Transferring LC Methods Between Different Instruments Using the ACE LC Translator Tool

AKN0028

ACE Knowledge Note #0028

Introduction

Transferring LC methods from one instrument to another is common, but maintaining the separation selectivity and peak resolution can be challenging. For reliable method transfer, careful consideration of the instrument characteristics and settings is required, including any change in dwell volume for gradient methods. Errors in method transfer can easily lead to detrimental changes in selectivity or peak resolution. This Knowledge Note discusses the key parameters which require attention and shows how the **ACE LC Translator** can assist in simplifying the process to help achieve reliable method transfer.

Method Transfer

The transfer of UHPLC/HPLC methods between instruments is a common activity. This may be within the same lab or a different lab in the same organisation, or even to an external organization e.g. a CRO. It is rare to find the originating and receiving instruments are the same, with different instrument vendors commonplace. Even if the instrument is the same vendor and model it is not unusual to find configuration differences that affect system parameters, such as dwell volume and extra column volume, which may affect the chromatography of the method being transferred. This Knowledge Note discusses the underlying principles of how to accurately transfer gradient LC methods and highlights instrument parameters and characteristics that should be considered to help achieve a successful transfer.

To help simplify the process of transferring LC methods, the free **ACE LC Translator** is available to download (www.ace-hplc.com). This Microsoft Excel based spreadsheet automatically performs all calculations required for a variety of method transfer activities. The calculator also allows the translation of methods between different column dimensions whilst maintaining chromatographic performance. This article discusses method transfer (migration of a method to a new instrument without changing the column dimensions or method conditions). Method translation (changing the column format and method conditions, e.g. translating a method from HPLC to UHPLC) is not covered by this article. A detailed discussion of method translation using the **ACE LC Translator** can be found in a previous Knowledge Note [1].

The **ACE LC Translator** also includes several other tools useful for everyday practical chromatography such as dwell volume determination, mobile phase consumption, buffer make up and column equilibration calculators (Figure 1).

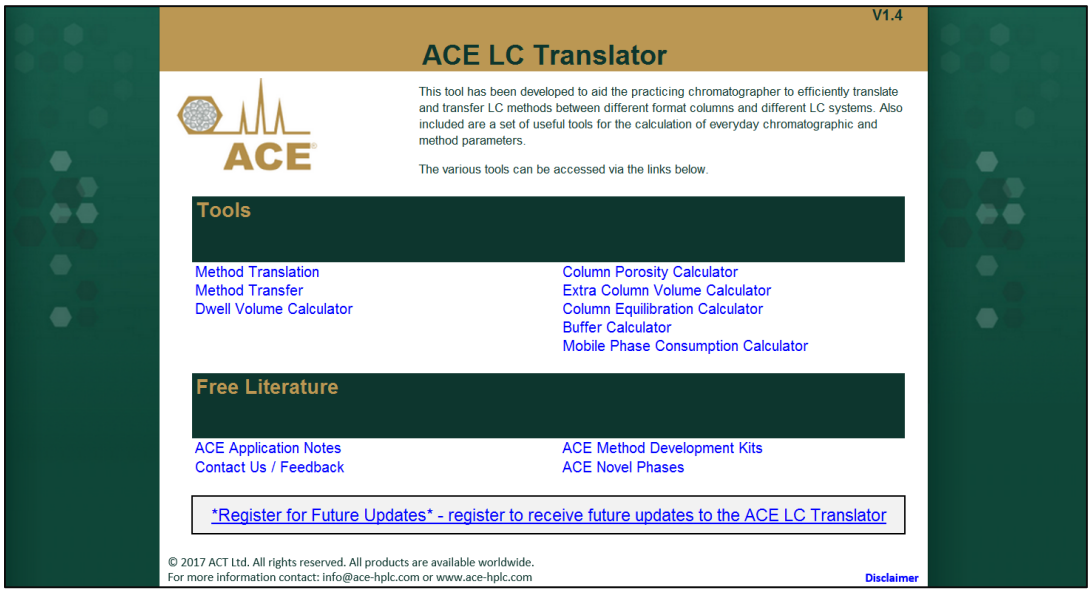

Figure 1: Screenshot of the **ACE LC Translator** index page.

