

Using Narrow Bore Columns to Enhance Sensitivity for LC-MS Analyses.

INTRODUCTION

Scaling down the inner diameter of the analytical column for a method can bring multiple benefits to the overall analysis workflow. Whilst it is an attractive option, there are a few notes of caution to ensure optimal and robust method performance. This short article examines different column diameters and the benefits they may provide for UV and MS analyses. Commonly observed reductions in method performance with smaller ID columns are also illustrated, and the proposed solutions to recapture method performance are highlighted and discussed.

COLUMN DIMENSIONS

Analytical LC columns are available in a range of different column geometries, with varying lengths and internal diameters (IDs). LC column ID varies considerably and popular dimensions can be broadly categorised according to Table 1. In many LC-UV-based analytical laboratories, the 4.6 mm ID column is the de facto standard and is suitable for a wide variety of applications. For those laboratories routinely operating LC-MS, typical column IDs can vary depending upon the main application areas. Bioanalysis labs may use larger ID columns for robustness purposes whilst other LC-MS

Table 1: Broad classification of LC columns by internal diameter.

Preparative	Semi-Preparative	Analytical	Microbore	Capillary/Nano
50.0 mm	21.2 mm	4.6 mm	2.1 mm	300 µm
30.0 mm	10.0 mm	4.0 mm	1.0 mm	100 µm
	7.75 mm	3.0 mm	0.5 mm	75 µm

labs may be advocates of the lower flow rates of 2.1 mm ID columns to enhance sensitivity of electrospray sources.

In general, for LC-UV, smaller ID columns will provide the same separation and response as larger ID columns, for isocratic or gradient methods, provided that the flow rate and injection volume are scaled accordingly.

REDUCING SOLVENT CONSUMPTION

If a smaller ID column is selected and the flow rate scaled to maintain the same linear velocity, then solvent consumption can be dramatically reduced. Figure 1 shows the isocratic separation of three analytes on a high performing 2 μm C18 column. The separation was run using a 50 x 4.6 mm column and then scaled to a 50 x 3.0 mm column to achieve identical retention and separation. To ensure the same retention and peak height are obtained, both the flow rate (F) and injection Volume (V) are scaled to the new column dimensions according to equations 1 and 2. The original flow rate of 1.0 mL/min is reduced to 0.43 mL/min to maintain a constant linear velocity of mobile phase flowing through the column, a reduction of 57%. In this case, the total

amount of solvent used per injection has been reduced from 6 mL to 2.6 mL.

$$F_2 = F_1 \times \frac{d_{c2}^2}{d_{c1}^2} \quad (1)$$

$$V_{i2} = V_{i1} \times \frac{V_{M2}}{V_{M1}} \quad (2)$$

where d_c is the column internal diameter and V_M is the column dead volume.

INCREASING SENSITIVITY

Another benefit that can be obtained by using smaller column IDs is increased sensitivity. To achieve this, the analysis is moved to a narrow bore column, and the flow rate scaled, as in Figure 1. However, unlike the previous example, the injection volume remains unchanged. The same sample volume is injected, with the net effect of increasing the concentration of the sample on column, leading to an increase in peak height and signal to noise ratio. Figure 2 (parts A & B) shows the same separation as Figure 1, this time with the injection volume kept constant. The peak height has increased approximately 2 fold when changing from a 4.6 mm to 3.0 mm ID.

