SPECIFICATIONS OF C18 BONDED RP MATERIALS

Octadecyl (ODS) or C18 bonded phases are the most widely used reversed-phase materials. Table 1 lists the physical characteristics of a range of C18 bonded small pore silica phases.

Table 1. Octadecylsilyl-bonded silica phases

Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Endcapped	Page
Acclaim C18	2.2, 3, 5	120	300	18	Yes	152
Accucore C18[1]	2.6	80	130	9	Yes	149
Accucore XL C18[1]	4	80	90	7	Yes	149
ACE C18[2]	2, 3, 5, 10	100	300	15.5	Yes	58, 71, 72
ACE C18-HL	3, 5, 10, 15	90	400	20	Yes	58, 64, 71, 72
ACE C18-AR[2]	2, 3, 5, 10	100	300	15.5	Yes	58, 60, 71, 72
ACE C18-PFP[2]	2, 3, 5, 10	100	300	14.3	Yes	58, 61, 71, 72
ACE SuperC18[2,4]	2, 3, 5, 10	90	400	14.8	Yes	58, 59, 71, 72
Brownlee Spheri RP-18	5, 10	80	180	11	Yes	126
Brownlee Spheri ODS	5	80	180	14	Yes	126
Brownlee SPP C18[1]	2.7	90	150	8	Yes	-
CAPCELL PAK ACR	3, 5	80	340	18	Yes	-
CAPCELL PAK AG C18	5	120	300	15	Yes	-
CAPCELL PAK MG III C18	3, 5	100	260	15	Yes	-
CAPCELL PAK SG C18	5	120	300	14	Yes	-
CAPCELL PAK UG C18	3, 5	120	300	15	Yes	-
Chromegabond BAS-C18	5	120	180	12	No	85
Chromegabond C18	5	100	300	16	No	85
Chromegabond MC-18	5	60	475	-	Yes	85
Chromolith RP-18e	-	-	300	18	Yes	110
Cogent Bidentate C18	4	100	350	16.5	No	115, 117, 120
COSMOSIL C18-AR-II	3, 5	120	300	17	Yes	76, 79
COSMOSIL C18-MS-II	3, 5	120	300	16	Yes	76,79
Develosil ODS-UG	3, 5	140	300	18	Yes	80, 81
Develosil ODS-MG	3, 5	100	450	15	Yes	80, 81
Develosil ODS-HG	3, 5	140	300	18	Yes	80, 81
Develosil ODS-SR	3, 5	80	-	18	Yes	80, 81
Endeavorsil C18	1.8	120	300	20	Yes	82, 83
Epic C18	1.8, 3, 5, 10	120	230	18	Yes	85
Epic C18-MS	1.8, 3, 5, 10	120	350	22	Yes	85
Epic C18-SD	1.8, 3, 5, 10	120	350	24	Yes	85
Exsil ODS	3, 5, 10	100	200	11	Yes	86
Exsil ODS1	3, 5	100	200	7	No	86
Exsil ODSB	3, 5	100	200	12	Yes	86
Genesis C18	3, 4, 7	120	300	18	Yes	90
GraceSmart C18	3, 5	120	220	10	Yes	90
HALO C18[1]	2.7	90	150	8	Yes	91
HALO-5 C18[1]	5	90	90	5.5	Yes	91
HALO Peptide ES-C18[1]	2.7	160	80	4.6	Yes	91
HECTOR-M C18	3, 5, 10	100	320	17	Yes	2
Hichrom C18	3.5, 5	150	250	15	Yes	92-96
Hichrom RPB[3]	3.5, 5, 10	110	340	14	Yes	97, 98
Hydrosphere C18	2, 3, 5	120	340	12	Yes	-
Hypersil ODS	3, 5	120	170	10	Yes	151
Hypersil BDS C18	2.4, 3, 5	130	170	7	Yes	151
Inertsil ODS	5	100	350	14	Yes	88
Inertsil ODS-2	5	150	320	18.5	Yes	88, 89
Inertsil ODS-3	2, 3, 4, 5	100	450	15	Yes	87, 88
Inertsil ODS-4	2, 3, 5	100	450	11	Yes	87
Inertsil ODS-P	3, 5	100	450	29	No	87
Inertsil Peptide C18	5	100	450	15	Yes	87
Inertsil ODS-Sprint	3, 5	100	450	8.5	Yes	87
Inertsil Sulfa C18	3, 5	100	450	15	Yes	87
InertSustain C18	2, 3, 5	100	350	14	Yes	87
Inspire C18	3, 5, 10	100	440	27	Yes	-
Kromasil C18	2.5, 3.5, 5, 10	100	320	20	Yes	-
Kromasil Eternity C18	2.5, 5	100	330	14	Yes	-
L-column ODS	3, 5	120	340	17	Yes	101
L-column2 ODS	2, 3, 5	120	340	17	Yes	101

[1] Superficially porous phase [2] UHPLC compatible columns available as ACE Excel [3] Mixed alkyl mode C18/C8 [4] Superficially porous 2.5µm and 5µm also available

Specifications of C18 Bonded RP Materials (continued)

Table 1. Octadecylsilyl-bonded silicas (continued)

Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Endcapped	Page
Leapsil C18	2.7	100	440	27	Yes	82, 83
LiChrosorb RP-18	5, 10	100	300	16.2	No	111
LiChrospher RP-18	5	100	350	21.0	No	112, 113
LiChrospher RP-18e	5	100	350	21.6	Yes	112, 113
NUCLEODUR C18 Gravity	1.8, 3, 5	110	340	18	Yes	102
NUCLEODUR C18 ec	3, 5	110	340	17.5	Yes	102
NUCLEODUR C18 Isis	1.8, 3, 5	110	340	20	Yes	102
NUCLEODUR C18 PAH	1.8, 3	110	340	proprietary	Yes	102
NUCLEODUR C18 HTec	1.8, 3, 5, 7, 10	110	340	18	Yes	102
NUCLEOSHELL RP 18[1]	2.7	90	130	7.5	Yes	102
NUCLEOSIL C18	3, 5, 7, 10	100	350	15	Yes	103, 104
NUCLEOSIL C18	3, 5, 7, 10	120	200	11	Yes	103, 105
NUCLEOSIL C18 AB	5	100	350	25	Yes	103, 104
NUCLEOSIL C18 HD	3, 5	100	-	20	Yes	-
Partisil ODS	10	-	-	-	-	121-125
Partisil ODS2	10	-	-	-	-	121-125
Partisil ODS3	5, 10	-	-	-	-	121-125
Partisphere C18	5	-	-	-	-	122, 123
PrincetonSPHER C18	3, 5, 10	60, 100	500, 325	23, 19	Yes	134, 135
Purospher RP-18	5	90	500	18.5	Yes	110
Purospher RP-18e	5	120	350	18	Yes	110
Purospher STAR RP-18e	2, 3, 5	120	330	17	Yes	110
Spursil C18	3, 5, 10	100	440	25	Yes	82, 83
Spursil C18-EP	3, 5, 10	100	440	24	Yes	82, 83
Symmetry C18	3.5, 5	100	335	19	Yes	-
Syncronis C18	1.7, 3, 5	100	320	16	Yes	151
TSKgel ODS-140HTP	2.3	140	-	8	Yes	154
TSKgel ODS-100V	3, 5	100	-	15	Yes	154
TSKgel ODS-100Z	3, 5	100	-	20	Yes	154
TSKgel ODS-80TM	5, 10	80	-	15	Yes	-
TSKgel ODS-80TS	5, 10	80	-	15	Yes	-
TSKgel Super-ODS	2.3	110	-	8	Yes	154
TSKgel ODS-120T	5, 10	120	-	22	Yes	-
TSKgel ODS-120A	5, 10	120	-	20	Yes	-
Ultrasphere ODS	3, 5	80	-	-	Yes	156, 157
Vydac Denali	3, 5, 10	120	-	20	Yes	-
Waters µBondapak C18	10	125	330	10	Yes	-
Waters Nova-Pak C18	4	60	120	7	Yes	-
Waters Spherisorb ODS1	3, 5, 10	80	220	6.2	No	160, 161
Waters Spherisorb ODS2	3, 5, 10	80	220	11.5	Yes	160, 161
Waters Spherisorb ODSB	5	80	220	11.5	Yes	160, 161
YMC J-sphere ODS-L80	4	80	510	9	Yes	-
YMC J-sphere ODS-M80	4	80	510	14	Yes	-
YMC J-sphere ODS-H80	4	80	510	22	Yes	-
YMC ODS-A	3, 5	120	330	17	Yes	-
YMC ODS-AL	3, 5	120	330	17	No	-
YMC ODS-AM	3, 5	120	330	17	Yes	-
YMC ODS-AQ	3, 5	120	330	14	Yes	-
YMC ProC18	2, 3, 5	120	330	17	Yes	-
YMC ProC18RS	3, 5	80	510	22	Yes	-
YMC-Triart C18	1.9, 3, 5	120	-	16	Yes	-
ZORBAX ODS	5	70	330	20	Yes	165, 166
ZORBAX SB-C18	1.8, 3.5, 5, 7	80	180	10	No	-
ZORBAX Extend-C18	1.8, 3.5, 5	80	180	12.5	Yes	-
ZORBAX Eclipse XDB-C18	1.8, 3.5, 5	80	180	10	Yes	-
ZORBAX Eclipse Plus C18	1.8, 3.5, 5	95	160	8	Yes	-

[1] Superficially porous phase

SPECIFICATIONS OF C1 TO C8 & C30 BONDED REVERSED-PHASE MATERIALS

Octyl-bonded phases are the most common medium polarity alternative to C18 bonded phases. Very short chain alkyl-bonded phases are less stable. The shorter the alkyl chain the greater the vulnerability of the material to aqueous dissolution at high pH or loss of bonded phase at low pH. For wide pore silicas the C4 chemistry retains high popularity. Table 1 lists the physical characteristics of a range of C1 to C8 bonded and C30 bonded narrow pore silica phases. For wide pore (300Å) phases see page 38.

Table 1. Short chain alkyl (C1 to C8) and C30 bonded silica phases

Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Endcapped	Page
C1 Bonded						
CAPCELL PAK C1 UG	5	120	300	7	No	-
Chromegabond TMS	5	60	475	-	No	-
Develosil TMS-UG	3, 5	140	300	4.5	Yes	80, 81
Exsil C1	3, 5	100	200	3	No	86
Hypersil SAS	5	120	170	2.5	No	151
Kromasil C1	5	100	320	4.7	Yes	-
ProntoSIL C1	3, 5	120	300	2	No	-
Waters Spherisorb C1	3, 5	80	220	2.2	No	160, 161
YMC TMS	3, 5	120	330	4	No	-
ZORBAX TMS	5	70	330	4	Yes	165, 166
C2 Bonded						
Chromegabond C2	5	60	480	-	No	85
NUCLEOSIL C2	7	100	350	3.5	No	103, 104
C3 Bonded						
ZORBAX SB-C3	5	80	180	4	No	-
C4 Bonded						
ACE C4	2[2], 3, 5, 10	100	300	5.5	Yes	58, 71, 72
Epic C4-SD	1.8, 3, 5, 10	120	350	8	Yes	85
HECTOR-M C4	3, 5, 10	100	320	3	Yes	2
Inertsil C4	5	150	320	7.5	Yes	88, 89
Kromasil C4	2.5, 3.5, 5, 7, 10	100	320	8	Yes	-
PrincetonSPHER C4	3, 5, 10	60, 100	500, 325	8, 6	No	134, 135
ProntoSIL C4	3, 5	120	300	4	No	-
YMC C4	3, 5	120	330	7	Yes	-
YMC ProC4	3, 5	120	340	7	Yes	-
C6 Bonded						
Chromegabond C6	5	60	220	6	No	85
Chromegabond MC-CC6	5	60	475	7	Yes	-
PrincetonSPHER C6	3, 5, 10	60, 100	500, 325	10, 8	Yes	134, 135
Waters Spherisorb C6	3, 5	80	220	4.7	Yes	160, 161
C8 Bonded						
Acclaim C8	2.2, 3, 5	120	300	11	Yes	152
Accucore C8[1]	2.6	80	130	5	Yes	149
ACE C8	2[2], 3, 5, 10	100	300	9.0	Yes	58, 71, 72
AquaSep	3, 5	100	450	16	-	85
Brownlee Spheri RP-8	5, 10	80	180	6	Yes	126
Brownlee SPP[1]	2.7	90	150	7.7	Yes	-
CAPCELL PAK C8 UG	5	120	300	10	Yes	-
CAPCELL PAK C8 AG	5	120	300	10	Yes	-
CAPCELL PAK C8 DD	5	80	300	11	Yes	-
CAPCELL PAK C8 SG	5	120	300	10	Yes	-
Chromegabond BAS-C8	5	100	300	8	No	-
Chromegabond C8	5	100	300	8	No	85
Chromegabond C8-BD	5	100	475	12	No	-
Chromolith RP-8e	-	-	300	11	Yes	110
Cogent Bidentate C8	4	100	350	7	No	115, 117, 120
Develosil UG C8	5	140	300	11	Yes	80, 81
Epic C8	1.8, 3, 5, 10	120	230	10	Yes	85
Exsil C8	3, 5	100	200	6	Yes	86
Genesis C8	3, 4, 7	120	300	11	No	90
Genesis C8 e/c	3, 4, 7	120	300	11	Yes	90
HALO C8[1]	2.7	90	150	5.4	Yes	91
HALO-5 C8[1]	5	90	90	3.7	Yes	91
HECTOR-M C8	3, 5, 10	100	320	10	Yes	2

