Chromatography Solutions

Chromatography white paper

By Dr Matt James, Senior Research Scientist

Achieving successful method translations in liquid chromatography

In today's analytical laboratory, an ever-increasing emphasis is placed on achieving shorter chromatographic run times to drive improvements in laboratory productivity. Newly developed liquid chromatography (LC) methods will often utilise newer generation low dispersion, higher pressure rated HPLC/UHPLC systems, along with sub-2 micron or solid-core particles packed into small format columns to achieve highly efficient, high throughput separations. For existing LC separations, many opportunities exist to increase sample throughput by translating the methods to smaller dimension columns packed with smaller particles. This approach has become a common laboratory activity and allows substantial improvements in laboratory efficiency to be achieved. This practice has been driven by the development and availability of sub-2 micron particles packed into small column formats and Ultra High Pressure Liquid Chromatography (UHPLC) systems with operational pressures up to 1,400 bar. Through careful selection of the correct column dimensions and particle size, the original method performance can be maintained with substantially reduced run time.

The aim of this white paper is to bring together the theory, principles and key equations that are required for method translation and demonstrate their use for the translation of isocratic and gradient LC methods. The Avantor® ACE® Method Translator is introduced as a powerful tool for simplifying method translation activities. Additionally, common pitfalls and key considerations of LC system characteristics are discussed, to help maximise the chances of successful method translation.

Avantor® ACE®

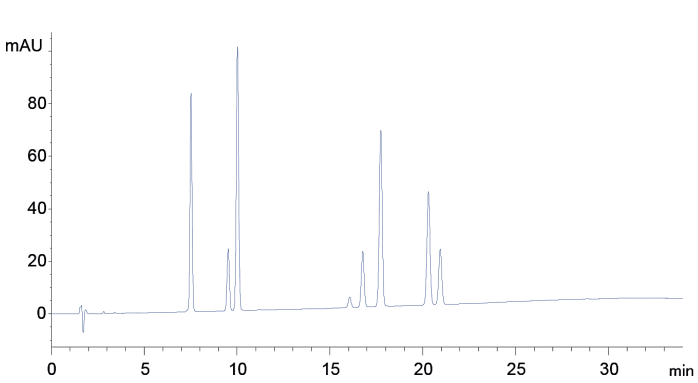
METHOD TRANSLATION: NOT LIMITED TO UHPLC

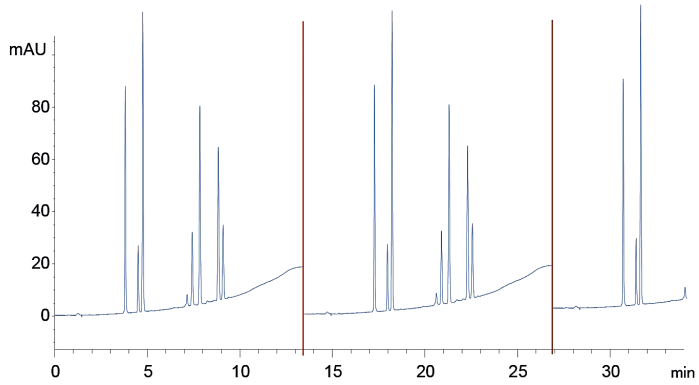
Although method translation is often discussed with an emphasis on converting methods to UHPLC, it is important to realise that method translation is not just limited to this approach. Many legacy HPLC methods present opportunities for improvement, without the need to employ UHPLC technology. Decreases in particle size allow substantial reduction in column length and therefore run time, whilst keeping pressures below 400 bar (to be compatible with standard HPLC systems). Figure 1 demonstrates how a gradient method can be translated to UHPLC to achieve an almost 10-fold increase in sample throughput. However, a 2.5-fold increase in throughput can also be obtained by moving the method to a column format that is compatible with a standard HPLC system. The resulting separation is well within the pressure limits of the HPLC system and could be used to provide a beneficial increase in throughput. Similarly, the adjustment of pharmacopoeial methods within allowable regulatory guidance can provide substantial time and cost savings, without the need for method re-validation or expensive capital expenditure in UHPLC equipment.[1]

A **Instrument: 400 Bar HPLC**
ACE 5 C18-AR, 150 x 4.6 mm
1 mL/min, P_{MAX} = 64 bar
26 samples in 24 hours