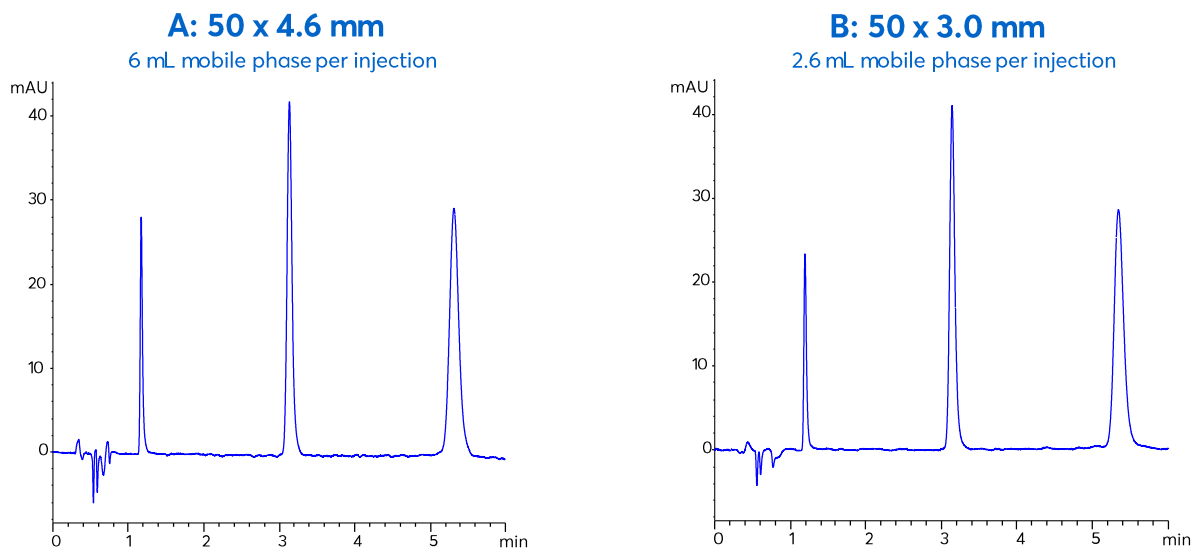


Figure 1: Translating an isocratic method to a smaller column ID to reduce solvent use. Column: Avantor® ACE® Excel 2 C18; Mobile phase: 0.1% formic acid in MeOH/H₂O 35:65 (v/v); Flow rate: A=1.00 mL/min, B=0.43 mL/min; Injection volume: A=1 μL, B=0.45 μL; Detection: UV, 235 nm; Sample: 1. caffeine, 2. aspirin, 3. 2-hydroxybenzoic acid.

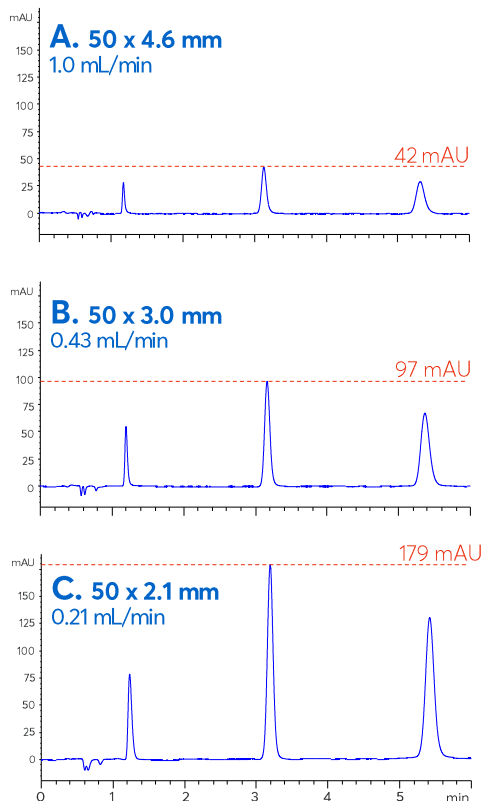


Figure 2: Increasing sensitivity by decreasing column ID. Column: Avantor® ACE® Excel 2 C18; Mobile phase: 0.1% formic acid in MeOH/H₂O 35:65 (v/v); Flow rate: 1.00 mL/min (4.6 mm ID), 0.43 mL/min (3.0 mm ID), 0.21 mL/min (2.1 mm ID); Injection volume: 1 µL; Detection: UV, 235 nm; LC system: binary UHPLC; Sample: 1: caffeine, 2: aspirin, 3: 2-hydroxybenzoic acid.

Taking this approach further, the sensitivity can be increased again using a 50 x 2.1 mm column (Figure 2C), with up to 4.3 times the peak height compared to the 4.6 mm column. This is attractive for applications with limited samples, or those requiring a sensitivity boost for low-level quantitation (the increased peak height response will improve the limits of detection and quantitation). Figure 3 illustrates a related substances analysis where improved visibility of the impurities present in the sample at 0.05%w/w, 0.10%w/w and 0.50%w/w are observed when scaling the flow rate from a 4.6 mm ID column to a 2.1 mm ID column but keeping a constant injection volume of 1 µL. Figure 4 shows how this approach can also be applied to LC-MS methods. In addition, for electrospray LC-MS applications, ionisation

efficiency and sensitivity are typically optimal at low flow rates used with narrow bore columns and so further benefits may be observed.

SOME CAVEATS FOR USING SMALL ID COLUMNS

Smaller ID columns such as 2.1 mm and 3.0 mm have lower sample loading capacity than larger columns so it is possible that poor peak shape may be experienced if using the same injection volume as the 4.6 mm ID column. In this case, the injection volume will need to be adjusted down on the smaller ID column. As a further practical note, the injection of samples in a diluent stronger (e.g. higher organic content in reversed-phase) than the mobile phase is more likely to result in distorted peak shape with smaller ID columns.

