Thermo Scientific
Technical Resources Document

LC Columns and Accessories

Thermo SCIENTIFIC

Section Contents

LC Column Selection

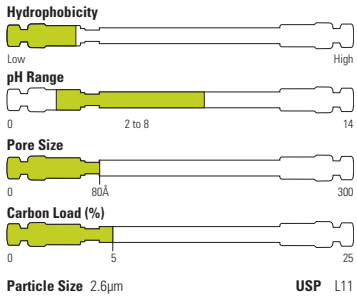
Information in the following section will help you make an informed decision on the appropriate HPLC column for your application, based on stationary phase use, analyte properties, LC-MS requirements or USP specifications. You will also find a useful table of Thermo Scientific phases with specifications, as well as recommended Thermo Scientific alternatives for other popular columns.

Refer to the **Advanced User Graphic** (AUG) on the corresponding product page (illustrated to the right) for more help and information on general purpose column selection.

The AUG will show you Hydrophobicity which gives the relative retention on the column. Generally, the higher the hydrophobicity, the greater the retention of neutral compounds and the higher the organic content in the mobile phase. A lower value indicates a need for higher aqueous mobile phases to achieve comparable retention and resolution. The recommended pH Range for the column is illustrated, outside of which column lifetimes will diminish.

The Pore Size is shown, with larger pore size columns being more applicable to larger analytes such as proteins or peptides. The percentage Carbon Load is related to the hydrophobicity. Below the icon, you will see the particle sizes available, as well as the USP code. These graphics are designed to allow you to quickly compare the main characteristics of multiple stationary phases, allowing you to choose quickly the most appropriate stationary phase for your analysis.

For additional help in column selection, please see the back cover to contact our expert Technical Support and tap into our expertise to help make the best choice for your application.



Advanced User Graphic (AUG)

Common HPLC Phases and Their Uses

Common Name	Alternative Name	Functional Group	Normal Phase	Reverse Phase	Ion Exchange	HILIC	Application
Silica	Silica	-OH	•			•	Non-polar and moderately polar organic compounds.
C1	SAS	$-(CH_3)_3$		•			Least retentive of all alkyl group bonded phases for non-polar solutes. Typically used for moderately polar and multi-functional compounds.
C4	Butyl	$-C_4H_9$		•			Shorter retention than C8, C18. Separation of peptides and proteins.
C8	MOS	$-C_8H_{17}$		•			Less retentive than C18; normally used for small peptides and proteins, pharmaceuticals, steroids, environmental samples.
C18	ODS	$-C_{18}H_{37}$		•			Most retentive of the alkyl-bonded phases. Used widely for pharmaceuticals, steroids, fatty acids, phthalates, environmental etc.
Cyano	CPS, CN	$-(CH_2)_3CN$	•	•			Unique selectivity for polar compounds, more suitable than base silica for normal phase gradient separations. When used in reversed phase, the selectivity is different to that of the C8 and C18 phases. Useful for a wide range of pharmaceutical applications and for mixtures of very different solutes.
Amino	APS	$-(CH_2)_3NH_2$	•	•	•	•	**HILIC:** Carbohydrate analysis and other polar compounds. **Weak anion exchange:** anions and organic acids. **Normal Phase:** Alternative selectivity to silica. Good for aromatics.
Phenyl		$-(CH_3)C_6H_5$		•			Aromatic compounds and moderately polar compounds.
Pentafluo-rophenyl	PFP	$-C_6F_5$		•			Extra selectivity and retention for halogenated, polar compounds and isomers.
Diol		$-(CH_2)_{20}$ $CH_2(CH_2OH)_2$	•	•		•	**Reversed Phase:** Proteins, peptides. **Normal Phase:** Similar selectivity to silica, but less polar.
SCX	Strong Cation Exchanger	$-RSO_3H-$			•		Organic bases.
SAX	Strong Anion Exchanger	$-RN+(CH_3)_3$			•		Organic acids, nucleotides and nucleosides.
AX	Anion Exchanger Polyethyleneimine (PEI)	$-(CH_2CH_2NH-)_n$			•		Organic acids, nucleotides and oligonucleotides.
Porous graphitic carbon	PGC	100% carbon	•	•			Particularly useful for the separation of highly polar compounds that are difficult to retain using conventional silica based columns; separation of structurally similar compounds (e.g., isomers, diastereoisomers).

HPLC Stationary Phase Column Selection

Before beginning a new analysis, consider the physical and chemical properties of the analyte(s), the mode of analysis and how the analyte(s) will interact with the surface of the chromatographic phase. To aid column selection, the following guide may be useful.