HPLC →

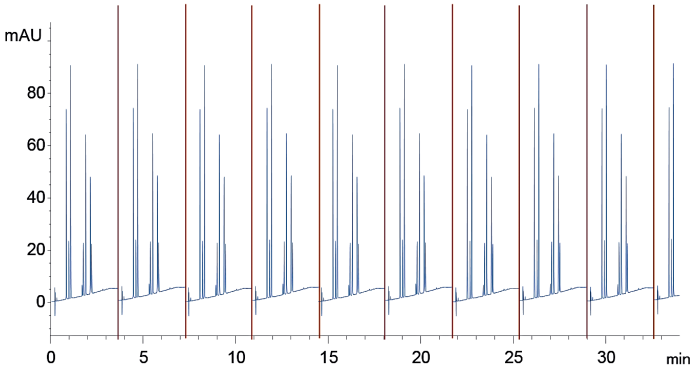
C **Instrument: 1,400 Bar UHPLC**
ACE Excel 1.7 C18-AR, 50 x 3.0 mm
1.25 mL/min, P_{MAX} = 510 bar
250 samples in 24 hours





UHPLC ↓

B **Instrument: 400 Bar HPLC**
ACE 3 C18-AR, 100 x 3.0 mm
0.71 mL/min, P_{MAX} = 193 bar
67 samples in 24 hours



Gradient time (mins)			
150 x 4.6 mm, 5 µm	100 x 3.0 mm, 3 µm	50 x 3.0 mm, 1.7 µm	%B
0	0	0	35
28	11.06	2.98	65
33	13.04	3.51	65
34	13.44	3.61	35
54	21.34	5.74	35

FIGURE 1: **Increasing sample throughput of a gradient HPLC method (A) using UHPLC (B) and modified rapid HPLC (C) options.** Mobile phase: A: 0.1% formic acid (aq), B: 0.1% formic acid in MeCN, Gradient: 35-65%B, Injection volume: (A) 5 µL, (B) 0.7 µL, (C) 1.4 µL, Detection: UV (254 nm). Sample: 1. Sulindac, 2. Bendroflumethiazide, 3. Ketoprofen, 4. Ibuprofen, 5. Diclofenac, 6. Indomethacin, 7. Mefenamic acid, 8. Meclofenamic acid. Note: post-gradient equilibration times are not shown but are detailed in the table.

SELECTING THE NEW COLUMN DIMENSIONS AND PARTICLE SIZE

One of the most fundamental requirements for method translation is that the selectivity or peak spacing should be similar before and after method translation. It is therefore important that stationary phase chemistry is kept the same, ideally using the same bonded phase and base silica. With modern stationary phase materials this should not be a problem as most high-quality column vendors (e.g. Avantor® ACE®) can provide complete scalability across the particle size range, ensuring selectivity is maintained. Additionally, it is recommended that all new method development is performed on such phases to future-proof method translation activities.

When translating an LC method, it is important to carefully consider the dimensions and particle size of the new column to ensure that acceptable performance of the translated method is achieved. Selecting a column format that doesn't provide the required column performance or isn't suitable for use on the LC system available will mean that the separation obtained may differ when compared to the original method. To ensure that the translated method maintains the same separation efficiency and resolution as the original method, the new column must match the efficiency of the original column. Column efficiency at a specified flow rate is primarily determined by the particle size of the packing material and the length of the column. If the column length is reduced, then the particle size must also be reduced to achieve a similar column efficiency. The approach of maintaining column efficiency is a useful starting point for any method translation and can be conveniently assessed by considering the column length (L) to particle size (d_P) ratio (L/d_P). The selection of a shorter column packed with smaller particles can be expected to deliver similar efficiency and resolution at optimum linear velocity, provided the L/d_P ratio is maintained.[2] Table 1 summarises some commonly available column formats and their corresponding L/d_P ratios. From this table, a 300 mm column packed with 10 µm particles has an identical L/d_P ratio as a 150 mm column packed with 5 µm particles and, in turn, a 50 mm column packed with 1.7 µm particles. All three columns can therefore be expected to deliver similar performance, with the shorter columns providing substantially reduced run times (Table 2). The application of L/d_P for method translation is now used by the US Pharmacopoeia in the General Chapter on Chromatography (USP <621>) for determining allowable changes to monograph methods. This guidance can be used to reduce analysis time and now facilitates the use of sub-2 micron and solid-core particles.[1, 3]

Whilst many translations aim to maintain efficiency and resolution, it is possible to accept a reduction in the L/d_P ratio (and therefore a reduction in resolution) for the translated column to achieve an even faster separation, if excess resolution is available in the original method (and regulatory guidance permits). Conversely, it may be desirable to increase L/d_P to enhance efficiency and analyte resolution. In some applications, this concept can be taken further by coupling together multiple columns to obtain ultra-resolution separations. This approach may be particularly beneficial for profiling complex samples, such as natural products and peptide digests.[4]

The use of a smaller column internal diameter (ID) with a reduced flow rate for the translated method is a popular approach for decreasing solvent consumption. Large reductions in solvent use are possible (>90% reported) [1, 5], making this approach highly attractive (Table 3).

