Chromatography Solutions

Knowledge note #0034

Approaches for Reducing the Environmental Impact and Increasing Throughput of LC Separations

INTRODUCTION

For many analytical laboratories there is increasing pressure to reduce the amount of solvents purchased and the amount of waste solvents generated. This is particularly important in high-throughput laboratories where the large number of samples analysed results in a large amount of solvent usage and consequently, waste solvent disposal. This is both detrimental to the environment and also to running costs. As well as solvents, there is also pressure to reduce energy consumption within these laboratories, and although the electrical supply associated with one LC system may not be considered too troubling, when running multiple LC-MS systems the electrical load will be considerable.

Significant reductions in environmental footprint can be made by moving to UHPLC instrumentation, since the use of shorter, narrower internal diameter columns reduces mobile phase/solvent consumption. There are additional benefits, such as improving the overall laboratory efficiency, better instrument utilisation, increased analytical capacity and reducing overall running costs. Often, it is perceived that significant gains can only be made by upgrading to UHPLC instrumentation, however, it is often feasible to adapt established methods and better utilise existing equipment to realise substantial improvements. This article demonstrates how environmental impact for isocratic and gradient analytical LC methods can be decreased using both existing HPLC instruments and through upgrading to UHPLC, with the added advantage of increasing sample throughput.

REDUCING SOLVENT CONSUMPTION

Minimising environmental impact for LC applications is highly desirable for many laboratories and can help to not only reduce solvent and energy consumption, but also reduce sample turnaround times and overall running costs. Significant reductions in solvent usage can be achieved by quantitatively translating methods to a shorter length column, packed with smaller particles (either fully porous or solid-core). The principle for reducing solvent consumption, whilst maintaining the original method performance, is to ensure the column

length (L) to particle size (d_P) ratio (L/d_P) is kept consistent. Additionally, the column stationary phase chemistry should be kept the same. This results in similar separation performance being obtained in a reduced time. These principles are demonstrated in Table 1. All three columns are bonded with the same C18 stationary phase and therefore will provide identical separation selectivity (peak spacing). By selecting appropriate column lengths and particle sizes, all three columns have an L/d_P ratio of approximately 30,000 and will therefore provide similar separation efficiencies. Guidance from the USP (Chapter <621>) states that the L/d_P rule can be applied to guide allowable changes to the column length and particle size for monograph methods.[1] The guidance is due to be extended to gradient separations from December 2022.[2] In addition, the Eu. Ph. has recently published a revision of its general chapter 2.2.46, which incorporates the L/d_P approach for both isocratic and gradient method adjustments, which will be implemented from January 2023.[3]

Once a new column format has been identified, the analytical method will require scaling. Software LC method translation tools (e.g. the Avantor® ACE® LC Translator) include all the necessary equations to accurately scale method parameters, including injection volume, flow rate and gradient profile. Additionally, percentage reductions in solvent consumption and run time are calculated. The Avantor® ACE® LC Translator is described elsewhere[4] and can be downloaded for free.[5]

Using these approaches, significant reductions in solvent consumption can be realised without sacrificing method performance and/or robustness, together with compelling increases in sample throughput due to significantly reduced run times. The reduced run times per sample have the added benefit that energy consumption per sample is reduced. The translation of methods to shorter columns is often discussed in the context of migrating methods to UHPLC, however, impressive improvements can also be realised using standard HPLC instrumentation, thus improving the utilisation of existing equipment platforms.