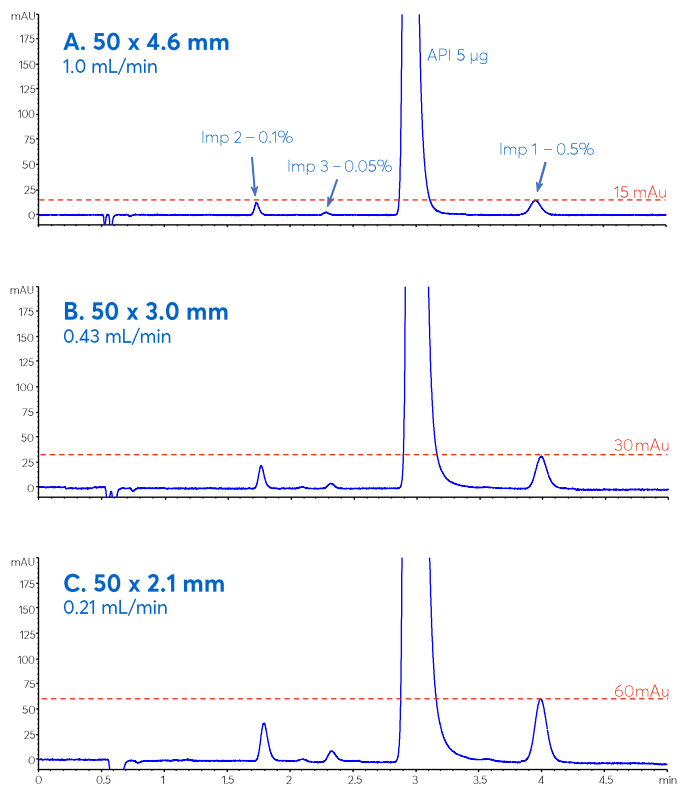


Figure 3: Increasing sensitivity for impurity detection by decreasing column ID. Column: Avantor® ACE® Excel 2 C18; Mobile phase: 0.1% formic acid in MeOH/H₂O 35:65 (v/v); Injection volume: 1 µL; Detection: UV, 214 nm; LC system: binary UHPLC; Sample: API: aspirin, Imp 1: 3-nitrophenol, Imp 2: 3-hydroxybenzoic acid, Imp 3: phenol.

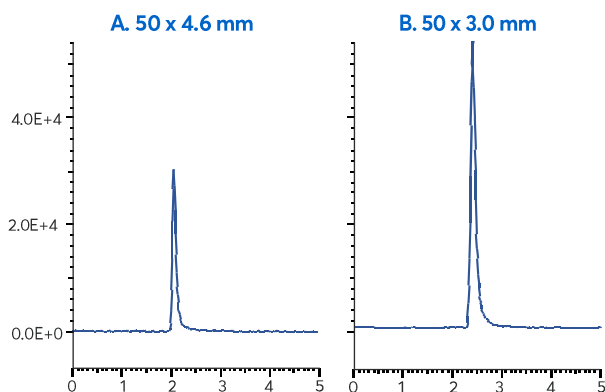


Figure 4: Increasing LC-MS sensitivity by decreasing column ID. Column: Avantor® ACE® Excel 2 C18; Mobile phase: 0.1% formic acid in MeCN/H₂O 40:60 (v/v); Flow rate: A=1.00 mL/min, B=0.43 mL/min; Injection volume: 1 µL; Detection: MS, positive mode, SIM (*m/z* 332.1); Sample: piroxicam.

THE DELETERIOUS EFFECTS OF DISPERSION AND EXTRA COLUMN BAND BROADENING WITH SMALL ID COLUMNS

The LC system used must have acceptable extra column volume for small ID columns (or more accurately the reduced column volumes found with small ID columns). Simple procedures for measuring extra column volume and system dispersion are available for those interested [1]. The effects of extra column dispersion (and resulting band broadening) becomes increasingly significant as column ID (and therefore column volume) decreases. This is especially significant for analytes with low retention (or small retention factor (*k*) values) commonly seen in rapid analysis LC-UV and/or LC-MS methods. To obtain the benefits of increased sensitivity with narrow bore columns (i.e. 3.0 and 2.1 mm ID), an optimised LC system, with low volume tubing and injector assembly and optimised flow cell geometry, should be used.

Even in the example shown in Figure 2, which was generated using an optimised UHPLC system, the impact of extra column dispersion on the rapidly eluting analyte can be observed. Figure 5 shows the peak height data from Figure 2, normalised to the peak heights obtained on the 4.6 mm ID column. For aspirin (*k* = 4.8) and 2-hydroxybenzoic acid (*k* = 8.8), a 2.3-fold increase in peak height is obtained for a 1 µL injection when the ID is reduced to 3.0 mm, whilst reducing the ID further to 2.1 mm provided a 4.3-fold increase over the 4.6 mm data.

For caffeine (*k* = 1.1), the same performance boost is not seen. On the 3.0 mm ID column, a similar 2.0 fold increase in peak height was observed. On the 2.1 mm column however, the peak height improvement is only 2.7-fold when compared to the 4.6 mm column. This smaller than expected increase in peak height is due to the effect of peak band broadening from the system dispersion on the less retained caffeine peak when using the 50 x 2.1 mm column.

