Chromatography Solutions

Knowledge note #0023

Gradient Method Translation Using the Avantor® ACE® LC Translator

INTRODUCTION

Translating gradient LC methods from one column dimension to another is common, however, maintaining the separation selectivity and peak resolution can be challenging. For the most reliable translation of gradient methods between column formats, careful consideration of fundamental chromatographic principles is required. Omitting key parameters in calculations can lead to detrimental changes in selectivity or peak resolution of the translated gradient method. The ACE LC Translator is based upon fundamental principles and includes all key parameters and calculations required for an accurate gradient method translation process.

SIMPLIFIED METHOD TRANSLATION

Method translation involves moving an LC method from one column dimension to another. Additionally, the method may be moved to a new LC system at the same time. With the increased popularity of sub-2 micron particles, many legacy LC methods are being translated from large column formats (e.g. 150 x 4.6 mm) to smaller ones (e.g. 50 x 3.0 mm). For example, a legacy method run on a large column may be translated to a small UHPLC format column to realise cost/efficiency improvements. Conversely, whilst new method development activities in the R&D lab may exploit the speed of UHPLC, there may be a need to translate the method to HPLC for routine use in the QC or production laboratory.

The principal goal for method translation is to maintain the chromatographic selectivity and performance of the original method with the newly translated method.

Accurate gradient method translation requires scaling flow rate, injection volume and gradient segment times to the new column format. Additionally, it is often helpful to correct the translated method for any changes to the column volume and system dwell volume. The free-to-download ACE LC Translator has been developed to help achieve successful method translation by automatically calculating the appropriate method conditions using the original method, system dwell volumes and target column details as input variables.

EXAMPLE: TRANSLATING A METHOD FROM HPLC TO UHPLC

Figure 1 shows an HPLC separation of a pharmaceutical API and related impurities on an ACE Excel 5 C18-PFP, 150 x 4.6 mm column. This example demonstrates translation of the method to a 50 x 3.0 mm, 1.7 µm UHPLC format column.

Step 1: Column Information

The first stage of translating the method is to enter the details of both the column used for the original method and the target column for translation (Figure 2). The ACE LC Translator uses this information to generate the L/d_P value (column length / particle size), a measurement of the column efficiency. Note, to maintain resolution and separation performance, it is necessary to translate to a column of similar efficiency.

Column porosity is also available as an input value in order to calculate the column volume (V_M). Accurate values of 0.63 for ACE fully porous particles or 0.55 for ACE UltraCore particles can be used. Other vendor columns may have different values. If accurate values are unavailable, V_M can readily be experimentally determined by the chromatographer and inputted directly (see the column porosity and V_M tab within the ACE LC Translator). The column volume is used to translate the injection volume and is also used to translate the gradient profile.

Step 2: Original Method Details

The next stage is to enter the details of the original analytical method (Figure 3). For gradient translations, an accurate experimental determination of LC dwell volume is helpful (see ACE Knowledge Note AKN0001 for details). For this example, the original method was run on a binary HPLC system with a dwell volume of 1.098 mL. An input box is also available to enter the backpressure recorded for the original method. Whilst this is not essential, the **ACE LC Translator** can use this value to assess the likely backpressure expected for the new method. Finally, the gradient profile of the original method is entered into the table provided, including the column re-equilibration time. From the original method the ACE LC Translator will also estimate efficiency savings that can be achieved for the translated method.

Step 3: Generating the New Method

After entering the details of the original method, the ACE LC Translator automatically generates the new gradient method (Figure 3). Firstly, the injection volume is scaled to the new column format.

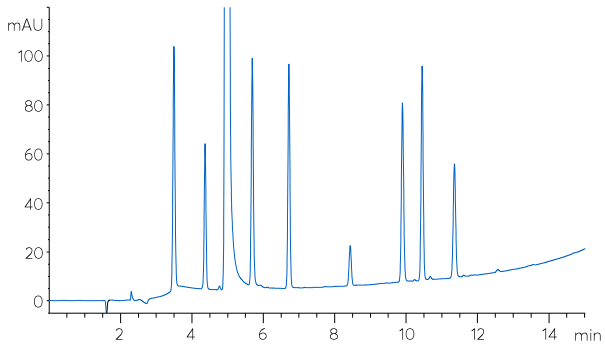

Figure 1: Separation of acetaminophen and related impurities on an ACE Excel 5 C18-PFP, 150 x 4.6 mm (flow rate: 1 mL/min, injection volume: 5 µL). See Avantor ACE Application Note AN4750 for full method and sample details.

