Column Dimensions & Related HPLC Parameters

Flow Rate Reduction, Solvent Savings

The Waters Alliance[®] Separations Module is the heart of an Alliance System. It has the capability to deliver accurate and precise flow rates and gradients from 50 uL/min to several mL/min (see WPP201 and WPP202). This makes it possible to use a wide range of chromatographic columns of different diameters and lengths. Some of the considerations for choosing a certain size column are listed below.

	Narrower Columns	Shorter Columns
Analytical Method Requirements		
Increased sensitivity	х	
Decreased solvent consumption and disposal	х	х
Decreased sample requirements	х	
Shorter analysis time		х
HPLC Instrument Considerations		
Precise flow rate	х	х
Accurate and precise solvent proportioning	х	х
System volume	х	х
Extracolumn band broadening	х	х

Standard columns for reversed phase, normal phase, and ion exchange chromatography typically range from 3.9 to 4.6 mm internal diameter and 15, 25, and 30 cm in length. Standard columns for gel permeation chromatography (GPC) are 7.8 mm by 30 cm. Flow rates are typically 1 to 2 mL/min. Narrowbore columns have smaller diameters, typically 2 and 3 mm for LC and 4.6 mm for GPC. Flow rates are typically 200 to 300 mL/min. Microbore columns are usually 1 mm or less in diameter with flow rates about 50 mL/min.

When using narrower diameter columns, the performance requirements of the HPLC solvent delivery system are more stringent because the flow rates are reduced. Therefore, small changes in flow have a more significant influence on chromatographic performance. Greater precision of flow rate and solvent proportioning are required at the lower flow rates for good retention time reproducibility.

Also with narrow diameter columns, extracolumn bandspreading must be minimized to maintain the chromatographic resolution. For isocratic separations, the bandspread must be minimal before and after the column. For gradient separations, the bandspreading must be minimal and is more important after the column.

Scaling factors for different size columns

Column Diameters

The calculations are relative to a 4 mm i.d. for LC and 7.8 mm for GPC. This is the scaling equation: $(I.D. 1)^2 / (I.D. 2)^2$. A relatively large savings in solvent can be gained by moving from a 4.6 mm to a 3.0 mm column. The increases in sensitivity are theoretical. The actual gain will depend on the extracolumn bandspreading of the peak caused by tubing volume and flow cell geometry.

Туре	Column Diameter, mm	Relative Flow Rate	Relative Sensitivity per same sample load	Relative Sample Load	Relative Solvent Savings,%
LC	4.6	1.32	0.7	1.32	-30
LC	4	1	1	1	0
LC	3.9	0.95	1.1	0.95	5
LC	3	0.56	1.8	0.56	44
LC	2.1	0.28	3.6	0.28	72
LC	2	0.25	4	0.25	75
LC	1	0.06	16	0.06	94
GPC	7.8	1	1	1	0
GPC	4.6	0.25	4	0.25	75

Assume the same column length containing the same packing.

Column Length

Values are referenced to 15 cm length. The scaling equation is: Length1/ Length 2. The decrease in pressure with shorter columns allow flow rates to be increased, further shortening analysis times.

Column Length cm	Relative Analysis Time	Relative Backpressure	Relative Solvent Savings,%
30	2	2	-100
25	1.67	1.67	-83
15	1	1	0
10	0.67	0.67	33
5	0.33	0.33	67
3	0.20	0.20	80
2	0.13	0.13	87

Assume the same column diameter, flow rate and packing.

With traditional HPLC instruments, modifications are generally required to obtain adequate performance with narrower or shorter columns (e.g. add or remove mixers, pulse dampeners, tubing, etc.). With advances in solvent management technology, the Waters Alliance Separations Module can now take advantage of different size columns to optimize separations without instrument modifications to achieve superior performance.