[1] Superficially porous phases [2] As ACE Excel column

Specifications of C1 to C8 & C30 Bonded Reversed-Phase Materials (continued)

Table 1. Short chain alkyl (C1 to C8) and C30 bonded silica phases (continued)

Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Endcapped	Page
C8 Bonded						
Hichrom C8	3.5, 5	150	250	8	Yes	92-96
Hypersil MOS	3, 5	120	170	6.5	No	151
Hypersil MOS-2	5	120	170	6.5	Yes	151
Hypersil BDS C8	2.4, 3, 5	130	170	11	Yes	151
Hypersil GOLD C8	1.9, 3, 5	175	220	8	Yes	150
Inertsil C8	5	150	320	10.5	Yes	88
Inertsil C8-3	2, 3, 5	100	450	9	Yes	87, 88
Inertsil C8-4	2, 3, 5	100	450	5	Yes	87
InertSustain C8	2, 3, 5	100	350	8	Yes	87
Inspire C8	3, 5, 10	100	440	17	Yes	82, 83
Kromasil C8	2.5, 3.5, 5, 10	100	320	12	Yes	-
L-column C8	5	120	340	10	Yes	101
L-column2 C8	5	120	340	10	Yes	101
LiChrosorb RP-8	5, 10	100	300	9.5	No	111
LiChrospher RP-8	5	100	350	12.5	No	112, 113
LiChrospher RP-8e	5	100	350	13	Yes	112, 113
Nova-Pak C8	4	60	120	4	Yes	-
NUCLEODUR C8 Gravity	1.8, 5	110	340	11	Yes	102
NUCLEODUR C8 ec	3, 5	110	340	10.5	Yes	102
NUCLEOSIL C8	5, 7, 10	100	350	8.5	No	103, 104
NUCLEOSIL C8	3, 5, 7, 10	120	200	6.5	No	103, 105
NUCLEOSIL C8 HD	5	100	-	13	Yes	-
Partisil C8	5, 10	-	-	-	-	121-125
Partisphere C8	5	-	-	-	-	122, 123
PrincetonSPHER C8	3, 5, 10	60, 100	500, 325	15, 11	Yes	134, 135
Symmetry C8	3.5, 5	100	335	12	Yes	-
Syncronis C8	1.7, 3, 5	100	320	10	Yes	151
TSKgel Octyl-80Ts	5	80	-	11	Yes	-
TSKgel Super-Octyl	2.3	110	-	5	Yes	154
Ultrasphere C8	3, 5	80	-	-	Yes	156, 157
Waters Spherisorb C8	3, 5, 10	80	220	5.8	Yes	160, 161
YMC Basic	3, 5	proprietary	proprietary	8	Yes	-
YMC C8	3, 5	120	330	10	Yes	-
YMC ProC8	3, 5	120	340	10	Yes	-
YMC-Triart C8	1.9, 3, 5	120	-	7	Yes	-
ZORBAX C8	5	70	330	12	Yes	165, 166
ZORBAX Eclipse Plus C8	1.8, 3.5, 5	95	160	7	Yes	-
ZORBAX Eclipse XDB-C8	1.8, 3.5, 5	80	180	7.6	Yes	-
ZORBAX Rx-C8	5	80	180	5.5	No	-
ZORBAX SB-C8	1.8, 3.5, 5	80	180	5.5	No	-
C30 Bonded						
Acclaim C30	3, 5	200	200	13	Yes	152
Accucore C30[1]	2.6	150	80	5	Yes	149
Cogent C30[2]	3, 5	200	-	18	No	119
Develosil RPAQUEOUS	3, 5	140	300	18	Yes	80, 81
Develosil RPAQUEOUS-AR	3, 5	140	300	18	Yes	80, 81
Develosil XG-C30	3, 5	140	300	19.5	Yes	80, 81
ProntoSil C30	3, 5, 10	200	200	20	No	-
PrincetonSPHER C30[2]	3, 5, 10	200	200	19	No	134, 135
YMC Carotenoid	3, 5	proprietary	proprietary	-	-	-

[1] Superficially porous phases

[2] C27 phase also available

POLAR EMBEDDED AND OTHER 'AQ' TYPE PHASES

Introduction

When separating very polar, water-soluble compounds, eluents containing less than 5% organic modifier are commonly used to achieve sufficient retention. However, operation under such highly aqueous conditions can lead to poor chromatographic reproducibility and decreasing retention times. Conventional C8 and C18 phases undergo dewetting or 'phase collapse' under these conditions, resulting in a reduction of accessible bonded phase. This phenomenon may either occur very quickly or more gradually.

'High Aqueous' Phases

Approaches to address this problem include embedding a polar group in the alkyl chain or using hydrophilic (polar) endcapping reagents (see Figure 1). Both these approaches, or the use of a C30 phase, have the effect of maintaining the phase surface under fully wetted conditions, even when using 100% aqueous eluent. Polar embedded phases are also used to obtain different selectivity from conventional C18 phases.

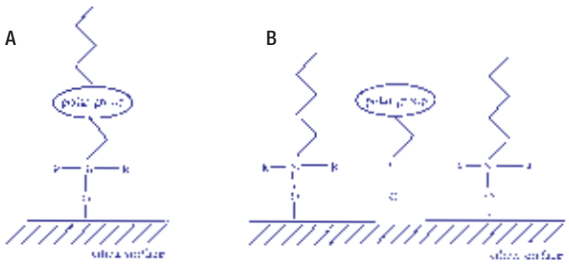


Figure 1. Polar embedded (A) and hydrophilic endcapped (B) phases

Good Retention and Resolution for Polar Compounds

Compared to traditional alkyl phases these 'high aqueous' phases are resistant to retention loss when using highly aqueous eluents, even after several days or weeks. Reproducible retention times and improved peak shapes are achieved for acidic, basic and zwitterionic analytes.

Alternative Selectivity

Conventional C18 phases depend primarily on differing hydrophobic interactions between analytes and the stationary phase to provide separation. 'AQ' type phases may also show hydrophilic (polar) interactions via H-bonding and dipole-dipole forces. This can influence retention time and improve selectivity for polar analytes.

Eliminate Need for Ion-pair Additives

Many separations of very polar analytes are performed using ion-pair chromatography in order to provide adequate retention. The use of an 'AQ' phase generally enables reproducible results to be obtained using conventional aqueous/organic eluents.

Typical Applications

Typical applications of these 'AQ' type phases include carboxylic acids, water soluble vitamins, catecholamines, nucleic acid bases and various polar pharmaceuticals.

'AQ' Type Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Comments	Page
Acclaim PolarAdvantage	Thermo Scientific	2.2, 3, 5	120	Embedded sulphonamide group	152
Acclaim PolarAdvantage II		2.2, 3, 5	120	Embedded amide group	152
Accucore AQ[1]		2.6	80	C18 with polar endcapping	149
Accucore Polar Premium[1]		2.6	150	Amide embedded C18	149
ACE AQ	Advanced Chromatography Technologies (ACT)	2[2], 3, 5, 10	100	C18 with integral polar functionality	58, 64, 71, 72
ACE C18-Amide		2[2], 3, 5, 10	100	C18 with integral amide polar group	58, 62, 71, 72
ACE C18-AR		2[2], 3, 5, 10	100	C18 with integral phenyl group	58, 60, 71, 72
AquaSep	ES Industries	3, 5	100	C8 with embedded ether group	85
Chromegabond ODS-PI		3, 5	120	Ureide embedded polar group	85
CAPCELL PAK C18 AQ	Shiseido	3, 5	80	C18	-
COSMOSIL C18-PAQ	Nacalai Tesque	5	120	C18 phase with polymeric linkage	76, 79
Develosil RPAQUEOUS	Nomura	3, 5	140	C30, monofunctional	80, 81
Develosil RPAQUEOUS-AR		3, 5	140	C30, trifunctional	80, 81
Epic Polar	ES Industries	1.8, 3, 5, 10	120	Embedded ether group	85
HALO RP-Amide[1]	Advanced Materials Technology	2.7	90	Polar embedded amide	91
Hydrosphere C18	YMC	2, 3, 5	120	Hydrophilic C18 surface	-
Hypersil GOLD AQ	Thermo Scientific	1.9, 3, 5, 8	175	Alkyl chain with polar endcapping	150
Inertsil ODS-EP	GL Sciences	5	100	C18 phase with polar embedded group	87
NUCLEODUR C18 Pyramid	Macherey-Nagel	1.8, 3, 5	110	C18 with hydrophilic endcapping	102
NUCLEODUR PolarTec		1.8, 3, 5	110	Polar embedded group	102
NUCLEOSIL Nautilus		3, 5	100	C18 with polar embedded group	-
NUCLEOSIL Protect 1		5	100	Protective polar group	-
Princeton ULTIMA C18, C8 & Phenyl	Princeton Chromatography	3, 5, 10	-	Embedded polar amide functionality	134, 135
ProntoSil C18 (or C8) ace-EPS	Bischoff	3, 5	120	C18 or C8 with embedded amide group	-
ProTec-RP	ES Industries	3, 5	100	C8, C18 or Phenyl with embedded amide group	-
Spursil C18 and C18-EP	Dikma Technologies	3, 5, 10	100	C18 with proprietary polar modification	82, 83
SymmetryShield	Waters	3.5, 5	100	C18 or C8 with polar embedded group	-
Syncronis aQ	Thermo Scientific	1.7, 3, 5	100	C18 with polar endcapping	151
YMC ODS-AQ	YMC	3, 5	120	C18 with hydrophilic endcapping	-
ZORBAX Bonus-RP	Agilent Technologies	1.8, 3.5, 5	80	C14 chain with embedded amide group	-
ZORBAX SB-Aq		1.8, 3.5, 5	80	Proprietary	-

[1] Superficially porous phases [2] As ACE Excel column

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.

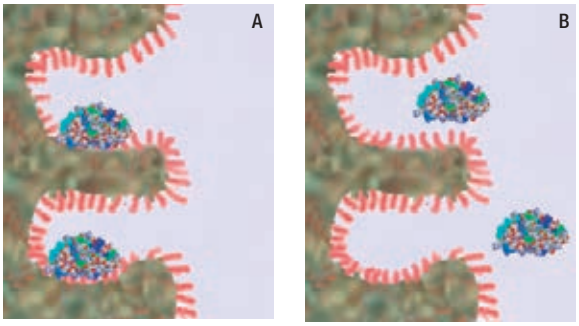


Figure 1. Adsorption and desorption of protein molecules

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	152
ACE[1] C4-300, C8-300, C18-300	ACT	3, 5, 10	100	2.6, 5, 9	58, 67, 73
Aquapore Butyl, Octyl, ODS	Perkin Elmer	7	100	3, 5, 10	126
BioBasic 4, 8, 18	Thermo Scientific	5	100	4, 5, 9	153
Bio-Bond C4, C8, C18	Dikma Technologies	3, 5, 10	100	3, 5, 8	82, 83
Cogent Bidentate C8 300	MicroSolv	5	150	5	115, 117, 120
COSMOSIL C18-AR-300, C8-AR-300, C4-AR-300	Nacalai Tesque	5	150	12, 7, 6	79
Eprogen RP8	Eprogen	5	-	-	84
HECTOR-W C-18, C8, C4, NH_2	RStech Corporation	3, 5, 10	-	7, 4, 3, -	2
Inertsil[1] WP300-C4, C8, C18	GL Sciences	5	150	3, 8, 9	-
Kromasil[1] C4, C8, C18	Akzo Nobel	5, 10, 16	110	2.9, 4.7, 8.7	-
NUCLEOSIL 300 C4, C8, C18	Macherey-Nagel	5, 7, 10	100	2, 3, 6.5	103, 106
PrincetonSPHER-300 C18, C8, C4, Phenyl, CN, NH_2, Diol, Silica	Princeton Chromatography	5, 10	100	-	134, 135
TSKgel Protein C4-300	Tosoh Bioscience	3	100	3	154
Vydac 201TP	Grace	3, 5, 10	-	8	90
Vydac 202TP		3, 5, 10	-	9	90
Vydac[1] 208TP, 208MS, 214TP, 214MS, 218TP, 218MS		3, 5, 10	-	-	90
Vydac Everest C18		5, 10	-	6	90
YMC[1] C4, C8, ODS-A	YMC	5	100	3, 4, 7	-
ZORBAX 300SB-C3, C8, C18	Agilent Technologies	3.5, 5, 7	45	1.1, 1.5, 2.8	-
ZORBAX 300-Extend		3.5, 5	45	4	-
ZORBAX Poroshell 300SB-C3, C8, C18		5	-	-	-

[1] Other wide pore bonded phases available

HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC) PHASES

Introduction

Hydrophobic Interaction Chromatography (HIC) is a powerful technique for the separation and purification of proteins and peptides. Separations are based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. Although the separation mechanism of HIC has similarities with that of standard reversed-phase HPLC, the density of the bonded phase ligands on the surface of the HIC packing material is much lower. HIC therefore involves weaker interactions and weaker eluents can be used. Samples are adsorbed to the HIC resin at relatively high salt concentrations and eluted by applying a linear or stepwise decreasing salt gradient. The mild conditions used in HIC typically maintain tertiary protein structure and thus biological activity (ie. no denaturation).

