ACE LC Translator

Contents

1. Introduction

The ACE LC translator has been designed to help simplify the process of HPLC method translation and method transfer. The tool uses the original method and column details as input along with the new column format and additional parameters such as dwell volume. Translated methods are then automatically generated with no need for the user to manually carry out numerous calculations.

For the purposes of this tool, method translation is defined as migrating an LC method from one LC system/LC column format to another (for example, migrating a gradient method from a 150 x 4.6 mm, 5 µm column on an HPLC system to a 50 x 3.0 mm, 1.7 µm column run on a UHPLC system). Method transfer deals with moving a method from one HPLC system to another with no change to the column format or method fundamentals.

Also included are a number of useful tools for determining parameters such as dwell volumes and column volumes that are required for method translation activities, along with other tools useful for everyday practical chromatography.

2. Disclaimer

The ACE LC Translator Excel spreadsheet tool and this document are provided 'as is'. All users do so at their own risk and without any acceptance of liability by ACT Ltd for damages, incorrect information or any regulatory body implications. Use of this spreadsheet tool indicates acceptance of these conditions.

It is important to note that even when a method is correctly translated, many factors such as frictional heating from the use of elevated flow rates and pressure induced changes in selectivity may mean that the exact chromatography of the original method may not be fully reproduced.

3. Index Page

The Index page of the LC translator appears as shown in Figure 1 and is divided into a “Tools” section and a “Free Literature” section. The tools listed can be accessed by clicking on the relevant link on the index page or can be accessed via the tabs at the bottom of the spreadsheet. Users can navigate back to the Index Page by clicking on the **Main Menu** link at the bottom left of each page.

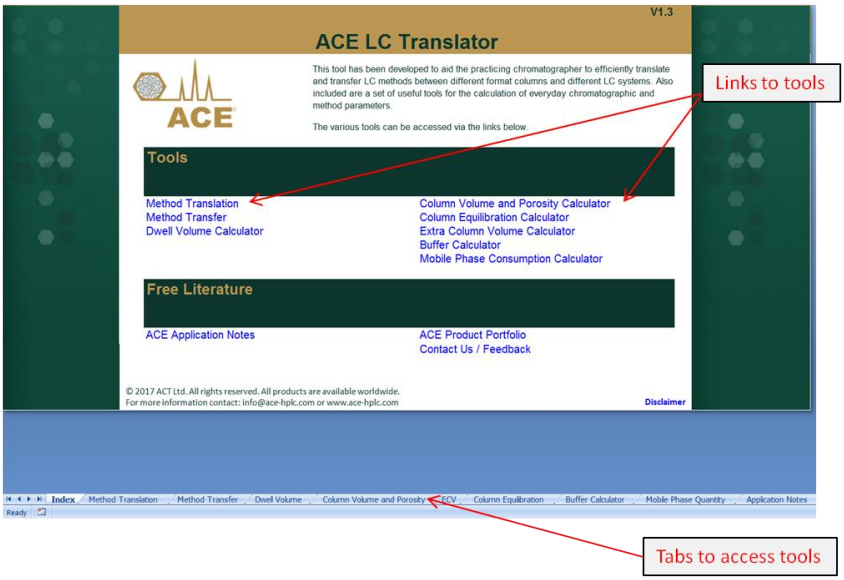


Figure 1: LC Translator – Index Page.

4. General Guidance

All of the tools follow a similar formatting style. In order to carry out a calculation, all of the input boxes (displayed as) must be completed. Once all input fields are completed, calculated values are displayed (Figure 2).

