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Reducing the LC Column's Internal Diameter to Drive Sustainable Chromatography

LC 컬럼의 내경을 줄여 분석 효율을 높이고 환경 친화적인 크로마토그래피를 수행하는 방법

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Abstract

The white paper provides detailed guidance on optimizing chromatographic separations with Avantor ACE® columns. It emphasizes key factors for improving method development in HPLC, including the selection of stationary phases, understanding particle size effects, and adjusting operating parameters like flow rate and temperature to maximize resolution and efficiency. The document addresses challenges in method scalability and transfer, offering practical solutions for translating methods between instruments with differing column dimensions or flow rates. It includes mathematical equations and examples to ensure accurate method adjustments during translation. Additionally, the paper underscores the importance of selecting appropriate mobile phase compositions to minimize retention time variability and maximize peak sharpness. With a focus on achieving reproducibility and robustness in separations, this resource is tailored for chromatographers aiming to streamline complex analytical workflows while maintaining high performance across diverse applications.

Keywords

Liquid Chromatography, LC column, Column's Internal Diameter, Analytic Method Adjustment, Reproducibility in Separation

Chromatography Solutions

Knowledge note #0035

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INTRODUCTION

In many industry sectors, sustainability has become an important aspect. At Avantor, sustainability is focused on four key pillars most meaningful to our business:[1]

- People and culture
- Innovation and the environment
- Governance and integrity
- Community engagement

This short article focuses on one of these aspects: reducing the environmental impact of analytical laboratories; a topic that has become increasingly important as organisations strive to reduce their environmental footprint. Key focus points include reducing energy use, solvent use and waste created, as well as implementing practices to source responsibly, e.g. sourcing from suppliers with clear and measurable sustainability goals that are reported publicly for transparency and integrity.[1]

From the analyst's perspective, sustainable laboratory practices are tightly coupled to their daily operational tasks, which include purchasing and consumption of solvents, reagents, and waste generation. In the case of liquid chromatography (LC), significant reductions in solvent use can be readily achieved with narrower column internal diameters (IDs), which are operated at reduced flow rates.

Adopting narrow-bore columns is an attractive option to downscale the methodology and consumables, particularly for expensive and/or sample limited assays. Understanding the balance between the advantages and disadvantages of reducing the column's ID can lead to methods that do not compromise significantly on resolution and can provide impressive gains in sensitivity. This article demonstrates how narrow-bore columns can decrease the laboratory's environmental impact and increase economic sustainability, whilst also maintaining the separation performance. The use of these columns to increase assay sensitivity is also discussed. Whilst reducing the ID is an attractive option, there are a

few notes of caution to ensure optimal and robust method performance, which are covered later in this article.

REDUCING THE COLUMN INTERNAL DIAMETER

As organisations begin to focus on promoting environmental sustainability goals by reducing their environmental impact, downscaling the column's ID is one of the simplest ways to implement environmentally friendlier practices for LC analyses. Reducing the ID results in the decrease of solvent consumption and waste production because the column is operated at significantly lower flow rates compared to wider bore columns. Additionally, the manufacturing process of a narrow-bore column itself uses less materials compared to the analytical-scale column (with respect to stationary phase and stainless steel). It is important to note that the cost of manufacture is still not relatively cheap due to the increased difficulty of packing a smaller column where the wall effect becomes more prominent.[2]

In many LC-UV-based analytical laboratories, 4.6 mm ID columns are commonly used and are suitable for a wide variety of applications. Relative to the 2.1 mm ID column, the 4.6 mm ID is more robust and the sample loading is higher. Scaling down the ID of the analytical column for a method can bring multiple benefits to the overall analysis workflow for both LC-UV and LC-MS methods. Table 1 and Figure 1 clearly demonstrate how reducing the column ID, coupled with running at a reduced flowrate, can significantly impact solvent consumption and drive improved laboratory sustainability. For LC-MS laboratories, 2.1 mm ID columns are ideally suited to the low volumetric flowrates required to enhance the ionisation process at the ESI interface.