Isocratic and Gradient Method Transfer

In principle, isocratic method transfer is straightforward. The method requires no adjustment for any change in system dwell volume and if the receiving instrument is suitably configured for the column format (e.g. low dispersion for small column formats such as 50 x 2.1 mm) and instrument settings (e.g. detector settings) are appropriate, then few issues should arise.

Gradient methods are more complicated to accurately transfer, as any change in dwell volume between the original system and the receiving instrument can affect the selectivity of the separation. Challenges are more likely to arise when a large change in dwell volume is encountered (e.g. when transferring a method from a high-dwell HPLC system to a low-dwell UHPLC system) and when transferring methods using small column formats (e.g. 2.1 or 3.0 mm i.d. UHPLC columns). Both scenarios are considered further in this article.

Accurate determination of dwell volume is simple and involves replacing the column with a zero-dead-volume connector and running an appropriate linear gradient. The **ACE LC Translator** includes a convenient, step-by-step method for measuring dwell volume (Figure 2) which has been described in full for a previous Knowledge Note [2]. The user is required to run the prescribed gradient and the resulting data is then simply entered into the calculation tool to determine the dwell volume. When transferring gradient methods, determination of dwell volumes is necessary as the values are used to accurately adjust the gradient table, to ensure the same chromatography is achieved on both instruments.

Several solutions are possible to account for a change in dwell volume during gradient transfer. Firstly, the receiving instrument can be physically modified to provide the same dwell as the originator instrument (e.g. by changing mixers and connecting tubing). Whilst effective and consistent with USP guidelines, this approach may mean that multiple instrument configurations will be required for different methods.

A second approach is to adjust the gradient start point relative to the injection point. When transferring a method from a high-dwell system to a low-dwell system (e.g. running a legacy HPLC method on a UHPLC system), this involves introducing (or extending the duration of) an initial isocratic pre-gradient hold. If the method is transferred to a higher-dwell system, the injection should be delayed until