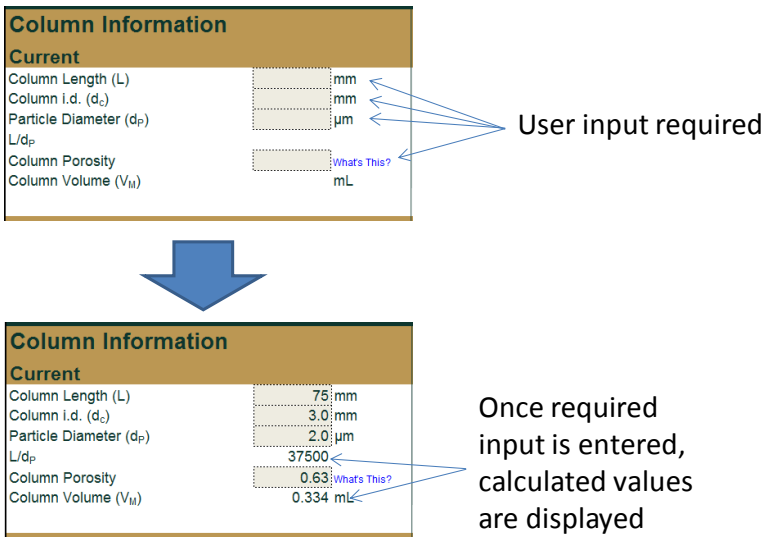


Figure 2.

Where necessary, further details for some input parameters can be accessed by clicking on the "What's This?" link (as shown in Figure 2).

The Index page can be accessed by clicking on the **Main Menu** link which can be found in the lower left corner of each page in the spreadsheet.

5. Method Translation Calculator

5.1 General

The method translation tab is divided into two sections, the first to translate isocratic methods (Figure 3) and the second to translate gradient methods (Figure 4). Both sections have the same format; the left-hand side of the screen displays the current method, whilst the right-hand side displays the new translated method. Once all input fields ( ) have been completed, the translated method is automatically displayed.

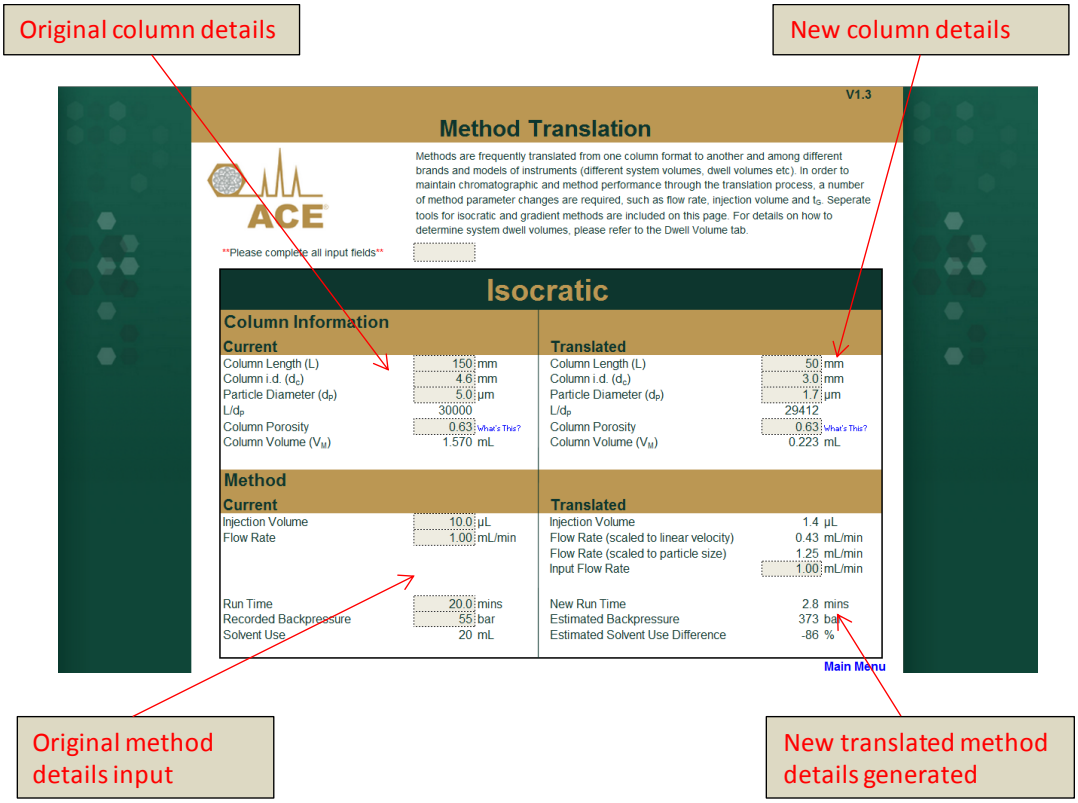


Figure 3: Isocratic method translation section.