Table 1: Solvent consumption and waste reduction, for different ID columns with a fixed analysis time of 10 min, column length (L) of 15 cm, particle size (d_P) of 3 µm, and L/d_P ratio of 50,000.

Column ID (mm)	Flow (mL/min)	Solvent use per injection (mL)	Reduction in waste
4.6	1.0	10	-
3.0	0.4	4	60%
2.1	**0.2**	**2**	**80%**

Highlighted in bold is a drastic 80% reduction in solvent consumption and waste production that can be achieved when reducing the ID from 4.6 to 2.1 mm, significantly impacting environmental and business sustainability. For separations which may be impacted by the effects of post column dispersion, the 3.0 mm ID is still an optimistic alternative with a 60% reduction of solvent consumption and waste production, relative to the 4.6 ID column of the same L/d_P ratio.

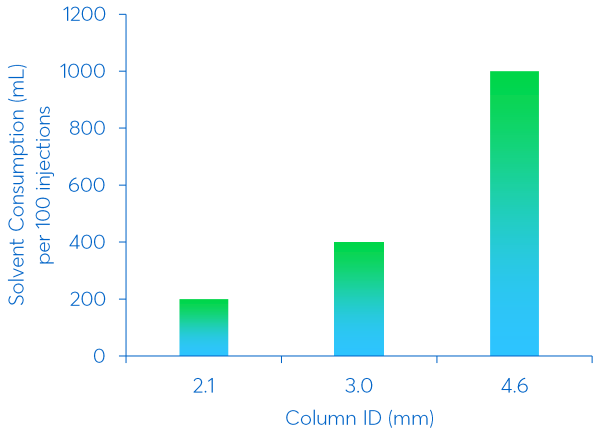

Figure 1: Solvent consumption (mL) for 100 injections for columns with differing IDs (other column properties as per Table 1).

METHOD TRANSLATION

To translate a method to a smaller column ID, the flowrate must be scaled to maintain a constant linear velocity of mobile phase through the column. This will ensure that separation performance, selectivity and retention remain approximately the same. The flow rate for the narrower ID column (F_2) can be easily determined through use of equation 1, where F_1 and d_{c1} is the flow rate of the original method and d_{c2} is the new columns ID. The Avantor® ACE® LC Translator Tool can also be used to automatically determine the required flow rate.[3]

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

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 (equation 2). As a further practical note, the injection of samples in a diluent that is stronger (with respect to eluotropic strength, 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.

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

Where V_i is the injection volume and V_M is the column dead volume.

Figure 2 demonstrates the use of these two equations to reduce the column ID, firstly from 4.6 mm to 3.0 mm and then to 2.1 mm for a complex peptide mapping separation. As shown, the selectivity and resolution of the separation is well maintained on the different ID columns. Similarly, despite the reduced injection volume, the sensitivity obtained is equivalent, highlighting the applicability of narrow bore columns to sample limited assays. A small reduction in peak capacity was observed for the 2.1 mm ID column compared to the 3.0 and 4.6 mm ID columns, due to the more significant impact of extra column volume and wall effects on this narrower