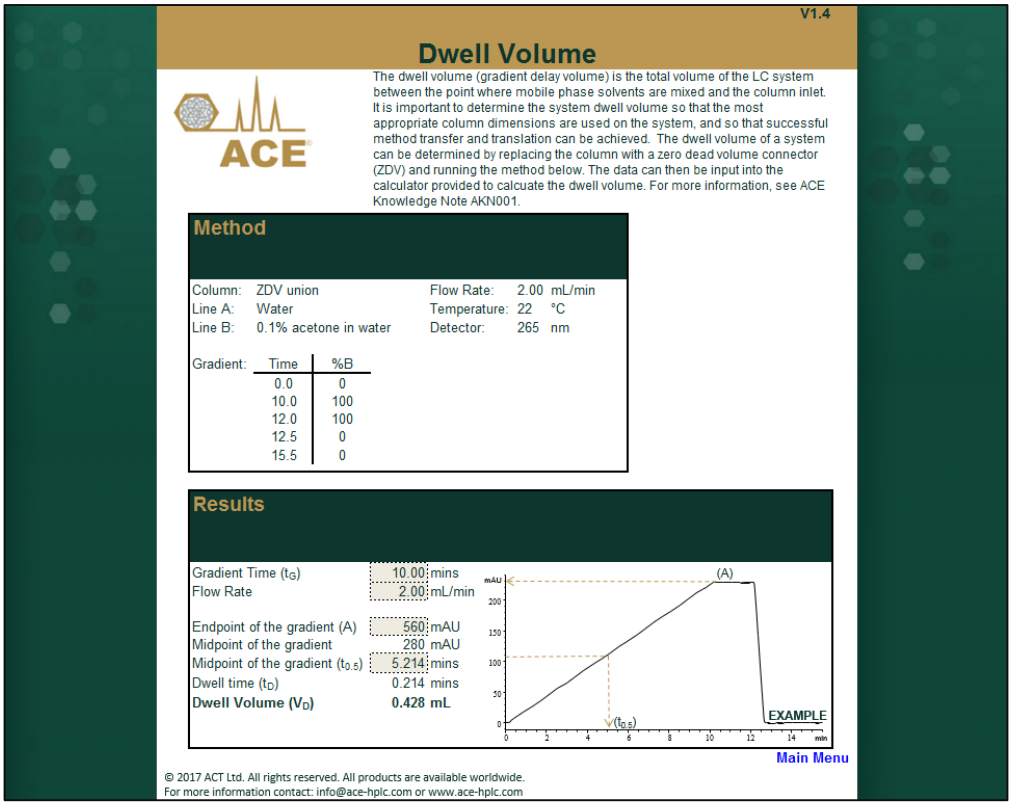

Figure 2: Determining system dwell volume using the **ACE LC Translator**.

after the gradient program starts (many instrument software packages include a feature for this), or the duration of any isocratic hold prescribed in the method should be reduced.

Figure 3 shows an example of how a large difference in dwell volume can affect the separation when transferring a UHPLC method. In this example, the method was transferred from a low dispersion 600 bar HPLC system (equipped with a large volume mixer) to a low dwell volume 1400 bar VWR Hitachi ChromasterUltra Rs UHPLC system. The separation was first transferred without correcting for the change in dwell volume (B), resulting in a large change in selectivity for early eluting peaks and a loss of resolution. The **ACE LC Translator** was then used to correct the gradient table for the change in dwell volume, enabling the original separation to be successfully reproduced (C). To determine the correction required, the user simply inputs the original method and column details, along with the dwell volumes of both systems; any adjustments to the gradient table are automatically generated (Figure 4). In this case, a 1.35 minute pre-gradient hold is added to the gradient table and all successive gradient time points are automatically adjusted.