HPLC Stationary Phase Column Selection *continued*

Polar

Analyte Solubilty	Analyte Chemical Properties		Mode of Separation	Recommended Phase
Polar solvents	Acidic	$pK_a < 2$	Polar retention effect on graphite	Hypercarb
			Anion exchange	Hypersil GOLD AX
				Hypersil GOLD SAX
			HILIC	Accucore Urea-HILIC
				Accucore 150-Amide-HILIC
				Acclaim HILIC-10
				Syncronis HILIC
	Neutral		Polar retention effect on graphite	Hypercarb
			HILIC	Accucore HILIC
				Acclaim Mixed-Mode HILIC-1
				Acclaim Mixed-Mode WAX-1
				Acclaim Mixed-Mode WCX-1
				Acclaim Trinity P1
				Hypersil GOLD Amino
				Hypersil GOLD HILIC
				Syncronis Amino
				Syncronis HILIC
	Basic	$pK_a > 10$	Polar retention effect on graphite	Hypercarb
			Cation exchange	Acclaim Mixed-Mode WCX-1
				Acclaim Trinity P1
			HILIC	Accucore Urea-HILIC
				Accucore 150-Amide-HILIC
				Acclaim HILIC-10
				Syncronis HILIC
Non-polar solvents			Normal phase	Hypersil GOLD Amino
				Hypersil GOLD CN
				Hypersil GOLD Silica
				Syncronis Amino
				Syncronis Silica

The above table recommends columns for the separation of small molecule analytes.

Column Format Selection for LC-MS

The Thermo Scientific range offers a broad array of column designs and stationary phases optimized for LC-MS applications. Use the following table to help you choose your column format to best meet your application needs. A variety of HPLC column hardware configurations are available, designed to give superior results for high speed, high sensitivity, high efficiency and convenience. A wide range of particle and monolithic stationary phases allows choices for optimized selectivity.

Column Hardware Selection for LC-MS

LC-MS Application	Column Hardware Design	Description
High throughput analysis	Javelin HTS columns	Direct-connection columns Slim design, 20mm length, 1mm to 4.6mm ID
High sensitivity analysis	Acclaim PepMap nano, capillary and micro columns	Nano, capillary and micro columns 0.075mm to 1mm ID; 50 to 250mm length nanoViper format offers fingertight dead volume free connection to 1000 bar
Proteomics analysis	EASY-Spray columns	Combined column/emitter design with nanoViper connection. Heated flexible silica columns 50µm and 75µm ID; 150 to 500mm length
	Acclaim PepMap nano columns, Accucore nano columns, nanoViper	nanoViper offers fingertight dead volume free connection to 1000 bar Flexible silica columns 50µm and 75µm ID; 50 to 500mm length Trap column 20mm x 100µm ID
	Acclaim PepMap nano columns, classic	Flexible silica columns 50µm and 75µm ID; 50 to 500mm length Trap column 20mm x 100µm ID
	EASY-Column	Flexible silica columns 100mm x 75µm Trap column 20mm x 100µm ID
	PepSwift monolithic columns	Flexible fused silica columns, nanoViper connections 100µm to 500µm ID, 50 to 250mm length Trap columns 5mm x 200µm ID

Various HPLC columns, throughout this LC section, can also be used for LC-MS application. Typical flow rates and MS source compatibility for these columns are shown in the table on the next page

Column Selection for LC-MS *continued*

Packed column selection for LC-MS

Analyte Molecular Weight	Sample Polarity	Interface Ionization	Relative Sensitivity	Column ID (mm)	Flow Rate (µL/min)	Column Hardware
< 1000 Da	Low	APCI	Low	4.6, 4.0, 3.0	2000 – 200	Javelin HTS, Analytical
			High	2.1, 1.0	200 – 50	Analytical, Javelin HTS
	Medium	APCI	Low	4.6, 4.0, 3.0	2000 – 200	Javelin HTS, Analytical
			High	2.1, 1.0	200 – 50	Analytical, Javelin HTS
		ESI	Low	2.1, 1.0	200 – 50	Analytical, Javelin HTS
			High	1.0 – 0.3	50 – 5	Acclaim PepMap (RSLC) capillary and micro
				0.2 – 0.05	2 – 0.2	EASY-Spray column, EASY-Column, Acclaim PepMap (RSLC) nano, Accucore nano
	High (or ionizable)	ESI	Low	2.1, 1.0	200 – 50	Analytical, Javelin HTS
			High	1.0 – 0.3	50 – 5	Acclaim PepMap (RSLC) capillary and micro
				0.2 – 0.05	2 – 0.2	EASY-Spray column, EASY-Column, Acclaim PepMap (RSLC) nano, Accucore nano
> 1000 Da		ESI	Low	2.1, 1.0	200 – 50	Analytical, Javelin HTS
			High	1.0 – 0.3	50 – 5	Acclaim PepMap (RSLC) capillary and micro
				0.2 – 0.05	2 – 0.2	EASY-Spray column, EASY-Column, Acclaim PepMap (RSLC) nano, Accucore nano

Monolith columns for LC-MS

Analyte Molecular Weight	Column ID (mm)	Flow Rate (µL/min)	Column Hardware
< 1000 Da	0.1, 0.2, 0.5	0.7 – 25	PepSwift Monolith
> 1000 Da	1.0	40 – 200	ProSwift Monolith

ProSwift is also available in larger IDs for high throughput applications.