Particle Size (µm)	Column Length (mm)							
	30	50	75	100	125	150	250	300
1.7	17,647	29,412	44,118	58,824	-	-	-	-
2	15,000	25,000	37,500	50,000	62,500	75,000	-	-
2.5	12,000	20,000	30,000	40,000	50,000	60,000	-	-
3	10,000	16,667	25,000	33,333	41,667	50,000	83,333	-
5	6,000	10,000	15,000	20,000	25,000	30,000	50,000	-
10	3,000	5,000	7,500	10,000	12,500	15,000	25,000	30,000

TABLE 1: Summary of L/d_P ratios for a selection of common commercially available column formats.

Dimensions (mm)	Particle size (µm)	L/d_P	Flow rate (mL/min)	Run time (min.)	Time saving
300 x 4.6	10	30,000	1.00	40	-
150 x 4.6	5	30,000	1.00	20	50%
50 x 4.6	1.7	29,412	1.00	6.7	83%

TABLE 2: Time saving calculations for a 40-minute isocratic method translated to a smaller column / particle size combination whilst keeping L/d_P and flow rate constant.

Column dimension (mm)	Particle size (µm)	L/d_P	Flow rate (mL/min)	Run time (min.)	Solvent use (mL)	Solvent saving
250 x 4.6	5	50,000	1.0	30	30	-
150 x 4.6	3	50,000	1.0	18	18	40%
150 x 3.0	3	50,000	0.43	18	7.7	74%
150 x 2.1	3	50,000	0.21	18	3.8	87%
75 x 2.1	1.7	44,118	0.21	9	1.9	94%

TABLE 3: Solvent savings achievable by translating methods to shorter columns with smaller ID.

An important consideration when scaling down column ID (and therefore column volume) is the negative impact of instrument dispersion and band broadening on observed peak efficiency. Instrument dispersion has a proportionally greater negative impact on column performance as the column dimensions are reduced. This is discussed in more detail later in this paper. Additional factors that should be considered when translating methods to smaller ID columns include: the accuracy of flow rate delivery, injection repeatability at lower injection volumes, detector sampling rates and detection mode of the new method.

TRANSLATING ISOCRATIC METHODS

Isocratic methods are relatively straightforward to translate and require only simple volumetric scaling of the injection volume and flow rate. The injection volume (V_i) is simply calculated from the original injection volume and internal volumes of the new and original columns, V_{M2} and V_{M1}, according to equation 1.

1

$$V_{i2} = \frac{V_{i1} \times V_{M2}}{V_{M1}}$$

The column volume can be determined experimentally by injection of an appropriate dead time marker or estimated using equation 2. For Avantor® ACE® columns, the column porosity (ε) can be taken as 0.63 for fully porous particles and 0.55 for UltraCore solid-core particles, whilst d_C and L correspond to the column's ID and length respectively.

2

$$V_M = \pi \left(\frac{d_c}{2}\right)^2 L\varepsilon$$

If a different ID column is used for the translated method, the flow rate will require scaling accordingly. If the particle size does not change, the flow rate is scaled to maintain a constant linear velocity of mobile phase flowing through the column (equation 3). If the particle size changes during translation, a different equation can be used to estimate the new flow rate (equation 4).[5, 6, 7] This equation attempts to account for differences in plate heights between the original and translated method, to provide approximately similar efficiency for the separation.

3

$$F_2 = \frac{F_1 \times d^2_{c2}}{d^2_{c1}}$$

4

$$F_2 = \frac{F_1 \times d^2_{c2} \times d_{p1}}{d^2_{c1} \times d_{p2}}$$

In practice, a translated method may deliver peak efficiency values lower than anticipated when using equation 4. It is therefore acceptable to further adjust or reduce the translated flow rate to achieve performance similar to that provided by the original method. This approach is advised within the US Pharmacopoeia and is accepted practice. [1, 3]

Finally, the run time of the new method can be calculated using equation 5, whilst the back pressure can be estimated using equation 6.