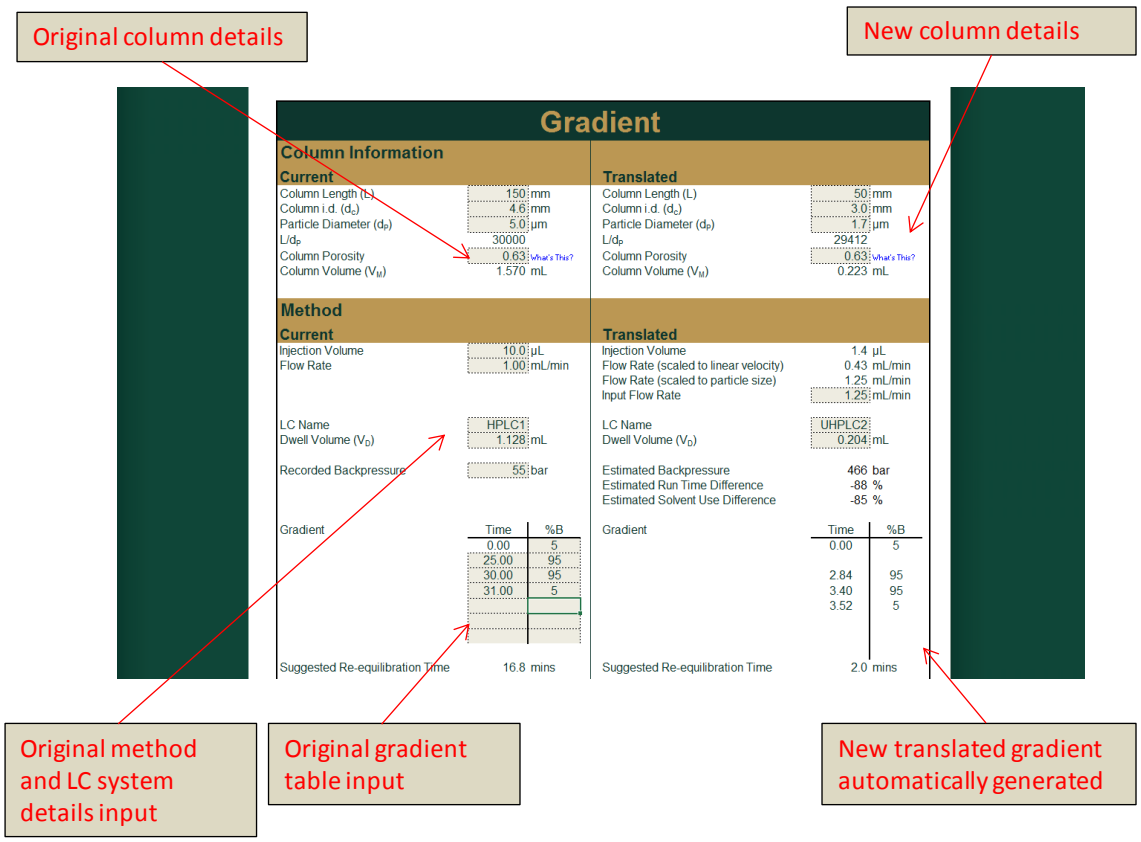


Figure 4: Gradient method translation section.

5.2 Entering column details

The user is required to enter the details of both the original column and the column that the method will be translated to (Figure 5). The column length, internal diameter and column porosity are used to calculate the column volumes. For ACE fully porous particles, a porosity value of 0.63 can be used, whereas for UltraCore solid core particles, a porosity value of 0.55 can be used. The porosity values for other vendors' columns will vary from these values; however, they can be used as an approximation, particularly if translating between particles from the same vendor with identical particle architectures (i.e. fully porous or solid core).

