



# HICHROM

Chromatography Columns and Supplies

## LC COLUMN INFORMATION Preparative and Process Scale Columns

Catalogue 9

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- Wide range of bulk silicas
- Particle sizes 5 - 50µm
- Column internal diameters 10 - 100mm (4")
- Analytical matched test columns
- High purity products
- Good recoveries

## Introduction

Preparative HPLC is used to isolate and purify milligram to kilogram amounts of compound. The technique uses larger particle size silica materials and wider internal diameter columns than in analytical HPLC. Column efficiency can be preserved on scale-up from analytical to preparative separations. However, broader lower efficiency chromatographic peaks are more often observed when the column is used in an overload state.

## Separation Criteria

The criteria governing preparative separations are very similar to those influencing analytical HPLC. However, economic considerations become more important. They are governed by four factors.

- 1) Resolution** – By optimising the separation between the peak of interest and the nearest contaminant, high sample loads can be achieved without compromising product purity.
- 2) Loadability** – Loadability is controlled by the silica's pore size and available surface area. The smaller the pore size the larger the surface area and the higher the potential loadability. The comparative loadability of different pore size silicas is shown in Figure 1. However, application of the smaller pore size silicas is limited by the range of molecular weight materials they can separate.
- 3) Chemical stability** – The lifetime of a column is often dependent on the silica's chemical stability. Conditions of use are very important.
- 4) Physical stability** – Larger preparative and process scale columns are often repacked during their lifetime. The robustness of a silica will determine how many times a material can be successfully repacked into a column.

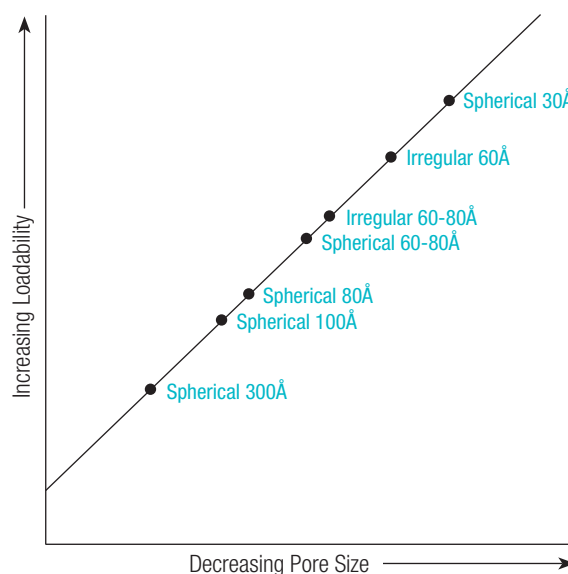


Figure 1. Comparative loadability of different pore size silicas

## Separation Strategy

Reversed-phase is the dominant technique used in analytical HPLC. However, normal-phase HPLC is still often used in preparative separations due to the high cost of reversed-phase materials and the easier recovery of solute from the organic solvents used.

Two strategies dominate the approach to preparative HPLC. In the 'scale-up' approach, a method developed for analytical purposes is directly applied to a larger i.d. column. Although typical 3 - 5µm particles may be replaced with 10µm material of identical selectivity, high preparative efficiencies are maintained. Such an approach is particularly suitable for purifying gram quantities of material with low k values.

In the alternative 'overload' approach, resolution is sacrificed by operating the column in an overload situation. Resolution needs only to be maintained between the peak of interest and the nearest contaminant. Such high loadings maximise column capacity. Separations are poorer but gram to kilogram amounts of material may be purified.

Figure 2 illustrates a typical preparative method development strategy. The extent to which the analytical column can be overloaded, whilst still maintaining adequate resolution and peak shape, is first determined (Figure 2(b)). The injection volume is then scaled up in line with the flow rate increase, used to maintain the same linear velocity through the preparative column (Figure 2(c)).