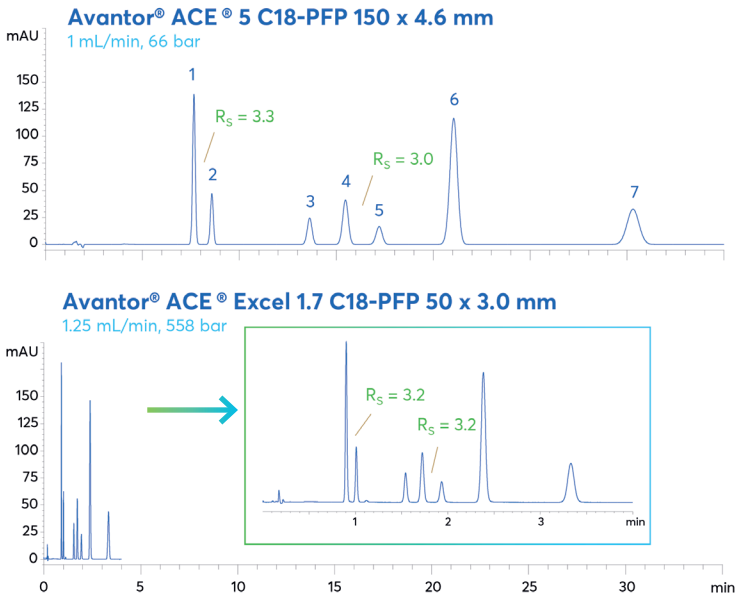
5

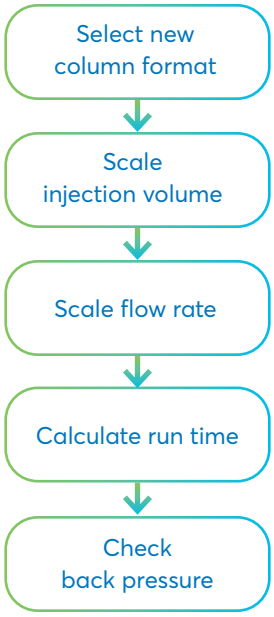
$$t_2 = \frac{t_1 F_1 V_{M2}}{F_2 V_{M1}}$$

6

$$P_2 = \frac{P_1 \times F_2 \times L_2 \times d^2_{c1} \times d^2_{p1}}{F_1 \times L_1 \times d^2_{c2} \times d^2_{p2}}$$

Figure 2 shows how the application of these key equations can be used to translate an isocratic method from HPLC to UHPLC. In this example, the efficiency and resolution of the original separation are maintained by keeping a constant L/d_P ratio, whilst scaling of the flow rate to the 1.7 µm particle size provides an 88.6% reduction in run time.





	Original method	Translated method	Difference
Efficiency of last peak	12,387	12,357	
Run Time	35 mins.	4 mins	-88.6%
Solvent consumption	35.0 mL	5.0 mL	-85.7%

FIGURE 2: Translation of an isocratic method from HPLC to UHPLC. Method conditions: Isocratic MeCN:H_2O 30:70 v/v, column temperature: 40 °C, detector: UV, 214 nm. Sample: 1. 1,2,3-trimethoxybenzene, 2. 1,2,4-trimethoxybenzene, 3. 1,4-dimethoxybenzene, 4. methoxybenzene, 5. 1,3-dimethoxybenzene, 6. 1,3,5-trimethoxybenzene, 7. Toluene.

GRADIENT METHOD TRANSLATION

The translation of gradient methods is somewhat more complex, as the gradient profile requires scaling to the new column dead volume and flow rate. The gradient profile must be deconstructed, and each segment translated individually. Gradient segments can be translated using equation 7, whilst isocratic holds can be translated using equation 8.

7

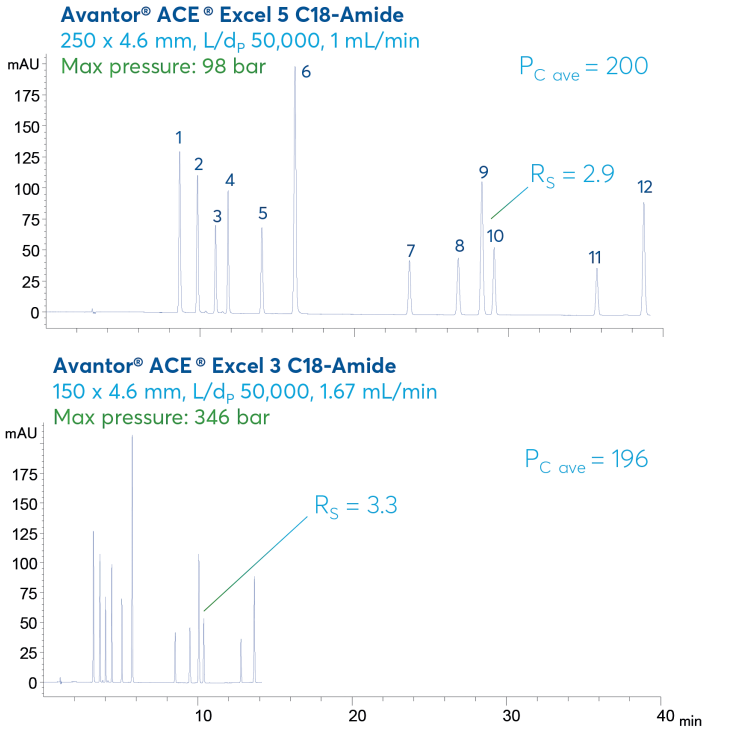
$$t_{G2} = \frac{t_{G1} \times F_1 \times V_{M2}}{F_2 \, V_{M1}}$$