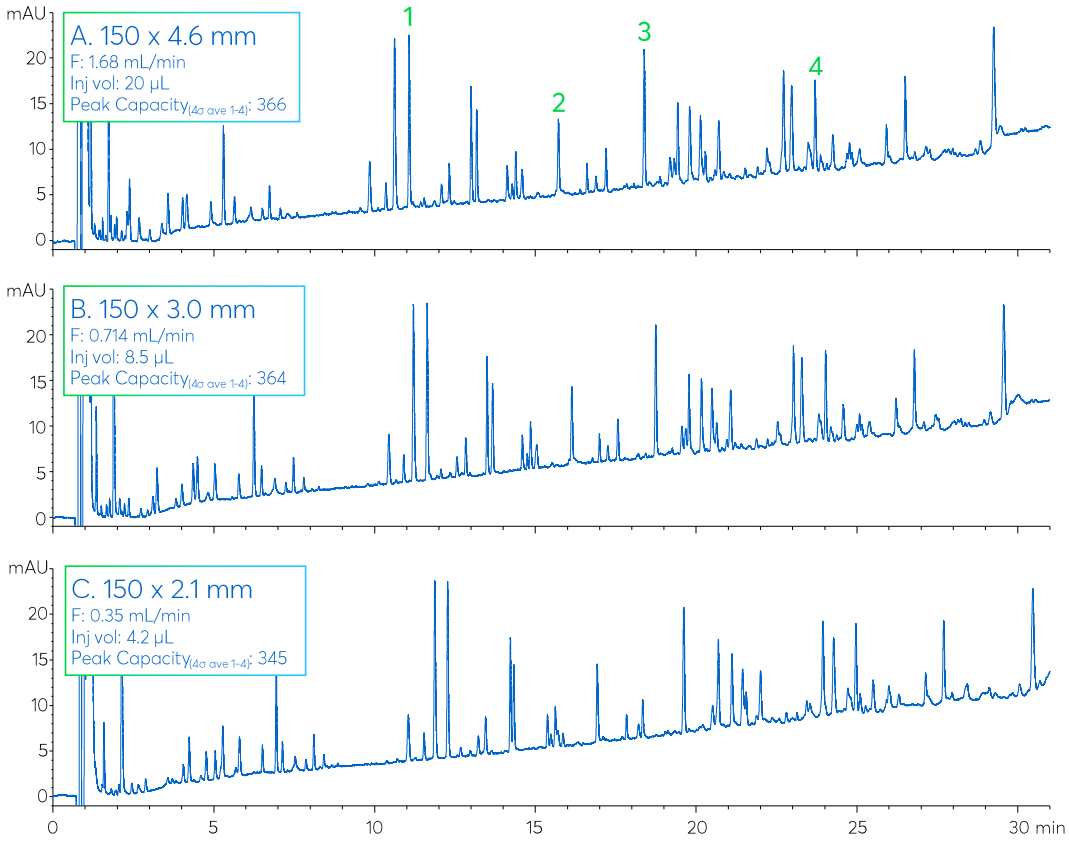

Figure 2: Scaling the ID of the analytical column used for the analysis of a bovine serum albumen peptide digest sample from (A) 4.6 mm to (B) 3.0 mm and (C) 2.1 mm using equations 1 and 2. Peak capacities stated are the average determined for peaks 1-4 in each chromatogram. Columns: Avantor® ACE® UltraCore 2.5 SuperC18; Mobile phase A: 0.05% TFA in water, B: 0.05% TFA in MeCN; Gradient: hold at 5% B for 1.5 minutes* then 5-35 %B in 30 minutes, then 35-96 %B in 5 minutes; Temperature: 60°C; Detection: UV, 214 nm.

*The isocratic hold duration was adjusted to compensate for the change in the VD/VM ratio when translating to a smaller ID column (see Reference 4 and 5 for further details).

column ID, but this had minimal impact on the separation and sensitivity obtained. Table 2 highlights the considerable reductions in mobile phase consumption that are achieved when translating this separation to narrower bore columns, which are comparable to those stated in Table 1.

INCREASING SENSITIVITY

Another benefit that can be obtained by using smaller ID columns is increased sensitivity. To achieve this, the same sample volume is injected as on the wider bore column, 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 3

Table 2: Reduction in mobile phase consumption and waste generated that was achieved per injection by translating the peptide mapping example shown in Figure 2 to narrower ID columns.