HPLC Column Selection by U.S. Pharmacopeia Specifications*

USP Code	Description	Recommended Phase
L1	Octadecyl silane (C18) chemically bonded to porous or ceramic microparticles, 1.5 to 10µm in diameter, or a monolithic rod	Acclaim 120 C18
		Acclaim 300 C18
		Accucore C18
		Accucore aQ
		Accucore 150-C18
		Accucore XL C18
		BioBasic 18
		Hypersil GOLD
		Hypersil GOLD aQ
		Acclaim PepMap 100 C18
		Syncronis C18
		Syncronis aQ
L3	Porous silica particles, 1.5 to 10µm in diameter, or a monolithic rod	Accucore HILIC
		Hypersil GOLD Silica
		Syncronis Silica
L7	Octyl silane chemically bonded to totally or superficially porous silica particles, 1.5 to 10µm in diameter, or a monolithic rod	Acclaim 120 C8
		Accucore C8
		Accucore XL C8
		BioBasic 8
		Hypersil GOLD C8
		Acclaim PepMap 100 C8
		Syncronis C8
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10µm in diameter	Hypersil GOLD Amino
		Syncronis Amino
L10	Nitrile groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter	Hypersil GOLD CN
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10µm in diameter	Accucore Phenyl-Hexyl
		Hypersil GOLD Phenyl
		Syncronis Phenyl
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10µm in diameter	BETASIL C1
		Hypersil SAS
L14	Silica gel having a chemically bonded, strongly basic quaternary ammonium anion exchange coating, 5 to 10µm in diameter	Hypersil GOLD SAX
		Hypersil SAX
L15	Hexylsilane (C6) chemically bonded to totally porous silica particles, 3 to 10µm in diameter	BETASIL C6

HPLC Column Selection by U.S. Pharmacopeia Specifications* *continued*

USP Code	Description	Recommended Phase
L17	Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12µm in diameter	HyperREZ XP Carbohydrate H^+
		HyperREZ XP Organic Acids
L19	Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, about 9µm in diameter	HyperREZ XP Carbohydrate Ca^{2+}
		HyperREZ XP Sugar Alcohols
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10µm in diameter	BETASIL Diol
L26	Butyl silane chemically bonded to totally porous silica particles, 1.5 to 10µm in diameter	Accucore 150-C4
		BioBasic 4
		Hypersil GOLD C4
		Acclaim PepMap 300 C4
L33	Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 daltons. It is spherical, silica-based, and processed to provide pH stability	BioBasic SEC 120
		BioBasic SEC 300
		BioBasic SEC 1000
		MAbPac SEC-1
L34	Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, 7 to 9µm in diameter	HyperREZ XP Carbohydrate Pb^{2+}
L43	Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10µm in diameter	Accucore PFP
		Hypersil GOLD PFP
L46	Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads about 9 to 11um in diameter	OmniPac PAX-100
L50	Multifunction resin with reverse-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 350m^2 per g. Substrate is coated with quaternary ammonium functionalized latex particles consisting of styrene cross-linked with divinylbenzene	OmniPac PAX-500
L52	A strong cation exchange resin made of porous silica with sulfopropyl groups 5 to 10µm in diameter	BioBasic SCX
L58	Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30µm in diameter	HyperREZ XP Carbohydrate Na^+
L59	Packing for the size exclusion separation of proteins (separation by molecular weight) over the range of 5 to 7,000 kDa. The packing is spherical, 1.5 to 10um, silica or hybrid packing with a hydrophilic coating	BioBasic SEC 300 (5µm)
		MAbPac SEC-1
L60	Spherical, porous silica gel, 10µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped	Acclaim PolarAdvantage
		Acclaim PolarAdvantage II
		Accucore Polar Premium
L62	C30 silane bonded phase on a fully porous spherical silica, 3 to 15µm in diameter	Acclaim C30
		Accucore C30
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 groups) functional groups chemically bonded to porous or non-porous or ceramic micro-particles, 1.0 to 50µm in diameter or a monolithic rod	Acclaim Mixed-Mode WAX-1

*These are the recommended Thermo Scientific HPLC columns for various USP categories although other columns for each category are also available.