8

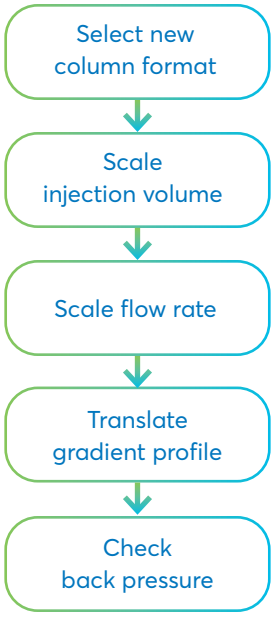
$$t_{ISO2} = \frac{t_{ISO1} \times F_1 \times V_{M2}}{F_2 \times V_{M1}}$$

As with isocratic translations, the injection volume can be scaled using equation 1, whilst the flow rate can be selected using equations 3 and 4. Figure 3 demonstrates how these translation principles can be used to translate a gradient method from the original 250 mm column, packed with 5 µm particles, to a 150 mm column packed with 3 µm particles. Both columns have an L/d_P ratio of 50,000 and, therefore, the peak capacity and resolution of the separation is maintained, whilst an overall reduction in run time of 64.7% is achieved. Despite the elevated flow rate and decrease in particle size, this translated method is fully compatible with standard 400 bar HPLC instrumentation.

Ideally, a translated gradient method should be corrected for any change in the system dwell volume (V_D) and/or column dead volume (V_M) to most accurately replicate the original separation. However, many gradient translations, such as that shown in Figure 3, yield acceptable separations without applying this correction. If the translated gradient method is assessed experimentally and found to provide the desired separation, then it may be



t (min)	%B		t (min)	%B
0	5		0	5
45	100	← Equation 7 →	15.87	100
50	100	← Equation 8 →	17.63	100
52	5	← Equation 7 →	18.34	5
Post time: 30 mins		← Equation 8 →	Post time: 10.6 mins	



	Original method	Translated method	Difference
Cycle Time	82.0 mins	28.96 mins	-64.7%
Solvent consumption	82.0 mL	48.4 mL	-41.0%

FIGURE 3: HPLC gradient translation. Conditions: A: 20 mM KH_2PO_4 pH 2.7 (aq), B: 20 mM KH_2PO_4 pH 2.7 in MeCN:H_2O 65:35 v/v, column temperature: 40 °C, detector: UV, 254 nm. Sample: 1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid. Note: experimentally determined values of 2.668 mL and 1.571 mL were used for V_{M1} and V_{M2} to translate this method.

acceptable to omit this correction. If a change in selectivity is observed compared to the original method, as is shown in Figure 4B, then the dwell and column volumes should be corrected for as follows. The difference in the ratio of the system dwell volume to the column dead volume (V_D/V_M) between the original and translated methods should approach zero for accurate translation, [5, 8, 9] i.e.

$$\left(\frac{V_D}{V_M}\right)_{Original} - \left(\frac{V_D}{V_M}\right)_{Translated} \approx 0$$

If this is not the case, then a pre-gradient hold or a delayed injection (usually possible to program through the LC instrument software) is required to restore the desired separation (Figure 4C). This is particularly important when translating to small volume column formats, where the ratio of system dwell to column dead volume can be much higher than for HPLC column formats and can result in significant changes in selectivity. Alternatively, it may be possible to manually correct by adjusting the LC system dwell volume through the use of a different volume mixer in the LC pump configuration.

To apply this correction, it is necessary to accurately determine the dwell volumes of the original LC system together with the new system to which the method will be translated (refer to reference 10 for details).

Using the V_D/V_M ratio for both the original and translated methods, the magnitude of the pre-gradient hold or injection delay time in minutes can be determined by equation 9. A negative value indicates that the injection should be delayed until x minutes

after the gradient starts. A positive value indicates an isocratic hold of x minutes should be inserted at the start of the gradient timetable.

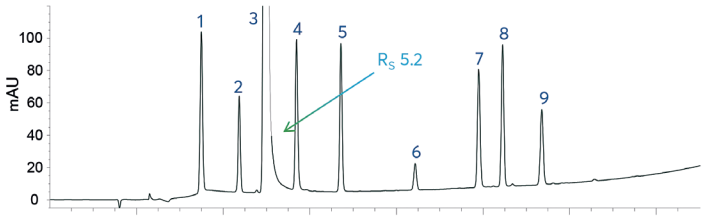
An alternative approach to minimise the effect of V_D/V_M on separations is to use a column with a larger ID (e.g. 50 x 3.0 mm rather than 50 x 2.1 mm) to minimise the impact of system dwell on the separation.