Selectivity

An optimum HIC separation will combine high dynamic binding capacity (DBC), adequate selectivity, good mass recovery and retention of biological activity. Proteins show varying degrees of hydrophobicity depending on their amino acid composition, structure and size. Separation can be optimised by varying the nature of the HIC phase or by varying the eluent. Very hydrophilic proteins are generally purified using highly hydrophobic stationary phases, whereas very hydrophobic proteins are separated using the least hydrophobic phases.

Method Development

In addition to the hydrophobicity of the phase ligand, several parameters affect HIC separations. These include salt type, pH, buffer concentration, temperature and gradient. Ammonium sulphate (1 or 2M) or sodium chloride (3M) salts are most commonly used for HIC applications. The pH of the salt solution will influence retention; pH 7.0 is a good starting point. Figure 1 shows the influence of pH for various salts on the DBCs of lysozyme.

Applications

Hydrophobic interaction chromatography is suitable for the separation and purification of a wide range of biomolecules. In addition to proteins, antibody fragments, RNAs, antibiotics etc. can be analysed by HIC. HIC can also be used for protein desalting. Figure 2 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column.

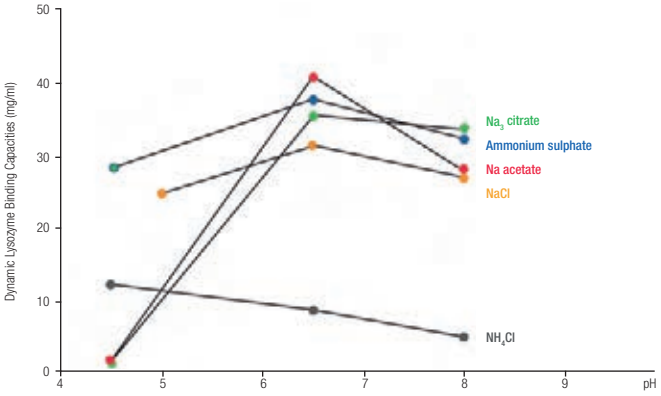
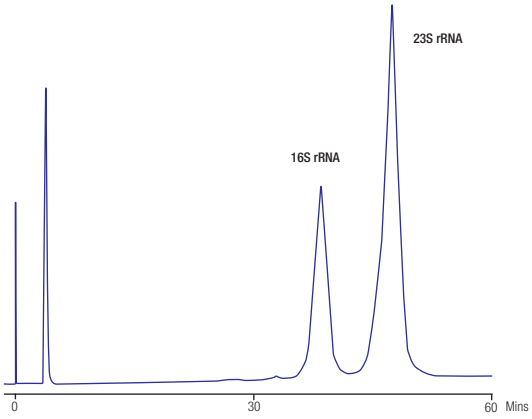


Figure 1. Influence of pH



Column: TSKgel Phenyl-5PW (75 x 7.5mm)
Eluent: Linear gradient from 2mol/l to 0mol/l $(NH_4)_2SO_4$ in 0.1mol/l phosphate buffer, pH 7.0
Flow rate: 0.5ml/min
Detection: UV, 280nm

Figure 2. Separation of RNAs on TSKgel Phenyl-5PW

HIC Phases

Phase	Manufacturer	Base material	Bonding	Particle Size (µm)	Pore Size (Å)	Page
COSMOSIL HIC	Nacalai Tesque	Silica	Diol	5	300	76, 79
HIC PH-814	Shodex	Polyhydroxymethacrylate	Phenyl	10	2,000	142
MCI GEL CQH Series	Mitsubishi Chemicals	Polyhydroxymethacrylate	Ether, Butyl, Phenyl	10	600	108
PolyPROPYL A	PolyLC	Silica	Propylaspartamide	3, 5, 12	300, 1000, 1500	127, 130
PolyETHYL A			Ethylaspartamide			127, 130
PolyMETHYL A			Methylaspartamide			127, 130
ProPac HIC-10	Thermo Scientific	Silica	Amide/ethyl	5	300	153
TSKgel	Tosoh Bioscience	Methacrylate	Ether, Phenyl	10, 13, 20	1,000	155
			Butyl-NPR	2.5	-	155

PHENYL BONDED PHASES

Phenyl bonded silica phases offer an alternative reversed-phase selectivity to alkyl bonded phases. They show lower hydrophobic retention than their C18 counterparts, with similar retention characteristics to C8-bonded phases. Phenyl stationary phases interact with compounds containing aromatic groups or unsaturated bonds through the involvement of π-π interactions. For aromatic solutes containing an electronegative atom or group (e.g. F, NO_2), the degree of π-π interactions with the phenyl phase will increase.

Due to the rigid nature of the phenyl ring, solute shape can also influence selectivity.

Traditional phenyl phases tend to be less stable than the corresponding C8 or C18 reversed-phases. Additionally, the larger steric size of the phenyl group reduces surface coverage, leaving a greater number of exposed silanol sites. More recently introduced phenyl phases show greater stability. The use of a purer silica base, more effective and reproducible bonding procedures and the availability of a sterically protected phenylsilane all contribute to greater phase robustness and reduced column bleed.

Conventional phenyl phases are bonded to the silica through a propyl spacer. The incorporation of the longer chain hexyl spacer results in increased hydrophobic retention and aromatic selectivity. Phenyl bonded (mainly with propyl linker) and Phenyl-Hexyl bonded phases are listed in separate tables below.

Phenyl Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
Acclaim Phenyl-1	Thermo Scientific	3	120	300	152
Accucore Phenyl-X[1]		2.6	80	130	149
ACE C18-AR[2]	Advanced Chromatography Technologies (ACT)	2[3], 3, 5, 10	100	300	58, 60, 71, 72
ACE Phenyl		2[3], 3, 5, 10	100	300	58, 71, 72
CAPCELL PAK UG Phenyl	Shiseido	5	120	300	-
Chromegabond Alkyl Phenyl	ES Industries	3, 5, 10	60, 80, 100	475, 200, 190	85
Cogent Phenyl Hydride	MicroSolv	4	100	350	115, 118, 120
Develosil Phenyl-UG	Nomura	3, 5	140	300	80, 81
Genesis Phenyl	Grace	4	120	300	90
HECTOR-M Phenyl	RStech Corporation	3, 5, 10	100	320	2
Hypersil GOLD Phenyl	Thermo Scientific	1.9, 3, 5	175	220	150
Hypersil Phenyl		5	120	170	151
Hypersil Phenyl-2		5	120	170	151
Hypersil BDS Phenyl		3, 5	130	170	151
Inertsil Phenyl	GL Sciences	5	150	320	88, 89
Inertsil Phenyl-3		2, 3, 5	100	450	87, 88
InertSustain Phenyl		3, 5	100	350	87
Kromasil Phenyl	Akzo Nobel	5, 10, 16	100	320	-
NUCLEOSIL Phenyl	Macherey-Nagel	5, 7	100, 120	350, 200	103-105
PrincetonSPHER Phenyl	Princeton Chromatography	3, 5, 10	60, 100	500, 325	134, 135
ProntoSIL Phenyl	Bischoff	3, 5	120	300	-
Syncronis Phenyl	Thermo Scientific	1.7, 3, 5	100	320	151
TSKgel Super-Phenyl	Tosoh Bioscience	2.3	110	-	154
Vydac 219MS[4]	Grace	5	300	-	90
Waters µBondapak Phenyl	Waters	10	125	330	-
Waters Nova-Pak Phenyl		4	60	120	-
Waters Spherisorb Phenyl		3, 5	80	220	160, 161
YMC Phenyl	YMC	3, 5	120	330	-
YMC-Triart Phenyl		1.9, 3, 5	120	-	-
ZORBAX Phenyl	Agilent Technologies	5	70	330	165, 166
ZORBAX SB-Phenyl		3.5, 5	80	180	-
ZORBAX Eclipse XDB-Phenyl		3.5, 5	80	180	-

[1] Superficially porous phase [2] C18 with integral Phenyl, classed as L1 [3] As ACE Excel column [4] Diphenyl phase

Phenyl-Hexyl Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
Accucore Phenyl-Hexyl[1]	Thermo Scientific	2.6	80	130	149
ACE UltraCore SuperPhenylHexyl[1]	Advanced Chromatography Technologies (ACT)	2.5, 5	95	130, 100	1
Brownlee SPP Phenyl-Hexyl[1]	Perkin Elmer	2.7	90	150	-
Epic Phenyl-Hexyl	ES Industries	1.8, 3, 5, 10	120	350	85
HALO Phenyl-Hexyl[1]	Advanced Materials Technology	2.7	90	150	91
HALO-5 Phenyl-Hexyl[1]		5	90	90	91
Kromasil Eternity Phenyl-Hexyl	Akzo Nobel	2.5, 5	100	330	-
NUCLEODUR Phenyl-Hexyl	Macherey-Nagel	1.8, 3, 5	110	340	102
NUCLEOSHELL Phenyl-Hexyl[1]		2.7	90	130	102

[1] Superficially porous phase

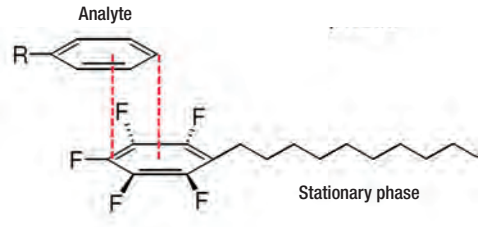
PENTAFLUOROPHENYL (PFP) BONDED PHASES

Fluorinated stationary phases have shown novel selectivity for several classes of compounds and in many cases have proved useful as an alternative to traditional C18 and C8 phases. In particular, pentafluorophenyl (PFP) bonded phases are becoming increasingly popular when alternative selectivity is required. Details of these PFP phases are listed on this page. For other fluorinated phases please see individual manufacturer's pages (eg. Fluophase page 151, Wakopak Fluofix page 159, PrincetonSPHER Fluoropropyl and Fluorooctyl page 135).

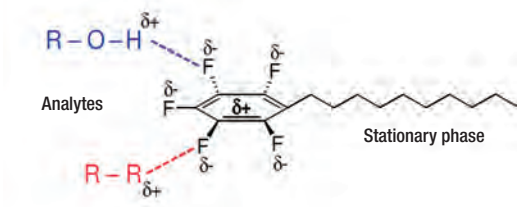
Separation Mechanisms

PFP-bonded phases use multiple retention mechanisms for separation of challenging compounds. These interactions include hydrophobic, π-π interaction, dipole-dipole, H-bonding and shape selectivity. The predominance of each retention mechanism will be influenced by the solute's physicochemical properties, its structure and the chromatographic conditions utilised.

The electronegative fluorine atoms produce an electron deficient phenyl ring, so that the PFP phase acts as a Lewis acid or electron acceptor. This is the opposite of phenyl phases, which contain an electron rich aromatic ring. π-π interaction can occur with solutes that are rich in electrons (Lewis bases). The carbon-fluorine bonds of the PFP ring are very polar, thus enabling analytes to also be retained by dipole-dipole and H-bonding interactions, resulting in increased analyte retention.



π–π interaction



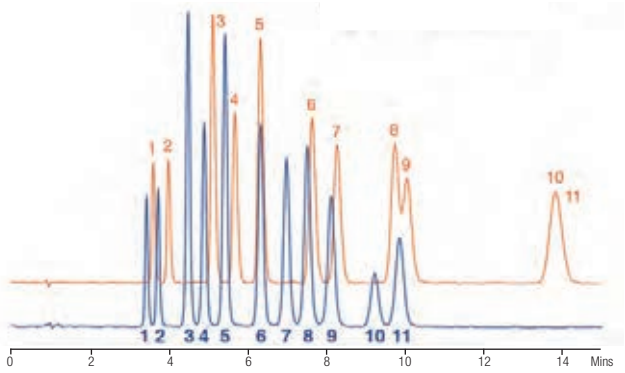
Dipole-dipole and H-bonding

Applications

PFP phases show excellent selectivity especially for highly polar analytes, aromatic and unsaturated compounds, phenols, halogenated compounds and taxanes. In addition, positional isomers show increased separation on PFP phases.