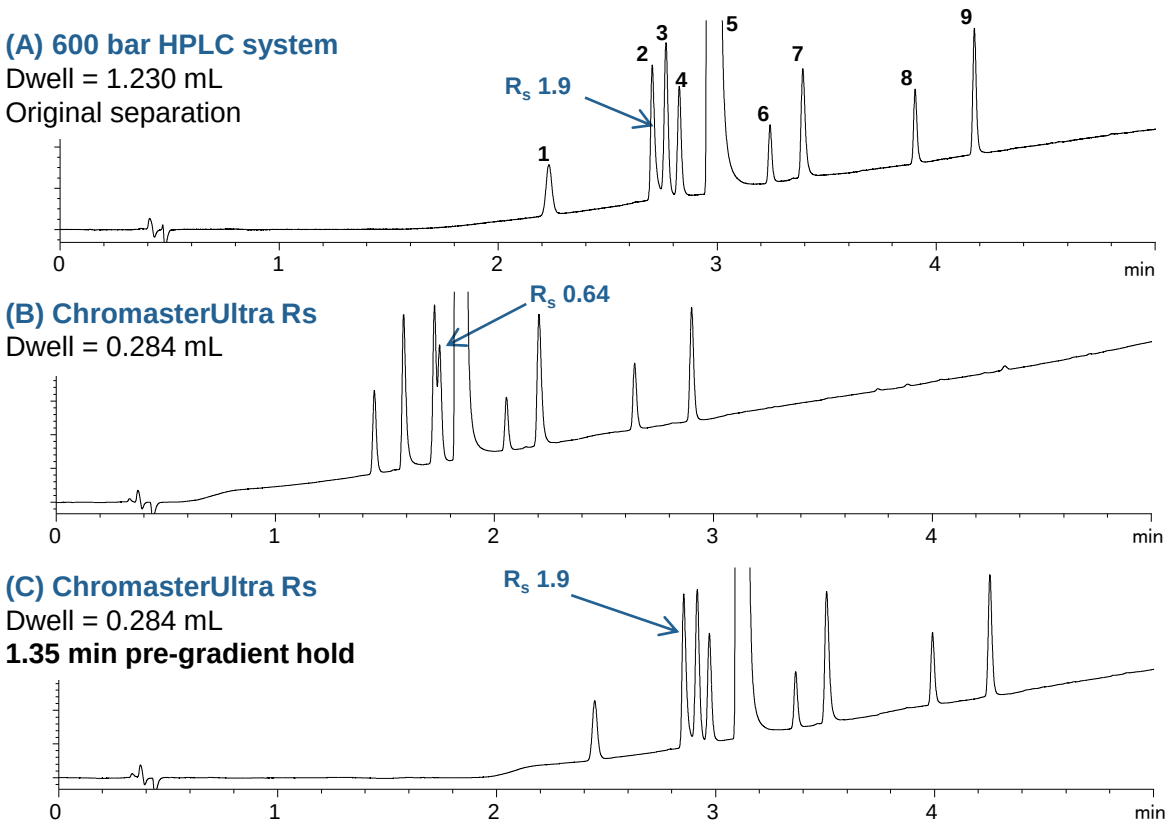

Figure 3: Transfer of an aspirin and related substances gradient method using the ACE LC Translator

Column:	ACE Excel 1.7 CN-ES (P/N: EXL-1713-0503U)
Mobile phase:	A: 0.1% formic acid (aq.); B: 0.1% formic acid in MeCN
Gradient:	5 to 100% in 5 minutes
Flow rate:	0.7 mL/min
Temperature:	30 °C
Injection volume:	1 µL
Detection:	UV, 230 nm

1. 2-Acetamidophenol
2. 4-Hydroxyisophthalic acid
3. Acetanilide
4. Salicylamide
5. Acetylsalicylic acid (aspirin)
6. Phenacetin
7. Salicylic acid8.
8. Acetylsalicylsalicylic acid
9. Salsalate

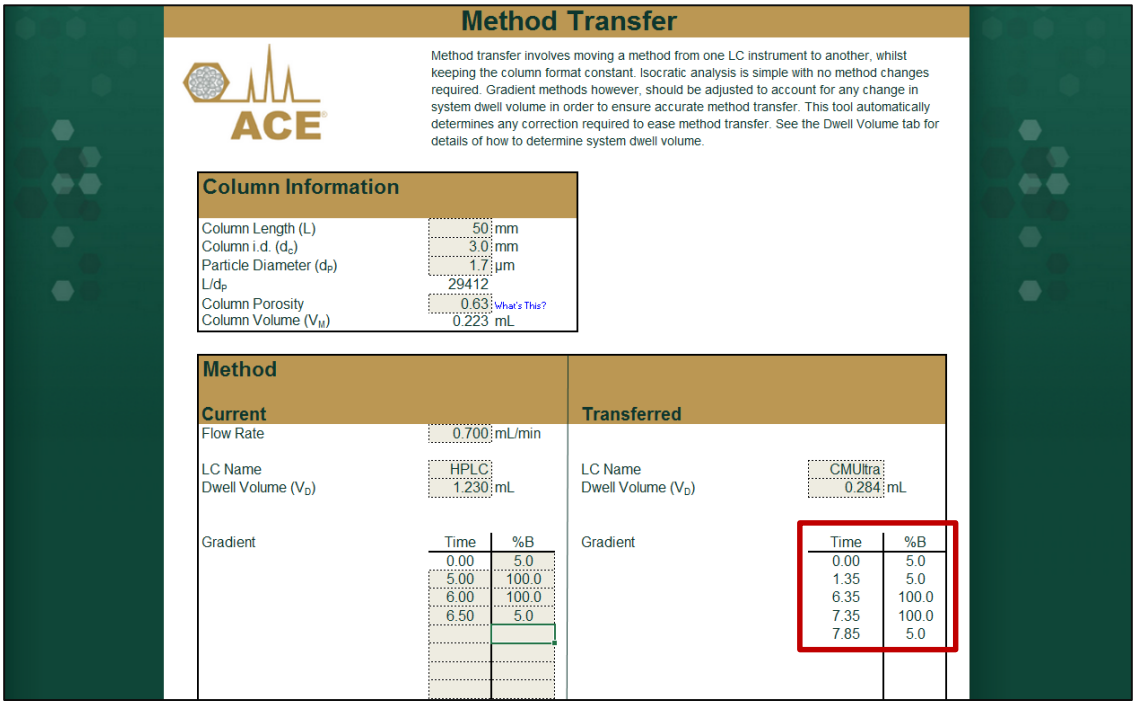

Figure 4: ACE LC Translator screenshot for the transfer of the separation shown in Figure 3. The corrected gradient is highlighted in red.

