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SUPELCO

Supelco Park • 595 North Harrison Road
Bellefonte, PA 16823-0048 USA
Telephone 800-247-6628 • 814-359-3441
Fax 800-447-3044 • 814-359-3044
email: supelco@sial.com
sigma-aldrich.com

Application Note 167

Increase Sensitivity and Decrease Sample Consumption Using HPLC Microbore and Capillary Column Dimensions

In the world of modern HPLC separations, smaller is often better. Columns with narrow ID give taller peaks for enhanced sensitivity, and consume less sample. These two features make them ideal for applications where the need is to see compounds that exist at very low concentration in small sample volumes. The low flow rates and miniscule solvent consumption also makes narrow ID columns ideal for LC/MS applications because of the lower desolvation volume. This short article provides some background information and the practical application of small ID columns and capillaries.

Key Words:
● column ID ● LC/MS ● gradient elution ● sensitivity

Why is There a Trend Toward Smaller ID Columns?

Proteomics and other areas of modern biological research often generate large numbers of samples containing very small volumes that need to be analyzed in a minimal amount of time. Additionally, compounds of interest in these samples may exist at very low concentrations. When sample concentrations and volumes are sufficiently small, injection onto conventional internal diameter (ID) columns (4.6mm), and even narrowbore (2.1mm), immediately reveals that current means of detection lack adequate sensitivity for satisfactory analysis. This may be the case whether detection is by UV light absorption or mass spectrometry where inlet systems can be concentration dependent as well. This problem of detection sensitivity with conventional ID columns is a simple result of sample dilution within the relatively large volume comprised by the column and tubing. A direct approach to reducing the extent of dilution and to increase sensitivity is to reduce the column volume. When less diluted by the column volume, the peaks are narrower and taller as opposed to broader and more diffuse. Reducing column ID is one method to reduce column volume. One can detect levels thousands of times lower by decreasing the column ID. The use of smaller ID columns is also a valid strategy simply to increase sensitivity even where there are no sample volume restrictions. The data in Table 1 shows the flow rates, injection volume, sensitivity, and mobile phase consumption of small ID relative to conventional 4.6mm ID columns. These values were calculated by multiplying the ratio of the cross-sectional areas, which reduces to the ratio of the square of the internal diameters.

Increased Sensitivity Demonstrated

Figure A (on reverse) shows the observed behavior of increased sensitivity on columns of decreasing ID. The same sample was injected onto Discovery BIO Wide Pore C18 columns of equal length (10cm) but varying ID from 0.32mm to 2.1mm, on the same chromatographic system. Linear velocity (L(cm)/t_0(min) was held constant, an important consideration when comparing columns of different diameters. The relative corresponding peak heights closely approximate what is mathematically predicted in Table 1. The small differences in retention times of corresponding peaks between the columns are due to differences in system dead volumes (the volume of tubing from and including the injector to the column inlet, plus the volume from the column outlet to the detector) relative to the volumetric flow rate. For instance, tubing ID used in the case of the 0.32 and 0.50mm ID columns was 25µm, for the 1mm ID column was 50µm, and for the 2.1mm ID column was 75µm. Ideally, tubing ID would scale with column ID, but that becomes impractical. If 75µm tubing is used for a 2.1mm ID column, then a corresponding tubing ID for a 0.32mm ID column would be <2µm. Not only is such tubing not readily available, but it would be difficult to keep such a fluid path adequately free of obstruction.

Table 1. Effect of Column ID on Relative Sensitivity and Sample Consumption

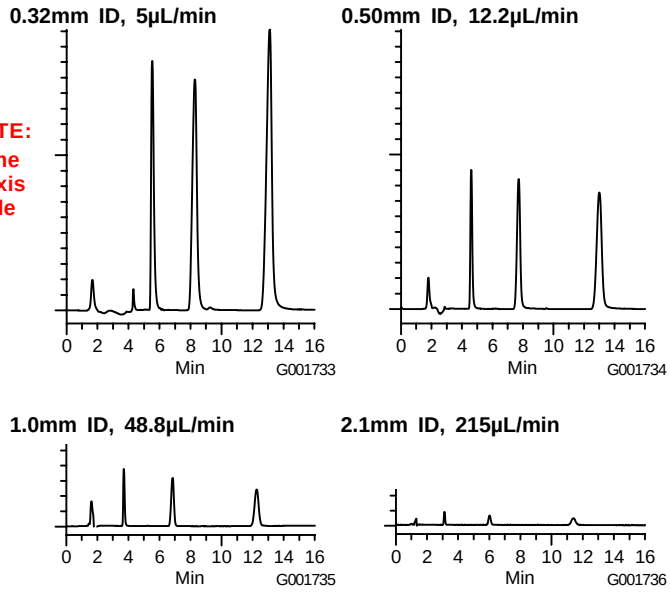
ID*	Flow Rates	Injection Volume	Sensitivity	Mobile Phase Consumption	Typical Flow Rates	Typical Column Composition
4.6mm	1.0	1.0	1	1.0	1 - 2 mL/min	stainless steel
3mm	0.42	0.42	2	0.42	0.4 - 0.1 mL/min	"
2.1mm	0.21	0.21	5	0.21	0.2 - 0.4 mL/min	"
1mm	0.047	0.047	21	0.047	50 - 100 µL/min	glass-lined stainless steel
0.5mm	0.012	0.012	85	0.012	10 - 20 µL/min	"
0.32mm	0.0048	0.0048	207	0.0048	5 - 10 µL/min	fused silica
0.18mm	0.001531	0.001531	653	0.001531	1 - 5 µL/min	"
75µm	0.000266	0.000266	3,762	0.000266	0.2 - 1 µL/min	"
50µm	0.000118	0.000118	8,464	0.000118	0.1 - 0.2 µL/min	"
25µm	0.000030	0.000030	33,856	0.000030	< 0.1 µL/min	"