Due to the low bleed characteristics of many of the newer PFP phases, they are ideally suited for low UV wavelength and LC-MS applications. PFP phases are generally resistant to dewetting and can be used under highly aqueous conditions.

Figure 1 illustrates the orthogonal selectivity shown by a PFP compared to a C18 phase for the separation of phenol isomers.



1. o-Cresol
2. m-Cresol
3. 3,4-Dimethylphenol
4. 3,5-Dimethylphenol
5. 2,5-Dimethylphenol
6. 2,6-Dichlorophenol
7. 2,3-Dichlorophenol
8. 2,4-Dichlorophenol
9. 3,4-Dichlorophenol
10. 2,4-Dibromophenol
11. 3,5-Dibromophenol

Columns: NUCLEODUR PFP (blue) and C18 HTec (red)
Dimensions: 5µm, 125 x 4mm
Eluent: 0.1% formic acid in CH_3CN – 0.1% formic acid in H_2O (35:65)
Flow rate: 1ml/min
Temperature: 35°C
Detection: UV, 280nm

Figure 1. Separation of phenol isomers

PFP Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
Accucore PFP[1]	Thermo Scientific	2.6	80	130	149
ACE C18-PFP[2]	ACT	2[3], 3, 5, 10	100	300	58, 61, 70, 71
Epic PFP-LB	ES Industries	1.8, 3, 5, 10	120	230	85
FluoroSep-RP Phenyl (FSP)		3, 5	60	350	85
HALO PFP[1]	Advanced Materials Technology	2.7	90	150	91
HALO-5 PFP[1]		5	90	90	91
Hypersil GOLD PFP	Thermo Scientific	1.9, 3, 5, 8, 12	175	220	150
NUCLEODUR PFP	Macherey-Nagel	1.8, 3, 5	110	340	102
NUCLEOSHELL PFP[1]		2.7	90	130	102
Partisphere TAC-1	Hichrom	5	-	-	122, 123
PrincetonSPHER PFP	Princeton	3, 5	60, 100	-	134, 135
YMC-Triart PFP	YMC	1.9, 3, 5	120	-	-

[1] Superficially porous phase [2] C18 with integral PFP, classed as USP L1 [3] As ACE Excel column

POLAR BONDED PHASES

Introduction

Polar bonded silica phases offer an alternative selectivity to alkyl bonded materials (see p.31-36). In general they have a lower hydrophobicity but higher polarity. Cyano, amino and diol bonded phases can be used in both normal- and reversed-phase modes. In normal-phase they equilibrate more rapidly with the eluent than silica itself and are not deactivated by traces of water.

Availability

Cyano bonded phases show unique selectivity for polar compounds and are more suitable than bare silica for normal-phase gradient separations. The cyano functional group is a strong dipole that can interact with other dipoles or induce dipoles on solutes. These phases also exhibit moderate hydrophobicity due to the alkyl linker.

Amino bonded phases show alternative normal-phase selectivity to unbonded silica, especially for aromatics. Amino columns are also used in the HILIC mode for carbohydrate analysis and for other polar compounds. Their weak anion-exchange properties can be used in the analysis of anions and organic acids.

Diol bonded phases are a versatile alternative to unbonded silica for normal-phase separations. The hydroxyl groups provide good selectivity without excessive retention, since H-bonding with the diol layer is weaker than with silanols. Some diol bonded phases have been developed specifically for HILIC applications. Differing pore size materials are used in size-exclusion separations.

Cyano Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
ACE CN	Advanced Chromatography Technologies (ACT)	2[1], 3, 5, 10	100	300	58, 71, 72
ACE CN-ES		2[1], 3, 5, 10	100	300	58, 63, 71, 72
CAPCELL PAK CN UG	Shiseido	5	120	300	-
Chromegabond BAS-CN	ES Industries	3, 5, 10	120	180	85
Chromegabond CN-BD		3, 5, 10	100	475	85
Chromegabond CN-HS		3, 5, 10	60	550	85
COSMOSIL CN-MS	Nacalai Tesque	5	120	300	76
Develosil CN-UG	Nomura	5	140	300	80, 81
Develosil XG-CN		3, 5	140	300	80
Exsil CN	Grace	3, 5	100	200	86
Genesis CN		4	120	300	90
HALO ES-CN[2]	Advanced Materials Technology	2.7	90	150	91
HALO-5 ES-CN[2]		5	90	90	91
HECTOR-M CN	RStech Corporation	3, 5, 10	100	320	2
Hypersil GOLD CN	Thermo Scientific	1.9, 3, 5	175	220	150
Hypersil CPS		3, 5	120	170	151
Hypersil CPS-2		5	120	170	151
Hypersil BDS CPS		3, 5	130	170	151
Inertsil CN-3	GL Sciences	3, 5	100	450	87, 88
Kromasil CN	Akzo Nobel	5, 10, 16	60	540	-
LiChrosorb CN	Merck	5	100	300	111
LiChrospher CN		5	100	350	112, 113
NUCLEODUR CN and CN-RP	Macherey-Nagel	3, 5	110	340	102
NUCLEOSIL CN		5, 10	100	350	103, 104
		7	120	200	103, 105
PrincetonSPHER CN	Princeton Chromatography	3, 5, 10	60, 100	500, 325	134, 135
ProntoSIL CN	Bischoff	3, 5	120	300	-
TSKgel CN-80Ts	Tosoh Bioscience	5	80	-	-
Ultrasphere CN	Hichrom	3, 5	80	-	156, 157
Waters µBondapak CN	Waters	10	125	-	-
Waters Nova-Pak CN HP		4	60	-	-
Waters Spherisorb CN		3, 5	80	220	160, 161
YMC CN	YMC	3, 5	120	330	-
ZORBAX CN	Agilent Technologies	5	70	330	165, 166

[1] As ACE Excel column [2] Superficially porous phase

Polar Bonded Phases (continued)

Amino Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
CAPCELL PAK NH_2 UG	Shiseido	5	80	-	-
Chromegabond A/RP	ES Industries	3, 5, 10	60, 100	475, 330	-
Chromolith NH_2	Merck	-	-	300	110
COSMOSIL NH2-MS	Nacalai Tesque	5	120	300	76
Exsil NH_2	Grace	3, 5	100	200	86
Genesis NH_2		3	120	300	90
HECTOR-M NH_2	RStech Corporation	3, 5, 10	100	320	2
Hypersil GOLD Amino	Thermo Scientific	1.9, 3, 5	175	220	150
Hypersil APS-2		3, 5	120	170	151
Inertsil NH_2	GL Sciences	3, 5	100	450	87
InertSustain NH_2		3, 5	100	350	87
Kromasil NH_2	Akzo Nobel	3.5, 5, 7, 10	100	320	-
LiChrosorb NH_2	Merck	5, 10	100	300	111
LiChrospher NH_2		5	100	350	112, 113
NUCLEODUR NH_2 and NH_2-RP	Macherey-Nagel	3, 5, 7	110	340	102
NUCLEOSIL NH_2		5	100	350	103, 104
		7	120	200	103, 105
PrincetonSPHER NH_2	Princeton Chromatography	3, 5, 10	60, 100	500, 300	134, 135
Purospher STAR NH_2	Merck	5	120	330	110
Syncronis NH_2	Thermo Scientific	1.7, 3, 5	100	320	151
TSKgel NH2-100	Tosoh Bioscience	3	100	450	155
Waters µBondapak NH_2	Waters	10	125	330	-
Waters Spherisorb NH_2		3, 5, 10	80	220	160, 161
YMC NH_2	YMC	3, 5	120	330	-
ZORBAX NH_2	Agilent Technologies	5	70	330	165, 166

Diol Bonded Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
Chromegabond D/RP	ES Industries	3, 5	60, 100	475, 330	85
COSMOSIL Diol	Nacalai Tesque	5	120	300	76
HECTOR-M Diol	RStech Corporation	3, 5, 10	100	320	2
Inertsil Diol	GL Sciences	3, 5	100	450	88
Inertsil WP Diol		5	300	150	-
Kromasil Diol	Akzo Nobel	5, 10, 16	60	540	-
Kromasil HILIC-D		5	60	540	-
LiChrosorb Diol	Merck	5, 10	100	300	111
LiChrospher Diol		5	100	350	112, 113
NUCLEOSIL Diol	Macherey-Nagel	5, 7	100	350	103, 104
PrincetonSPHER Diol	Princeton Chromatography	3, 5, 10	60, 100	500, 325	134, 135
ProntoSIL Diol	Bischoff	3, 5	120	300	-
YMC Diol	YMC	5	120	330	-
YMC-Triart Diol-HILIC		1.9, 3, 5	120	-	-

Figures 1 and 2 show typical applications on amino and diol bonded columns respectively.

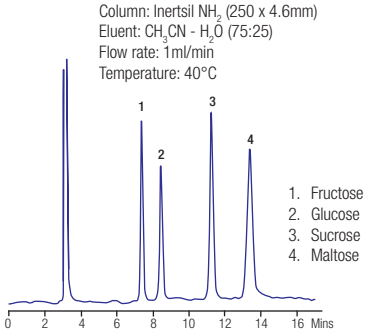


Figure 1. Separation of sugars on amino column

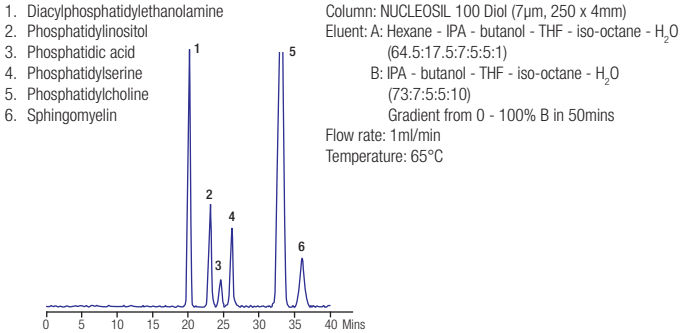
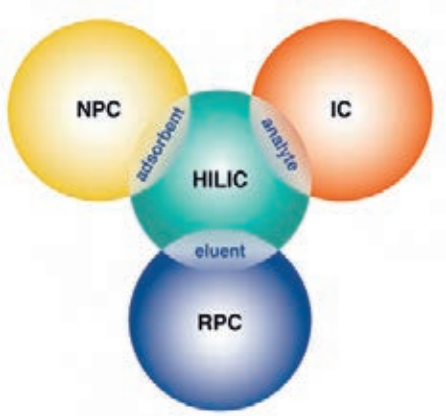


Figure 2. Separation of phospholipids on diol column

HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) PHASES

Introduction

Hydrophilic Interaction Chromatography (HILIC) is a variant of normal-phase chromatography which is performed using polar stationary phases with partially aqueous eluents. The technique combines the characteristics of 3 major liquid chromatography techniques – reversed-phase, normal-phase and ion chromatography. HILIC is an alternative approach to reversed-phase for the effective separation of polar compounds. Solutes elute in the order of increasing hydrophilicity (polarity), the opposite of reversed-phase, thus providing an orthogonal selectivity.



Mode of Operation

Retention in HILIC is proportional to the amount of organic solvent in the eluent. Typical HILIC eluents contain 65-90% acetonitrile or methanol. The low proportion of water in the eluent generates a water-rich layer on the surface of the polar stationary phase. This enables solutes to partition between the eluent and this water-rich layer (Figure 1). In addition, weak electrostatic interactions between solute and stationary phase contribute to overall selectivity. Gradient elution may be performed either with a decreasing organic or increasing salt gradient. Salt is not required for uncharged solutes such as carbohydrates, but typically 10mM salt is necessary with charged solutes such as peptides. Ammonium formate and acetate are suitable volatile buffers for LC-MS.

Several types of HILIC phases have been developed, including unbonded silica, neutral bonded ligands (eg. amide, diol), charged ligands (eg. amino), zwitterionic phases and mixed reversed-phase/HILIC phases. A wide selection of HILIC phases is summarised in the table below.

ERLIC, also referred to as eHILIC, is a subset of HILIC separations which employs charged interactions and their subsequent orientation effects (see PolyLC section for further details).

Aqueous normal-phase (ANP) is a further technique related to HILIC (see pages 115-120 for further details).