Thermo Scientific HPLC Phases

The tables below list Thermo Scientific HPLC sorbents offered for small molecules separations.
Please also refer to the Advanced User Graphic (AUG) for each HPLC phase on the pages indicated.

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m^2/g)	% Carbon	Endcapping	USP Code	Phase Code
Acclaim Phases								
120 C18	Spherical, fully porous silica	2.2, 3, 5	120	300	18	Yes	L1	–
300 C18	Spherical, fully porous silica	3	300	100	8	Yes	L1	–
120 C8	Spherical, fully porous silica	2.2, 3, 5	120	300	11	Yes	L7	–
Phenyl-1	Spherical, fully porous silica	3	120	300	13	Yes	L11	–
C30	Spherical, fully porous silica	3, 5	200	200	13	Proprietary	L62	–
PA	Spherical, fully porous silica	2.2, 3, 5	120	300	16	Yes	L60	–
PA II	Spherical, fully porous silica	2.2, 3, 5	120	300	16	Yes	L60	–
HILIC-10	Spherical, fully porous silica	3	120	300	8	Yes	–	–
Trinity P1	Nano polymer silica hybrid	3	300	100	–	Proprietary	–	–
Trinity P2	Nano polymer silica hybrid	3	300	100	–	Proprietary	–	–
Trinity Q1	Nano polymer silica hybrid	3	300	300	–	Proprietary	–	–
Mixed Mode HILIC-1	Spherical, fully porous silica	3	120	300	–	Proprietary	–	–
Mixed Mode WAX-1	Spherical, fully porous silica	3, 5	120	300	–	Proprietary	–	–
Mixed Mode WCX-1	Spherical, fully porous silica	3, 5	120	300	–	Proprietary	L78	–
Organic Acid	Spherical, fully porous silica	3, 5	120	300	–	Yes	–	–
Surfactant	Spherical, fully porous silica	3, 5	120	300	–	Yes	–	–
Surfactant Plus	Spherical, fully porous silica	3, 5	120	300	–	Yes	–	–
Explosives	Spherical, fully porous silica	3, 5	120	300	–	Yes	–	–
Carbamate	Spherical, fully porous silica	3, 5	120	300	–	Yes	–	–
Carbonyl C18	Spherical, fully porous silica	2.2	120	300	–	Yes	–	–
SEC	Spherical, resin	5, 7	300	1000	–	–	–	–
Accucore Phases								
RP-MS	Spherical, solid core silica	2.6	80	130	7	Yes	–	176
C18	Spherical, solid core silica	2.6	80	130	9	Yes	L1	171
C8	Spherical, solid core silica	2.6	80	130	5	Yes	L7	172
aQ	Spherical, solid core silica	2.6	80	130	9	Polar	L1	173
Polar Premium	Spherical, solid core silica	2.6	150	90	8	Yes	L60	280
Phenyl-Hexyl	Spherical, solid core silica	2.6	80	130	5	Yes	L11	179
Phenyl-X	Spherical, solid core silica	2.6	80	130	6	Yes	–	279
PFP	Spherical, solid core silica	2.6	80	130	5	Yes	L43	174
C30	Spherical, solid core silica	2.6	150	90	5	Yes	L62	278
HILIC	Spherical, solid core silica	2.6	80	130	–	–	L3	175
Urea-HILIC	Spherical, solid core silica	2.6	80	130	–	–	–	277
Accucore XL Phases								
C18	Spherical, solid core silica	4	80	90	7	Yes	L1	741
C8	Spherical, solid core silica	4	80	90	4	Yes	L7	742