ISOCRATIC LC METHODS

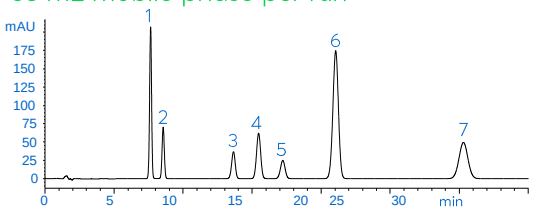
Figure 1 gives a simple overview of how mobile phase requirements for an isocratic LC method can be decreased, and sample throughput increased, using two options. The original separation, using a 150 x 4.6, 5 µm column has a run time of 35 minutes, with a back pressure of 66 bar. By translating the method to a shorter UHPLC column with narrower ID (50 x 3.0 mm, 1.7 µm) and operating at a higher mobile phase linear velocity, the run time is reduced to 4 minutes with a moderate pressure of 558 bar. This equates to a significant reduction in the volume of mobile phase consumed per injection, along with an 8 times increase in sample throughput and >88% reduction in the runtime (Figure 2).

However, if UHPLC instrumentation is not available, mobile phase consumption could still be reduced by >70% and sample throughput more than doubled by using the existing HPLC instrument with a shorter, narrower HPLC column and smaller particle size (100 x 3.0 mm, 3 µm). In this case, sample throughput is more than doubled, with run time reduced by 60% to 14 minutes at a reasonable 215 bar. From a solvent perspective, analysing 100 samples would require

Table 1: L/d_P ratios for three LC columns that will provide similar separation performance.

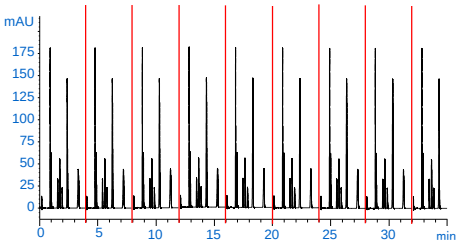
Stationary phase	Avantor® ACE® 5 C18	Avantor® ACE® 3 C18	Avantor® ACE® 1.7 C18
Dimensions	150 x 4.6 mm	100 x 3.0 mm	50 x 2.1 mm
Particle size	5 µm	3 µm	1.7 µm
L/d_P	30,000	33,333	29,412

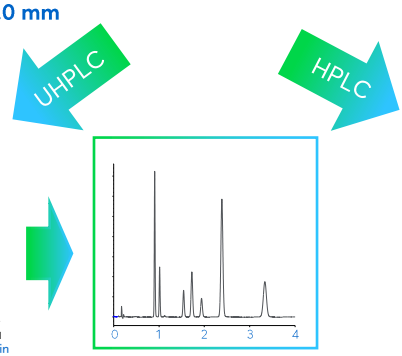
A Avantor® ACE® 5 C18-PFP, 150 x 4.6 mm
Instrument: 400 Bar HPLC
1 mL/min, 66 bar
35 mL mobile phase per run



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

B Avantor® ACE® Excel® 1.7 C18-PFP, 50 x 3.0 mm
Instrument: 1,400 Bar UHPLC
1.25 mL/min, 558 bar
5 mL mobile phase per run





C Avantor® ACE® 3 C18-PFP, 100 x 3.0 mm
Instrument: 400 Bar HPLC
0.71 mL/min, 215 bar
9.9 mL mobile phase per run

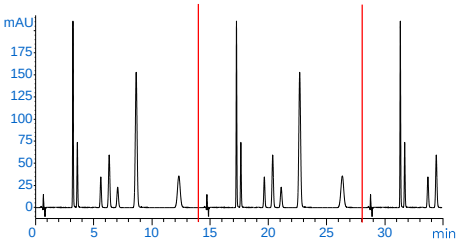
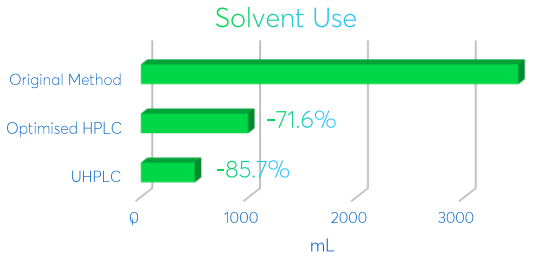
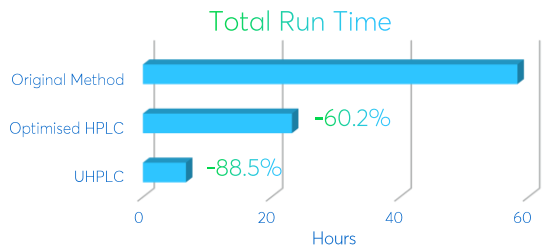