Column ID (mm)	Flow (mL/min)	Mobile phase per injection (mL)	Reduction in waste
4.6	1.68	92.0	-
3.0	0.714	38.3	58%
2.1	**0.35**	**18.4**	**80%**

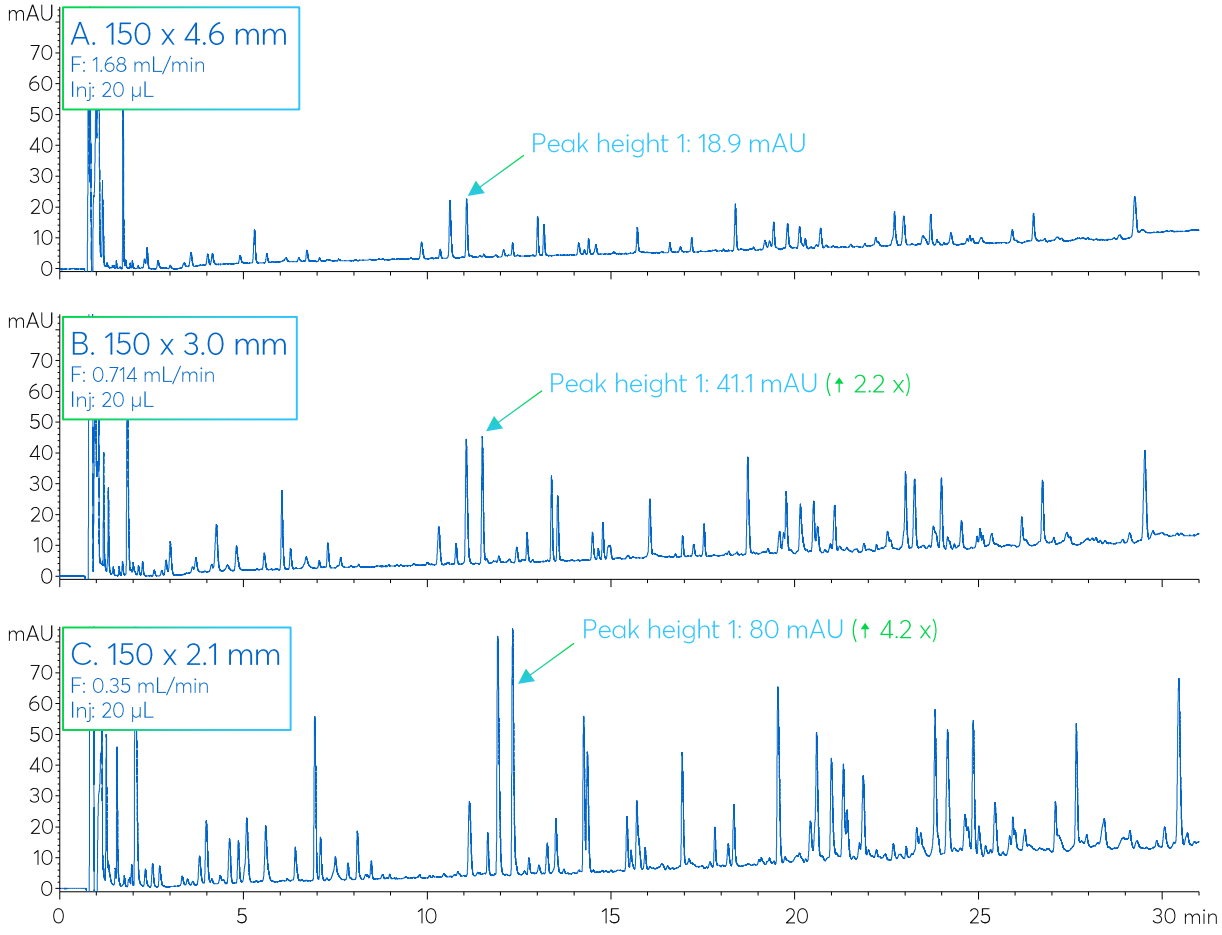

Figure 3: Increasing assay sensitivity by decreasing the column ID whilst maintaining injection volume (A) 4.6 mm to (B) 3.0 mm and (C) 2.1 mm using equation 1. Method conditions as per Figure 2.

