Increasing HPLC / UHPLC Sample Throughput: Doing More in Less Time

In many analytical laboratories, options to increase sample throughput for LC analyses are highly desirable. This is especially pertinent in high sample volume or time critical environments such as in-process testing, clinical, forensic or doping laboratories. As well as getting more done and outputting data quicker, there are additional benefits, such as improving the overall laboratory efficiency, better instrument utilisation, increased analytical capacity and reduced solvent consumption. This short article demonstrates how throughput for existing isocratic and gradient analytical LC methods can be increased using existing HPLC instruments and where applicable, by upgrading to UHPLC.

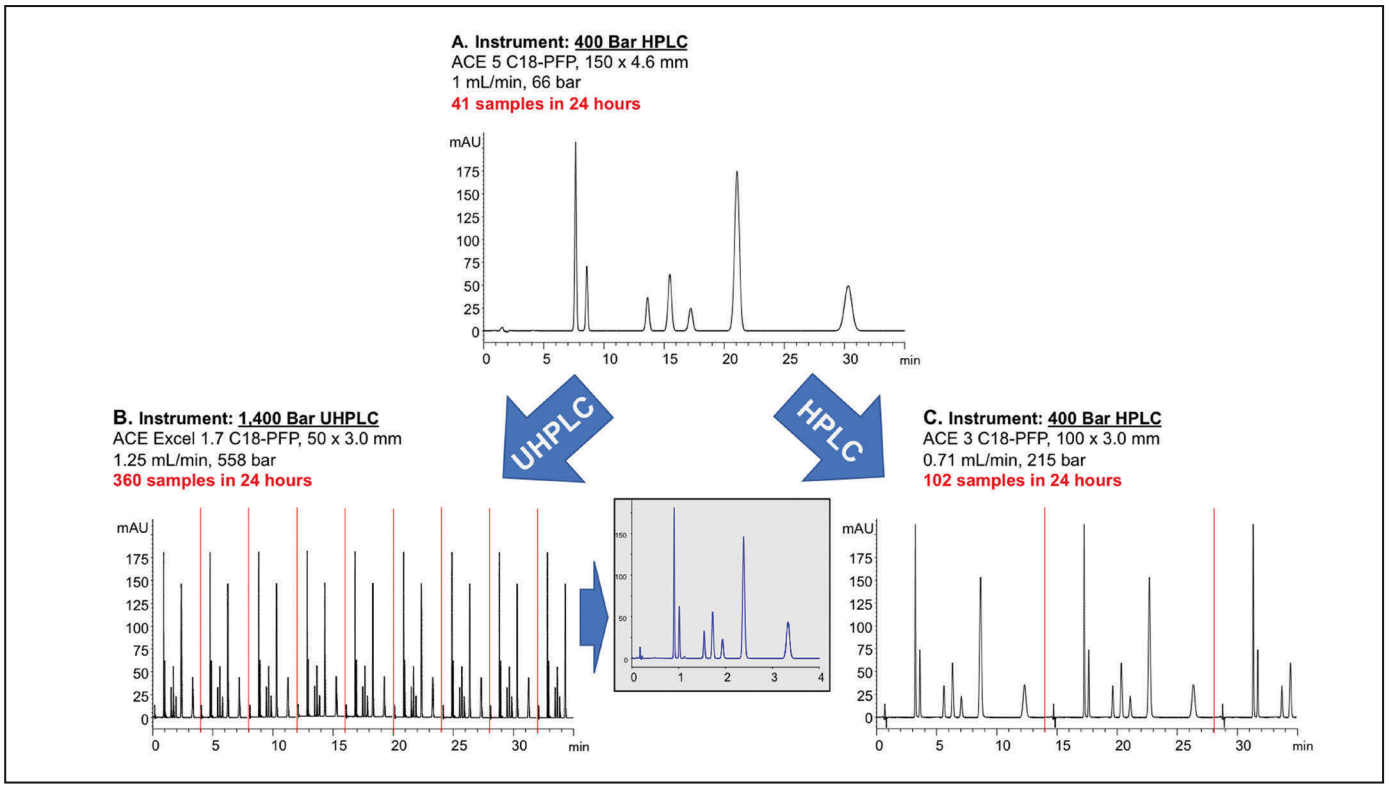

Figure 1: Increasing sample throughput of an existing isocratic HPLC method (A) using UHPLC (B) and modified HPLC (C) options. Mobile phase: MeCN:H_2O (3:7 v/v), Injection volume: (A) 5 µL, (B) 0.7 µL, (C) 1.4 µL, Detection: UV (214 nm), Sample: 1. 1,2,3-Trimethoxybenzene, 2. 1,2,4-Trimethoxybenzene, 3. 1,4-Dimethoxybenzene, 4. Methoxybenzene, 5. 1,3-Dimethoxybenzene, 6. 1,3,5-Trimethoxybenzene, 7. Toluene.

Introduction

Maximising sample throughput for LC applications is useful for many laboratories. It is possible to obtain significant reductions in method run times by quantitatively translating the method to a shorter length column with smaller particles (either fully porous or solid core). The principle for increasing sample throughput, 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. This results in similar separation performance being observed in a reduced time. Software LC method translation tools (e.g. the ACE Translation Tool) include all the necessary equations to accurately scale method parameters including injection volume, flow rate, gradient profile etc. and are described elsewhere [1] and can be downloaded for free [2]. Significant improvements in throughput can be realised, without sacrificing method performance and / or robustness. In addition to reducing analysis time, compelling reductions in solvent consumption are also achievable. 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.

