



Flexar UHPLC Time Saver Method Calculator

A method converter tool for easy migration from conventional HPLC to UHPLC

Quick start and Help documentation

Step by step guide

Step 1 – Enter the column used for the reference analysis

The first step is to specify the column dimensions of your current HPLC method (Reference analysis).

Original method - Conventional HPLC
Reference Analysis Parameters

1 Enter column dimensions used for Reference Analysis

Column I.D. 4.60 mm

Particle size 5.00 μm

Column length 10.00 cm

- Specify the column inner diameter in mm by moving the slider to the appropriate value.
- Specify the particle size in μm.
- Specify the column length in cm.

Step 2 – Enter settings used with Reference Analysis

In the second step you specify additional settings of the reference analysis.

2 Enter settings used with Reference Analysis

Resolution factor, R_s 2 Optional

Injected volume: 10.00 μL

Flow rate: 1.00 mL/min

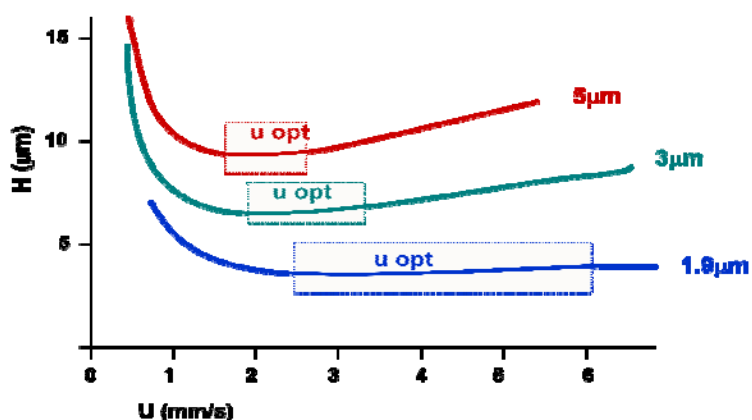
Pressure: 1200 psi

- In the “**Resolution factor, R_s** ” box you can specify the chromatographic resolution between two peaks (if you have two components eluting close together and you want a certain resolution between these two peaks).
The entry in this field is optional.
This tool will calculate the theoretical resolution you will obtain with the column you specified for the new UHPLC method. The resolution between two peaks will depend on the particle size and on the column length.

Tip:

If you find that your conventional HPLC method gives you a typical resolution of 2.0 between a critical pair of peaks, and the transferred UHPLC method gives you a resolution of 1.8, you can increase the length of the UHPLC column or reduce the particle size of the stationary phase in the UHPLC column to improve the resolution. Remember that the analysis time is proportional to the column length.

- b. In the “**Injected volume**” field, enter the injection volume you use today.
The tool will suggest a new injection volume for the UHPLC method based on the volume of the UHPLC column.
- c. Specify the **flow rate** in mL/min for your current HPLC method. The tool calculates a new flow rate suitable for your UHPLC method.



Tip:

According to chromatographic theory (see VanDeemter plot above) there is an optimal mobile phase flow (u_{opt}). For typical HPLC columns with particle size of $5\mu\text{m}$, the optimal flow is about 1 mL/min . The optimal flow will depend on the particle size (the smaller the particle size the wider the range of the optimal flow.) Thus, it is usually possible to increase the flow rate to speed up the analysis without a significant decrease in chromatographic resolution.

- d. Specify the **pressure** in psi for your current HPLC method and the tool will calculate the corresponding expected pressure for the UHPLC method. This entry is optional. The back pressure in the system will depend on column length and the particle size. If the calculated expected UHPLC pressure is too high, you can shorten the column or increase the particle size. The calculation of the pressure is an estimate and will also depend on factors such as mobile phase composition and temperature.

Step 3 – Enter the gradient

In the third step you specify the gradient of your current HPLC method.

3 Enter the gradient
(For isocratic : fill out Step 1 only)

Step	Duration (min)	%A	%B
0 (Equilibration)	5	100	0
Step 1	2	100	0
Step 2	5	50	50
Step 3	5	20	80
Step 4	5	20	80
Step 5			
Step 6			

Analysis time 17.00 min
Cycle time 22.00 min
Solvent consumption 44.00 mL

Show Plots Hide Plots

Display or hide the gradient profile plots

Please note that you need to have macros enabled in excel for this function to work.