Alternatively, the Column Porosity tool (accessed from the index page or navigation tabs) provides a method for determining the porosity and hence column volume experimentally. This approach is recommended for maximum accuracy of the translated method.

The L/d_P (column length/particle diameter) ratio, an indicator of column efficiency, is also calculated. To maintain chromatographic resolution through method translation, the L/d_P ratio for both columns should be similar.

Column Information					
Current			**Translated**		
Column Length (L)	75	mm	Column Length (L)	50	mm
Column i.d. (d_c)	3.0	mm	Column i.d. (d_c)	2.1	mm
Particle Diameter (d_P)	5.0	µm	Particle Diameter (d_P)	3.0	µm
L/d_P	15000		L/d_P	16667	
Column Porosity	0.63	What's This?	Column Porosity	0.63	What's This?
Column Volume (V_M)	0.334	mL	Column Volume (V_M)	0.109	mL

Figure 5: Entering the column details.

5.3 Selecting flow rate

For maximum flexibility, the user must manually enter a flow rate for the translated method (Figure 5). The ACE LC Translator will automatically generate a new translation for any flow rate entered, allowing the user to assess the effect on run time, solvent use and predicted backpressure. ***It is important for the user to ensure that the flow rate and backpressure of the new method are compatible with the HPLC column and LC system specifications.*** The LC Translator provides two suggestions for a translated flow rate; firstly, the flow rate is scaled geometrically to the new column format to maintain a constant linear velocity of mobile phase through the column. As a second option, the flow rate is scaled to the new particle size to take into account that higher flow rates can be used for smaller particles to achieve optimum efficiency. In practice, it may be necessary to translate to a range of different flow rates and run the corresponding methods to establish experimentally the optimum flow rate for the translated method.

5.3 Corrections for dwell and column volumes in gradient methods

For gradient methods, it is important to correct for the change in the LC system dwell volume/column volume ratio (V_D/V_M) when a method is translated. This correction is automatically calculated by the ACE LC Translator in the form of either incorporating a pre-gradient hold in the gradient table of the translated method (as shown in Figure 7), or by delaying the injection until after the start of the gradient (Figure 8). A delayed injection can often be included within a method using the LC systems operating software, for further details please contact your instrument manufacturer.

