

Exploiting particle size to reduce solvent consumption in analytical HPLC

The global shortage of acetonitrile is causing concern for many HPLC operators. In this article, the use of sub-2 μm particle packed columns as a strategy to help reduce or replace acetonitrile consumption in analytical test methods is presented.



Introduction

As the supply of acetonitrile becomes more limited, high performance liquid chromatography (HPLC) operators are coming under increasing pressure to reduce or replace this solvent in their analytical methods. One simple way to achieve a reduction in solvent usage is to use a shorter column. If the resolution of a separation allows, then simply shortening the column length from 250 mm to 150 mm will reduce solvent usage by 40%.

A more dramatic saving in solvent usage can be made by reducing the internal diameter of a column, together with an appropriate scaling down in the flow rate. Separation efficiencies can be recovered by also reducing particle size; for example, columns packed with sub-2 μm particles offer advantages over the more traditional systems containing 3 μm and 5 μm particles by allowing operation at higher flow rates without compromising efficiency. Consequently, this results in shorter analysis times and a reduction in solvent consumption, together with associated improvements in resolving power, sensitivity and peak capacity.

Experimental Conditions

HPLC conditions: Instrument, Accela U-HPLC system; column, Hypersil GOLD 5 μm , 150 x 4.6 mm; mobile phase, A = 0.05% H_3PO_4 in $\text{H}_2\text{O}/\text{ACN}$ (66.34), B = ACN; gradient, 0 mins = 0% B, 25 mins = 0% B, 55 mins = 85% B, 70 mins = 85% B; flow rate, 1.0 mL/min; injection volume, 10 μL ; detection, UV at 214 nm (0.1 s rise time; 20 Hz); temperature, 30 $^\circ\text{C}$.

Fast U-HPLC conditions: Instrument, Accela U-HPLC system; column, Hypersil GOLD 1.9 μm , 50 x 2.1 mm; mobile phase, A = 0.05% H_3PO_4 in $\text{H}_2\text{O}/\text{ACN}$ (66.34), B = ACN; gradient, 0 mins = 0% B, 3.2 mins = 0% B, 7.1 mins = 85% B, 8.9 mins = 85% B; flow rate, 0.55 mL/min; injection volume, 0.7 μL ; detection, UV at 214 nm (0.1 s rise time; 20 Hz); temperature, 30 $^\circ\text{C}$.

Results and Discussion

When transferring methods, whether from HPLC to HPLC or to U-HPLC, an understanding of some practical calculations can help to achieve the correct scaling and maintain a consistent assay profile between the original and transferred method. There are two main considerations for isocratic methods: scaling the flow rate and adjusting the injection volume. When a gradient method is transferred, it also becomes necessary to adjust the gradient profile. This is discussed in more detail below.

1. Scale the flow rate: To maintain an equivalent separation when transferring a method it is important to keep the reduced linear velocity constant between the original and new method. The reduced linear velocity is related to the flow rate, internal diameter of the column and particle size. A simple equation can be derived to calculate the flow rate (F_2) required for the new method. This is shown below:

$$F_2 = F_1 \times (d_{c2}^2 / d_{c1}^2) \times (d_{p1} / d_{p2})$$

where F_1 = original flow rate (mL/min), d_{c1} = original column internal diameter (mm), d_{p1} = original column particle size (μm), d_{c2} = new column internal diameter (mm), and d_{p2} = new column particle size (μm).

2. Adjust the injection volume: When a method is transferred to smaller volume columns, the same injection volume as used in the original method will take up a larger proportion of the new column, possibly leading to band broadening or potentially overloading the column. It is, therefore, important to scale down the injection volume to match the change in column volume. Once again, a simple equation can be used to calculate the injection volume (V_{i2}) required for the new method.

$$V_{i2} = V_{i1} \times (d_{c2}^2 \times L_2 / d_{c1}^2 \times L_1)$$

where V_{i1} = original injection volume (μL), d_{c1} = original column internal diameter (mm), L_1 = original column length (mm), V_{i2} = new injection volume (μL), d_{c2} = new column internal diameter (mm), and L_2 = new column length (mm).