The USP and European Pharmacopeia (EP) both permit the second approach discussed (i.e. isocratic hold) to be used to correct a gradient method for a change in dwell volume when an isocratic hold is prescribed in the monograph method[3,4]. This will necessarily involve adaptation of all gradient time points accordingly. The inclusion of an isocratic hold when developing new gradient methods allows a method to be easily adapted for changes in dwell volume and is an approach that is adopted by many laboratories. Figure 5 shows an example UHPLC method on an ACE Excel C18-Amide 50 x 3.0 mm column, which was transferred to a variety of different UHPLC systems from multiple instrument vendors (Table 1). Additionally, both binary and quaternary pump designs were also assessed, thereby covering a comprehensive set of potential transfer scenarios that could be encountered during the method lifecycle. The original method includes an initial isocratic hold of 0.3 minutes, which can be extended when the method is transferred to a lower dwell volume system, or reduced for transfer to a higher dwell system. In this example, the **ACE LC Translator** was used to determine the necessary adjustment to the isocratic hold and achieve a successful method transfer to all systems in all circumstances.

Table 1: System specifications for the UHPLC systems utilised in Figures 5 and 6.

System	Vendor	Pump	Dwell Volume
UHPLC 1	1	Quaternary	0.574 mL
UHPLC 2	2	Binary	0.284 mL
UHPLC 3	1	Binary	0.202 mL
UHPLC 4	1	Quaternary	0.964 mL
UHPLC 5	3	Quaternary	0.372 mL

