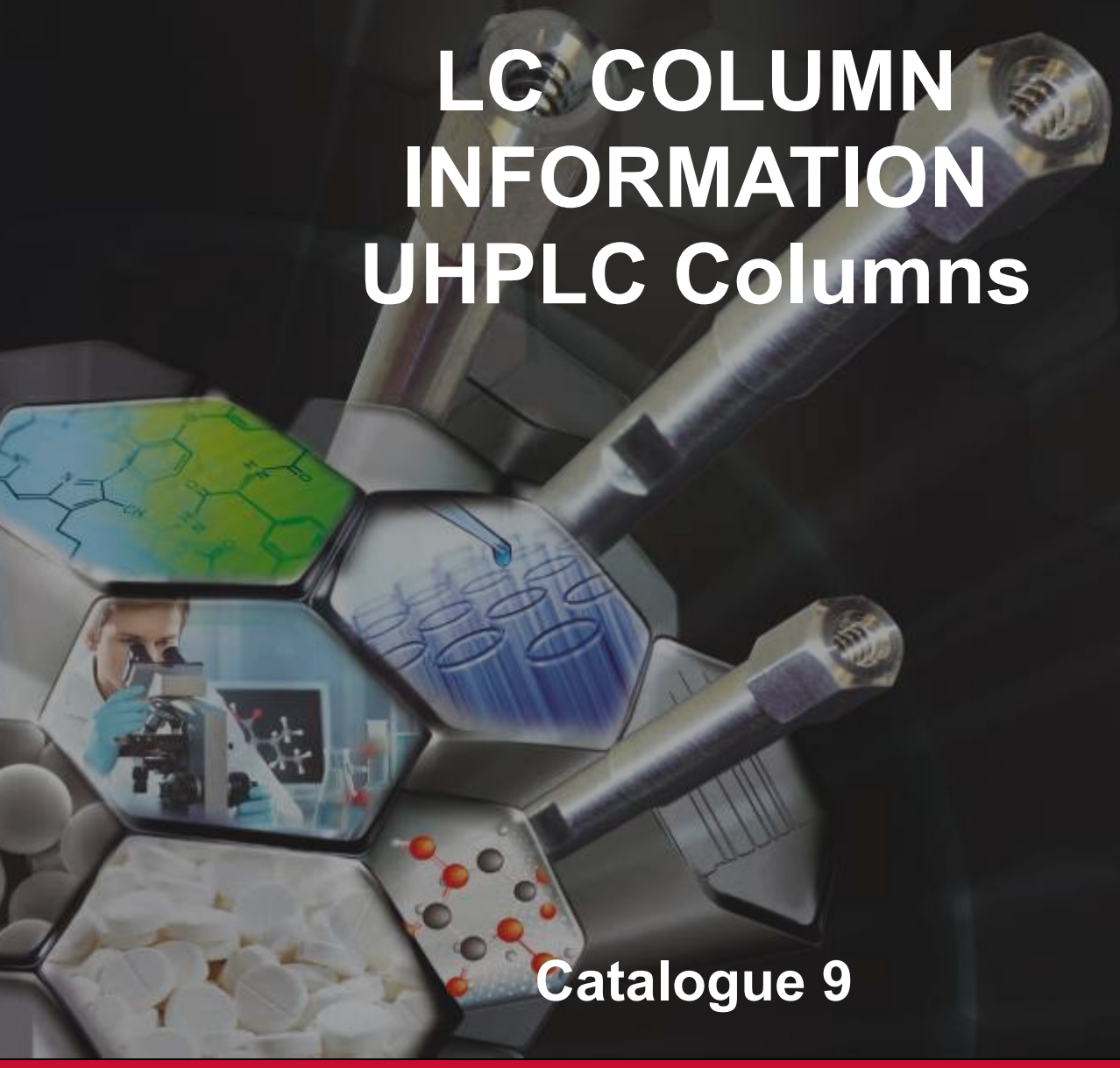
HICHROM

Chromatography Columns and Supplies

LC COLUMN INFORMATION UHPLC Columns



Catalogue 9

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UHPLC COLUMNS (≤ 2µm Particle Size)

- Decrease in analysis time
- Shorter columns with high separation efficiency
- Significant improvement in resolution
- Increased detection sensitivity

Introduction

There is an increasing demand for high throughput analysis and columns capable of ultra fast run times. A requirement for increased column efficiency has led to the development of smaller particle size materials. The introduction of ultra high pressure pumps and low dispersion hardware has enabled the use of smaller particle sizes to become more practical. However, many smaller particle columns have now been specifically designed so that they may still be used on conventional HPLC equipment, in addition to high pressure UHPLC systems.

