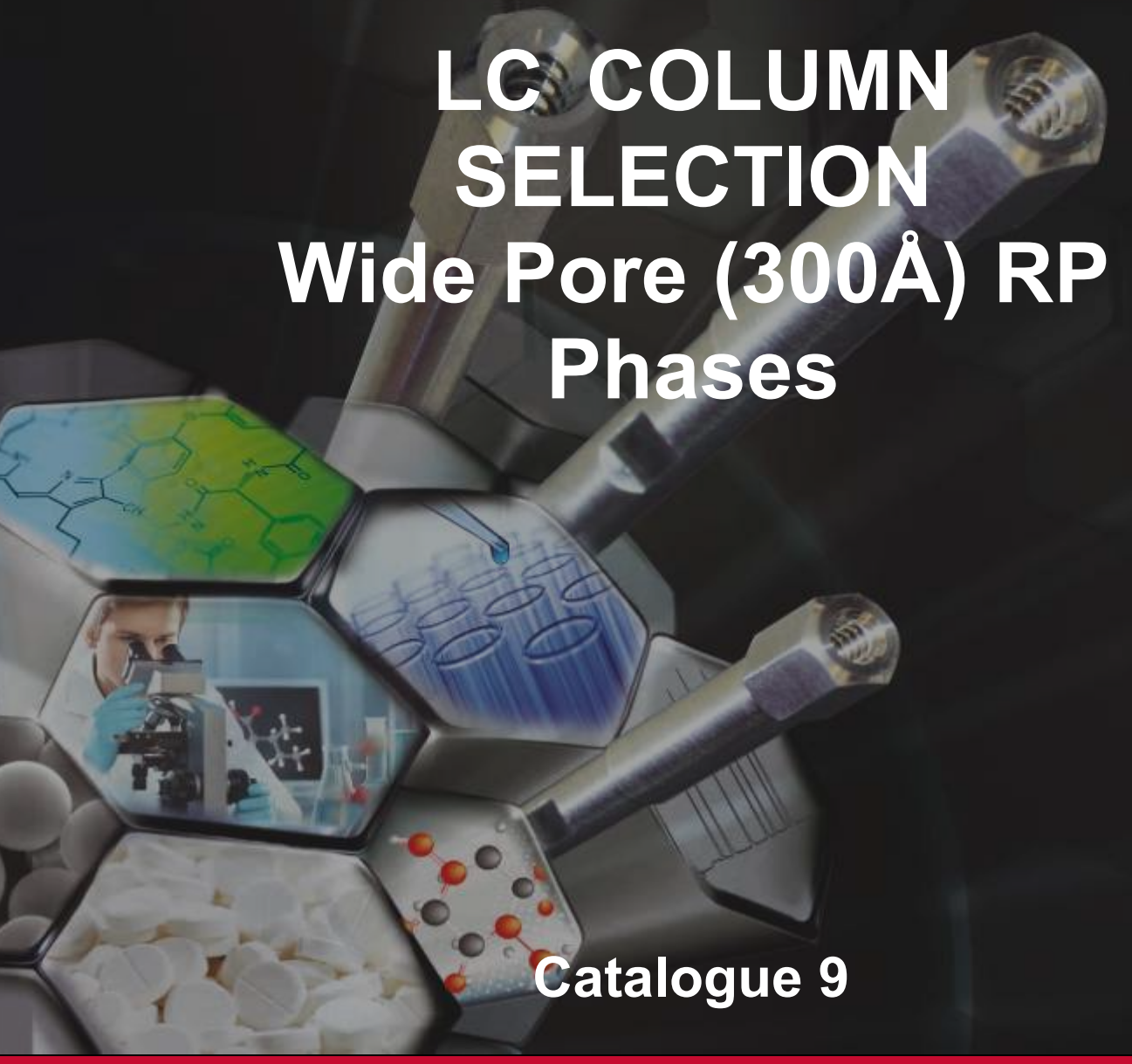
HICHROM

Chromatography Columns and Supplies

LC COLUMN SELECTION
Wide Pore (300Å) RP Phases



Catalogue 9

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WIDE PORE (300Å) REVERSED-PHASE MATERIALS

Introduction

In order for a sample molecule to freely access the interior of the pores of the packing material, its diameter must be smaller than the average pore diameter. For high molecular weight solutes, the use of lower pore size materials of 60-120Å may result in frictional drag within the pore, leading to restricted diffusion and reduced column efficiency.