Gradient Method Translation

Original Method			Translated Method		
Column Details			**Column Details**		
Column Length (L)	150	mm	**Column Length (L)**	50	mm
Column i.d. (d_c)	4.6	mm	**Column i.d. (d_c)**	3.0	mm
Particle Diameter (d_P)	5.0	µm	**Particle Diameter (d_P)**	1.7	µm
L/d_P	30000		**L/d_P**	29412	
Column Porosity	0.63	What's This?	**Column Porosity**	0.63	What's This?
Column Volume (V_M)	1.570	mL	**Column Volume (V_M)**	0.223	mL

Figure 2: Screenshot of the Column Information section, showing the original column input details on the left and the new column input details for the translated method on the right.

Next, two options for the flow rate of the translated method are provided. The flow may be scaled to the new column i.d. to maintain a constant linear velocity of mobile phase through the column. Alternatively, when translating a method to a smaller particle size, the flow rate can be scaled to increase the effective flow rate to take into account the fact that small particles provide their maximum efficiency at higher flow rates than larger particles (see AKN0012 for details). The ACE LC Translator allows the user to manually set the flow rate of the new method. In practice, it is worth assessing several flow rates between the two extremes to ensure that method backpressure and performance are suitable.

Finally, the dwell volume of the LC system that the new method uses is required; in this case a quaternary UHPLC system with a dwell of 0.926 mL. Once all of the required information has been entered, the ACE LC Translator generates a correctly translated gradient method. It is important that each gradient segment is accurately translated according to equation 1 and corresponds to the same number of column volumes of mobile phase as the original method in order to preserve the chromatographic selectivity.

$$t_{G2} = \frac{t_{G1} \times F_1 \times V_{M2}}{F_2 \times V_{M1}}$$

Equation 1: t_G is the gradient segment time, F the flow rate and V_M the column volume. The subscripts 1 and 2 refer to the original and translated methods respectively.

CORRECTING FOR THE CHANGE IN SYSTEM DWELL VOLUME AND COLUMN VOLUME

For gradient chromatography, the dwell volume of the system and flow rate determine how long it takes for a gradient mixed in the pump to reach the column (dwell time). When translating a method, the dwell volume should be equal in the original and translated methods when normalised to V_M. If this is not the case, large

Method Details

Injection Volume	5.0	µL
Flow Rate	1.000	mL/min
LC Name	HPLC 1	
Dwell Volume (V_D)	1.098	mL
Recorded Backpressure	67	bar

Gradient	Time	%B
	0.00	5
	15.00	95
	17.00	95
	17.50	5

Suggested Re-equilibration Time	16.8	mins
Total run time	34.3	mins
Solvent use	34.3	mL

Method Details

Injection Volume	0.7	µL
Flow Rate (scaled to linear velocity)	0.425	mL/min
Flow Rate (scaled to particle size)	1.251	mL/min
Input Flow Rate	1.250	mL/min
LC Name	UHPLC 2	
Dwell Volume (V_D)	0.926	mL
Estimated Backpressure	568	bar
Estimated Run Time Difference	-87	%
Estimated Solvent Use Difference	-83	%

Gradient	Time	%B
	0.00	5
	1.70	95
	1.93	95
	1.98	5

Suggested Re-equilibration Time	2.6	mins
Time to delay injection after the gradient begins	0.62	mins (770 µL)

Correct translation of this method requires that the injection is delayed until after the gradient begins by this time. A delayed injection can be added to a method in many LC instrument software packages.

Figure 3: Screenshot of the gradient method section of the Avantor ACE LC Translator. The original method details are entered on the left hand side, whilst the new translated method is automatically displayed on the right.

differences in selectivity can result. The ACE LC Translator overcomes this potential problem by calculating a correction factor in the form of either incorporating a pre-gradient hold into the translated method, or a delayed injection (Figure 3), which is readily implemented in most CDS software packages. If this correction is not included in the final method, the selectivity of the separation may be altered compared to the original method, ultimately leading to poor performance of the translated method.