Table 1: Original and recalculated gradient profiles for the three methods in Figure 2. Calculations were performed using the ACE Translation Tool [2]. Note real experimentally determined column volumes were used in calculations [3].

%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

Improving sample throughput for isocratic LC methods

Figure 1 gives a simple overview of how sample throughput for an isocratic LC method can be 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 UHPLC column (50 x 3.0 mm, 1.7 µm) on a UHPLC instrument, the run time is reduced to 4 minutes with a moderate pressure of 558 bar. This equates to a >8 times increase in sample throughput and a >88% reduction in the runtime. However, if UHPLC instrumentation is not available, sample throughput could still be more than doubled by using the existing HPLC instrument with a shorter HPLC column and smaller particle size (100 x 3.0 mm, 3 µm). In this case, sample throughput is >2 times better than the original method with run time reduced by 60% to 14 minutes at a reasonable 215 bar. From a solvent perspective, analysing 100 samples (excluding equilibration times, cleaning, shutdown methods etc.) would require 3,500 mL with the original method, 500 mL for the UHPLC method and 994 mL for the modified rapid HPLC option.

Improving sample throughput for gradient LC methods

Figure 2A 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 is 20 minutes (or ~13 column volumes). It is possible to translate the gradient method to a new UHPLC format, or a modified rapid HPLC format to understand the impact on sample throughput. Using similar principles as in Figure 1, along with a software translation tool [2], it is possible to quantitatively translate the gradient method to the two new column formats. For gradient methods, it is also 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 formats. Table 1 shows the original and recalculated gradient times for the separation on each column format.

The original HPLC separation in Figure 2, 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-equilibration, with P_{MAX} of 64 bar. Translating this to the UHPLC format gives a 3.6 minute run time