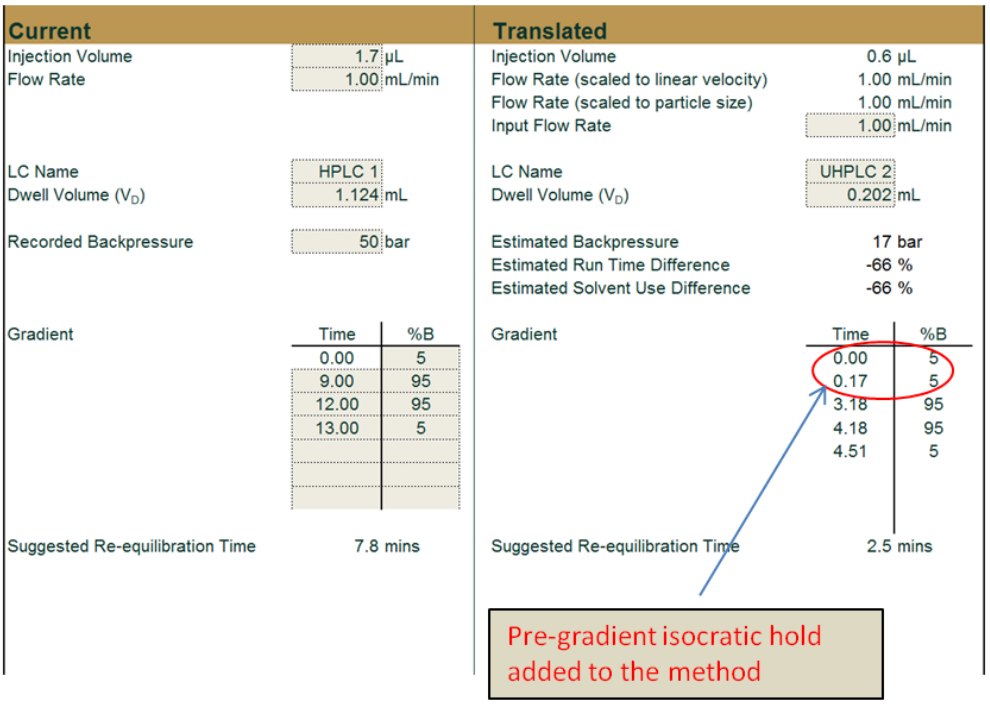


Figure 7: Example of the new translated method requiring a pre-gradient isocratic hold to correct for the change in V_D/V_M.

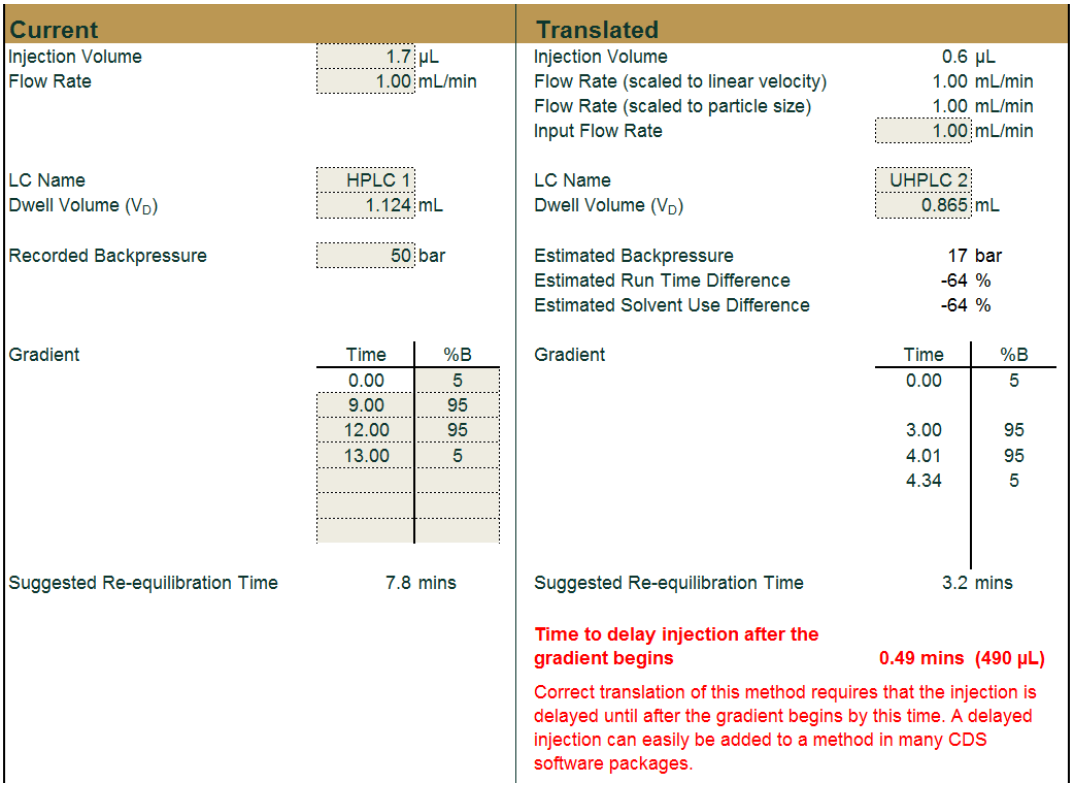


Figure 8: Example of the new translated method requiring delayed injection to correct for the change in V_D/V_M.