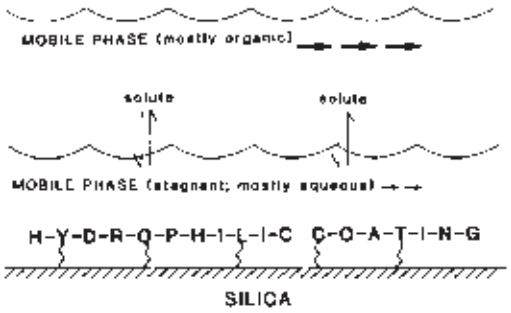


Figure 1. Hypothetical partition mechanism of hydrophilic interaction chromatography (HILIC)

Applications

HILIC phases are particularly useful for compounds that are weakly retained by reversed-phase columns. Typical application areas include carbohydrates, oligonucleotides, peptides and proteins, amino acids and natural products.

HILIC Phases

Phase	Manufacturer	Functional Group	Particle Size (µm)	Pore Size (Å)	Page
Acclaim HILIC-10	Thermo Scientific	Proprietary	3	120	152
Accucore HILIC[1]		Proprietary	2.6	80	149
Accucore Urea-HILIC[1]		Urea	2.6	80	149
Accucore 150-Amide-HILIC[1]		Amide	2.6	80	149
BioBasic AX		Polyethyleneimine	5	300	153
Brownlee SPP HILIC[1]	Perkin Elmer	Unbonded silica	2.7	90	-
COSMOSIL HILIC	Nacalai Tesque	Triazole	2.5, 5	120	76, 78, 79
Epic HILIC-HC	ES Industries	Polyhydroxylated polymer	1.8, 3, 5, 10	120	85
HALO and HALO-5 HILIC[1]	Advanced Materials Technology	Unbonded silica	2.7, 5	90	91
HALO and HALO-5 Penta-HILIC[1]		Penta-hydroxy	2.7, 5	90	91
Hypersil GOLD HILIC	Thermo Scientific	Polyethyleneimine	1.9, 3, 5	175	150
Inertsil HILIC	GL Sciences	Propyl alcohol	3, 5	100	87, 88
Kromasil HILIC-D	Akzo Nobel	Diol	5	60	-
NUCLEODUR HILIC	Macherey-Nagel	Zwitterionic ammonium sulphonic acid	1.8, 3, 5	110	102
NUCLEOSHELL HILIC[1]			2.7	90	102
Obelisc N	SIELC	Proprietary	5	100	147
PolyGLYCOPLEX	PolyLC	-	5, 12	-	127, 128, 130
Syncronis HILIC	Thermo Scientific	Zwitterionic	1.7, 3, 5	100	151
TSKgel Amide-80	Tosoh Bioscience	Carbamoyl	3, 5	100	155
TSKgel NH2-100		Ethylamino	3	100	155
VisionHT HILIC	Grace	-	1.5, 3, 5, 10	120	90
YMC-Triart Diol-HILIC	YMC	Diol	1.9, 3, 5	120	-
ZIC-HILIC	Merck	Zwitterionic sulphobetaine	3.5, 5	100, 200	109
ZIC-*p*HILIC		Zwitterionic sulphobetaine	5	-	109
ZIC-cHILIC		Zwitterionic phosphorylcholine	3	100	109

[1] Superficially porous phase

SILICA PHASES

Introduction

Despite its porosity, spherical porous HPLC silica exhibits a high mechanical strength compared with other materials. Additionally, it is readily chemically modified. A wide range of porous silicas is available for normal-phase HPLC, characterised by surface area, pore size and particle size measurements.

The use of normal-phase HPLC has not been limited by the silica dissolution or peak tailing problems associated with reversed-phase HPLC. Hence traditional silicas are still commonly used. As an alternative to normal-phase HPLC, some unbonded silica phases are promoted for use as HILIC phases (see page 44).

Particle Size

For analytical work, as the quality and reproducibility of porous silica improves, the use of 3, 3.5 or 4µm particle size materials increases. 10µm particles are less commonly used but remain a key particle size for preparative applications. For economic reasons irregular silicas still share some of this latter market.

Physical Characteristics

The physical characteristics of the newer silica particles have been improved in several ways. However, a number of them are not readily available as they are principally used as a base material for the manufacture of new reversed-phase silicas.

- **Surface Activity**
  A lower level of the unwanted, free and isolated silanol groups is observed. The lower metal ion contaminant level partly contributes to this drop in surface activity. Basic compounds interact less strongly with the silica surface resulting in improved chromatography.
- **Physical Properties**
  Improved control of physical properties such as surface area, pore volume, mean pore diameter and particle size have given the new silicas better lot-to-lot reproducibility.
- **Purity**
  The level of metal ion impurities has in some cases been reduced to cumulative figures < 10ppm. Undesirable chelation of metal ion and solute has been minimised.

Silica Phases

Phase	Manufacturer	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Page
ACE SIL	ACT	2[1], 3, 5, 10	100	300	58, 71, 72
Chromolith Si	Merck	-	-	300	110
Cogent Silica-C	MicroSolv	4	100	350	115, 119, 120
COSMOSIL SL-II	Nacalai Tesque	3, 5	120	300	76, 79
Exsil Silica	Grace	5, 10	100	200	86
Genesis Silica		4, 7, 15	120	300	90
HECTOR-M Sil	RStech Corporation	3, 5, 10	100	320	2
Hypersil Silica	Thermo Scientific	3, 5	120	170	151
Hypersil GOLD Silica		1.9, 3, 5	175	220	150
Inertsil SIL	GL Sciences	3, 5	100	450	87
Kromasil Silica	Akzo Nobel	3.5, 5, 7, 10	60, 100	540, 320	-
LiChrosorb Silica	Merck	5, 10	60, 100	500, 300	111
LiChrospher Si		5	60, 100	700, 400	112, 113
NUCLEODUR Silica	Macherey-Nagel	3, 5	110	340	102
NUCLEOSIL Silica		3, 5, 7, 10	100, 120	350, 200	103-105
Partisil Silica	Hichrom	5, 10	-	-	121-125
Partisphere Silica		5	-	-	122, 123
PrincetonSPHER Silica	Princeton Chromatography	3, 5, 10	60, 100	500, 325	134, 135
ProntoSIL Si	Bischoff	3, 5, 10	120	300	-
Purospher STAR Si	Merck	5	120	330	110
Syncronis Silica	Thermo Scientific	1.7, 3, 5	100	320	151
Ultrasphere Silica	Hichrom	3, 5	80	-	156, 157
VisionHT Silica	Grace	1.5, 3, 5, 10	120	220	90
Waters Nova-Pak Silica	Waters	4	60	120	-
Waters Spherisorb Silica		3, 5, 10	80	220	160, 161
YMC Silica	YMC	3, 5	120	300	-
ZORBAX Silica	Agilent Technologies	5	70	330	165, 166

[1] As ACE Excel column

ION-EXCHANGE PHASES

Introduction

Ion-exchange phases separate solutes on the basis of differences in ionic charge. Retention in ion-exchange chromatography is determined by the pH of the eluent, the nature and ionic strength of the buffer and temperature. Column efficiencies are lower than in reversed-phase HPLC. Eluents are normally aqueous but can contain some organic component.

Base Material

Both silica based and polymer based ion-exchangers are available. For the former, ionic species are attached to the silica surface, whereas for the latter the ion-exchange groups are distributed throughout the matrix. Silica based materials maintain a mechanical strength and higher efficiency advantage, whereas the polymer based materials have greater pH stability.

Applications

Ion-exchange is used for the analysis of small ions but the key application area of the technique is the separation of biomolecules such as peptides, proteins and oligonucleotides. Weak ion-exchangers are used for the analysis of inorganic ions, a technique more specifically termed ion chromatography (see page 48).

Ion-Exchange Capacity

The exchange capacity of an ion-exchanger is an important measure of its retentivity (typically measured in milliequivalents per gram material). For any one column the packing density of the phase must also be taken into account. Wide pore materials will typically have lower ion-exchange capacities.

Cation-exchange phases contain negatively charged functional groups and retain positively charged cations. Conversely, anion-exchange phases retain negatively charged analytes by their positively charged functional groups. In the schematics below, the ion strength of the counter ions can be adjusted to shift the equilibrium position and thus the retention times of the analytes.

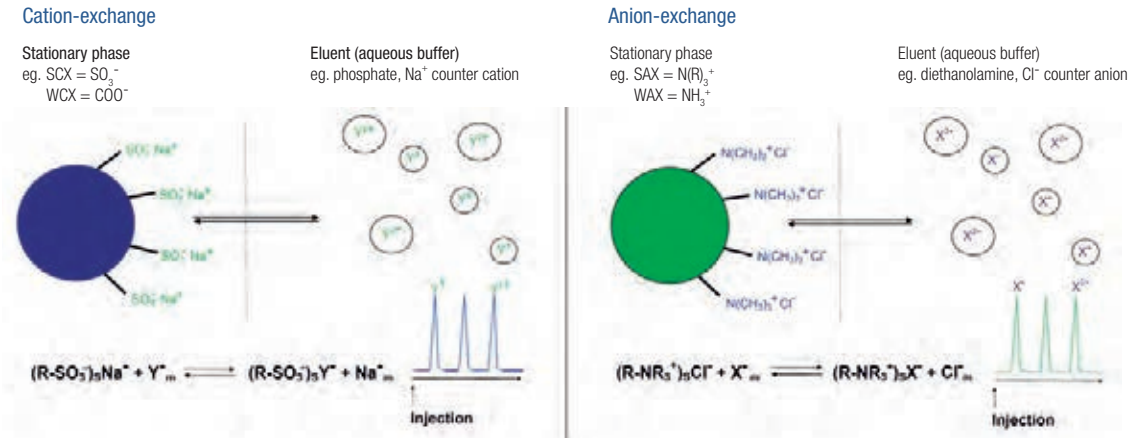


Figure 1. Mechanisms of ion-exchange

Classification

	Type	Strength	Nomenclature	Typical Functionality	pH Ionisation Range
Ion-Exchange	Anion	Weak	WAX	Amine	Ionised at specific pH
		Strong	SAX	Quaternary Ammonium	Ionised over complete pH range
	Cation	Strong	SCX	Sulphonic Acid	
		Weak	WCX	Carboxylic Acid	Ionised at specific pH

Ion-Exchange Phases

Phase	Manufacturer	Base Material	Classification	Particle Size (µm)	Pore Size (Å)	Applications and Features	Page
BioBasic AX, SCX	Thermo Scientific	Silica	SAX, SCX	5	300	Proteins, peptides, nucleic acids	153
CAPCELL PAK UG SCX	Shiseido	Silica	SCX	5	80	Small molecules	-
COSMOGEL IEX Type Q, Type S	Nacalai Tesque	Polymer	SAX, SCX	5	1000	Proteins, DNA	79
COSMOGEL IEX Type Q-N, Type S-N			SAX, SCX	5	Non-porous		

Ion-Exchange Phases (continued)

Ion-Exchange Phases (continued)