Figure 1: Reducing mobile phase consumption of an existing isocratic LC method (A), using (B) UHPLC and (C) modified HPLC options. Mobile phase: MeCN/H_2O (3:7 v/v); Injection volume: (A) 5 µL, (B) 0.7 µL, (C) 1.4 µL; Detection: UV (214 nm).





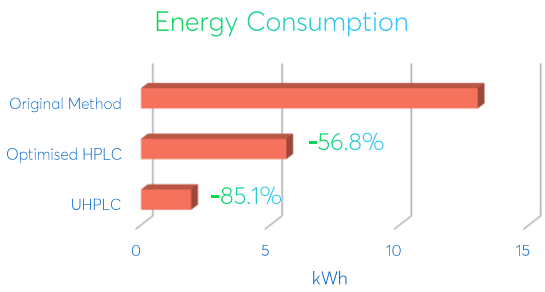


Figure 2: Solvent savings, power consumption and reduction in total run time for 100 injections using the methods in Figure 1. Electrical consumption was calculated using measured electrical usage values of 0.1302, 0.0562 and 0.0194 kWh per sample for the HPLC, optimised HPLC and UHPLC methods.

3,500 mL with the original method, 500 mL for the UHPLC method and 994 mL for the modified rapid HPLC option. As well as significant solvent savings, substantial reductions in electrical consumption were also achieved, the optimised HPLC methodology resulted in a 56.8% reduction, whilst the use of UHPLC provided an 85% reduction in energy use for 100 samples. Additionally, there are also efficiency savings (excluding equilibration times, cleaning, shutdown methods etc.), which are highlighted in Figure 2.

GRADIENT LC METHODS

Figure 3A uses a 150 x 4.6 mm, 5 µm column and shows a gradient analysis of non-steroidal anti-inflammatory drugs. The post-gradient re-equilibration time from the gradient table (Table 2) is 20 minutes (or ~13 column volumes), giving a total run time per injection of 54 minutes. This gradient method was translated to UHPLC and rapid HPLC formats, to demonstrate the improvements that can be achieved. Using similar principles as in Figure 1, along with the Avantor® ACE® LC Translator,[5] the gradient method can be quantitatively translated to the two new column formats. For gradient methods, it is necessary to scale the gradient profile and correct for differences in instrument dwell volume to ensure the same gradient separation and resolution is obtained with the new column format.[6] Table 2 shows the original and recalculated gradient times for the separation on each column format.

The original HPLC separation in Figure 3A, using a 150 x 4.6 mm, 5 µm column, has a run time of 34 minutes, but a total cycle time of 54 minutes due to gradient re-