5.4 Column Equilibration

The ACE LC Translator gives a suggested equilibration time for the original and translated methods (10 x V_M + V_D). This is a generic recommendation for reversed phase methods. ***It is important for the user to establish whether this is appropriate for the method, as some methods may require longer equilibration times (e.g. gradient HILIC methods).***

6. Method Transfer Calculator

Method transfer involves moving a method from one LC system to another with no changes to the basic method or column dimensions. Isocratic methods require no changes to be made, as long as the new LC system is fit for purpose and correctly setup for the method.

To transfer a gradient method, the column details are first entered under “Column Information”, see Figure 9 (see section 5.2 for column volume input). The LC method and gradient is then entered. Gradient methods often require a correction for a change in system dwell volume. If a pre-gradient isocratic hold or delayed injection is required for the transferred method, this is automatically generated as outlined in section 5.3.

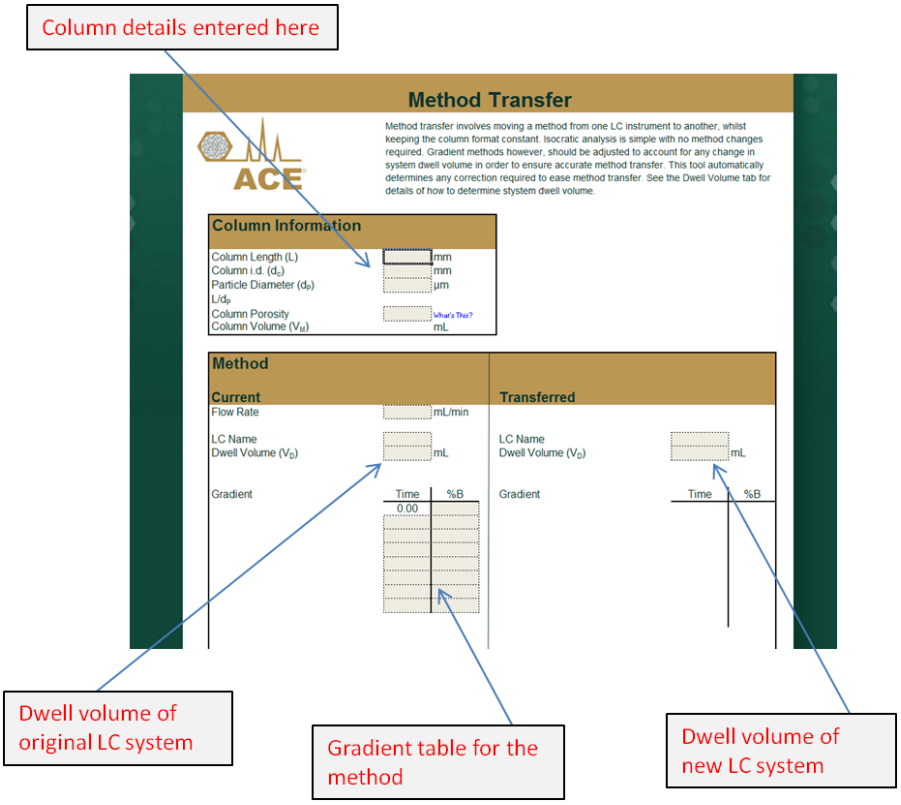


Figure 9: The method transfer tool.

7. Dwell Volume Calculator

This tool allows accurate determination of LC system dwell volumes, which is essential for accurate gradient method transfer and translation. This tool provides an experimental approach for dwell volume determination and calculates the system dwell volume based on the experimental results. To obtain an accurate result, it is recommended that the gradient is performed at least twice and the first run omitted to ensure full system equilibration.

After running the gradient, determine the maximum absorbance at the gradient end point from the chromatogram and enter the value into the calculator as shown in Figure 10. The calculator then determines the gradient mid-point. This value should then be used to determine the time of the gradient midpoint from the chromatogram. The system dwell volume is then calculated.