Phase	Manufacturer	Base Material	Classification	Particle Size (µm)	Pore Size (Å)	Applications and Features	Page
Epic SCX	ES Industries	Silica	SCX	1.8, 3, 5, 10	120	Small molecules	85
Eprogen AX300, CM300	Eprogen	Silica	WAX, WCX	6	300	Small molecules	84
Eprogen Q300, S300			SAX, SCX	6	300		84
Exsil SAX, SCX	Grace	Silica	SAX, SCX	5	100	Small molecules	86
Hamilton PRP-X100, PRP-X200	Hamilton	Polymer	WAX, WCX	10	100	Inorganic ion analysis	-
Hamilton PRP-X500, PRP-X600		Polymer	SAX, WAX	7	-	Proteins, DNA oligomers	-
HECTOR-ACD WCX, SCX	RStech Corporation	Silica	WCX, SCX	3, 5, 10	100	Separation of acidic compounds	2
Hypersil GOLD AX, SAX	Thermo Scientific	Silica	WAX, SAX	1.9, 3, 5	175	AX – small proteins and peptides SAX- small molecules	150
Inertsil AX, CX	GL Sciences	Silica	SAX, SCX	5	100	Small molecules	87, 88
MCI GEL ProtEx-DEAE, -SP	Mitsubishi Chemicals	Polymer	WAX, SCX	5	-	Proteins	-
MCI GEL CQA Series			SAX, WAX	10			108
MCI GEL CQK Series			SCX, WCX	10			108
NUCLEOSIL SA, SB	Macherey-Nagel	Silica	SCX, SAX	5, 10	100	Small molecule analysis	103, 104
NUCLEOGEN DEAE			WAX	7	60, 500, 4000	Bioanalytical	102
Partisil SAX, SCX	Hichrom	Silica	SAX, SCX	5, 10	-	Small molecule analysis	121-125
Partisphere SAX, SCX			SAX, SCX	5	-		122, 123
PolyCAT A	PolyLC	Silica	WCX	3, 5, 12	300, 1000, 1500	Aspartic acid functionality	127, 128, 130
PolySULFOETHYL A			SCX		200, 300, 1000	Sulfoethylaspartamide	127, 129, 130
PolyWAX			WAX		100, 300, 1000, 1500	Proteins with isoelectric point <6.0	127-130
PL-SAX, PL-SCX	Agilent Technologies	Polymer	SAX, SCX	8, 10	1000	Protein applications	-
ProPac WCX-10, SCX-10, WAX-10, SAX-10	Thermo Scientific	Polymer	WCX, SCX, WAX, SAX	10	Non-porous	Proteins variants	153
Asahipak ES-502N, ES-502C	Shodex	Polymer	WAX, WCX	9	-	Proteins, peptides, oligonucleotides	140
Shodex IEC QA			SAX	12	-		140
Shodex IEC DEAE, SP, CM			WAX, SCX, WCX	8	-		140
TSKgel DEAE-2SW, CM-2SW	Tosoh Bioscience	Silica	WAX, WCX	5	125	Nucleotides, drug molecules, catecholamines	155
TSKgel DEAE-3SW, CM-3SW			WAX, WCX	10	250		155
TSKgel SuperQ-5PW, DEAE-5PW, SP-5PW, CM-5PW		Polymer	SAX, WAX, SCX, WCX	10, 13	1000	Enzymes, proteins, DNA, nucleic acids	155
TSKgel BioAssist Q		Polymer	SAX	10, 13	~4000	Plasmids, antibodies and other large proteins	155
TSKgel BioAssist S		Polymer	SCX	7, 13	~1300		155
TSKgel Q-STAT, CM-STAT, SP-STAT		Non-porous resin	SAX, WCX, SCX	7, 10	Non-porous	Nucleic acids, mAb variants, protein aggregates	155
TSKgel DNA-STAT			SAX	5			155
Waters Spherisorb SAX, SCX	Waters	Silica	SAX, SCX	5	80	Small molecules	160, 161
YMC-BioPro QA, SP	YMC	Hydrophilic polymer	SAX, SCX	5	1000	Peptides, proteins, nucleic acids, other biomolecules	-
YMC-BioPro QA-F, SP-F			SAX, SCX	5	Non-porous		-
ZirChrom SAX, WAX	ZirChrom Separations	Zirconia	SAX, WAX	3, 5	300	Inorganic and organic anions, biomolecules	162-164
ZirChrom SHAX, WCX			SAX, WCX			Proteins	162-164

ION CHROMATOGRAPHY PHASES

Introduction

Ion chromatography (IC) is a special form of ion-exchange chromatography developed as a means of separating the ions of strong acids and bases. The most important form of ion chromatography involves a combination of specific ion-exchange phases with conductivity detection. It is a sensitive technique, in some cases being able to detect ppb levels of ions.

Suppressed or Non-Suppressed Detection

Eluents used in IC contain a relatively high level of salt ions and therefore exhibit high conductivity. This leads to a high background signal which could inhibit the detection of low level analytes. Suppression of eluent conductivity post column is necessary for efficient detection of sample ions and is the most common method for anion analyses. Although isocratic elution is more commonly used, the use of suppressors enables gradient elution to be used for complex samples.

Phases

Silica and polymer based phases are available for anion and cation analyses. Silica based columns, although showing better efficiency, have a limited pH range. As a result, they are not compatible with anion IC methods requiring suppressed detection, due to the high pH of the eluents required. Polymer based materials are stable over a wider pH range and have higher capacities.

Tables 1 and 2 show typical base materials and bonding for anion and cation chromatography phases respectively. The majority of phases for anion chromatography are bonded with quaternary ammonium groups, with a permanent cationic charge. For cation chromatography phases, sulphonate is the most common functionality. Columns optimised for non-suppressed IC generally have lower capacity than those for suppressed detection, in order to achieve a relatively low background conductivity. They are therefore not suitable for suppressed detection.

Eluents

The choice of eluent for IC depends on whether the method is suppressed or non-suppressed. For suppressed anion IC, carbonate/bicarbonate or hydroxide are the most common eluents. Hydroxide eluents have the advantage of producing water only in the suppressor and thereby giving a very low background conductivity. However, some phases are not stable at the high pH (12) of this eluent. For non-suppressed anion IC analyses, typical eluents include p-hydroxybenzoic acid and phthalic acid. Typical eluents for suppressed and non-suppressed cation IC include HCl, HNO_3, tartaric acid and succinic acid.

Applications

High sensitivity ion analyses are important in a wide spectrum of industries including pharmaceutical, food, water, semiconductor etc. In addition to the common inorganic anions eg. F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, acid salts can also be analysed by IC eg. formate, acetate. Quantitative analysis of anions at the ppb level can be achieved. Cation chromatography is used for the separation and detection of Group I and II metal ions, in addition to some transition metal ions, ammonium ions and ethanolamines. Small ions are generally eluted before larger ions and monovalent ions before di- and trivalent ions.

Table 1. Anion Chromatography Phases

Base Material	Functional Group	Typical pH Range
Silica	Quaternary ammonium	2 – 5.5
Polystyrene-divinylbenzene	Quaternary ammonium	2 – 12
Polymethacrylate	Quaternary ammonium	2 – 10
Polyvinyl alcohol	Quaternary ammonium	3 – 12

Table 2. Cation Chromatography Phases

Base Material	Functional Group	Typical pH Range
Silica	Carboxylate, sulphonate	2 – 7
Silica	Polybutadiene/maleic acid coating	2 – 7
Polystyrene-divinylbenzene	Sulphonate	2 – 12

A selection of ion chromatography columns can be found in the following sections:

MCI GEL page 107
Shodex page 140

Please contact Hichrom for advice on further IC column selection.

SIZE EXCLUSION CHROMATOGRAPHY (SEC) PHASES

Introduction

SEC columns separate components according to their molecular size in solution, larger molecules eluting first. Separation is achieved by the differential exclusion or inclusion of components within the packing material particles. In addition to the separation of discrete components, the technique is used for characterising the molecular weight distribution of polymers.

Base Material

Silica based SEC materials generally exhibit higher resolving power than polymer based materials. However, polymer based materials show greater stability for use with high pH eluents. Polymeric packing materials are generally available in larger particle sizes, which may be more practical for large-scale preparative separations.

Modes of Operation

Gel permeation chromatography (GPC) refers to the SEC separation of organic soluble polymers using an organic solvent as the eluent. Gel filtration chromatography (GFC) refers to the SEC separation of water soluble polymers in aqueous eluents. SEC separations exhibit lower resolving power and capacity compared with adsorptive HPLC techniques.

Applications

SEC analyses do not normally result in the denaturation of samples, making the technique a suitable choice for biological samples where activity must be retained. A wide range of biomolecules and organic polymers are separated by SEC. For samples of wide molecular weight distribution, it can be useful to use a mixed pore size phase or to couple columns of one or more pore sizes in series.

Size Exclusion Chromatography Phases

Phase / Series	Manufacturer	Base Material / Bonding	Mode	Pore Sizes (Å)	Typical Applications	Page
GPC PEPTIDE	Eprogen	Glycerol bonded silica	GFC	50	Small peptides	84
GPC100, 300, 500, 1000, 4000			GFC	100, 300, 500, 1000, 4000	Proteins, carbohydrates, nucleic acids, water soluble polymers	84
GPC LINEAR			GFC	100-1000	Organic polymers, denatured proteins	84
CATSEC		Silica with polymerised polyamine coating	GFC	100, 300, 1000	Cationic polymers	84
MCI GEL CQP	Mitsubishi Chemical Corp.	Polyhydroxymethacrylate	GFC	120, 200, 600	Proteins, peptides, enzymes and other biomolecules	108
PLgel	Agilent Technologies	Polystyrene-divinylbenzene	GPC	50, 100, 500, 1000, 10,000, 100,000, MIXED	Oils, oligomers, high MW synthetic polymers, starches, polystyrenes, resins	-
PL aquagel-OH 30, 40, 50, 60, MIXED		Polystyrene-divinylbenzene with polyhydroxyl functionality	GFC	-	Surfactants, polysaccharides, polyacrylamides, starches, gum	-
PolyHYDROXYETHYL A	PolyLC	Silica with hydroxyethylaspartamide coating	GFC	60, 100, 200, 300, 500, 1000, 1500	Peptides, proteins, carbohydrates, small molecules	127, 130
Asahipak GF	Showa Denko	Polyvinyl alcohol	GFC/ GPC	400, 2000, 10000	Hydrophilic and hydrophobic compounds	142
Shodex GPC		Styrene-divinylbenzene	GPC	Various	Polymers, plastics	142
Shodex OHpak SB		Polyhydroxymethacrylate	GFC	Various	Water soluble samples	141
Shodex PROTEIN KW		Silica	GFC	400, 1000, 1500	Proteins	141
Acclaim SEC	Thermo Scientific	Hydrophilic polymethacrylate	GFC	300, 1000	Water soluble polymers	-
BioBasic SEC		Silica	GFC	60, 120, 300, 1000	Peptides and proteins	153
MAbPac SEC-1		Silica	GFC	300	Monoclonal antibodies and aggregates	153
TSKgel SW	Tosoh Bioscience	Silica	GFC	125, 250, 450	Proteins, antibodies, enzymes, nucleic acids	154
TSKgel PW		Polymethacrylate	GFC	<100, 125, <200, 200, 500, 1000, >1000	Water soluble organic polymers, polysaccharides, DNA	154
TSKgel H		Polystyrene-divinylbenzene	GPC	-	Oligomers, polymers and polymer additives	154
TSKgel Alpha and SuperAW		Hydrophilic polyvinyl	GFC/ GPC	-	Organic and water soluble polymers	154
ZORBAX GF Series	Agilent Technologies	Zirconia-clad silica	GFC	150, 300	Proteins, peptides	-

AFFINITY CHROMATOGRAPHY PHASES

Introduction

Affinity chromatography offers the highest specificity and selectivity in biomolecular separations and purifications. Purifications up to several orders of magnitude can be achieved in a single step. Affinity separations can often remove contaminants difficult to eliminate using conventional chromatographic procedures.

Mechanism

The basis of affinity chromatography is a 'lock and key' type mechanism. An affinity ligand, specific for a binding site on the target molecule, is coupled to an inert chromatography matrix. Using suitable binding conditions, target molecules are bound to the affinity ligand according to its specificity. Unbound solutes are washed through the column. The adsorbed target molecules are then desorbed and eluted from the column. Purification of several thousand-fold may be obtained due to the high selectivity of the affinity interactions.

Group specific affinity resins (eg. Tosoh Bioscience) bind molecules sharing specific structural features. Alternatively, if greater specificity is required, ligands with precise specificity for the target molecule can be used. For example, the Protein A phase has specific affinity for the Fc region of immunoglobulins.

Immunoaffinity Chromatography

Immunoaffinity chromatography is a specialised form of affinity chromatography, utilising an antibody or antibody fragment as the ligand immobilised on to a solid support in such a manner that its binding capacity is retained.

Figure 1 shows a schematic diagram of the stages involved in a typical immunoaffinity analysis: loading the sample, washing the column to remove matrix components and impurities and eluting the target compound.

Applications

Affinity and immunoaffinity chromatography techniques are applicable in a variety of disciplines including biochemistry, immunochemistry, virology and molecular biology. Due to the increasing availability of a variety of antibodies, separations based on immunoaffinity techniques are being increasingly used in a wide range of applications involving the purification of complex biological samples.

The main application areas of immunoaffinity chromatography are proteins and enzymes. Method selectivity can be enhanced by combining the pre-concentration and pre-treatment of samples offered by immunoaffinity phases, with the separation capabilities of reversed-phase HPLC. The technique can also be used prior to MS analyses in proteomics.

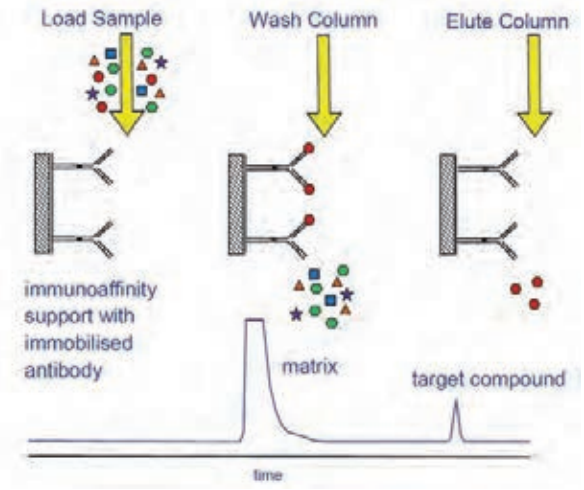


Figure 1. Typical Immunoaffinity Analysis

Affinity and Immunoaffinity Phases

Phase Range	Manufacturer	Base Material	Particle Size (µm)	Features	Page
AFpak	Shodex	Polyhydroxymethacrylate	18	6 different ligands available	142
ProPac IMAC-10	Thermo Scientific	Non-porous polystyrene-divinylbenzene	10	Used for separation of proteins and peptides by immobilised metal affinity chromatography	153
ProSwift ConA-1S	Thermo Scientific	Polymethacrylate monolith	-	Used for purification of Concanavalin A binding glycans, glycopeptides and glycoproteins	153
TSKgel	Tosoh Bioscience	Polymethacrylate	10	Group specific affinity phases for analysis of peptides, proteins and nucleic acids	155
TOYOPEARL	Tosoh Bioscience	Polymethacrylate	40 – 90 (30 – 60 for Protein A)	Various ligands. Bulk media	155

CHIRAL PHASES

In many biological processes, the activity of one member of an enantiomeric pair can be contrasted with the inactivity or even harmful activity of the other. The successful development of chiral stationary phases (CSPs) for HPLC and SFC now allows us to monitor the optical purity of a bulk drug and its presence in formulations or biological fluids. Further applications can be found within the agrochemical and related industries. The main types of HPLC/SFC CSPs are discussed below, with examples listed on pages 52-53. Please contact us for information on GC chiral phases.