The use of larger pore silica-based bonded phases therefore leads to improvements in resolution, capacity and recovery of proteins and other biomolecules, due to a reduction in size exclusion mechanism and enhanced molecular diffusion rates. A pore size of 300Å has become the accepted standard for wide pore silicas, and has been found to be suitable for a broad range of molecular weight proteins, peptides and oligonucleotides. In general, peptides exceeding approximately 50 amino acids and oligonucleotides greater than 25 residues are preferentially analysed on 300Å materials. Separations of very large biomolecules (MW >100,000Da) may require larger pore size packings (500 to 4000Å).

Bonded Phases

Alkyl-bonded silica phases are the most commonly used materials for the reversed-phase separation of biomolecules. The shorter C4 phases are generally recommended for large hydrophobic peptides and most proteins. Peptide maps, natural and synthetic peptides and small hydrophilic proteins are best chromatographed on C8 columns. C18 columns are often chosen for the analysis of small peptides. Other bonded wide pore phases, including cyano and phenyl, are available in some brands. The table below summarises a range of wide pore alkyl-bonded reversed-phase silica materials. Ion-exchange and size exclusion packings are also available as wider pore materials (please contact us for details).

Column Dimensions

Wide pore silica phases are available in a range of column dimensions from rapid analysis to preparative and process scale. Increased column capacity favours these wide pore materials for preparative separations of samples with molecular weight >5,000Da.

Separation Mechanism

In reversed-phase chromatography, proteins are retained by adsorption of the face of the protein (hydrophobic foot) to the hydrophobic surface of the packing material. The adsorption/desorption mechanism differs from that of small molecules, in that small changes in organic solvent composition can rapidly change the protein retention, thereby requiring use of shallow gradients. Proteins adsorb near the top of the column (Figure 1A) and remain adsorbed until the organic concentration reaches a high enough level for the protein to desorb (Figure 1B) and elute from the column.

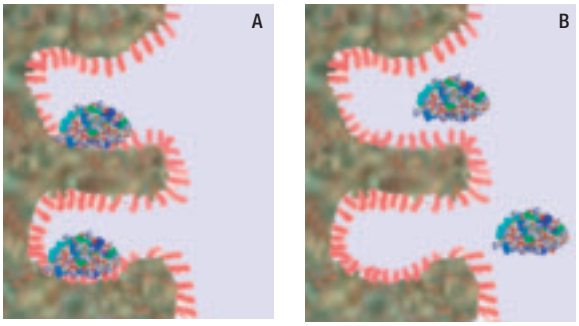

Figure 1. Adsorption and desorption of protein molecules

300Å Reversed-Phase Alkyl-Bonded Silica Phases

Phase	Manufacturer	Particle Size (µm)	Surface Area (m^2/g)	Carbon Load (%)	Page
Acclaim C18	Thermo Scientific	3	100	8	235
ACE[1] C4-300, C8-300, C18-300	ACT	3, 5, 10	100	2.6, 5, 9	64, 70, 75, 76
Aquapore Butyl, Octyl, ODS	Perkin Elmer	7	100	3, 5, 10	194
BioBasic 4, 8, 18	Thermo Scientific	5	100	4, 5, 9	239, 242
Bio-Bond C4, C8, C18	Dikma Technologies	3, 5, 10	100	3, 5, 8	97, 98
Cogent Bidentate C8 300	MicroSolv	5	150	5	186, 188
COSMOSIL C18-AR-300, C8-AR-300, C4-AR-300	Nacalai Tesque	5	150	12, 7, 6	94
Eprogen RP8	Eprogen	5	-	-	99
Inertsil[1] WP300-C4, C8, C18	GL Sciences	5	150	3, 8, 9	115
Kromasil[1] C4, C8, C18	Akzo Nobel	5, 10, 16	110	2.9, 4.7, 8.7	141, 148, 149
NUCLEOSIL 300 C4, C8, C18	Macherey-Nagel	5, 7, 10	100	2, 3, 6.5	163, 166
TSKgel Protein C4-300	Tosoh Bioscience	3	100	3	247, 248
Vydac 201TP	Grace	3, 5, 10	-	8	119, 122
Vydac 202TP		3, 5, 10	-	9	119, 122
Vydac[1] 208TP, 208MS, 214TP, 214MS, 218TP, 218MS		3, 5, 10	-	-	119, 120, 121
Vydac Everest C18		5, 10	-	6	119, 120, 121
YMC[1] C4, C8, ODS-A	YMC	5	100	3, 4, 7	270
ZORBAX 300SB-C3, C8, C18	Agilent Technologies	3.5, 5, 7	45	1.1, 1.5, 2.8	281
ZORBAX 300-Extend		3.5, 5	45	4	281, 282
ZORBAX Poroshell 300SB-C3, C8, C18		5	-	-	281, 282

[1] Other wide pore bonded phases available