The step “**0 (Equilibration)**” is the column conditioning step and is not a part of the run time. It is, however, a part of the overall analysis cycle time.

Enter your settings for the column conditioning step – the duration in minutes and the mobile phase composition as % A.

Isocratic run

For an **isocratic run** fill out the “Step 1” only and do not enter values in the other steps. You do not normally need to have an “0 (Equilibration)” step.

Gradient run

In the field “**Step 1**” to “**Step 6**” enter your current gradient. The time that you enter in minutes is the duration time of that step. For each step you also need to specify the mobile phase composition in % mobile phase A.

Example 1:

Step 1	2	100	0
Step 2	5	50	50

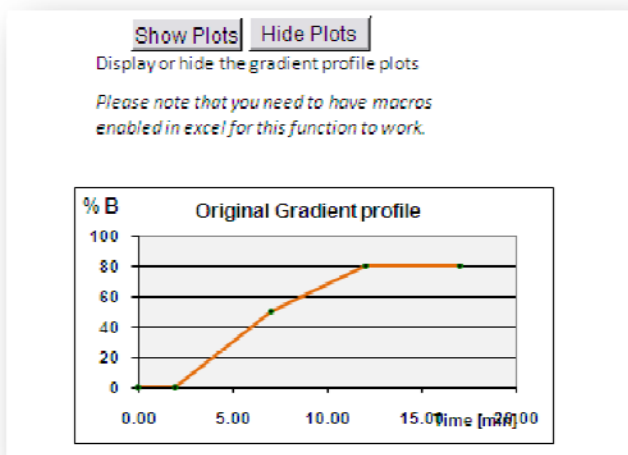
The gradient between Step 1 and 2 will change the mobile phase composition from 100 % A to 50 % A in 5 minutes (the duration of step 2).

Example 2:

Step 3	5	20	80
Step 4	5	20	80

To hold the mobile phase compositions at a certain value for a specified time, follow the example above. The mobile phase composition will not change between Step 3 and 4. Since the duration of step 4 is 5 minutes, this is how long the mobile phase composition will remain constant.

You can choose to display the gradient profile to see a graphical representation of your gradient by clicking “**Show plots**”.



Note: You need to have macros enabled for this function to work. See Appendix 1 for a guide on how to enable macros.

The tool will also calculate the **analysis time** and the **cycle time** (analysis time + equilibration time) in minutes of your current method. The **solvent consumption** in mL per cycle is also calculated.

Analysis time	17.00	min
Cycle time	22.00	min
Solvent consumption	22.00	mL

Step 4 – Select UHPLC column

In the fourth and final step of your method translation you need to specify the column dimensions of your UHPLC column – the new fast LC method.

Transferred method - UHPLC
New Analysis Parameters

4

Select UHPLC column:
Other - Specify dimensions below

Or specify dimensions:

UHPLC Column I.D.	2.10	mm
UHPLC Particle size	1.90	µm
UHPLC Column length	5.00	cm

Values used

Clear

You can select a predefined PerkinElmer Brownlee HRes column from the drop down list or enter the column dimensions manually. If you enter the column dimensions manually, these values will override the values for the selected predefined Brownlee HRes column. You can always see which values for the column dimension are being used for the calculation under “Values used” – see below.

Transferred method - UHPLC
New Analysis Parameters

4

Select UHPLC column:
Other - Specify dimensions below

Or specify dimensions:

UHPLC Column I.D.	2.10	mm
UHPLC Particle size	1.90	µm
UHPLC Column length	5.00	cm

Values used

Clear

You can use the “Clear” button to clear the manual entries.

Transferred method

New Settings

If you have completed step 1 through 4 you have now converted your conventional HPLC method to a fast UHPLC method. These new settings are described below.

New resolution factor, R''_s	2.29
New injection volume:	1.04 μ L
New flow rate:	0.55 mL/min
**New Pressure:	10935 psi

The “**New resolution factor, R''_s** ” is the estimated resolution for the UHPLC column you have specified. This value will depend on the “Original resolution” which you specify in Step 2 and the column length and particle size of your UHPLC column. Choosing a longer column and/or smaller particles will increase the resolution.