The original method and the translated UHPLC method are shown in Figures 4A and 4B respectively. As can be seen, the ACE LC Translator successfully generates a translated gradient method which accurately preserves the chromatographic separation and resolution of the original method, whilst delivering substantial efficiency savings. If the suggested correction for the change in dwell and column volume is not applied, the chromatographic selectivity is affected and the original separation is not accurately reproduced (Figure 4C).

CONCLUSION

This ACE Knowledge Note has demonstrated how the ACE LC Translator can be used to accurately translate a gradient method from HPLC to UHPLC. Through minimal user input, the new method is quickly generated without the requirement for the user to carry out numerous complex calculations. The validity of the calculated method is clearly evident in the final experimental results shown in Figures 4A and 4B.

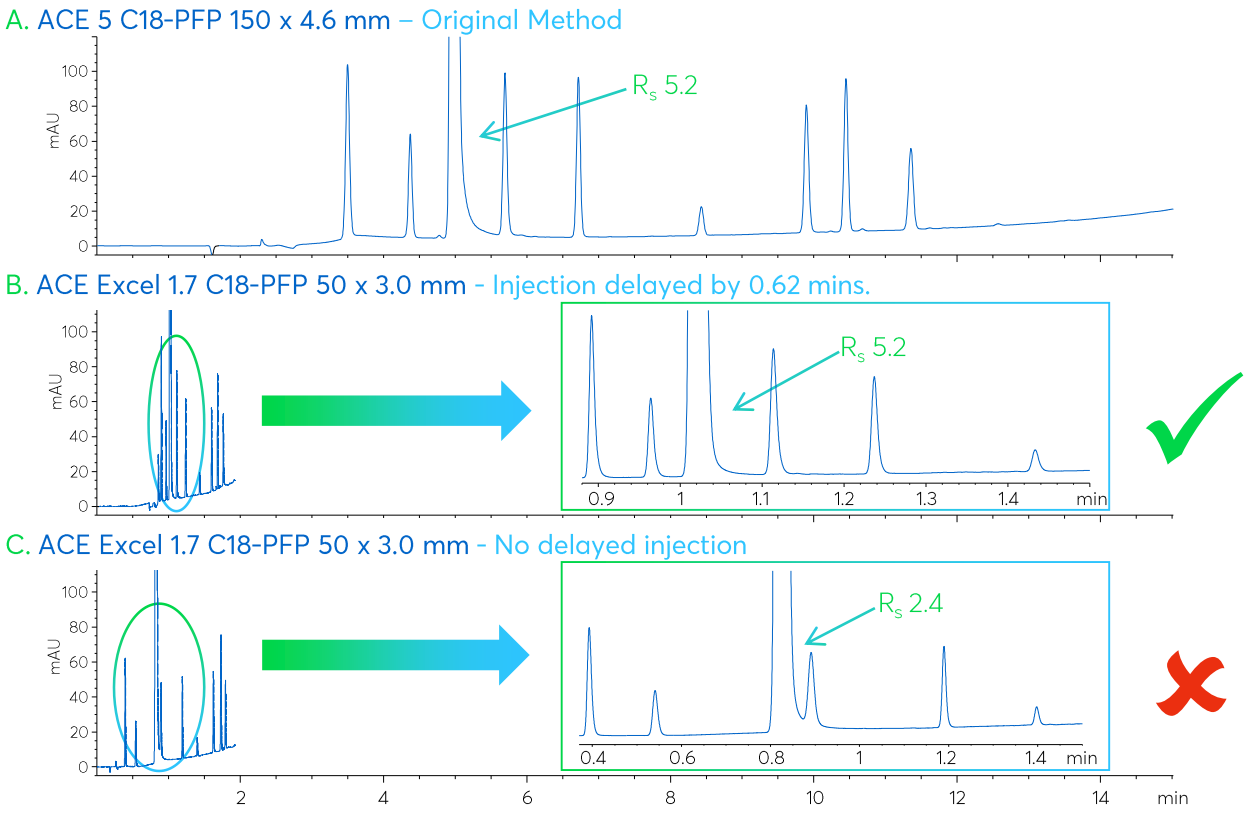

Figure 4: A: The original HPLC separation on the ACE Excel 5 C18-PFP. B: The translated method, as calculated by the Avantor ACE LC Translator showing excellent translation of the original method to the ACE Excel 1.7 C18-PFP, 50 x 3.0 mm at a flow rate of 1.25 mL/min. C: The translated method run without correcting for the change in system dwell volume and column volume.