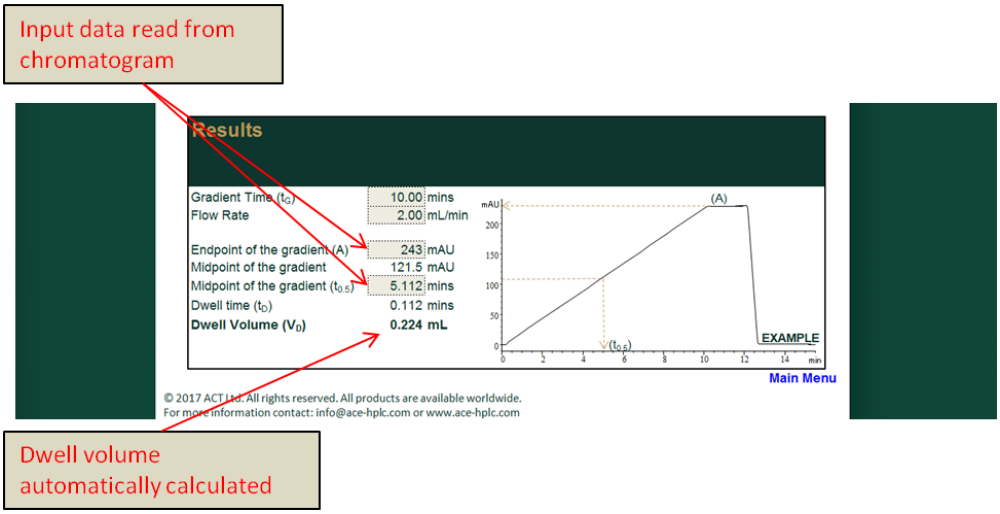


Figure 10: System dwell volume calculator.

8. Column Porosity Calculator

The column porosity, together with column length and internal diameter is used to calculate the internal volume of the columns used for the original and translated methods (see section 5.2). For ACE fully porous particles, a porosity value of 0.63 can be used, whereas for UltraCore solid core particles, a porosity value of 0.55 can be used. The porosity values for other vendors' columns will vary from these values. This tool provides a method for the accurate determination of column porosity by injection of a t_0 marker. The LC system extra column volume is also required (this can be estimated as follows or determined experimentally (see section 9).

Suggested approximate system extra column volumes*:

UHPLC system: 10-20 µL

Optimised HPLC system: 20-30 µL

Standard HPLC system: >40 µL

* These values are provided for guidance only and may vary significantly from experimentally determined values due to tubing sizes and configurations, flow cell volume, use of column switching valves etc.

9. Extra Column Volume (ECV) Calculator

This tool allows the LC system ECV to be quickly estimated experimentally from replicate injections of a 0.1% acetone solution with a zero dead volume union installed in place of the column. The retention time of acetone is entered into the calculator and the ECV is calculated.

Hint: If poor baseline or peak shape is obtained, try increasing the flow rate for a couple of minutes before returning to 0.1 mL/min to ensure no air bubbles are present in the detector cell. If the problem persists, try running the method at 0.2 or 0.3 mL/min.

10. Column Equilibration Calculator

Correct column equilibration is important to obtain reproducible chromatography. Reversed phase methods may only require short equilibration times, whereas other methods such as HILIC may require substantially longer times. This calculator determines equilibration times equating to 10, 20 or 30 column volumes for a given column/flow rate combination. It is important for the user to determine experimentally that sufficient column equilibration is achieved. See section 5.2 for tips on entering the column volume. For gradient separations, it is important to also add extra equilibration time to account for the system dwell volume.

11. Buffer Calculator

The Buffer calculator can be used to determine a recipe for commonly encountered HPLC mobile phases. ***It is important that the user checks whether their buffer purity, density and molecular weight match those displayed in the calculator otherwise incorrect values may be obtained.***

To use, select the buffer required from the drop-down menu and enter the amount of buffer required in Litres and the required buffer strength in mM units.