Thermo Scientific HPLC Phases *continued*

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m^2/g)	% Carbon	Endcapping	USP Code	Phase Code
Hypercarb Phase								
Hypercarb	Spherical, porous graphitic carbon	3, 5	250	120	100	–	–	350
Hypersil Phases								
ODS (C18)	Spherical, fully porous silica	3, 5	120	170	10	Yes	L1	301
ODS-2 (C18)	Spherical, fully porous silica	3, 5	80	220	11	Yes	L1	316
MOS (C8)	Spherical, fully porous silica	3, 5	120	170	6.5	No	L7	302
MOS-2 (C8)	Spherical, fully porous silica	5	120	170	6.5	Yes	L7	303
SAS (C1)	Spherical, fully porous silica	5	120	170	2.5	Yes	L13	305
Phenyl	Spherical, fully porous silica	5	120	170	5	No	L11	309
Phenyl-2	Spherical, fully porous silica	5	120	170	5	Yes	L11	319
CPS	Spherical, fully porous silica	3, 5	120	170	4	No	L10	308
CPS-2	Spherical, fully porous silica	5	120	170	4	Yes	L10	318
APS-2	Spherical, fully porous silica	3, 5	120	170	1.9	No	L8	307
Silica	Spherical, fully porous silica	3, 5	120	170	–	–	L3	300
SAX	Spherical, fully porous silica	5	120	170	2.5	Yes	L14	341
Hypersil BDS Phases								
C18	Spherical, fully porous silica	2.4, 3, 5	130	170	11	Yes	L1	281
C8	Spherical, fully porous silica	2.4, 3, 5	130	170	7	Yes	L7	282
Phenyl	Spherical, fully porous silica	3, 5	130	170	5	Yes	L11	289
Cyano	Spherical, fully porous silica	3, 5	130	170	4	Yes	L10	288
Hypersil GOLD Phases								
C18 selectivity	Spherical, fully porous silica	1.9, 3, 5, 12	175	220	10	Yes	L1	250
C8	Spherical, fully porous silica	1.9, 3, 5	175	220	8	Yes	L7	252
C4	Spherical, fully porous silica	1.9, 3, 5	175	220	5	Yes	L26	255
aQ	Spherical, fully porous silica	1.9, 3, 5	175	220	12	Polar	L1	253
PFP	Spherical, fully porous silica	1.9, 3, 5	175	220	8	Yes	L43	254
Phenyl	Spherical, fully porous silica	1.9, 3, 5	175	220	8.5	Yes	L11	259
CN (Cyano)	Spherical, fully porous silica	1.9, 3, 5	175	220	4	Yes	L10	258
Amino	Spherical, fully porous silica	1.9, 3, 5	175	220	2	Yes	L8	257
AX	Spherical, fully porous silica	1.9, 3, 5	175	220	6	No	–	261
SAX	Spherical, fully porous silica	1.9, 3, 5	175	220	2.5	Yes	L14	263
Silica	Spherical, fully porous silica	1.9, 3, 5	175	220	–	–	L3	251
HILIC	Spherical, fully porous silica	1.9, 3, 5	175	220	6	No	–	265
Hypersil Green Phase								
PAH	Spherical, fully porous silica	3, 5	120	170	13.5	Yes	–	311
Syncronis Phases								
C18	Spherical, fully porous silica	1.7, 3, 5	100	320	16	Yes	L1	971
C8	Spherical, fully porous silica	1.7, 3, 5	100	320	10	Yes	L7	972
aQ	Spherical, fully porous silica	1.7, 3, 5	100	320	19	Polar	L1	973
Phenyl	Spherical, fully porous silica	1.7, 3, 5	100	320	11	Yes	L11	979
Amino	Spherical, fully porous silica	1.7, 3, 5	100	320	4	Yes	L8	977
Silica	Spherical, fully porous silica	1.7, 3, 5	100	320	–	–	L3	970
HILIC	Spherical, fully porous silica	1.7, 3, 5	100	320	5	–	–	975

HPLC Column Selection by Manufacturer

To find a suitable Thermo Scientific alternative to another manufacturer's columns, refer to the selection guide below. The Thermo Scientific alternative phases are selected based on a combination of physical and chemical similarities as well as mode of retention. These alternatives are not guaranteed to provide the same retention or selectivity, but should be suitably similar in character to allow a similar or improved separation to be achieved with some method optimization. The user should refer to the individual phase information to ensure that the characteristics of the alternative match the requirements of their separation.

The following table is not complete in terms of manufacturer or products offered. Although every effort is made to ensure that the product information provided is as accurate as possible, some errors may occur in collation and transcription. We cannot accept any responsibility for the use of the following information.