shows the same separation as Figure 2, this time with the injection volume kept constant. The peak height increased >2-fold when changing from a 4.6 mm to 3.0 mm ID. Taking this approach further, the sensitivity can be increased again using a 150 x 2.1 mm column (Figure 3C), with up to 4.2 times the peak height compared to the 4.6 mm column. This is attractive for applications with limited sample volumes, or those requiring a sensitivity boost for low-level quantitation (the increased peak height response will improve the limits of detection and quantitation). For electrospray LC-MS applications, ionisation efficiency and sensitivity are typically optimal at the low flowrates used with narrow bore columns and so further benefits may be observed and has been shown previously.[6]

INSTRUMENT DISPERSION

An important consideration when scaling down column ID (or more accurately column volume) is the negative impact of instrument dispersion and band broadening on observed peak efficiency. Instrument dispersion has a proportionally greater negative impact on column performance as the column dimensions are reduced, as shown in Figure 4. As the column ID is reduced from 4.6 mm through 3.0 mm to 2.1 mm, the system variance (extra column volume) becomes more impactful on the efficiency that is obtained from the column. This effect 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.

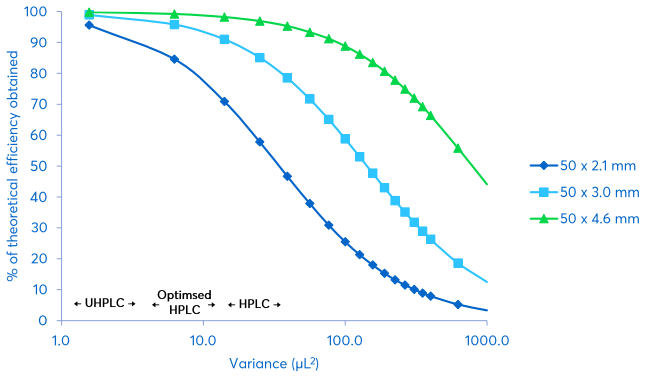

Figure 4: Plot of theoretical efficiency obtained for an analyte with retention factor (*k*) of 5 on columns with differing internal diameters (ID) as the system variance (i.e. extra column dispersion) is increased. Also shown are approximate variance ranges typical for HPLC, optimised HPLC and UHPLC systems.

Significant contributions to extra-column volume include system tubing in the flow path, injector tubing, detector flow cell volume and any poor-quality tubing connections (e.g. with the analytical column). For traditional larger format columns, such as 150 x 4.6 mm, the impact of system extra-column volume is negligible relative to the large column volume. However, in the case of smaller format columns (e.g. 2.1 and 3.0 mm ID), which have smaller column dead volumes, the effects of extra-column volume may be observed. It is therefore important to consider the potential impact of extra-column dispersion on the new separation and to ensure that lower-dead-volume, narrow-bore columns are only used with suitably optimised instrumentation.

If the separation performance (efficiency and resolution) is observed to decrease when moving a method to a smaller ID column, it is likely that optimisation of the system components outlined above may be required to restore the expected performance. In general, for isocratic separations in particular, 4.6 mm ID columns are recommended for use on HPLC systems, 3.0 mm ID for use on optimised HPLC and UHPLC systems and the 2.1 mm ID should be limited to use on low dispersion UHPLC systems. Columns with 1.0 mm ID and smaller should only be used with the appropriate capillary or 'nano' LC. For further details, please refer to references 4 and 7-9.

CONCLUSION

This Knowledge Note highlights how reducing the LC column internal diameter can help significantly reduce the environmental impact of liquid chromatography and move towards more sustainable laboratory practices. Solvent consumption and waste production can be reduced by around 60% when reducing the column's ID from 4.6 to 3.0 mm, whilst a drastic reduction of 80% is achieved on decreasing the ID from 4.6 to 2.1 mm. This article has demonstrated that the analytical performance of LC assays can readily be maintained when moving to smaller ID columns, providing the flowrate and injection volumes are correctly scaled. In addition, the approach of reducing column ID to boost sensitivity was also showcased, 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 LC systems with optimised extra column volumes, so a performance loss is not observed.

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