9

$$\chi = \left[\left(\frac{V_{D1}}{V_{M1}}\right) - \left(\frac{V_{D2}}{V_{M2}}\right)\right] \times \frac{V_{M2}}{F_2}$$

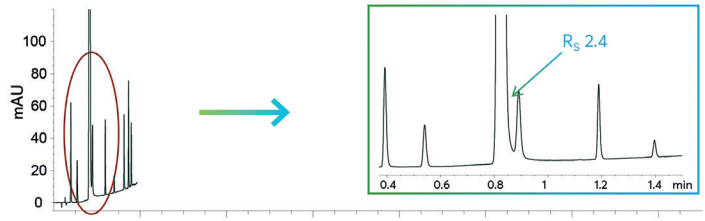
A

ACE 5 C18-PFP 150 x 4.6 mm



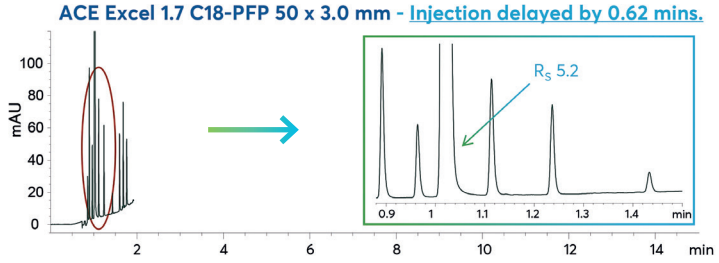
B

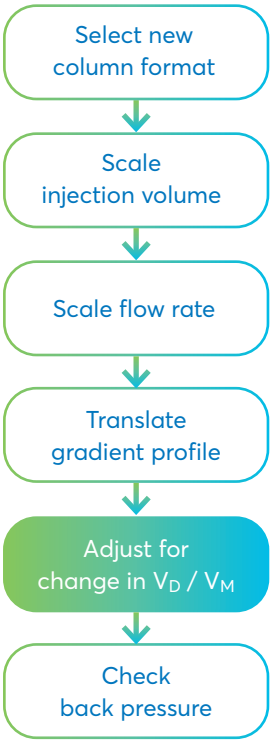
ACE Excel 1.7 C18-PFP 50 x 3.0 mm - No delayed injection



C

ACE Excel 1.7 C18-PFP 50 x 3.0 mm - Injection delayed by 0.62 mins.





t (min)	%B		t (min)	%B
0	5		0	5
15	95	← Equation 7 →	1.70	95
17	95	← Equation 8 →	1.93	95
17.5	5	← Equation 7 →	1.98	5
Post time: 20 mins		← Equation 8 →	Post time: 2.3 min	

FIGURE 4: Translation of an API and related substances gradient method from HPLC to UHPLC. A: Original separation (system V_D = 1.098 mL) B: Translated method not corrected for influence of system dwell volume C: Translated method corrected for influence of dwell volume. Method conditions: Line A: 20 mM ammonium acetate pH 6.0 (aq), Line B: 20 mM ammonium acetate pH 6.0 in MeCN:H_2O 80:20 v/v, column temperature: 40 °C, detector: UV, 230 nm. Sample: 1. 4-aminophenol, 2. hydroquinone, 3. 4-acetamidophenol (paracetamol), 4. 2-aminophenol, 5. 2-acetamidophenol, 6. phenol, 7. 4-nitrophenol, 8. 4-chloroacetanilide, 9. 2-nitrophenol. Impurities were spiked at 0.5% w/w.

SIMPLIFIED TRANSLATION USING THE AVANTOR® ACE® LC TRANSLATOR

Use of the principles outlined in this white paper can be a time-consuming process. The Avantor® ACE® LC Translator tool has been developed to automatically translate LC methods without the need to perform the numerous calculations required. It is Microsoft Excel-based and provides tools for the automatic translation of isocratic and gradient LC methods. Figure 5 shows a screenshot of the gradient method translator and its use to automatically generate an accurately translated gradient method for the application shown in Figure 4. The tool is easy to use: the user enters the column dimensions and conditions of an existing method, along with the dimensions of a target column and the translated method is automatically generated. Additionally, a variety of other tools are provided, including method transfer, a buffer calculator, a mobile phase quantity calculator and a dwell volume calculator.

The tool can be downloaded free at **vwr.com/ace**.

FIGURE 5: **From Avantor® ACE® LC Translator, used to translate the gradient separation shown in Figure 4.**

HINTS & TIPS FOR SUCCESSFUL METHOD TRANSLATION

To obtain the best possible outcome for method translations, several aspects of the receiving LC system should be considered; the most important of which are the system dispersion and detector capabilities. Extra-column band broadening results from dispersive effects impacting the analyte chromatographic band, resulting in lower method performance. LC system extra-column volume is a major contributor to dispersion. Significant contributions to extra-column volume include system tubing in the flow path, injector tubing, detector flow cell volume and any poor-quality tubing connections (e.g. with the analytical column).[11,12]