Features of UHPLC Silica Particles

Resolution Equation

$$R_s = \frac{1}{4}\frac{(\alpha - 1)}{\alpha}\sqrt{N}\,\frac{k}{1+k}$$

As the resolution equation shows, resolution (Rs) is proportional to the square root of separation efficiency (N). Efficiency is inversely proportional to particle size (dp). This means that when particle size is reduced, column length can also be reduced, whilst still maintaining constant efficiency. Using a shorter column with smaller particles, at the same flow rate as the larger particle column, means that analysis time can be considerably reduced. The optimal flow rates for particles of ≤2µm are higher than for 3 and 5µm particles (see Figure 1).

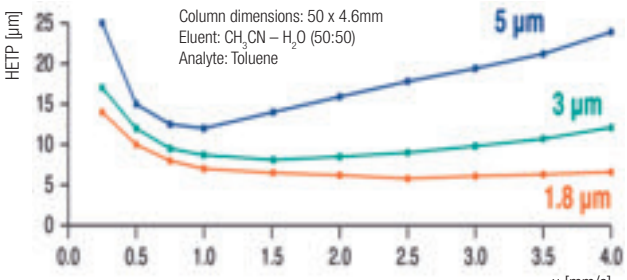

Figure 1. Van Deemter plot

Table 1. Particle size effects

Particle Size (µm)	Resolution (Rs)	Efficiency (N) (plates/m)	Pressure (psi)
Proportional to	$1/dp^{1/2}$	1/dp	$1/dp^2$
2	2.97	200,000	5000
3	2.57	150,000	2250
3.5	2.39	130,000	2000
5	2.00	90,000	1000
10	1.48	50,000	300

Column: 100 x 4.6mm Eluent: $CH_3OH – H_2O$ (85:15) Flow rate: 1.0ml/min Temperature: 22°C

Column Back Pressure

For columns with smaller particles the back pressure will increase inversely proportional to the square of the particle size. Table 1 illustrates that as particle size is reduced the column pressure is seen to increase more rapidly than resolution. For instance theoretically, when reducing the particle size from 5µm to 2µm, the efficiency will more than double. This results in a resolution increase of nearly 50%, but pressure can be expected to increase by a factor of 5. At higher operating pressures, frictional heating of the eluent can become a significant factor, with a corresponding reduction of column efficiency and change of peak resolution and selectivity.

Selectivity

From the resolution equation above it can be deduced that selectivity (α) has a greater influence on a separation than attempting to improve efficiency alone. Therefore, it is strongly recommended that when a decrease in analysis time is required, selectivity effects are always investigated in addition to efficiency effects. Please contact us for further advice and support.

Instrument Requirements

Generally sub 2µm columns are available in short column lengths and small column i.d.s, leading to low column volumes. To maximise performance, the dead volume of the HPLC system has to be carefully considered and optimised irrespective of whether or not the columns are being operated at ultra high pressure. The use of high pressure, low dead volume column connectors is recommended (see page 69, for example), alongside low volume tubing and flow cells. In addition, fast data recording is required to ensure that the chromatographic performance is accurately recorded.

UHPLC Phases[1]

Brand	Phase	Manufacturer	Particle Size (µm)	Page
ACE Excel	C18, C18-AR, C18-PFP, C8, C4, CN, Phenyl, AQ, SIL, SuperC18, C18-Amide	Advanced Chromatography Technologies	2	5, 7, 68, 69, 74
Endeavorsil	C18	Dikma	1.8	97, 98
Epic	C18, C18-MS, C18-SD, C8, C4-SD, Phenyl-Hexyl, HILIC-HC, PFP-LB, SCX	ES Industries	1.8	100
Hypersil GOLD	GOLD, C8, C4, aQ, CN, PFP, Phenyl, Amino, AX, Polar, SAX, Silica, HILIC	Thermo Scientific	1.9	228-230
Inertsil	ODS-4, C8-4, ODS-3, C8-3, Phenyl-3	GL Sciences	2	107, 108, 112, 113
InertSustain	C18		2	105, 106
NUCLEODUR	C18 Gravity, C8 Gravity, C18 Pyramid, C18 Isis, Sphinx RP, HILIC, PolarTec, C18 PAH, C18 HTec, PFP	Macherey-Nagel	1.8	157, 159, 160
ProntoPEARL sub2 TPP	C18 ace-EPS, C8 ace-EPS, NH2	Bischoff	1.8	78
Purospher STAR	RP-18e, RP-8e	Merck	2	180
Syncronis	C18, C8, aQ, Phenyl, Amino, Silica, HILIC	Thermo Scientific	1.7	234
VisionHT	C18-HL, C18-B, C18, C18-P, HILIC, Silica	Grace	1.5	123
YMC-UltraHT	ProC18, Hydrosphere C18	YMC	2	268, 269
YMC-Triart	C18, C8, Diol-HILIC, Phenyl, PFP	YMC	1.9	6, 266, 267

[1] For superficially porous phases please see page 20