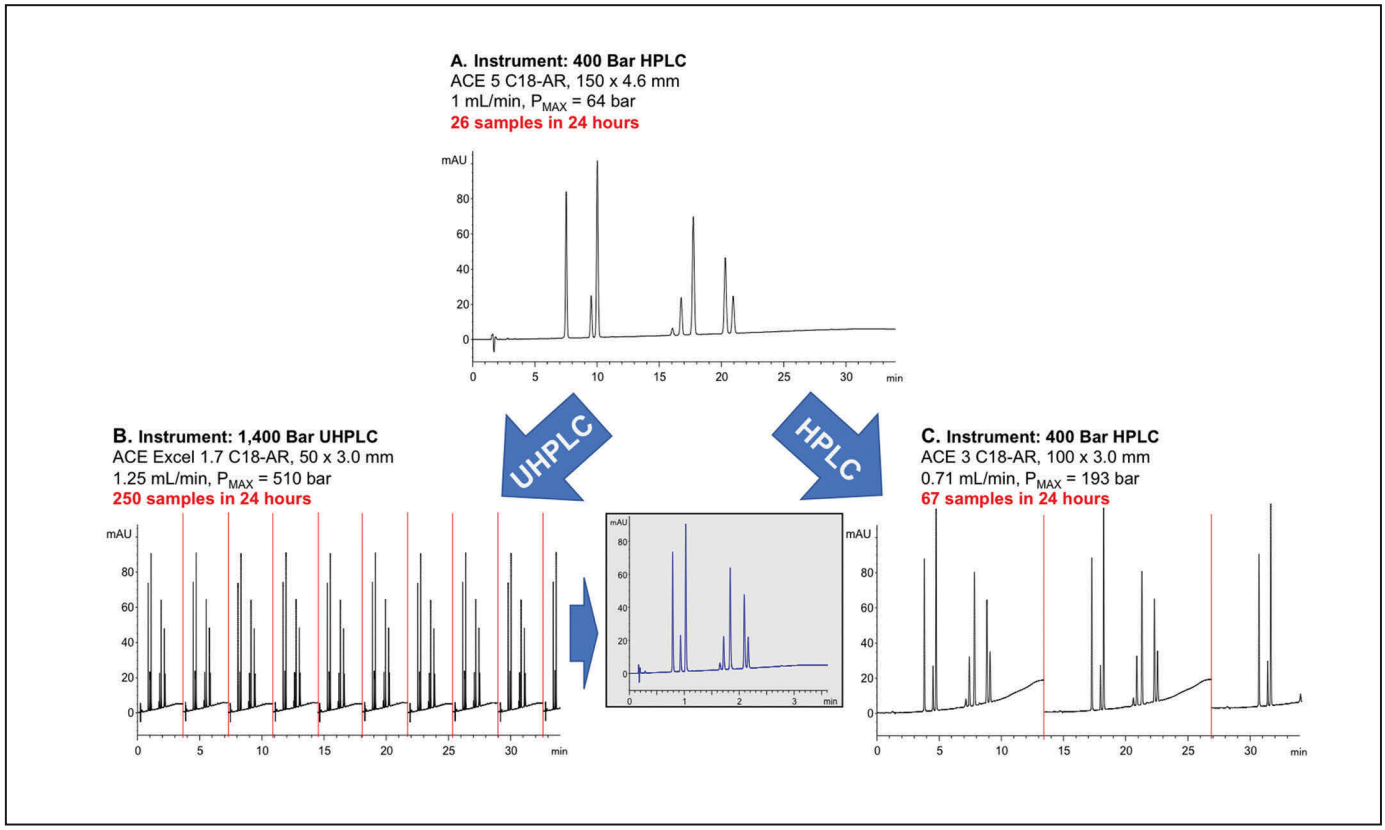

Figure 2: Increasing sample throughput of an existing gradient HPLC method (A) using UHPLC (B) and modified rapid HPLC (C) options. Mobile phase: A: 0.1% formic acid (aq), B: 0.1% formic acid in MeCN, Gradient: 35-65%B, Injection volume: (A) 5 µL, (B) 0.7 µL, (C) 1.4 µL, Detection: UV (254 nm), Sample: 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 Table 1.

and total method cycle time of 5.7 minutes with P_{MAX} of 510 bar. This provides a >9 times increase in sample throughput and a >89% reduction in runtime / cycle time. The modified rapid HPLC format data in Figure 2C, has a new run time of 13.4 minutes and a total cycle time of 21.3 minutes (including equilibration) with P_{MAX} of 193 bar. This represents a >2.5 times increase in sample throughput and a >60% reduction in runtime / cycle time. Solvent consumption for the total cycle times for each format (but excluding initial equilibration and shutdown methods) for 100 samples can be calculated as 5,400 mL, 718 mL and 1,515 mL for the original, UHPLC and modified rapid HPLC methods respectively.

Concluding remarks

Using simple first principles or free software tools, it is possible to significantly increase sample throughput and reduce solvent consumption for many legacy HPLC methods providing a boost to lab capacity and speed of data output. The magnitude of the improvement will depend 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 sample throughput, runtime savings and solvent 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 [4, 5]. These concepts and how they can affect isocratic and gradient method translations are also discussed elsewhere [6]. 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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