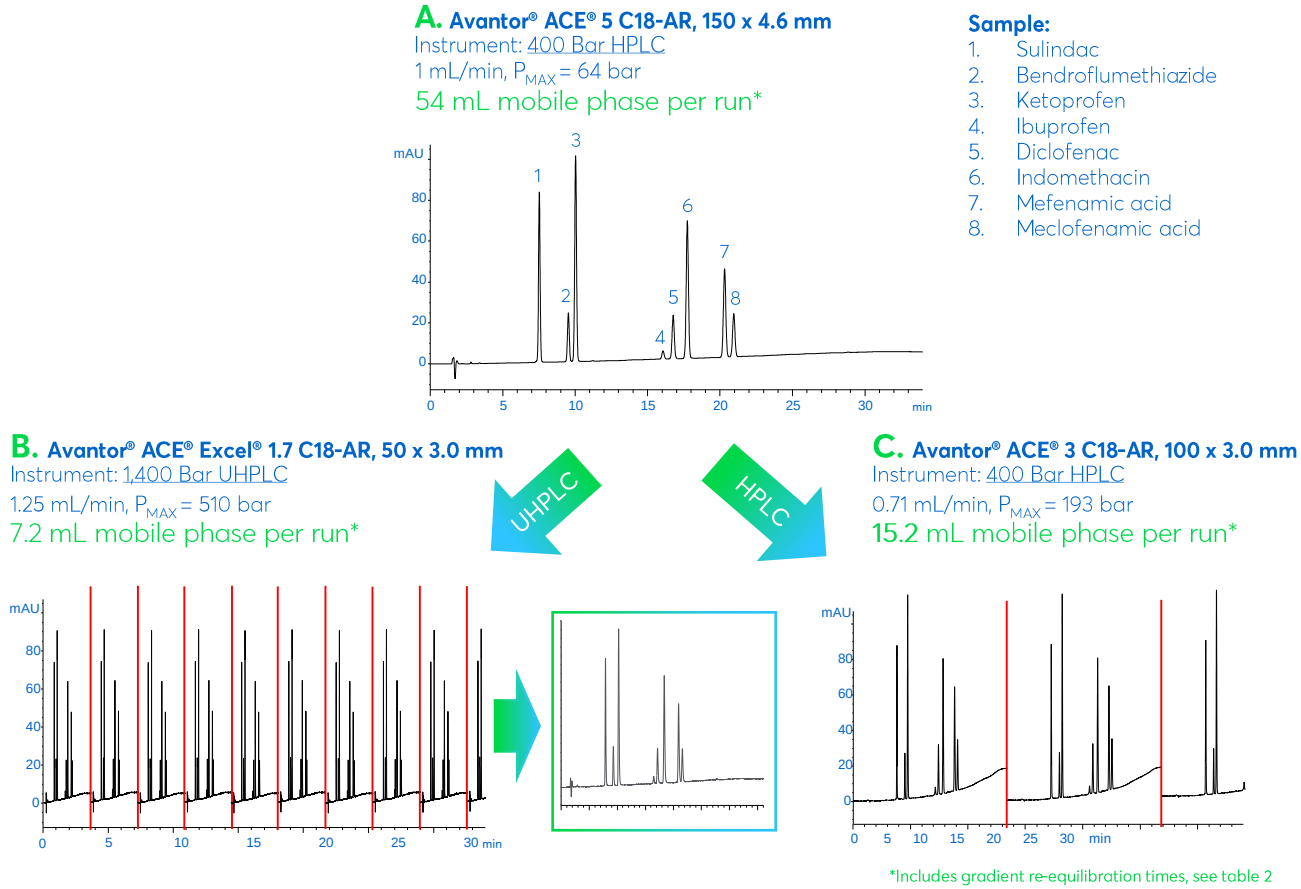


Figure 3: Reducing mobile phase consumption of a gradient LC method (A), existing HPLC method using (B) UHPLC and (C) modified rapid HPLC options. Mobile phase: A: 0.1% formic acid (aq), B: 0.1% formic acid in MeCN; Gradient: 35-65%B; Injection volume: (A) 5 µL, (B) 0.7 µL, (C) 1.4 µL; Detection: UV (254 nm). Note: post-gradient equilibration times are detailed in Table 2.