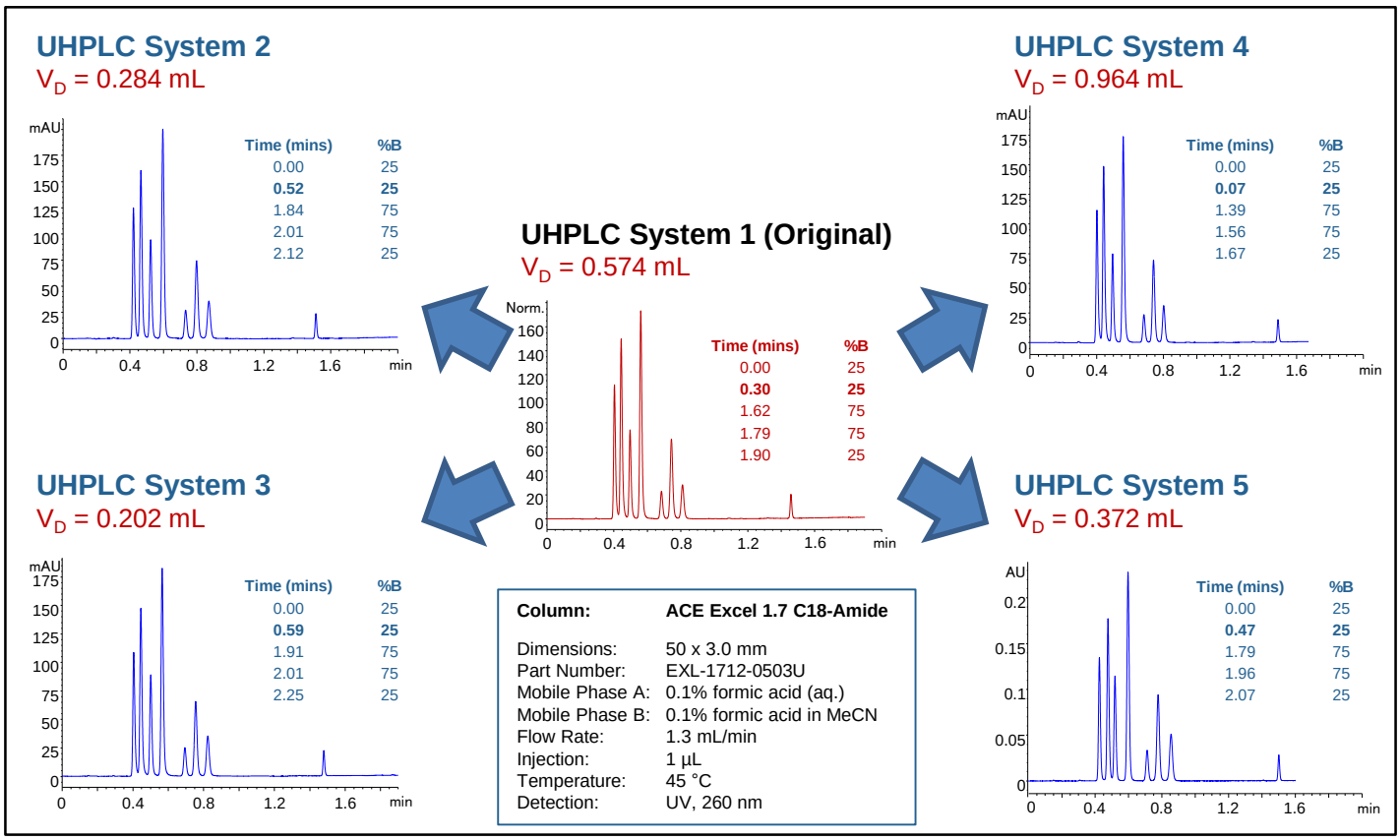

Figure 5: Transfer of a UHPLC method to four different UHPLC systems.
Details of each system are shown in Table 1.

Sample: 1. Vanillic acid, 2. 4-Hydroxybenzoic acid, 3. Vanillin, 4. 4-Hydroxybenzaldehyde, 5. Guaiacol, 6. *o*-Vanillin, 7. Ethylvanillin, 8. Eugenol.

Instrument characteristics and settings

It is important to ensure that the receiving instrument is fit for purpose. Physical characteristics such as pressure capabilities and injector type and settings should be checked. In general, methods developed on UHPLC systems on small format columns (e.g. 2.1 and 3.0 mm ID) should be transferred to similar specification systems. It is generally recommended that LC methods utilise a column oven to improve method robustness. When transferring methods, the accuracy of the column oven should be checked. Additionally, many column ovens have mobile phase pre-heating capabilities. The use of mobile phase pre-heating can have important implications for method transfer. Figure 6 demonstrates the effect that mobile phase pre-heating can have on an HPLC separation.

A general shift to longer retention time is observed when no pre-heating is used. Additionally, several changes in the band spacing are apparent, including co-elution of peaks 12 and 13. When transferring methods, it is therefore useful to verify the pre-heating method utilised and any accompanying settings that may be adjustable in the instrument method. This situation may be particularly relevant when transferring legacy methods, developed on older systems, to more modern systems.

Another instrument characteristic that requires consideration is system extra-column volume, which includes any system volume between the injector and detector (such as tubing and needle seat internal volumes). Excessive extra-column volume can result in analyte band dispersion, leading to peak

broadening and loss of efficiency. The negative impact of excessive extra-column volume is more pronounced for small format columns (e.g. 2.1 mm ID) and is also typically more detrimental to early eluting analytes. Therefore, if the method was developed on an optimised HPLC or UHPLC system then the receiving instrument should be similarly optimised to ensure method performance is maintained during method transfer. It is also important to check that the flow cell installed on the instrument is a suitable volume for the application. Detector settings including sampling rate, response time and bandwidth can also cause problems for method transfer if not set correctly.