The “**New injection volume**” is the calculated suggested injection volume for the UHPLC method. This value will depend on the injection volume in the original HPLC method and the column dimensions of the UHPLC method.

The “**New flow rate**” is the calculated suggested flow rate of the UHPLC method. This value will depend on the flow rate of the original HPLC method and the column dimensions of the column selected for the UHPLC method.

The “****New Pressure**” is the calculated estimated pressure for the UHPLC method. The calculation depends on the pressure specified for the original HPLC method specified in Step 2 and the column length and particle size of the UHPLC column.

New UHPLC Gradient

New UHPLC gradient

Step	Duration (min)	%A	%B
0 (Equilibration)	1.0	100	0
Step 1	0.38	100	0
Step 2	0.95	50	50
Step 3	1.0	20	80
Step 4	1.0	20	80
Step 5			
Step 6			

Analysis time 3.23 min 13.77 min saved
 Cycle time 4.18 min 17.82 min saved
 Solvent consumption 2.29 mL 19.71 mL saved

In the window above you see the calculated new UHPLC gradient. The Flexar UHPLC calculator translates the gradient volumes of the original HPLC method to the new UHPLC method. The gradient will depend on the original HPLC gradient and the volume of the UHPLC column.

The calculated UHPLC method analysis time and cycle time is displayed as well as the solvent consumption per analysis cycle.

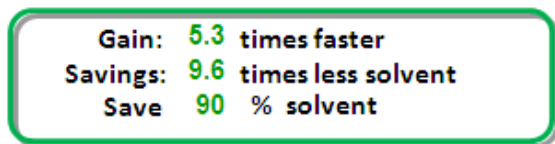
Analysis time	3.23 min	13.77 min saved
Cycle time	4.18 min	17.82 min saved
Solvent consumption	2.29 mL	19.71 mL saved

The gain in both analysis time and cycle time as well as the reduction in solvent consumption for the new UHPLC method compared to the original HPLC method is also displayed to the left.

Analysis time	3.23 min	13.77 min saved
Cycle time	4.18 min	17.82 min saved
Solvent consumption	2.29 mL	19.71 mL saved

Savings

In the window below you see the summary of the calculations and the gain in analysis time and the reduction in solvent consumption when you migrate your method from conventional HPLC to the new UHPLC method.



In this example the new UHPLC analysis is 5.3 times faster than your current HPLC method. The new UHPLC method consumes 9.6 times less solvent than your current HPLC method and you save 90% of your solvent.

Print report 

When you are happy with the translation of your current HPLC method to the new UHPLC method you can choose to print a report by clicking the print icon.

Help 

Clicking the ?-icon will open this document.

Further optimization

This tool has been designed to make the migration from conventional HPLC to UHPLC more convenient. The calculated values will provide a good starting point for your new UHPLC method but further optimization may improve the analysis even more. By increasing the mobile phase flow to a higher value than the calculated/suggested value for the new UHPLC method, the analysis times and cycle times can be reduced. Selecting a longer column will improve the chromatographic resolution, while a shorter column will speed up the analysis even further.

Appendix 1

1. Installation/set up

This tool does not require any installation. It does require that Microsoft® Excel® (version 95/2003 or 2007) is installed on the computer you want to use for this tool.

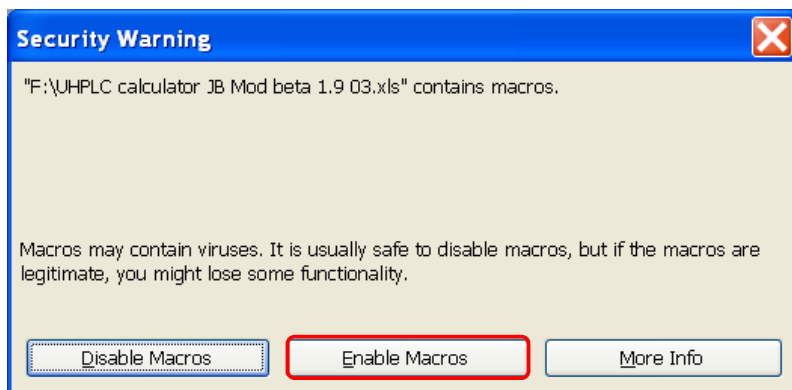
1.2 About macros

This tool utilizes Excel macros for certain operations such as show/hide the gradient profiles and the print preview function.