Immobilised Polysaccharide CSPs

Coated polysaccharide CSPs are limited in the solvents that may be used in the eluent and as sample diluents. Newer immobilised CSPs allow the use of a more robust and expanded range of solvents and bring new selectivity and higher sample solubility relative to conventionally coated CSPs.

Cellulose and Amylose Bound

Cellulose and amylose are linear polymers of optically active glucose units with molecular weights of 250,000 to 1,000,000. Cross-linked derivatives of these materials coated onto silica give unique chiral selectivity. Their chiral recognition properties depend on the 'steric fit' of guest enantiomers into the material's cavities. Choice of eluent is the key factor affecting chiral recognition.

'Brush-Type'

Although 'brush-type' (Pirkle) chiral selectors are relatively simple molecules, their well defined structure contains three types of functional groups capable of participating in charge transfer (π-π bonding), hydrogen bonding ('dipole stacking' interactions) and steric effects. The monolayer of chiral selector covalently bound to the silica surface usually gives a column of relatively high capacity and efficiency but often with limited chiral discrimination ability. Since the synthesis of the popular D-3,5-dinitrobenzoylphenylglycine phase, significant numbers of these multiple interaction CSPs have been synthesised. Polyaromatic hydrocarbon derivative CSPs are the most recent additions to the range. All 'brush-type' phases are typically used with normal-phase eluents.

Protein Bound

Proteins are high molecular weight polymers containing chiral sub-units. When bound to silica they act as very effective CSPs. The binding or complexation of small enantiomeric molecules is often stereospecific, especially for serum proteins such as α_1-acid glycoprotein (AGP) or human serum albumin (HSA). The additional stability of the Ultron ES-OVM and ES-Pepsin columns enable them to be used with high organic content eluents. Immobilised enzymes can similarly be used. Protein immobilised CSPs are typically used in buffered aqueous eluents compatible with many biological samples. They offer good selectivity. Enantiomer retention and stereoselectivity can often be significantly altered by changes in eluent pH or modifier concentration. Their low capacity makes them unsuitable for preparative applications.

Cyclodextrin Inclusion

Cyclodextrins are a class of oligosaccharides containing six to twelve optically active glucose units. They are covalently bound to silica to form the corresponding CSP. The physical shape of these molecules is that of a truncated cone, the internal diameter of which is proportional to the number of glucose units. The interior of the cavity is relatively hydrophobic. Secondary hydroxyl groups at the entrance to the cavity contribute to the separation process. The relative stability of the inclusion complexes formed by the enantiomers of the guest molecule at the edge of the cyclodextrin cavity dictates the degree of separation. β-Cyclodextrin and its derivatives are the most commonly used CSPs of this type. Cyclodextrin CSPs are used in reversed-phase and are suitable for preparative separations.

Crown Ether

Chiral recognition with crown ether phases is achieved when a complex is formed between the crown ether and an ammonium ion from the analyte. These phases are used for solutes with a primary amino group at or near its chiral centre, such as amino acids and amino alcohols.

Ligand Exchange

Ligand exchange chiral phases are characterised by the attachment of a chiral chelating ligand to the stationary support. In the presence of an appropriate transition metal cation such as copper (II), a molecular complex is formed with the chiral stationary phase ligand and the analyte. Compounds that are suitable for chiral ligand exchange are α-amino acids, hydroxy acids and small peptides.

Network Polymeric

In a network polymeric CSP the chiral selector is anchored into a network polymer by a cross-linking reaction which simultaneously bonds it to the silica. The aim is to combine in one CSP the efficiency and capacity of 'brush-type' structures with the chiral recognition power of those phases based on chiral polymers.

Chiral Phases (continued)

Chiral Phases

Phase	Manufacturer	Chiral Type	Chiral Selector	Particle Size (µm)	Features	Page
CHIRA-chrom-1	Hichrom	Brush	D-Phenylglycine	5	High efficiency and capacity. Low cost	100
			L-Phenylglycine	5		100
			L-Leucine	5		100
CHIRA-chrom-2			Dinitrophenyltartramide	5		100
ChiraDex	Merck	Cyclodextrin	β-Cyclodextrin	5	Forms inclusion complexes	110
CHIRALPAK AGP	Chiral Technologies[2]	Protein	α_1-Acid glycoprotein	5	Widely used. pH variation a useful tool	75
CHIRALPAK CBH		Enzyme	Cellobiohydrolase	5		75
CHIRALPAK HSA		Protein	Human serum albumin	5		75
CHIRALPAK IA		Amylose	Immobilised amylose derivative	3, 5	Broad application range	74
CHIRALPAK IB		Cellulose	Immobilised cellulose derivative	3, 5		74
CHIRALPAK IC		Cellulose		3, 5		74
CHIRALPAK ID		Amylose	Immobilised amylose derivative	3, 5		74
CHIRALPAK IE		Amylose		3, 5		74
CHIRALPAK IF		Amylose		3, 5		74
CHIRALPAK AD		Amylose	Amylose derivative	3, 5, 10	Unique separation applications. Very versatile	74
CHIRALPAK AS				3, 5, 10		74
CHIRALCEL OD		Cellulose	Cellulose derivative	3, 5, 10		74
CHIRALCEL OJ				3, 5, 10		74
CHIRALPAK QD-AX		Anion-exchange	Quinidine derivative	5	Useful for chiral acids	75
CHIRALPAK QN-AX			Quinine derivative	5		75
CROWNPAK		Crown ether	18-crown-6 type crown ether	5	Suitable for amino acids and primary amines	75
Chirobiotic R	Supelco[1]	Macrocyclic glycopeptide	Ristocetin A	5	Broad selectivity	-
Chirobiotic T			Teicoplanin	5		-
Chirobiotic V			Vancomycin	5		-
Cyclobond I		Cyclodextrin	β-Cyclodextrin	5	Forms inclusion complexes	-
Cyclobond II			γ-Cyclodextrin	5		-
ChiroSil	Regis/RStech	Crown ether	(18-crown-6)-tetracarboxylic acid	5, 10	Suitable for primary amines and amino acids	137
DACH-DNB	Regis	Brush	3,5-Dinitrobenzoyl derivatives	5	π-electron acceptor/donor. Widely used	136
ULMO				5		136
Whelk-01/Whelk-02				5, 10		136
α-Burke 2				5	π-electron acceptor	136
β-GEM 1				5		136
Leucine				5		136
Phenylglycine				5		136
Pirkle-1J			β-Lactam derivative	5		136
RegisCell		Cellulose	Cellulose derivative	5, 10	Broad application range	137
RegisPack		Amylose	Amylose derivative	5, 10		137
RegisPack CLA-1		Amylose	Chlorinated amylose derivative	10	Complementary selectivity to RegisCell and RegisPack	137
Kromasil AmyCoat	Akzo Nobel	Amylose	Amylose derivative	3, 5, 10	Broad application range	-
Kromasil CelluCoat		Cellulose	Cellulose derivative			-
Kromasil DMB		Network polymer	Acylated N,N'-diallyl-L-tartardiamide	5, 10	High stability and capacity. Suitable for preparative applications	-
Kromasil TBB				5, 10		-

[1] Please contact Hichrom for ordering information

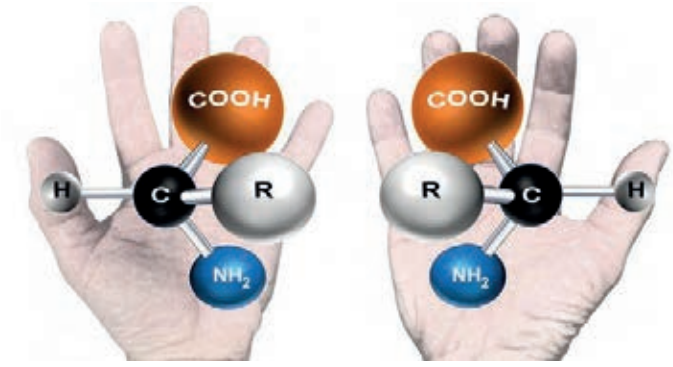
[2] CHIRALPAK ZWIX phases also available – see page 75

Chiral Phases (continued)

Chiral Phases (continued)

Phase	Manufacturer	Chiral Type	Chiral Selector	Particle Size (µm)	Features	Page
NUCLEODEX β-OH	Macherey-Nagel	Cyclodextrin	β-Cyclodextrin	5	Reversed-phase applications	102
NUCLEODEX α-PM			Permethylated α-, β- and γ-cyclodextrins respectively	5		102
NUCLEODEX β-PM				5		102
NUCLEODEX γ-PM				5		102
NUCLEOSIL CHIRAL-1		Ligand exchange	L-Hydroxyproline-Cu^{2+} complex	5	α-Amino acid applications	102
RESOLVOSIL BSA-7		Protein	Bovine serum albumin	7		102
NUCLEOCEL *DELTA*		Cellulose	Cellulose derivative	5	Broad application range	102
ORpak CDA	Shodex	Cyclodextrin	α-Cyclodextrin	6	Polyhydroxymethacrylate base material	142
ORpak CDB			β-Cyclodextrin	6		142
ORpak CDC			γ-Cyclodextrin	6		142
ORpak CDBS			β-Cyclodextrin	3	Silica base	142
ORpak CRX		Ligand exchange	L-Amino acid derivative	6	Suitable for underivatised amino acids	142
Ultron ES-OVM	Shinwa Chemical Industries	Protein	Ovomucoid	5, 10	USP L57 column	158
Ultron ES-Pepsin			Pepsin	5	Suitable for basic compounds	158
Ultron ES-CD		Cyclodextrin	β-Cyclodextrin	5	Suitable for hydrophobic cyclic compounds	158
Ultron ES-PhCD			Phenylcarbamated β-cyclodextrin	5		158
YMC Chiral CD BR	YMC[3]	Cyclodextrin	Bromide derivatives of cyclodextrin (α, β or γ)	5	Separates wide range of polar compounds	-
YMC Chiral NEA		Brush	α-Naphthylethylamine	5	NP or RP applications	-
YMC Sumichiral OA series		Various	Various	5	17 different phases	-
ZirChrom Chiral LEU	ZirChrom	Brush	Leucine derivative	3, 5	Zirconia base material	163
ZirChrom Chiral NESA			Naphthylethylsuccinamic acid derivative	3, 5		163
ZirChrom Chiral PG			Phenylglycine derivative	3, 5		163
ZirChrom CelluloZe		Cellulose	Cellulose derivative	3, 5		163

[3] YMC CHIRAL polysaccharide phases also available – please enquire



HPLC COLUMN SELECTION BY USP SPECIFICATIONS

The following list of USP (United States Pharmacopoeia) column specifications (USP 35) includes a selection of recommended columns within each category. In most cases there are several columns available within a given category, but in a few indicated instances a packing very closely fitting the specification has been included. Please contact us for further advice and assistance on selecting a suitable column by USP specification. Please also contact us for advice on column selection by EP (European Pharmacopoeia) specification. The USP monographs allow chromatographers flexibility to make method adjustments within specified limits in order to meet system suitability requirements. Please see page 57 for further details.