Table 2: Original and recalculated gradient profiles for the three methods in Figure 3. Calculations were performed using the Avantor® ACE® LC Translator.[5] Note that experimentally determined column volumes were used in calculations.[7]

%B	Gradient time (mins) 150 x 4.6, 5 µm	Gradient time (mins) 100 x 3.0, 3 µm	Gradient time (mins) 50 x 3.0, 1.7 µm
35	0	0	0
65	28	11.06	2.98
65	33	13.04	3.51
35	34	13.44	3.61
35	54	21.34	5.74
Mobile phase per run	54 mL	15.2 mL	7.2 mL

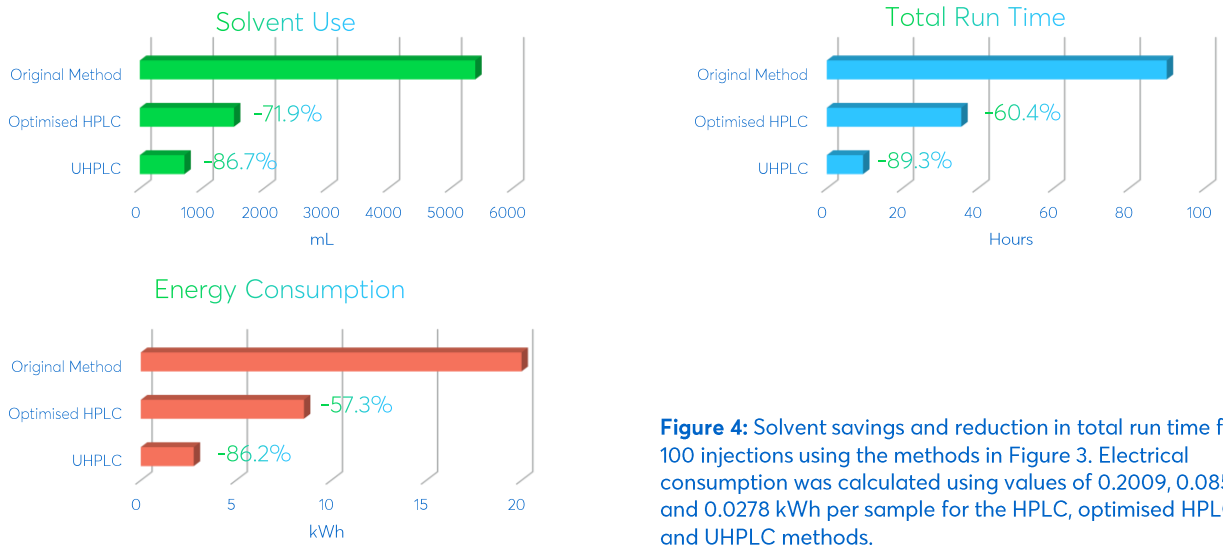


Figure 4: Solvent savings and reduction in total run time for 100 injections using the methods in Figure 3. Electrical consumption was calculated using values of 0.2009, 0.0857 and 0.0278 kWh per sample for the HPLC, optimised HPLC and UHPLC methods.

equilibration, with P_{MAX} of 64 bar. Translating this to the UHPLC format gives a total method cycle time of 5.7 minutes with P_{MAX} of 510 bar. This represents a >89% reduction in run time / cycle time (Figure 4), corresponding to a reduction in mobile phase requirements from 5,400 mL to just 718 mL (86.7% reduction). A >9 times increase in sample throughput is achieved. The modified rapid HPLC format data in Figure 3C, has a total cycle time of 21.3 minutes per sample (including equilibration) with a P_{MAX} of 193 bar and can therefore be run on standard, <400 bar HPLC systems. This represents a 79.1% reduction in mobile phase consumption, >60% reduction in runtime/cycle time and >2.5 times increase in sample throughput.

These efficiency savings are summarised for 100 injections in Figure 4. Solvent consumption for each format were calculated to be calculated as 5,400 mL, 718 mL and 1,515 mL for the original, UHPLC and modified rapid HPLC methods respectively, whilst energy consumption was reduced by 57.3% using the optimised HPLC methodology and by 86.2% when using a UHPLC approach.