Phase	Manufacturer	Pore Size (Å)	Area (m^2/g)	% Carbon	Recommended Thermo Scientific Alternative
ACE C18	ACT	100	300	15.5	Syncronis C18
ACE C8	ACT	100	300	9	Syncronis C8
ACE Phenyl	ACT	100	300	9.5	Syncronis Phenyl
ACE AQ	ACT	100	300	14	Syncronis aQ
ACE C18-300	ACT	300	100	9	BioBasic 18
ACE C8-300	ACT	300	100	5	BioBasic 8
ACE C4-300	ACT	300	100	2.6	BioBasic 4
ACQUITY UPLC™ BEH HILIC	Waters	130	185	–	Hypersil GOLD Silica (1.9µm)
ACQUITY UPLC HSS C18	Waters	100	230	15	Hypersil GOLD (1.9µm)
ACQUITY UPLC BEH C18	Waters	130	185	18	Hypersil GOLD (1.9µm)
ACQUITY UPLC BEH C8	Waters	130	185	13	Hypersil GOLD C8 (1.9µm)
ACQUITY UPLC BEH Phenyl	Waters	130	185	15	Hypersil GOLD Phenyl (1.9µm)
ACQUITY UPLC HSS T3	Waters	100	230	11	Hypersil GOLD aQ (1.9µm)
Aeris PEPTIDE XB-C18	Phenomenex	100	200	–	Accucore 150-C18
Aeris WIDEPORE XB-C18	Phenomenex	200	25	–	Accucore 150-C18
Aeris WIDEPORE XB-C8	Phenomenex	200	25	–	Accuccore 150-C4
Aeris WIDEPORE XB-C4	Phenomenex	200	25	–	Accuccore 150-C4
Alltima™ HP C18	Grace	190	200	12	Hypersil GOLD
Alltima HP C18 AQ	Grace	100	450	20	Hypersil GOLD aQ
Alltima HP C18 HiLoad	Grace	100	450	24	Syncronis C18
Alltima HP C8	Grace	190	200	8	Hypersil GOLD C8
Alltima HP CN	Grace	190	200	4	Hypersil GOLD CN
Alltima HP Silica	Grace	190	200	–	Hypersil GOLD Silica
Aminex™ HPX42C	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate Ca^{2+}
Aminex HPX72S	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate H^{+}
Aminex HPX87C	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate Ca^{2+}
Aminex HPX87H	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate H^{+}
Aminex HPX87N	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate Na^{+}
Aminex HPX87P	Bio-Rad	–	–	–	HyperREZ XP Carbohydrate Pb^{2+}
AQUA™ C18	Phenomenex	125	320	15	Hypersil GOLD aQ
Ascentis™ C18	Supelco	100	450	25	Syncronis C18
Ascentis C8	Supelco	100	450	15	Syncronis C8
Ascentis Express C18	Supelco	90	150	–	Accucore C18
Ascentis Express C8	Supelco	90	150	–	Accucore C8
Ascentis Express F5	Supelco	90	150	–	Accucore PFP
Ascentis Express HILIC	Supelco	90	150	–	Accucore HILIC
Ascentis Express Phenyl-Hexyl	Supelco	90	150	–	Accucore Phenyl-Hexyl
Ascentis Express RP-Amide	Supelco	90	150	–	Accucore Polar Premium
Ascentis Express Peptide ES-C18	Supelco	160	80	–	Accucore 150-C18
Ascentis Phenyl	Supelco	100	450	19	Syncronis Phenyl