L1	Octadecylsilane chemically bonded to porous or non-porous silica or ceramic microparticles, 1.5 to 10µm in diameter, or a monolithic rod *Widely available*
L2	Octadecylsilane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50µm in diameter *Pellicular ODS*
L3	Porous silica particles, 1.5 to 10µm in diameter, or a monolithic silica rod *Widely available*
L4	Silica gel of controlled surface porosity bonded to a solid spherical core, 30 to 50µm in diameter *Pellicular silica*
L5	Alumina of controlled surface porosity bonded to a solid spherical core, 30 to 50µm in diameter *Please enquire*
L6	Strong cation-exchange packing – sulphonated fluorocarbon polymer coated on a solid spherical core, 30 to 50µm in diameter *Please enquire*
L7	Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10µm in diameter, or a monolithic silica rod *Widely available*
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10µm in diameter *Widely available*
L9	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10µm in diameter *Partisil SCX* *NUCLEOSIL SA*
L10	Nitrile groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter *Widely available*
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter *Widely available*
L12	A strong anion-exchange packing made by chemically bonding a quaternary amine to a solid silica spherical core, 30 to 50µm in diameter *Please enquire*
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10µm in diameter *YMC TMS* *Exsil C1* *Develosil TMS-UG*
L14	Silica gel having a chemicallly bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10µm in diameter *NUCLEOSIL SB* *Exsil SAX* *Partisil SAX*
L15	Hexylsilane chemically bonded to totally porous silica particles, 3 to 10µm in diameter *Spherisorb C6* *Chromegabond C6*
L16	Dimethylsilane chemically bonded to porous silica particles, 5 to 10µm in diameter *NUCLEOSIL C2* *Chromegabond C2*
L17	Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12µm in diameter *Hamilton HC-75 H^+* *Shodex SUGAR SH1011* *NUCLEOGEL Sugar 810H*
L18	Amino and cyano groups chemically bonded to porous silica particles, 3 to 10µm in diameter *Partisil PAC* *Partisphere PAC*
L19	Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form, about 9µm in diameter *Hamilton HC-75 Ca^{2+}* *NUCLEOGEL Sugar 810 Ca* *Shodex SUGAR SC1011*
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10µm in diameter *LiChrospher Diol* *NUCLEOSIL Diol* *PrincetonSPHER Diol*
L21	A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30µm in diameter *Hamilton PRP-1* *PLRP-S* *Shodex RSpak RP18*
L22	A cation-exchange resin made of porous polystyrene gel with sulphonic acid groups, about 10µm in size *Hamilton PRP-X200* *Shodex SUGAR SH1011*
L23	An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, 7 to 12µm in size *TSKgel BioAssist Q* *COSMOGEL QA* *Shodex IEC QA-825*

HPLC Column Selection by USP Specifications (continued)

L24	Polyvinyl alcohol chemically bonded to porous silica particles, 5µm in diameter *YMC-Pack PVA-Sil*
L25	Packing having the capacity to separate compounds with a molecular weight range from 100-5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contained some residual carboxyl functional groups) was found suitable *TSKgel G2500PW$_{XL}$* *Shodex OHpak SB-802HQ*
L26	Butylsilane chemically bonded to totally porous silica particles, 1.5 to 10µm in diameter *Widely available*
L27	Porous silica particles, 30 to 50µm in diameter *YMC Silica* *LiChroprep Silica* *Develosil Silica* *NUCLEODUR Silica*
L28	A multifunctional support, which consists of a high purity, 100Å, spherical silica substrate that has been bonded with anionic exchanger, amine functionality in addition to a conventional reversed-phase C8 functionality *Alltech Mixed-Mode C8/Anion*
L29	Gamma alumina, reversed-phase, low carbon percentage by weight, alumina-based polybutadiene spherical particles, 5µm in diameter with a pore volume of 80Å units *GammaBond RP1*
L30	Ethylsilane chemically bonded to totally porous silica particles, 3 to 10µm in diameter *As for L16*[1]
L31	A hydroxide-selective, strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 8.5µm macroporous particles having a pore size of 2000Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene *IonPac AS11-HC*
L32	A chiral ligand-exchange resin packing - L-proline copper complex covalently bonded to irregularly shaped silica particles, 5 to 10µm in diameter *NUCLEOSIL Chiral-1* *CHIRALCEL WH*
L33	Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability *TSKgel G4000SW$_{XL}$* *Shodex PROTEIN KW-800 series*
L34	Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 7 to 9µm in diameter *Hamilton HC-75 Pb^{2+}* *Shodex SUGAR SP0810*
L35	A zirconium-stabilised spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase having a pore size of 150Å *ZORBAX GF-250*
L36	A 3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to 5µm aminopropyl silica *Hichrom CHIRA-chrom-1*
L37	Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 Da. It is a polymethacrylate gel *TSKgel G3000PW$_{XL}$* *Shodex OHpak SB-803HQ*
L38	A methacrylate-based size-exclusion packing for water-soluble samples *TSKgel G1000-G6000PW$_{XL}$* *Shodex OHpak SB-800HQ Series*
L39	A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin *TSKgel G1000-G6000PW$_{XL}$* *Shodex OHpak SB-800HQ Series*
L40	Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5µm to 20µm in diameter *CHIRALCEL OD-H* *RegisCell* *NUCLEOCEL DELTA*
L41	Immobilised α_1-acid glycoprotein on spherical silica particles, 5µm in diameter *CHIRALPAK AGP*
L42	Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5µm in diameter *Hichrom RPB*
L43	Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10µm in diameter *HALO PFP* *Hypersil GOLD PFP* *Partisphere TAC-1*
L44	A multifunctional support, which consists of a high purity, 60Å, spherical silica substrate that has been bonded with a cationic exchanger, sulphonic acid functionality in addition to a conventional reversed phase C_8 functionality *Chromegabond RP-SCX*
L45	Beta cyclodextrin, R,S-hydroxypropyl ether derivative, bonded to porous silica particles, 5 to 10µm in diameter *ChiraDex* *NUCLEODEX β-OH* *Ultron ES-CD*
L46	Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalised latex beads, about 9µm to 11µm in diameter *CarboPac PA1*
L47	High capacity anion-exchange microporous substrate, fully functionalised with a trimethylamine group, 8µm in diameter *CarboPac MA1*

[1] Column represents the closest match to USP specifications

HPLC Column Selection by USP Specifications (continued)

L48	Sulphonated, cross-linked polystyrene with an outer layer of sub-micron, porous, anion-exchange microbeads, 5 to 15µm in diameter *IonPac AS5*
L49	A reversed-phase packing made by coating a thin layer of polybutadiene on to spherical porous zirconia particles, 3 to 10µm in diameter *ZirChrom PBD*
L50	Multi-function resin with reversed-phase retention and strong anion-exchange functionalities. The resin consists of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15µm in diameter, and a surface area of not less than 350 m^2/g. Substrate is coated with quaternary ammonium functionalised latex particles consisting of styrene cross-linked with divinylbenzene *OmniPac PAX-500*
L51	Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 5 to 10µm in diameter *CHIRALPAK AD* *RegisPack*
L52	A strong cation-exchange resin made of porous silica with sulphopropyl groups, 5 to 10µm in diameter *BioBasic SCX* *TSKgel SP-2SW*
L53	Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15µm diameter. Substrate is surface grafted with carboxylic acid and/or phosphoric acid functionalised monomers. Capacity not less than 500 µEq/column *IonPac CS14*
L54	A size exclusion medium made of covalent bonding of dextran to highly cross-linked porous agarose beads, about 13µm in diameter *Please enquire*
L55	A strong cation-exchange resin made of porous silica coated with polybutadiene-maleic acid copolymer, about 5µm in diameter *Universal Cation*
L56	Propyl silane chemically bonded to totally porous silica particles, 3 to 10µm in diameter *Zorbax SB-C3*
L57	A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5µm in diameter, with a pore size of 120Å *Ultron ES-OVM*
L58	Strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30µm in diameter *PL Hi-Plex Na* *Shodex SUGAR KS series*
L59	Packing for the size exclusion separations of proteins (separation by molecular weight) over the range of 5 to 7000 kDa. It is spherical (1.5 to 10µm), silica or hybrid packing with a hydrophilic coating *TSKgel G3000SW_{XL}* *Shodex PROTEIN KW-803*
L60	Spherical, porous silica gel, 10µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped *HALO RP-Amide* *Discovery RP-Amide*
L61	A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 13µm microporous particles having a pore size less than 10Å units and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 85nm diameter microbeads bonded with alkanol quaternary ammonium ions (6%) *IonPac AS11*
L62	C30 silane bonded phase on a fully porous spherical silica, 3 to 15µm in diameter. *PrincetonSPHER C30* *Develosil C30* *Cogent C30*
L63	Glycopeptide teicoplanin linked through multiple covalent bonds to a 100Å units spherical silica *CHIROBIOTIC T*
L64	Strongly basic anion-exchange resin consisting of 8% crosslinked styrene-divinylbenzene copolymer with a quaternary ammonium group in the chloride form, 45 to 180µm in diameter *AG 1-X8*
L65	Strongly acidic cation-exchange resin consisting of 8% sulphonated crosslinked styrene-divinylbenzene copolymer with a sulphonic acid group in the hydrogen form, 63 to 250µm in diameter *AG 50W-X2*
L66	A crown ether coated on a 5µm particle size silica gel substrate. The active site is (S)-18-crown-6-ether *CROWNPAK CR(+)*
L67	Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10µm in diameter *Asahipak ODP-50* *apHera C18*
L68	Spherical, porous silica, 10µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped *Suplex pKb-100*
L69	Ethylvinylbenzene/divinylbenzene substrate agglomerated with quaternary amine functionalised 130nm latex beads, about 6.5µm in diameter *CarboPac PA20*
L70	Cellulose tris(phenyl carbamate) coated on 5µm silica *CHIRALCEL OC-H*
L71	A rigid, spherical polymethacrylate, 4 to 6µm in diameter *Shodex RSpak DE*

HPLC Column Selection by USP Specifications (continued)

L72	(S)-phenylglycine and 3,5-dinitroaniline urea linkage covalently bonded to silica *Sumichiral OA-3300 S*
L73	A rigid, spherical polydivinylbenzene particle, 5 to 10µm in diameter *Jordi-Gel DVB*
L74	A strong anion-exchange resin consisting of a highly cross-linked core of 7µm macroporous particles having a 100Å average pore size and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and an anion-exchange layer grafted to the surface, which is functionalised with alkyl quaternary ammonium ions *IonPac AS14A*
L75	A chiral-recognition protein, bovine serum albumin (BSA), chemically bonded to silica particles, about 7µm in diameter, with a pore size of 300Å *RESOLVOSIL BSA*
L76	Silica based weak cation-exchange material, 5µm in diameter. Substrate is surface polymerised polybutadiene-maleic acid to provide carboxylic acid functionalities. Capacity not less than 29 µEq/column *IonPac SCS-1*
L77	Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 6 to 9µm diameter. Substrate is surface grafted with carboxylic acid functionalised groups. Capacity not less than 500 µEq/column (4mm x 25cm) *IonPac CS17*
L78	A silane ligand that consists of both reversed-phase (an alkyl chain longer than C8) and anion-exchange (primary, secondary, tertiary or quaternary amino) functional groups chemically bonded to porous, non-porous or ceramic microparticles, 1.0 to 50µm in diameter or a monolithic rod *Acclaim Mixed-Mode WAX-1*
L79	A chiral-recognition protein, human serum albumin (HSA), chemically bonded to silica particles, about 5µm in diameter *CHIRALPAK HSA*
L80	Cellulose tris(4-methylbenzoate)-coated, porous spherical silica particles, 5µm in diameter *CHIRALCEL OJ-H*
L81	A hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 9µm porous particles having a pore size of 2000Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene with a latex coating composed of 70nm diameter microbeads (6% cross-linked) bonded with alkanol quaternary ammonium ions *IonPac AS11-HC*
L82	Polyamine chemically bonded to cross-linked polyvinyl alcohol polymer, 4-5µm in diameter *Asahipak NH2P-40 Asahipak NH2P-50*
L83	A hydroxide-selective, strong anion-exchange resin, quaternary amine bonded on latex particles attached to a core of 10.5µm microporous particles having a pore size of 10Å and consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene *IonPac AS17-C*
L84	Weak cation-exchange resin consisting of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 5µm diameter. Substrate is surface grafted with carboxylic acid functionalised groups. Capacity not less than 8400 µEq/column (5mm x 25cm) *IonPac CS16*

USP and EP Allowable Adjustments

Allowable adjustments that can be made to a USP or EP (European Pharmacopoeia) method, without the method requiring revalidation, are summarised in the table below. Please contact us for further advice and assistance.

Parameter	USP[1] Allowable Adjustment	EP[2] Allowable Adjustment
Column Length	±70%	±70%
Column i.d.	±25%	±25%
Particle Size	–50%	–50%
Column Temperature	±10°C	±10°C
Flow Rate	±50%	±50%
Eluent pH	±0.2 units	±0.2 units
Concentration of Buffer Salts	±10%	±10%
Solvent A:B Ratio	Minor ±30% relative, but ≤±10% absolute	Minor ±30% relative or ±2% absolute, ≤ ±10% absolute for other
Injection Volume	Any reduction	Any reduction
Change of UV Detector Wavelength	0, but ±3nm between detectors	No change

[1] United States Pharmacopoeia 34 (2011) Section 621 [2] European Pharmacopoeia 6.0 (2010) Section 2.2.4.6