3. Adjust the gradient profile: Geometrical transfer of the gradient requires calculation of the number of column volumes of mobile phase in each segment (time interval) of the gradient in the original method to ensure that the new calculated gradient takes place over the same number of column volumes, for the new column. The following calculation should be performed for each time segment of the gradient, including column re-equilibration. It takes into consideration the void volume of each column (V_c , calculation described below), the flow rate in the original method and the flow rate in the new method (calculated in step 1 above) and the time segment in the original method.

$$t_{g2} = t_{g1} \times (V_{c2}/V_{c1}) \times (F_1/F_2)$$

where t_{g1} = time segment in original gradient (min), t_{g2} = time segment in new gradient (min), V_{c1} = original column void volume (mL), V_{c2} = new column void volume (mL), F_1 = original flow rate (mL/min), and F_2 = new flow rate (mL/min).

The void volume of the column is the volume that is not taken up by the stationary phase (approximately 68% of the column volume):

$$V_c = 0.68 \times \pi \times r^2 \times L$$

where V_c = column volume (mL), L = column length (cm), and r = column radius (cm).

To demonstrate the savings that can be made, the method transfer process described above has been applied to the separation of ibuprofen and six impurities. The original HPLC method, which uses a 150 x 4.6 mm, 5 μ m column, is adapted from the *European Pharmacopoeia* and the simple calculation routines are used to transfer the method to use a 50 x 2.1 mm column packed with 1.9 μ m particles.

The chromatographic profiles obtained for the original HPLC method and the geometrically scaled U-HPLC methods on the smaller column packed with 1.9 μ m particles are shown in Figure 1(a) and 1(b), respectively. When the method is transferred to the 50 x 2.1 mm column the resolution of peaks 5 and 6 is maintained while analysis time is reduced approximately sevenfold.

The time and solvent savings that can be gained by transferring HPLC methods to U-HPLC are summarized in Table 3. Once column re-equilibration is taken into account, using the 50 mm column can result in an eightfold reduction in time, saving 76 mins per sample. The analyst can now run eight times as many samples. In terms of solvent saving, only 6 mL of solvent is used for the method utilizing the 50 mm column packed with 1.9 μ m particles compared with 87 mL for the method using the 150 mm column packed with 5 μ m particles. In such a way 14 samples can be acquired for the same solvent usage as one sample — saving money.