For traditional larger format columns, such as 150 x 4.6 mm, the impact of system extra-column volume is negligible relative to the large column volume. However, in the case of smaller format columns (e.g. 2.1 and 3.0 mm ID), which have much smaller column dead volumes, the effects of extra-column volume on chromatographic bands increases. When translating legacy methods to smaller format columns, it is therefore important to consider the potential impact of extra-column dispersion on the new separation and to ensure that lower-dead-volume, narrow-bore columns are only used with suitably optimised instrumentation. In general, for isocratic separations in particular, 4.6 mm ID columns are recommended for use on HPLC systems, 3.0 mm ID for use on optimised HPLC and UHPLC systems and the 2.1 mm ID should be limited to use only on low dispersion UHPLC systems. Columns with 1.0 mm ID and smaller should only be used with the appropriate capillary or 'nano' LC.

It is additionally worth considering that in isocratic analyses, analytes with low retention factors are considerably more prone to the effects of extra-column band broadening than well retained analytes. To reduce extra-column band broadening, improvements can be made by reducing the LC system tubing ID, flow cell path length and volume etc. where possible.[11] Increasing the column ID (e.g. translating to a 3.0 mm ID column rather than a 2.1 mm ID column) will also help reduce the impact, whilst still achieving the desired reduction in analysis time and significant reductions in mobile phase consumption.

In addition, it is also important to ensure that detector settings are appropriately optimised for translated methods. Generally, at least 20 data points should be collected across a chromatographic peak. When translating a method to a smaller format column packed with smaller particles, peak width is reduced; therefore, the data sampling rate needs to be increased. As with the effects of extra-column band broadening, early eluting analytes in isocratic methods are more prone to the detrimental effects of slow detector sampling rates. This is demonstrated in Figure 6, which shows the impact of detector sampling rate on efficiency and resolution for a fast isocratic UHPLC method. For the early eluting sample components (i.e. low retention factor), data capture rates of at least 40-80 Hz are required to fully describe the narrower peaks and improve method performance. The efficiency of the later eluting analytes is affected to a lesser degree. It is, however, worth bearing in mind that faster data sampling rates can increase baseline noise which can be problematic for measuring trace sample components, therefore a balance should be carefully considered on an application dependent basis.

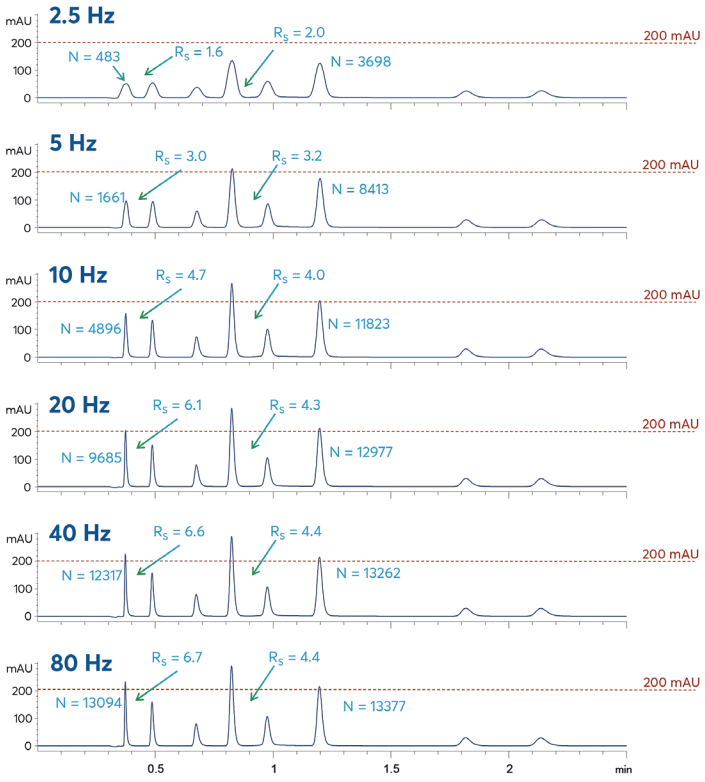


FIGURE 6: Isocratic separation of antihistamines on an Avantor® ACE® UltraCore 2.5 SuperC18 (75 x 3.0 mm) at different data capture rates. Method conditions: Isocratic 30 mM KH_2PO_4 pH 2.7 in MeOH:H_2O 40:60 v/v, flow rate: 0.85 mL/min, injection volume: 0.9 µL, column temperature: 30 °C, detector: UV, 214 nm. Sample (in order of elution): 1. maleic acid, 2. norephedrine, 3. doxylamine, 4. salicylamide, 5. guaifenesin, 6. guaiacol, 7. chlorpheniramine, 8. triprolidine.