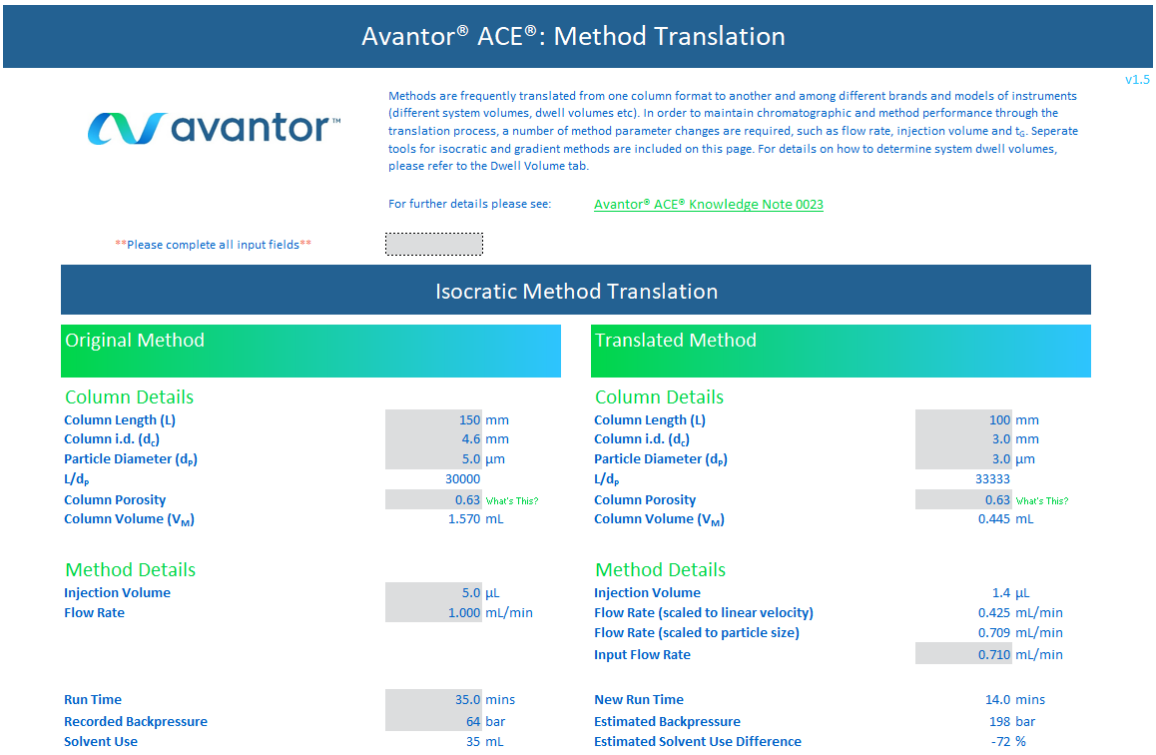
SIMPLIFIED METHOD TRANSLATION USING THE ACE LC TRANSLATOR TOOL

The translation of existing HPLC methods to smaller column formats is now routinely performed and involves geometric scaling of the original method to the new column format. Calculations for both isocratic and gradient translations can be automatically performed using the free-to-download Avantor® ACE® LC Translator Tool.[5] This MS Excel-based tool allows the user to input the HPLC column and method parameters, along with details of the new column that the method will be translated to. The LC conditions for the translated method are then automatically generated (Figure 5), from first principles formulae in the spreadsheet, including expected savings in solvent consumption, without the need to manually perform the calculations. These new conditions may then be inputted into the instrument and applied to the new column.

The ACE LC Translator Tool includes other useful chromatography calculators such as method transfer between instruments and for everyday, practical chromatography. For further details on method translation using the tool, please refer reference 4.