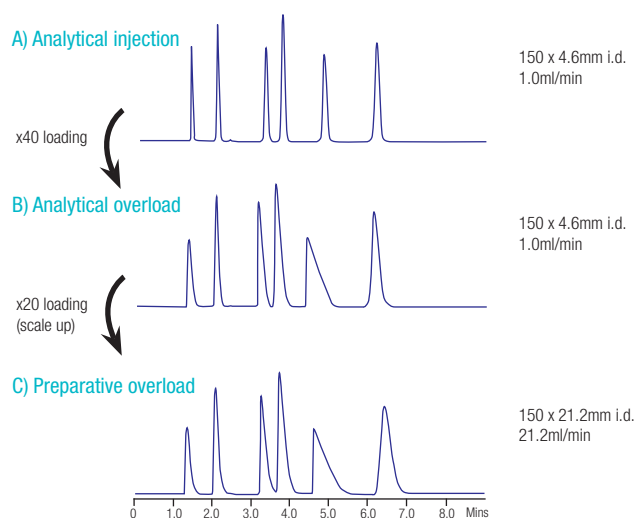


Figure 2. Scale-up strategy using C18 column

## PREPARATIVE AND PROCESS SCALE COLUMNS (continued)

A comparison of scale-up parameters is highlighted in Table 1.

**Table 1. Typical sample capacities**

Column Size	Column i.d. (mm)	Relative Flow Rate (ml/min)	Volume of 250mm Length Column (ml)	Weight of Phase <sup>1</sup> (g)	Typical Injection Volume (µl)	Maximum Column Capacity per Injection	
						Optimum	Overload
Analytical	4.6	1.0	4.2	2.5	10	2mg	85mg
Semi-preparative	7.75	2.8	12	7	30	6mg	240mg
Semi-preparative	10	4.7	20	12	50	10mg	400mg
Preparative	21.2	21	90	53	200	45mg	1.8g
Preparative	30	42.5	177	106	400	90mg	3.6g
Process	50	118	490	295	1200	250mg	10g
Process	100	473	1964	1182	4800	1g	40g

<sup>1</sup> Assumes 250 x 4.6mm column contains 2.5g material

## Bulk Preparative Materials

Hichrom distributes a large number of commercially available preparative HPLC bulk materials. The physical properties of a selection of these are listed below. Please note that for a given brand not all chemistries are available in all particle sizes and pore sizes.

Material <sup>1</sup>	Manufacturer	Particle Size (µm)	Particle Shape <sup>2</sup>	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Chemistry	Page
Daisogel	Daiso Co.	7, 10, 15, 20, 50	S	60, 100, 120, 200, 300, 1000, 2000	450, 450, 300, 200, 100, 25, 15	Sil, C18, C8, C4, C1, NH <sub>2</sub>	-
Develosil	Nomura Chemical Co.	10, 10-20, 15-30	S	30, 60, 100	760, 500, 320	Sil, C18	95
Impaq	Silicycle	5, 10, 20, 40	I	60, 100	500, 400	Sil, C18	-
		7, 10, 13, 16	S	60	540	Sil, CN, Diol	149
Kromasil	Akzo Nobel	7, 10, 13, 16	S	100	320	Sil, C18, C8, C4, NH <sub>2</sub> , Phenyl	149
		10, 16	S	300	110	Sil, C18, C8, C4	149
LiChroprep	Merck	15-25, 25-40, 40-63	I	60, 100	500, 300	Sil, C18, C8, NH <sub>2</sub> , CN, Diol	185
		10, 30	S	120, 250, 600	Polyhydroxymethacrylate based resins		172
MCI GEL	Mitsubishi Chemical Corp.	Various	S	250, 450	Non-functionalised styrene-divinylbenzene copolymer for RP		172
		Various	S	-	Styrene-divinylbenzene copolymer ion-exchange resins		172
SiliaSphere PC	Silicycle	20-45, 40-75, 75-200	S	70, 100, 300, 1000	500, 280, 100, 50	Sil, C18	-
Vydac	Grace	10-15, 15-20, 20-30	S	300	-	C18, C8, C4, Phenyl	-
YMC ProC18		10	S	120	-	C18	275
YMC HG Series	YMC	10, 15, 20, 50	S	120, 200, 300	330, 175, 100	Sil, C18, C8, C4, C1, Phenyl, NH <sub>2</sub> , CN, Diol	275
YMC Triart Prep		10, 15, 20, 50	S	120, 200	-	C18, C8	267, 275
YMC BioPro		10, 30, 75	S	1000	-	QA, SP	273, 275

<sup>1</sup> Not all chemistries available in all particle sizes and pore sizes

<sup>2</sup> S=Spherical, I=Irregular

A large range of bulk resins from Tosoh Bioscience is also available – see page 255-256 for further details.