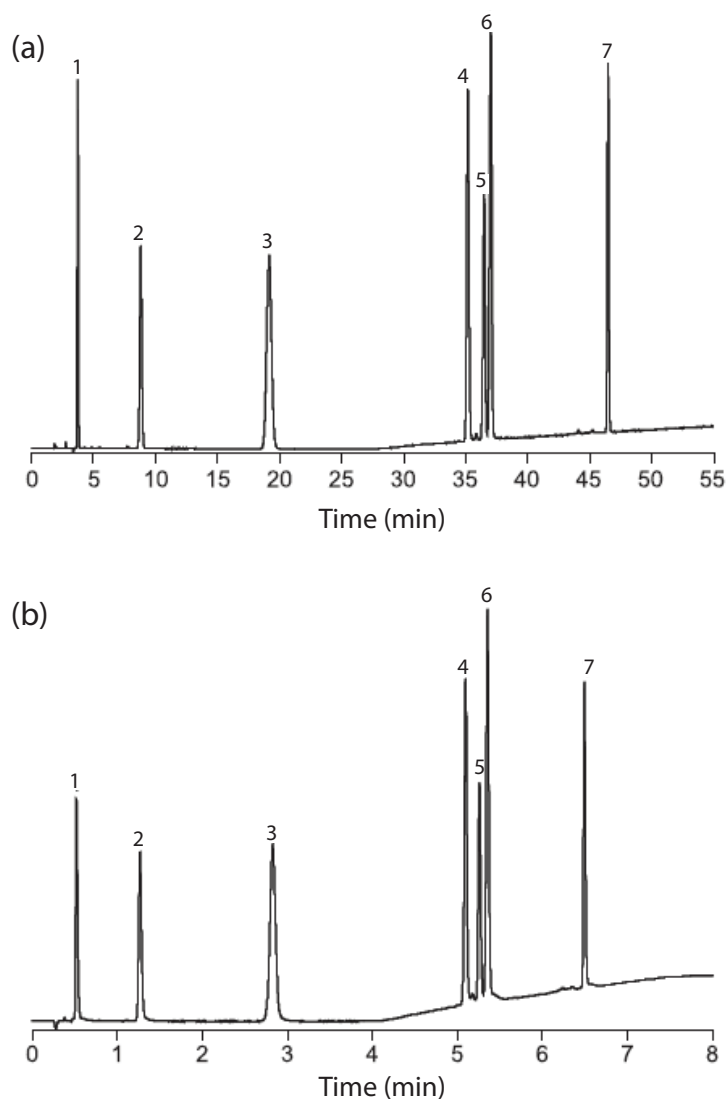
System considerations

To obtain the best data using fast chromatography it is critical that the liquid chromatography (LC) instrument system is optimized to operate under these conditions. All system components for the assay should be considered. System volume (connecting tubing i.d. and length, injection volume, flow cell volume in UV) must be minimized,

detector time constant and sampling rate need to be carefully selected, and when running fast gradients pump dwell volume needs to be minimized.

Excess system volume gives rise to band broadening, which has a detrimental effect on the chromatographic performance. This can arise from the column, the autosampler, the tubing connecting the column to injector and detector, and in the detector flow cell. The extra-column effects become more significant for scaled-down

Figure 1



Analytes: 1. Impurity K; 2. Impurity D; 3. Impurity C; 4. Ibuprofen; 5. Impurity A; 6. Impurity B; 7. Impurity E.

	HPLC 150 x 4.6 mm, 5 µm	U-HPLC 50 x 2.1 mm, 1.9 µm
Run time (min)	70	9
Total analysis time (min)	87	11
Time saved (min)		76
Flow rate (mL/min)	1.00	0.55
Solvent used (mL)	87	6
Solvent saved (mL)		81

separations because of the smaller column volumes and for less retained peaks, which have a lower peak volume making it even more critical to minimize extra column dispersion.

With sub-2 µm particles, operating parameters can be optimized to give fast analysis. This results in narrow chromatographic peaks which may be in the order of 1-2 s or less in width. It is important to scan the detector (whether it is UV or MS) fast enough to achieve optimum peak definition, otherwise resolution, efficiency and analytical accuracy will be compromised.

The HPLC pump dwell volume is particularly important when running high-speed applications using fast gradients, typical of high-throughput separations on small particle packed columns. This is because the pump dwell volume affects the time it takes for the gradient to reach the head of the column. If we consider a method using a flow rate of 0.4 mL/min and a fast gradient of 1 min, the theoretical gradient reaches the column immediately. A pump with a 65 µL dwell volume will get the gradient onto the column in 9.75 s. A traditional quaternary pump with a dwell volume

of 800 µL will take 2 mins to get the gradient to the column. When running rapid gradients this is too slow and it may become necessary to introduce an isocratic hold at the end of the gradient to allow elution of the analytes.

Conclusion

Transferring a method from a conventional 3 or 5 µm particle packed column to a sub-2 µm particle packed column can result in significant time and solvent savings. In this example, transfer of a method for the analysis of ibuprofen and impurities was successfully accomplished by geometrically scaling flow rate, injection volume and gradient profile. Analysis time was reduced from 87 mins to 11 mins (an eightfold saving), while at the same time maintaining equivalent resolution of the same critical pair. Solvent consumption was reduced from 87 mL per sample to 6 mL per sample — a considerable saving per analysis.

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This article was first published in Separation Science

Reprinted for

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driving analytical chemistry forwards

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