POTENTIAL CHANGES IN SELECTIVITY

When translating methods to UHPLC (i.e. sub-2 µm particles), the mobile phase flow rate is often scaled up (e.g. according to equation 4) to obtain further reductions in run time. When the flow rate is increased through a packed column, frictional forces operating between the mobile phase and the packed silica particles result in higher mobile phase temperatures.[2, 13, 14] At the higher linear velocities typically used with sub-2 micron particles, significant frictional heating may occur, leading to a change in selectivity compared to the original method. If frictional heating is suspected as the cause of a change in selectivity, it may be necessary to investigate varying the column oven temperature to compensate.

Recently, increasing attention has been given to the effect of increased back pressure on chromatographic selectivity. It has been widely reported that an increase in retention is generally observed when column back pressure is increased, with charged/polar analytes being more affected than neutral analytes. Some sample components are therefore more susceptible to the effects of increased pressure than others, which can result in unexpected changes in selectivity and resolution when translating HPLC methods to UHPLC methods and vice versa. [15, 16, 17] In practice it is difficult to compensate for any undesired pressure effects, other than to run the separation at modified flow rates until acceptable resolution is obtained. Ultimately, the method may require redevelopment through altering the gradient time or temperature to achieve the desired separation.

CONCLUSIONS

Method translation has become a commonplace activity as organisations continue to make improvements in laboratory efficiency and reduce costs. Although emphasis has often been placed on translating methods to UHPLC, where dramatic increases in sample throughput can be achieved, in many cases method translation also has the potential to significantly increase the utilisation of standard HPLC equipment. This white paper has sought to summarise the processes required to obtain translated LC methods that accurately replicate the selectivity, resolution and performance of the original methods.

The first stage of method translation requires the selection of an appropriate LC column, which can be readily determined by considering the L/d_P ratio. Accurate translation of isocratic method parameters can then be accomplished by careful volumetric scaling of the flow rate and injection volume using the fundamental equations outlined in this article. Gradient method translations present a more complex situation, where the gradient profile requires accurate scaling and system dwell volume can have a significant impact on the separation obtained. To aid practicing chromatographers in quickly and reliably translating methods, the Avantor® ACE® Translator spreadsheet has been developed and is freely available to download. This tool allows the user to simply enter the column details and method parameters for the original method, along with the target column format, to automatically generate the translated method. Finally, some of the most common issues encountered in translating methods and potential solutions have been discussed, including the effects of dwell volume, extra-column band broadening, detector settings and the potential impacts of frictional heating and increased pressure on the selectivity of translated methods.

1. A. P. McKeown, Chromatography Today 8, issue 1 (2015) 32-36.
2. J. R. Mazzeo, U. D. Neue, M. Kele, R. S. Plumb, Anal. Chem. 77 (2005) 460A-467A.
3. United States Pharmacopoeia General Chapter <621> "Chromatography" First Supplement to USP 37-NF 32 (United States Pharmacopoeial Convention, Rockville, MD, USA).
4. Chromatography Today 12, issue 4, (2020) 16-18.
5. P. Petersson, M.R. Euerby, M.A. James, LCGC Europe 28 (2015) 310-320.
6. D. Guillarme, D.T.T. Nguyen, S. Rudaz and J.L. Veuthey, Eur. J. Pharm. Biopharm. 66 (2007) 475-482.
7. D. Guillarme, D.T.T. Nguyen, S. Rudaz and J.L. Veuthey, Eur. J. Pharm. Biopharm. 68 (2008) 430-440.
8. J.W. Dolan and L.R. Snyder, J. Chromatogr. A 799 (1998) 21-34.
9. A.P. Schellinger and P.W. Carr, J. Chromatogr. A 1077 (2005) 110-119.
10. Avantor® ACE® Knowledge Note 0001 "How to determine dwell volume of an HPLC or UHPLC system" (https://uk.vwr.com/cms/ace_knowledge_notes).
11. A.J. Alexander, T.J. Waeghe, K.W. Himes, F.P. Tomasella, T.F. Hooker, J. Chromatogr. A 1218 (2011) 5456-5469.
12. K. J. Fountain, U. D. Neue, E. S. Grumbach, D. M. Diehl, J. Chromatogr. A 1216 (2009) 5979-5988.
13. A. Clarke, J. Nightingale, P. Mukherjee, P. Petersson, Chromatography Today 3, issue 2 (2010) 4-9.
14. A. de Villiers, H. Lauer, R. Szucs, S. Goodall, P. Sandra, J. Chromatogr. A 1113 (2006) 84-91.
15. M. M. Fallas, U. D. Neue, M. R. Hadley, D. V. McCalley J. Chromatogr. A 1209 (2008) 195-205.
16. M. R. Euerby, M. James, P. Petersson J. Chromatogr. A 1228 (2012) 165-174.
17. M. R. Euerby, M. James, P. Petersson Anal. Bioanal. Chem. 405 (2013) 5557-5569.

0820 LIT. NO. 931152W - ZPROVWRI27673-EN