12. Mobile Phase Quantity Calculator

This tool allows the amount of mobile phase required to run x injections using a given LC method to be estimated. The calculated values should be rounded up to convenient amounts, ensuring at least 10% overage. It is important that sufficient mobile phase is prepared to allow for any flushing of solvent lines, columns and column for equilibration.

The calculator allows up to 4 solvent lines to be specified (denoted A, B, C and D). The total % across the 4 lines should always add up to 100% (green) as shown in Figure 11. If this is not entered correctly, this is highlighted in red.

Tip: For gradient methods, the column re-equilibration time should be included as an additional step in the gradient table, as in Figure 11. For isocratic methods, include extra runs to factor in column equilibration.

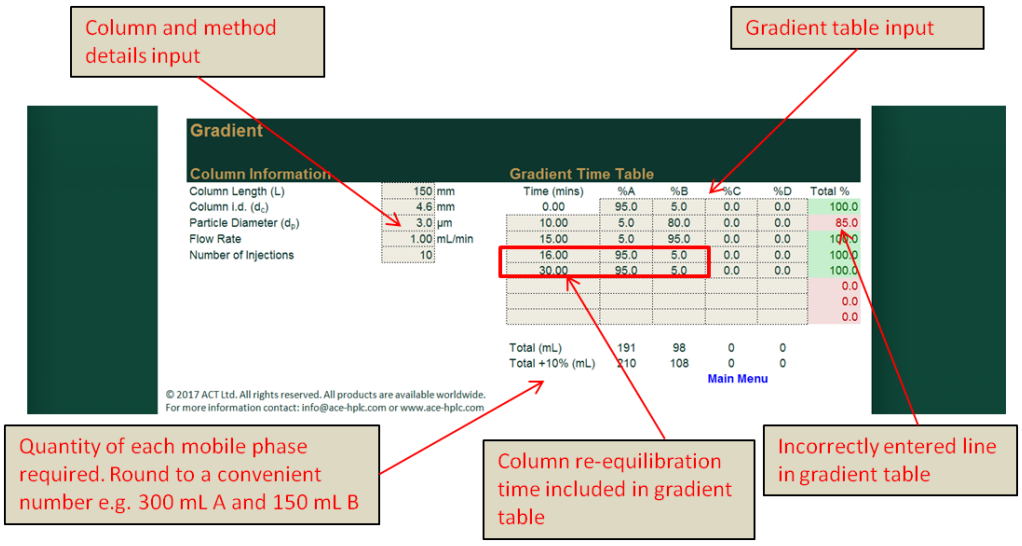
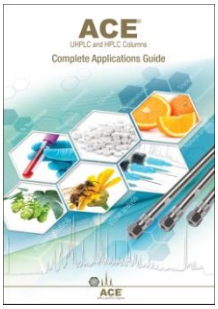


Figure 11: Mobile phase quantity calculation for a 10 minute gradient (5-95% in 10 minutes, hold for 5 minutes, ramp down to 5% in 1 minute and re-equilibrate for 14 minutes). Note that the second line of the gradient table is entered incorrectly.

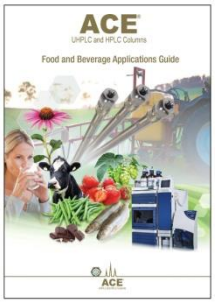
13. ACE Application Notes

This tab provides details of all currently available LC and LC/MS ACE application notes, from a diverse range of sectors including pharmaceutical, environmental, academia and food safety.

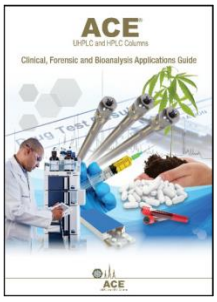
Also available are three application compilations, available by request (email info@ace-hplc.com):



ACE Complete Applications Guide



ACE Food and Beverage Applications Guide



ACE Clinical, Forensic and Bioanalysis Applications Guide

14. ACE Product Portfolio

This tab contains information on the entire range of ACE products, including a suite of novel selectivity stationary phases and Method Development Kits.

15. Contact Us

Use the links on this page for contact details and technical support for all ACE products and services.