Selecting an LC Column

Column Dimensions

Particle Size and Column Length

When choosing a column, the first two parameters that should be considered are the particle diameter and column length. These two parameters are the major contributors to separation efficiency (N), also known as theoretical plates. The number of theoretical plates is directly proportional to the length of the column over the diameter of the particle.

Equation 1 The resolution equation defines variables affecting separations.

$$R = 1/4 \sqrt{N} \times \left(k' / k'+1 \right) \times \left(\alpha-1/ \alpha \right)$$

Efficiency — Retention capacity — Selectivity

Particle Diameter

Particle diameter (dp), is commonly expressed in micrometers (µm), and has an inverse relationship to the efficiency of the separation. As the particle diameter decreases, the efficiency of the separation increases proportionately. If all other parameters remain equal, a 3 µm particle diameter offers an approximate 60% increase in efficiency over a 5 µm particle, and a 1.9 µm particle diameter offers an additional 60% over a 3 µm particle. System backpressure also increases proportionally as particle size decreases. Selecting the proper particle diameter is a way of controlling separation efficiency, and even analysis speed, but is limited by the pressure capabilities of the system. Often, particle diameters are determined by instrumentation. Table I is a guideline for selecting the optimal particle size, based upon pressure capability for common mobile phases.

Table I Emperically determined maximum pressures exhibited for acetonitrile and methanol gradients for various particle sizes and flow rates

Bold blue numbers represent optimal linear velocity for the given particle size and ID. For longer column lengths, the approximate pressure corresponds to the increase in column length. A 2-fold increase in column length yields a 2-fold increase in back pressure.

Flow rate (mL/min.)	Pressure (psi) Acetonitrile @ 25°C			Flow rate (mL/min.)	Pressure (psi) Methanol @ 25°C		
	1.9µm	2.2µm	3µm		1.9µm	2.2µm	3µm
0.2	2436	1755	1045	0.2	3198	2304	1371
0.3	3655	2633	**1567**	0.3	4797	3455	**2057**
0.4	4873	3510	2090	0.4	6395	4607	2743
0.5	6091	**4388**	2612	0.5	7994	**5759**	3429
0.55	**6700**	4826	2873	0.55	**8794**	6335	3771
0.6	7309	5265	3135	0.6	9593	6911	4114
0.7	8527	6143	3657	0.7	11192	8062	4800
0.8	9745	7020	4180	0.8	12791	9214	5486
0.9	10964	7898	4702	0.9	14390	10366	6171
1	12182	8775	5224	1	15989	11518	6857

Data are for 2.1 x 50 mm columns using a gradient of 5% B to 95% B (A: water, B: organic solvent). See Table II for optimal flow rates for alternate column internal diameters.

When choosing a particle diameter, it is not recommended to operate significantly below the optimal linear velocity, as losses in efficiency can be observed due to axial dispersion. As a quick estimate of particle diameter usability, check the optimal linear velocity for the organic solvent used and ensure maximum pressures observed are within the pressure specifications of your instrument. Please note that these are maximum pressures observed during gradient analyses. Isocratic mobile phases of lesser viscosity will operate with less back pressure.

Column Length

Column length (L) directly relates to efficiency. Increasing column length increases efficiency. It is important to note that column length is not an ideal way to increase resolution. Doubling the column length yields only a 1.4x gain in resolution (efficiency is a square root term in the resolution equation), while doubling both analysis time and system backpressure. Shorter column lengths are suitable for fast gradients and higher sample throughput, while longer column lengths are more suitable for higher peak capacity and shallow gradients.

Column Internal Diameter

Column internal diameter (ID) is the inner diameter of the column hardware holding the packing material, and is commonly expressed in millimeters (mm). Column ID is ultimately related to efficiency and flow rate through the van Deemter equation. This chromatographic concept relates column efficiency (often called band broadening) to linear velocity. Linear velocity is the distance mobile phase travels per unit time, while flow rate is the volume of mobile phase per unit time. A specific linear velocity has a flow rate that is dependent upon the internal diameter of the column. As column ID is lowered, a lower flow rate is needed to maintain the same linear velocity. Flow rate is the volume of mobile phase needed to create the desired liner velocity. It is important to note that as particle size decreases, optimal linear velocity increases. Columns with smaller particle sizes, namely 1.9 and 2.2 µm, are capable of running much higher flow rates and therefore creating higher sample throughput. Table II (next page) can be used to find the optimal flow rate, as it relates to particle size and internal diameter, and is a good starting point for method development.