PRACTICAL CONSIDERATIONS

When translating methods to smaller format columns packed with smaller particles, several additional factors may need consideration. The use of narrower ID columns, together with small particles, generates narrower, lower volume peaks. These smaller peak volumes will be more prone to extra-column band broadening effects caused by excessive system dispersion (usually a result of excessive dead volume being present within the system). Early eluting peaks are particularly prone to these dispersive effects. Low efficiency for early eluting analytes can therefore serve as an indicator that a



Avantor® ACE®: Method Translation

v1.5

avantor™

Methods are frequently translated from one column format to another and among different brands and models of instruments (different system volumes, dwell volumes etc). In order to maintain chromatographic and method performance through the translation process, a number of method parameter changes are required, such as flow rate, injection volume and t_G. Seperate tools for isocratic and gradient methods are included on this page. For details on how to determine system dwell volumes, please refer to the Dwell Volume tab.

For further details please see: Avantor® ACE® Knowledge Note 0023

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Isocratic Method Translation

Original Method			Translated Method		
Column Details			**Column Details**		
Column Length (L)	150	mm	Column Length (L)	100	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)	3.0	µm
L/d_p	30000		L/d_p	33333	
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)	0.445	mL
Method Details			**Method Details**		
Injection Volume	5.0	µL	Injection Volume	1.4	µL
Flow Rate	1.000	mL/min	Flow Rate (scaled to linear velocity)	0.425	mL/min
			Flow Rate (scaled to particle size)	0.709	mL/min
			Input Flow Rate	0.710	mL/min
Run Time	35.0	mins	New Run Time	14.0	mins
Recorded Backpressure	64	bar	Estimated Backpressure	198	bar
Solvent Use	35	mL	Estimated Solvent Use Difference	-72	%

Figure 5: Screenshot of the Avantor® ACE® LC Translator Tool showing translation of method A in Figure 1 to method C.

system is not appropriately optimised. In situations where extra-column band broadening is suspected, system dead volume may need to be reduced by e.g. using narrower ID tubing within the LC system and installation of a lower internal volume UV detector flow cell. Switching to a wider bore column (e.g. changing from 2.1 to a 3.0 mm ID or from a 3.0 mm to 4.0 or 4.6 mm ID) can also help to increase efficiency.

Narrow bore columns, such as 2.1 mm ID, should generally only be used with either UHPLC or highly optimised HPLC systems, as peaks eluted from such columns will be especially vulnerable to performance loss through extra column dispersion effects. Likewise, to achieve the full benefits of sub-2 micron UHPLC columns, it is essential to appreciate that the column must be used with a UHPLC system that is optimised to reduce peak dispersion and capable of ultra-high pressure separations (600-1,400 bar).

The narrower, low-volume peaks produced also require sufficiently fast data acquisition rates to provide enough data points to accurately describe the peaks. It is therefore important to pay close attention to detector settings and if necessary, increase the data capture rate. Figure 6 demonstrates the effect that insufficient detector rates can have on the chromatographic data obtained for an isocratic separation. In this example, six preservatives were separated in less than 3 minutes on a 2.5 µm solid-core column using a UHPLC instrument. When an 80 Hz detector rate was used, sufficient data points were recorded to accurately describe the narrow peaks. If too slow a detector rate is used, in this case 2.5 Hz, resolution and sensitivity (peak height) are negatively affected, with the earlier eluted analytes being more severely impacted.

