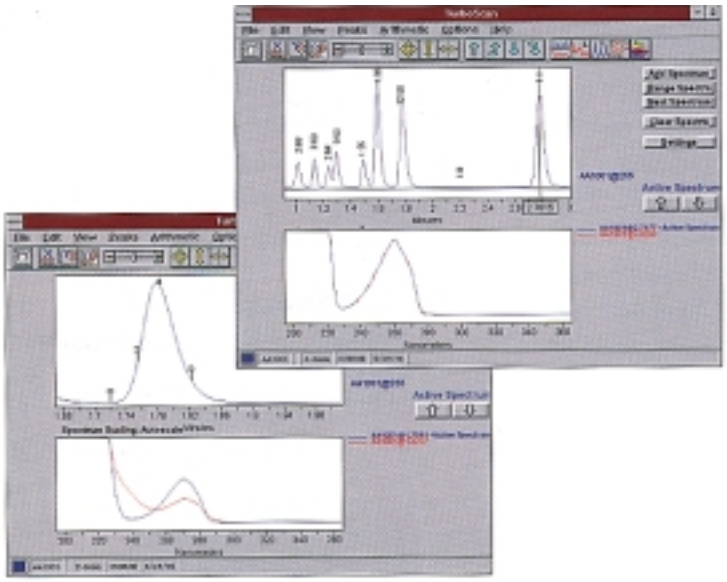


Figure 11. Chromatographic and spectral data generated during the survey help confirm peak identity and purity (i.e. homogeneity).

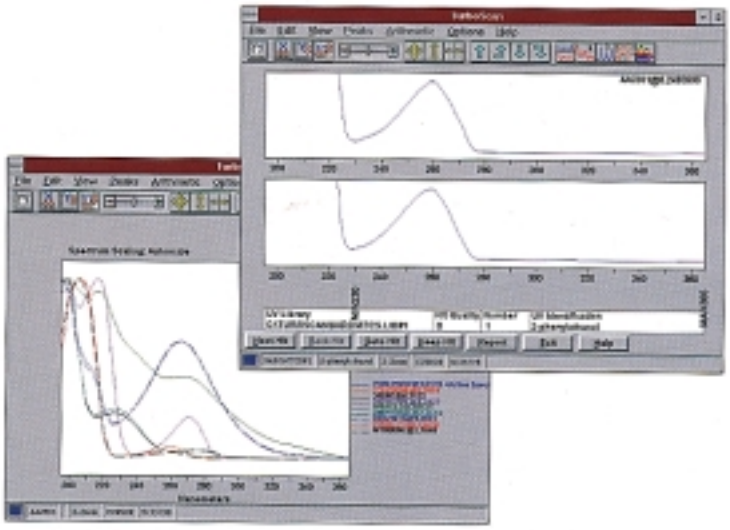


Figure 12. Spectral library matching can be used to facilitate identification of unknown peaks or further confirm identity.

In addition to viewing of the spectral data, TurboScan200 also performs automated, mathematical processing — yielding valuable qualitative information. Sufficient spectral data are always collected in order to describe the spectral purity and identity of the peak. The ratio of leading and trailing edge spectra is used by the system to calculate spectral peak purity. A value of 1.0 indicates high spectral purity while values > 2 indicate an impure peak.
Figure 11 shows one of our test separations (44% H2O/56% ACN) labeled with spectral peak purity values. All of the baseline-separated peaks have purity values near 1.0 except peak 7 (1.8 minutes), which has a large purity value, presumably due to a co-eluting spectral contaminant. The spectral overlays shown in the figure confirm that peak 7 contains two different spectral components. By contrast, peak 8, with a purity value of 1.1, contains upslope and downslope spectra, which overlay nearly perfectly.

Spectral library matching

Spectral library matching is another capability, which is often useful for method development and is part of TurboScan200. You can use diode array spectra to help confirm the identity of chromatographic

peaks. TurboScan200 contains a spectral library package, which allows users to construct libraries of standard spectra taken under specific conditions, which can be used for spectral matching. Figure 12 shows the library spectra assembled from the standards run in our nine-component aromatic separation. If standards were run using the same column and chromatographic conditions as unknowns, the spectral search can also be limited to a specific retention time window. In this example, the chromatographic peak eluting at 6.2 minutes yields a perfect spectral match with the 2-phenyl ethanol library standard. The matching algorithm gives "Hit Quality" results between 0 (perfect match) and 1.414 (poorest match). Thus, TurboScan200 has helped to confirm the spectral purity and identity of chromatographic peaks during our method development process.

Summary

Here we have seen an example of the application of PerkinElmer's integrated system approach to HPLC method development. TotalChrom control over the precise and proven performance of our LC instrumentation — coupled with our unique Turbo Method Development software — was used to find an optimum ternary mobile phase for a difficult nine-component separation. The search "area" was first automatically surveyed using a grid search, and then chromatographic interpolation was used to locate separation optima in regions not searched. The "ruggedness test" facility was then used to confirm the robustness of located optima. The Series 200 Diode Array detector and Turboscan200 spectral software helped to confirm the identity and spectral purity of chromatographic peaks during the search process.

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