To utilize the full functionality of the tool and to enable these functions, you need to allow macros being run for this application. The tool will work fine without macros enabled with the exception of the functions mentioned above. The procedure to enable macros in Excel will depend upon which version of Excel you use. Below is a guide on how to enable macros for different versions of Excel. Please check which version of Excel you are running and read the corresponding instructions below. Additional information on macros and security settings can be found in the Help section in Excel.

1.3 To enable macros in Excel 95/2003

When you open the tool in Excel 95 or 2003. Excel will recognize that the document utilizes macros and the following dialog box is displayed:



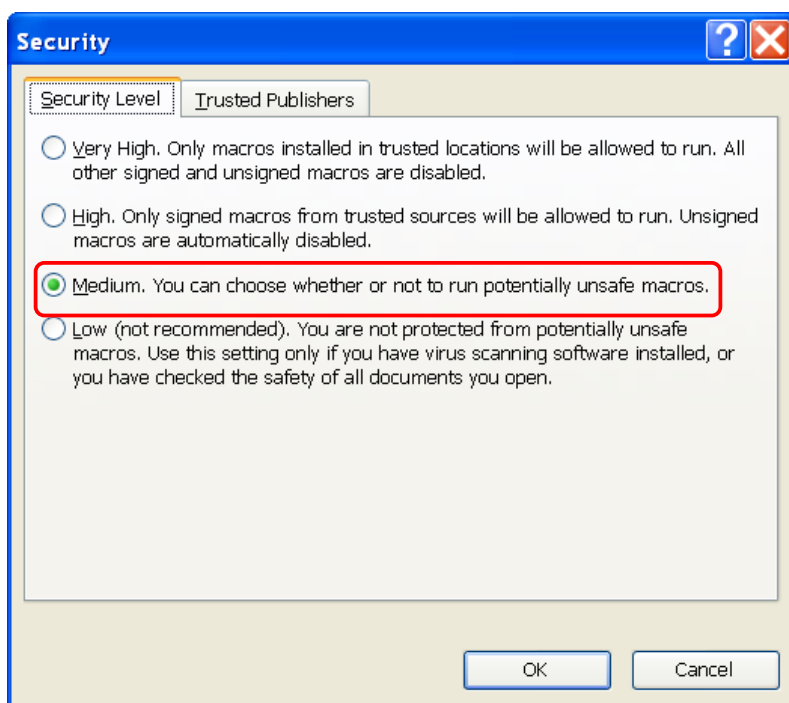
Since the tool utilizes Excel macros, you will get a Security Warning. This is normal and applies to all documents with macros enabled. To enable macros for this document, click "Enable Macros".

- You should now have full access to all functionalities of the tool.

1.3.1 Still got problems with macros in Excel 95/2003?

Excel 95/2003 can handle the macro security differently depending on your local security settings. To check your security settings, click “tools” select “Macro” and click “Security...”.

The following window appears:

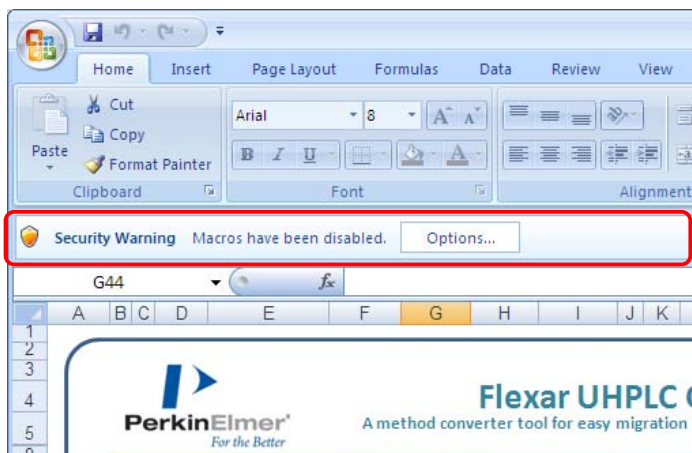


Make sure you have selected “Medium” and click “OK”.