HPLC Column Selection by Manufacturer *continued*

Phase	Manufacturer	Pore Size (Å)	Area (m^2/g)	% Carbon	Recommended Thermo Scientific Alternative
Atlantis™ dC18	Waters	100	330	12	Acclaim Polar Advantage II
Atlantis T3	Waters	100	300	14	Hypersil GOLD
Atlantis HILIC Silica	Waters	100	300	–	Hypersil GOLD Silica
Atlantis dC18	Waters	100	330	12	Hypersil GOLD aQ
Capcell Core C18	Shiseido	90	150	7	Accucore C18
Capcell Pak C18 AQ	Shiseido	120	300	11	Acclaim Polar Advantage II
Cortecs C18	Waters	90	100	6.6	Accucore C18
Cortecs C18+	Waters	90	100	5.7	Accucore Polar Premium
Cortecs HILIC	Waters	90	100	–	Accucore HILIC
Discovery™ BIO Wide Pore C18	Supelco	300	–	–	BioBasic 18
Discovery BIO Wide Pore C8	Supelco	300	–	–	BioBasic 8
Discovery C18	Supelco	180	200	14	Hypersil GOLD
Discovery C8	Supelco	180	200	–	Hypersil GOLD C8
Discovery Cyano	Supelco	180	200	–	Hypersil GOLD CN
Gemini™ C18	Phenomenex	110	375	14	Hypersil GOLD
Halo C18	AMT	90	150	–	Accucore C18
Halo C8	AMT	90	150	–	Accucore C8
Halo HILIC	AMT	90	150	–	Accucore HILIC
Halo PFP	AMT	90	150	–	Accucore PFP
Halo Phenyl-Hexyl	AMT	90	150	–	Accucore Phenyl-Hexyl
Halo RP-Amide	AMT	90	150	–	Accucore Polar Premium
HALO Peptide ES-C18	AMT	160	80	–	Accucore 150-C18
Inertsil™ C4	GL Sciences	150	320	8	Hypersil GOLD C4
Inertsil C8	GL Sciences	150	320	11	Syncronis C8
Inertsil ODS3V	GL Sciences	100	450	15	Syncronis C18
Inertsil Phenyl	GL Sciences	150	320	10	Syncronis Phenyl
Inertsil Silica	GL Sciences	150	320	–	Syncronis Silica
J'Sphere M80	YMC	80	–	14	Acclaim PolarAdvantage II
Jupiter™ C18	Phenomenex	300	170	13	BioBasic 18
Jupiter C4	Phenomenex	300	170	5	BioBasic C4
Kinetex C18	Phenomenex	100	–	12	Accucore C18
Kinetex C8	Phenomenex	100	–	10	Accucore C8
Kinetex HILIC	Phenomenex	100	–	–	Accucore HILIC
Kinetex PFP	Phenomenex	100	–	9	Accucore PFP
Kinetex Phenyl-Hexyl	Phenomenex	100	–	–	Accucore Phenyl-Hexyl
Kinetex XB-C18	Phenomenex	100	–	12	Accucore C18
Kromasil C18	Akzo-Nobel	100	340	19	Syncronis C18
Kromasil C4	Akzo-Nobel	100	340	8	Hypersil GOLD C4
Kromasil Silica	Akzo-Nobel	100	340	–	Syncronis Silica
LiChrospher™ CN	Merck	100	350	7	Hypersil GOLD CN
LiChrospher Diol	Merck	100	350	–	BETASIL Diol
LiChrospher NH_2	Merck	100	350	5	Syncronis Amino
LiChrospher RP 18	Merck	100	350	21	Syncronis C18
LiChrospher RP-18e	Merck	100	350	22	Syncronis C18
LiChrospher RP-8	Merck	100	350	13	Syncronis C8
LiChrospher RP-8e	Merck	100	350	13	Syncronis C8
Luna™ C18 (2)	Phenomenex	100	400	18	Syncronis C18
Luna C8 (2)	Phenomenex	100	400	14	Syncronis C8
Luna CN	Phenomenex	100	400	–	Hypersil GOLD CN
Luna HILIC	Phenomenex	200	200	5.7	BETASIL Diol
Luna NH2	Phenomenex	100	400	10	Syncronis Amino
Luna PFP (2)	Phenomenex	100	400	5.7	Hypersil GOLD PFP