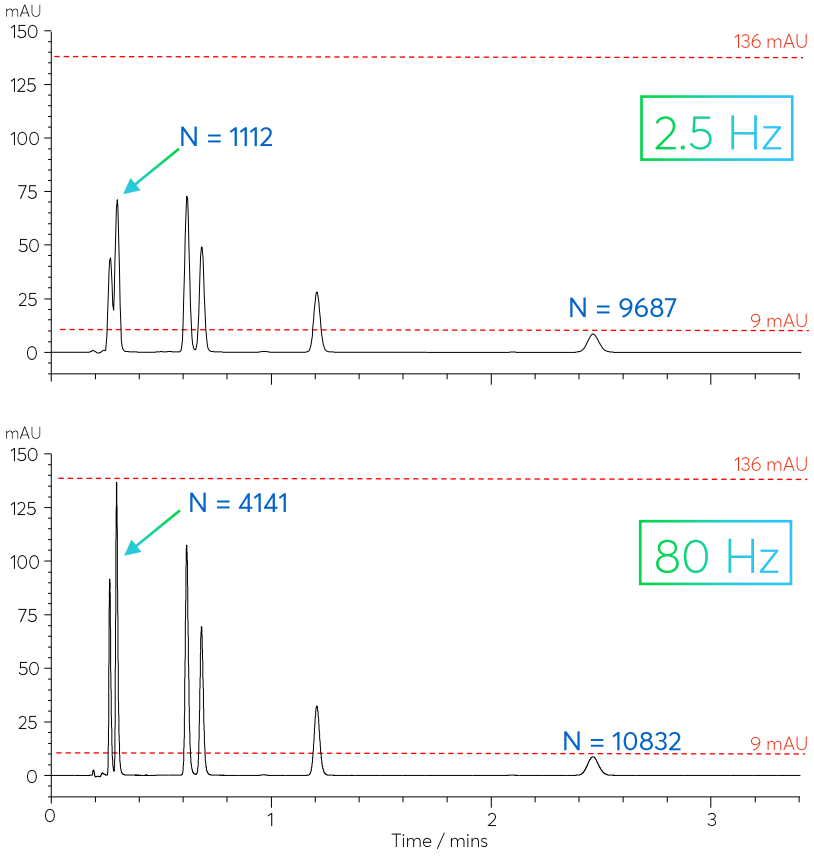

Figure 6: Effect of different detector sample rates on the fast separation of six preservatives. Column: Avantor® ACE® Excel 1.7 C18, 50 x 3.0 mm, Mobile phase: 20 mM KH_2PO_4 pH 2.5 in MeCN/H_2O (30:70 v/v), Flow rate: 1.25 mL/min, Injection volume: 0.7 µL Temperature: 20°C, Detection: UV, 230 nm. Sample: phthalic acid, 4-hydroxybenzoic acid, sorbic acid, methyl paraben, ethyl paraben, propyl paraben.

Finally, for gradient methods, it is important to assess the impact of system dwell volume on any translated method. If the ratio of the dwell volume to the column volume changes significantly between the original and translated method, significant differences in selectivity may be observed. If this is the case, corrections may be required, either to the gradient method, or to the physical system dwell volume to compensate. This topic is discussed in more detail in reference 6.

CONCLUSION

Using simple first principles or free software tools, significant reductions in mobile phase consumption and waste generated can be achieved by migrating existing methods to smaller format columns with narrower ID's, packed with smaller particles. Additionally, the faster run times that are obtained can help to reduce energy consumption on a per sample basis. These outcomes can assist laboratories in reducing the environmental impact of analytical testing. There is also the added benefit that reduced run times will improve laboratory throughput and efficiency and drive cost reductions. The magnitude of the improvement depends upon the laboratory instrumentation available; UHPLC offers impressive numbers but modified rapid HPLC options are still worthy of consideration.

In this article, isocratic and gradient examples have been used to demonstrate the approach with calculations of solvent, energy and run time savings included. When exploring these exciting potential improvements, it is important to remember that certain system characteristics (dispersion, dwell time, detector settings, etc.) should be determined and factored into experiments to ensure method translations are as accurate as possible.[8, 9] These concepts and how they can affect isocratic and gradient method translations are also discussed elsewhere.[6, 10] Although the method translation approach is often discussed in the context of migrating existing methods to UHPLC, this article demonstrates how significant improvements can also be made to maximise the utilisation of existing HPLC instrumentation.

REFERENCES

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8. Avantor® ACE® Knowledge Note AKN0001: How to Determine System Dwell Volume (https://uk.vwr.com/cms/ace_knowledge_notes)
9. Avantor® ACE® Knowledge Note AKN0017: How to Determine Extra Column Dispersion and Extra Column Volume (https://uk.vwr.com/cms/ace_knowledge_notes)
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