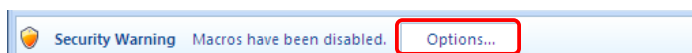
You may have to restart Excel and re-open the tool to apply the changes. Then follow the instructions at section 1.3 above.

1.4 To enable macros in Excel 2007

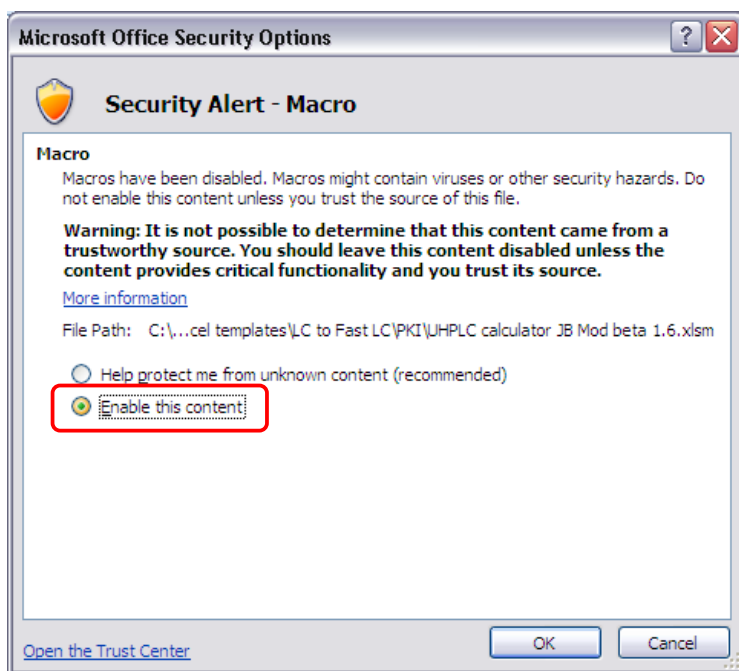
When you open the tool in Excel 2007, the following screen appears:



Since the tool utilizes Excel macros you will get a Security Warning. This is normal and applies to all documents with macros enabled.



By clicking the “Options...” button illustrated above the following window appears.

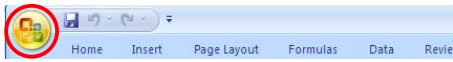


To enable macros for the tool, select “Enable this content” and click “OK”.

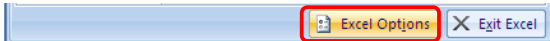
- You should now have full access to all functionalities of the tool.

1.4.1 Still got problems with macros in Excel 2007?

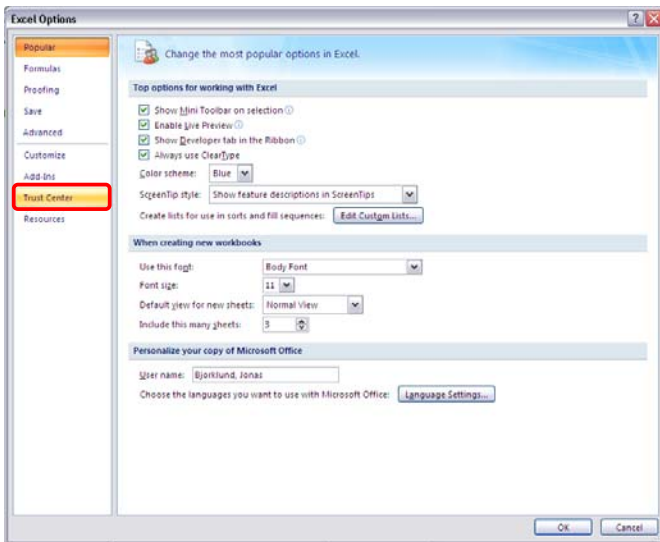
Excel 2007 handles the macro security differently depending on your local security settings. To check your security settings, click the “Office” button:



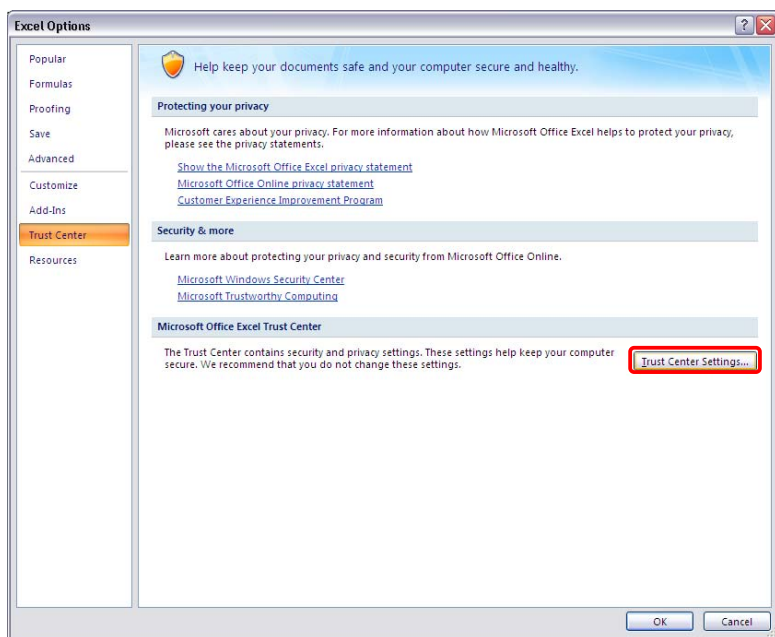
And click “Excel options” at the bottom of the menu list:



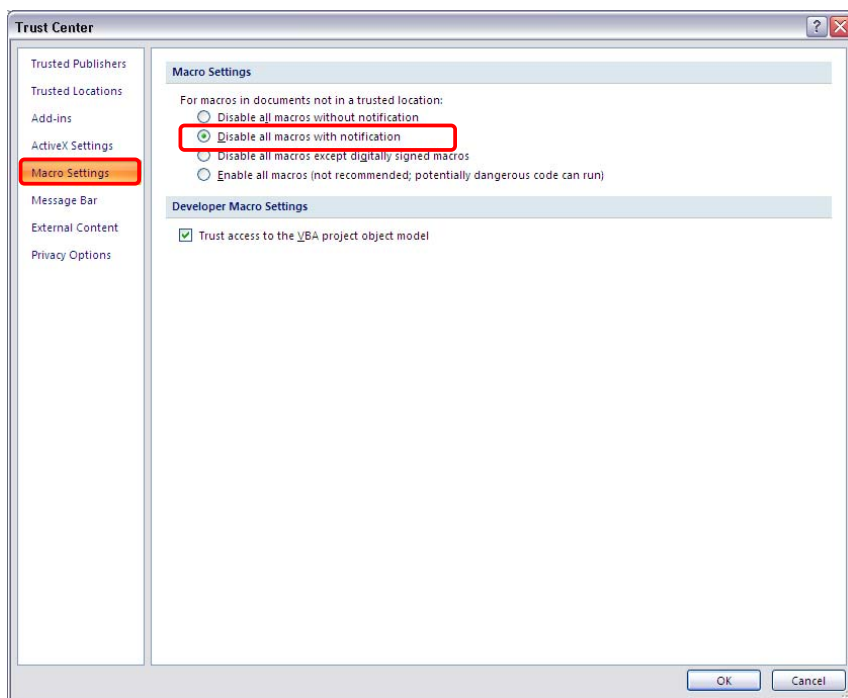
The following window appears:



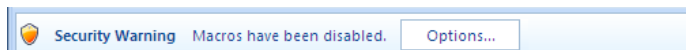
Click “Trust Center” and the following screen appears:



Click “Trust Center Settings” to display the following screen:



Select “Macro Settings” in the left column and inspect the Macro settings. Excel sets “disable all macros with notification” by default. This means you will see the “Security Warning” message whenever you open a document that contains a macro.



The macros will be disabled until you enable them.

Make sure the “Disable all macros with notification” is selected and click “OK”.

You may have to restart Excel and re-open the tool to apply the changes. Then follow the instructions at section 1.4 above.