* assumes columns are of the same length

Figure A. Comparison of Peak Height (Sensitivity) Between Columns of Different Internal Diameters

Column: **Discovery BIO Wide Pore C18, 300Å pores, 10cm length x 0.32mm ID, 0.50mm ID, 1.0mm ID or 2.1mm ID, 3µm**
Mobile Phase: (35:65) $CH_3CN:H_2O$
Flow Rate: shown on figures
Temp.: ambient
Det.: UV, 254nm
Inj.: 1µL
Sample: acetophenone (0.1µg), benzene (1µg), toluene (1µg)

NOTE: Same Y-axis scale



Instrument Considerations

When scaling down from standard bore columns to microbore (1mm ID) or less, modifications to system plumbing must be made. However, in the case of gradient elution methods, one system parameter which needs close attention is the system dwell volume. This is the volume from and including the mixer to the column inlet, and effectively represents a volume in which the gradient will be delayed before reaching the column. For instance, a system dwell volume of 500µL represents a 0.5 minute delay if pumping at 1mL/min on a standard bore column. However, if a 1mm ID column is used on that same system at the same linear flow rate, that 500µL represents at least a 10 minute delay. Furthermore, that gradient delay is also effectively an isocratic step prior to gradient elution and as such can significantly affect chromatographic results, usually negatively. For example, the 500µL dwell volume as an isocratic step is approximately 43% of the column volume of a 5µm material packed into a 10cm x 4.6mm ID column. With the same material packed into a 10cm x 1mm ID column, that 500µL is approximately 900% of the column volume. Thus it is easy to appreciate how this fixed system parameter can drastically affect column performance.

The effect is even more dramatic as one scales down column ID even further. Therefore, it is recommended that when scaling down the column ID, avoid the low-pressure mixing systems where the dwell volume is usually larger and fixed. Instead use a high pressure mixing system where the user has some control of the dwell volume in conjunction with tubing selection regarding length and ID. Binary or ternary mixing housings combined with mixing cartridges of volumes ranging from 2 to 250µL provide considerable flexibility in mixer configurations. These parts appear in the Ordering Information below.

One other subject is the issue of how low the HPLC pump can deliver such low flow rates reliably. New HPLC systems are available that are designed to operate at low flow rates. However, if the desired flow rate is below what the pump can reliably deliver, then flow splitting can be employed to modify the flow path so as to step down the flow rate prior to the injector to a suitable level.

In conclusion, if there is a need to increase sensitivity to see compounds lower levels or conserve precious samples, reducing column ID is a viable and effective option.

Ordering Information

(Other dimensions available. Please call or visit our web site.)

Description	Cat. No.
Discovery BIO Wide Pore C18	
10cm x 0.32mm ID, 3µm	**65527-U**
10cm x 0.5mm ID, 3µm	**65518-U**
10cm x 1mm ID, 3µm	**65506-U**
10cm x 2.1mm ID, 3µm	**567201-U**
ASI High-Pressure Mixing Chambers*	
ASI SS Binary Micro-Mixer Housing	**56666-U**
ASI SS Ternary Micro-Mixer Housing	**56667-U**
ASI High-Pressure Cartridges*	
ASI 2µL SS Micro-Mixer Cartridge	**56661-U**
ASI 5µL SS Micro-Mixer Cartridge	**56662-U**
ASI 10µL SS Micro-Mixer Cartridge	**56663-U**
ASI 25µL SS Micro-Mixer Cartridge	**56664-U**

* The highly-efficient cross-flow shearing mechanism in the ASI static mixer produces vortex mixing over a wide range of flow rates. Use the binary input housing to combine two flowpaths into one, such as in postcolumn applications. Use the in-line housing when additional mixing is needed in a single flowpath. Within each product series, the mixer cartridges are interchangeable.

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