To confirm the impact of dispersion, the 2.1 mm ID column separation was re-run on two 400-bar-rated HPLC systems with different configurations, and hence different amounts of system extra column volume. Figure 6A with 16 µL extra column volume shows the original UHPLC system separation. On the first HPLC system (Figure 6B, 62 µL extra column volume) the peak efficiencies, peak heights and signal to noise values are reduced compared to the UHPLC system. The early eluting caffeine peak (*k* = 1.1) is more affected than the later eluting peaks. On the second HPLC system (Figure 6C, 101 µL extra column volume) equipped with a column switching valve, the performance is even lower and the caffeine peak height was reduced by 88% to that obtained on the UHPLC system. It is additionally worth noting that if a closely eluting peak pair was present in the sample, analyte resolution would likely be compromised due to the loss in efficiency.

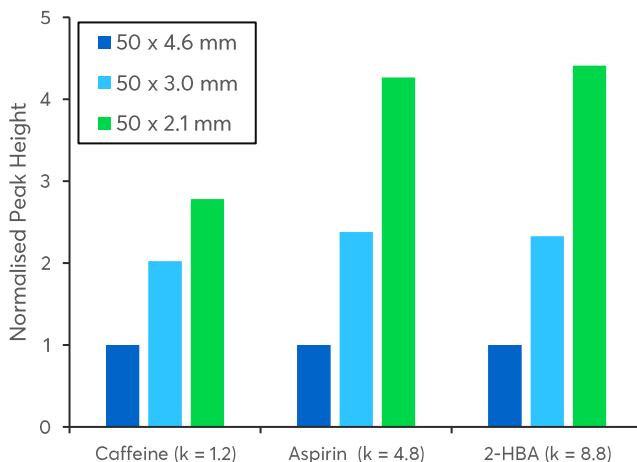
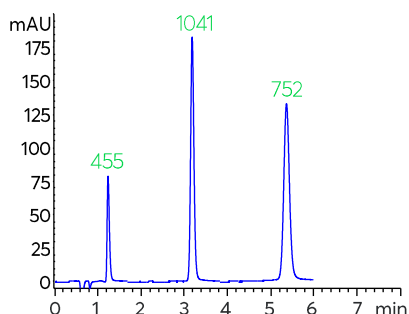


Figure 5: Peak height data for the three analytes on the three different column IDs shown in Figure 2. The peak heights for each analyte have been normalised to the peak height recorded on the 4.6 mm column ID.

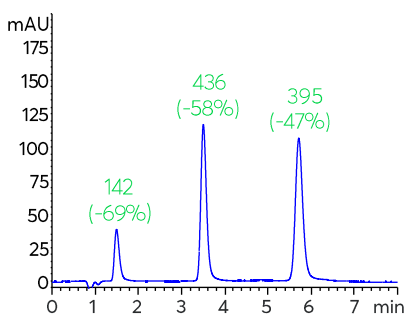
A. UHPLC system

16 μL extra column volume



B. HPLC system

62 μL extra column volume



C. HPLC system

101 μL extra column volume

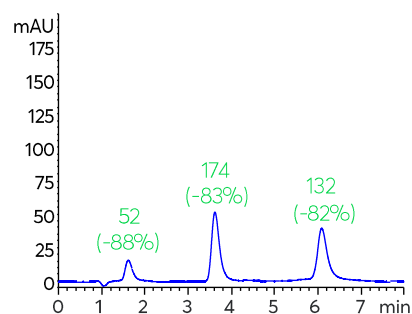


Figure 6: Comparison of performance for a 50 x 2.1 mm column used on a UHPLC instrument (A) and two differently configured HPLC instruments (B and C). The calculated signal to noise ratios are shown in green.

Column: Avantor® ACE® Excel 2 C18 50 x 2.1 mm; Mobile phase: 0.1% formic acid in MeOH/H₂O 35:65 (v/v); Flow rate: 0.21 mL/min; Injection volume: 1 μL ; Detection: UV, 235 nm; Sample: 1: caffeine, 2: aspirin, 3: 2-hydroxybenzoic acid.

CONCLUSION

By reducing the ID of the LC column, it is possible to both reduce mobile phase consumption and significantly boost sensitivity. This is a useful approach for low-level quantitation and LC-MS applications. However, it is important to consider that this approach can have limitations and that it is important to ensure that smaller ID columns are only used with suitably low extra-column volume or low volume optimised LC systems, so a performance loss is not observed.

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REFERENCES

1. Avantor® ACE® Knowledge Note #0017 "How to Determine Extra Column Dispersion and Extra Column Volume"