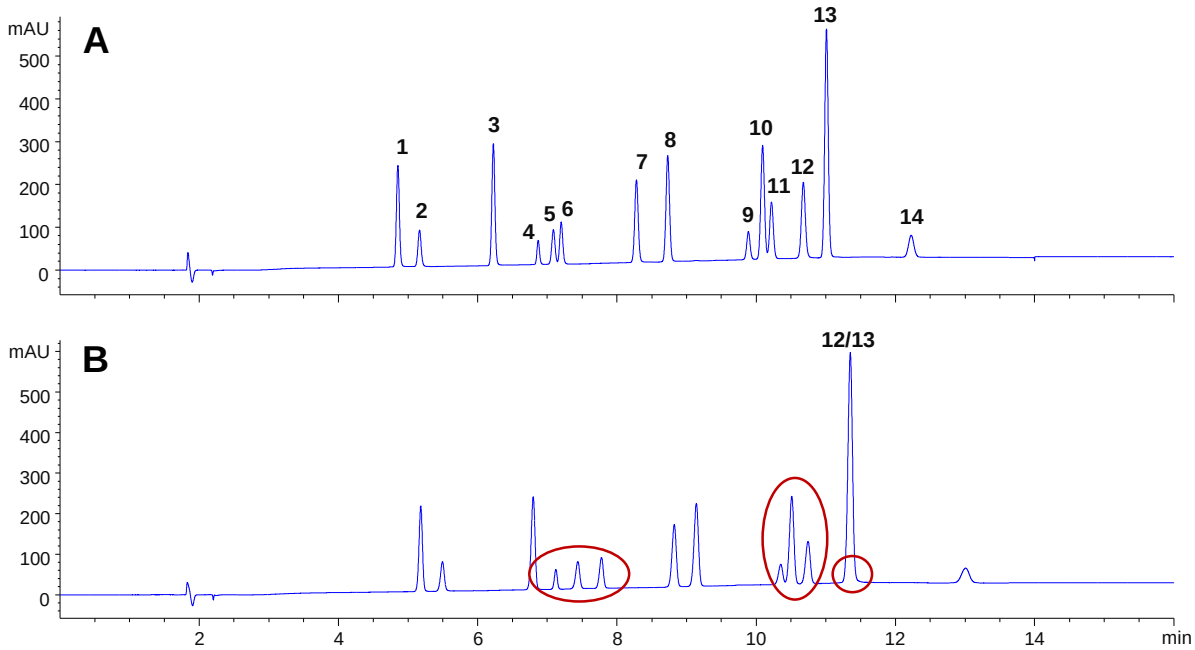

Figure 6: Separation of pharmaceutically relevant analytes on an ACE 3 C18-AR, (A) with mobile phase preheating and (B) without mobile phase pre-heating. The runs were performed on the same HPLC instrument.

Column dimensions:	150 x 4.6 mm
Mobile phase:	A: 0.1% formic acid (aq.), B: 0.1% formic acid in MeCN
Gradient:	0 to 35% B in 9 mins, hold for 5 mins, 35 to 95% B in 1 min
Flow rate:	1 mL/min
Column temperature:	40 °C
Detection:	UV, 240 nm

1. 4-Acetamidophenol
2. 4-Aminobenzoic acid
3. 4-Hydroxybenzoic acid
4. Caffeine
5. 2-Acetamidophenol
6. 3-Hydroxybenzoic acid
7. Salicylamide
8. Acetanilide
9. Acetylsalicylic acid
10. Benzoic acid
11. Sorbic acid
12. Salicylic acid
13. Phenacetin
14. Salicylaldehyde

Conclusion

This ACE Knowledge Note has discussed some of the considerations for transferring HPLC and UHPLC separations between instruments. **The ACE LC Translator** tool can assist with the transfer of LC methods and should improve success. Combining the use of the **ACE LC Translator** with a thorough assessment of instrument characteristics can help the analyst achieve more successful transfers of LC methods.

References

[1] ACE Knowledge Note #0023 “Gradient Method Translation Using the ACE LC Translator”

[2] ACE Knowledge Note #0001 “How to Determine System Dwell Volume: Theory and Practice”

[3] USP Chapter <621> “Chromatography”

[4] European Pharmacopoeia chapter 2.2.46 “Chromatographic separation techniques”