Phase	Manufacturer	Pore Size (Å)	Area (m^2/g)	% Carbon	Recommended Thermo Scientific Alternative
Luna SCX	Phenomenex	100	400	–	BioBasic SCX
Luna Silica (2)	Phenomenex	100	400	–	Syncronis Silica
µBondapak™ C18	Waters	125	330	10	Hypersil GOLD
µBondapak CN	Waters	125	330	–	Hypersil GOLD CN
µBondapak NH_2	Waters	125	330	4	Hypersil APS-2
µBondapak Phenyl	Waters	125	330	–	Hypersil GOLD Phenyl
Nova-Pak™ (HR) C18	Waters	60	120	7	Hypersil GOLD
Nova-Pak C8	Waters	60	120	–	Hypersil GOLD C8
Nova-Pak CN	Waters	60	120	–	Hypersil GOLD CN
Nova-Pak Phenyl	Waters	60	120	5	Hypersil GOLD Phenyl
Nova-Pak Silica	Waters	60	120	–	Hypersil GOLD Silica
NUCLEODUR™ C18 EC	Macherey-Nagel	110	340	18	Syncronis C18
NUCLEODUR C18 Gravity	Macherey-Nagel	110	340	18	Syncronis C18
NUCLEODUR CN	Macherey-Nagel	110	340	7	Hypersil GOLD CN
NUCLEODUR Pyramid	Macherey-Nagel	110	340	14	Syncronis aQ
Nucleoshell™ RP 18	Macherey-Nagel	90	130	7.5	Accucore C18
Nucleoshell Phenyl-Hexyl	Macherey-Nagel	90	130	4.5	Accucore Phenyl-Hexyl
Nucleoshell PFP	Macherey-Nagel	90	130	3	Accucore PFP
Nucleosil™ 100 C18	Macherey-Nagel	100	350	17	Syncronis C18
Nucleosil 100 C18 AB	Macherey-Nagel	100	350	24	Syncronis C18
Nucleosil 100 C_6H_5	Macherey-Nagel	100	350	–	Syncronis Phenyl
Nucleosil 100 C8	Macherey-Nagel	100	350	9	Syncronis C8
Nucleosil 100 CN	Macherey-Nagel	100	350	–	Hypersil GOLD CN
Nucleosil 100 $N(CH_3)_2$	Macherey-Nagel	100	350	–	Hypersil SAX
Nucleosil 100 NH_2	Macherey-Nagel	100	350	4	Syncronis Amino
Nucleosil 100 OH	Macherey-Nagel	100	350	–	BETASIL Diol
Nucleosil 100 SA	Macherey-Nagel	100	350	7	BioBasic SCX
Nucleosil 100 SB	Macherey-Nagel	100	350	10	Hypersil GOLD SAX
Nucleosil 300 C18	Macherey-Nagel	300	100	7	BioBasic 18
Nucleosil 300 C4	Macherey-Nagel	300	100	–	BioBasic 4
Nucleosil 300 C_6H_5	Macherey-Nagel	300	100	–	BioBasic Phenyl
Nucleosil 300 C8	Macherey-Nagel	300	100	–	BioBasic 8
Nucleosil 300 CN	Macherey-Nagel	300	100	–	BioBasic CN
Pinnacle™ C1	Restek	120	170	2	Hypersil SAS
Pinnacle C18	Restek	120	170	10	Hypersil GOLD
Pinnacle C4	Restek	120	170	4	Hypersil GOLD C4
Pinnacle CN	Restek	120	170	5	Hypersil GOLD CN
Pinnacle DB C18	Restek	140	–	11	Hypersil GOLD
Pinnacle DB C18 1.9µm	Restek	140	–	11	Hypersil GOLD (1.9µm)
Pinnacle DB C8	Restek	140	–	6	Hypersil GOLD C8
Pinnacle DB Cyano	Restek	140	–	4	Hypersil GOLD CN
Pinnacle DB Phenyl	Restek	140	–	5	Hypersil GOLD Phenyl
Pinnacle IBD	Restek	120	170	–	Hypersil GOLD
Pinnacle NH_2	Restek	120	170	2	Hypersil GOLD Amino
Pinnacle Phenyl	Restek	120	170	5	Hypersil GOLD Phenyl
Pinnacle SAX	Restek	120	170	3	Hypersil GOLD SAX
Pinnacle Silica	Restek	120	170	–	Hypersil GOLD Silica
Pinnacle Ultra C18	Restek	100	–	20	Syncronis C18
Pinnacle Wide Pore C4	Restek	300	–	2	BioBasic 4
Poroshell™ 120 EC-C18	Agilent	120	120	8	Accucore C18
Poroshell 120 EC-C8	Agilent	120	120	5	Accucore C8
Poroshell 120 SB-C18	Agilent	120	120	7.5	Accucore C18
Poroshell 120 SB-Aq	Agilent	120	130	–	Accucore aQ

HPLC Column Selection by Manufacturer *continued*

Phase	Manufacturer	Pore Size (Å)	Area (m^2/g)	% Carbon	Recommended Thermo Scientific Alternative
Poroshell 120 Phenyl-Hexyl	Agilent	120	130	9	Accucore Phenyl-Hexyl
Poroshell 120 Bonus-RP	Agilent	120	130	9.5	Accucore Polar Premium
Poroshell SB-C18	Agilent	300	45	2.8	Accucore 150-C18
Poroshell Extend-C18	Agilent	300	45	4	Accucore 150-C18
Poroshell 300 SB-C8	Agilent	300	45	1.5	Accucore 150-C4
Poroshell 300 SB-C3	Agilent	300	45	1.1	Accucore 150-C4
Primesep™	SieLC	–	–	–	Acclaim Mixed-Mode Columns
Prodigy™ C8	Phenomenex	150	310	13	Syncronis C8
Prodigy ODS2	Phenomenex	150	310	18	Syncronis C18
Prodigy ODS-3	Phenomenex	100	450	16	Syncronis C18
Prodigy ODS-3V	Phenomenex	100	450	16	Hypersil GOLD
Prodigy Phenyl-3	Phenomenex	100	450	10	Syncronis Phenyl
Purospher™ RP-18	Merck	60	500	–	Hypersil GOLD
Purospher STAR-8e	Merck	120	300	–	Hypersil GOLD C8
Purospher STAR RP-18e	Merck	120	300	–	Hypersil GOLD
Waters™ Spherisorb™ C1	Waters	80	200	2	Hypersil SAS
Waters Spherisorb C6	Waters	80	200	5	BETASIL C6
Waters Spherisorb C8	Waters	80	200	6	Hypersil GOLD C8
Waters Spherisorb CN	Waters	80	200	3	Hypersil GOLD CN
Waters Spherisorb NH_2	Waters	80	200	2	Hypersil APS-2
Waters Spherisorb ODS1	Waters	80	200	6	Hypersil GOLD
Waters Spherisorb ODS2	Waters	80	200	12	Hypersil GOLD
Waters Spherisorb ODSB	Waters	80	200	12	Hypersil GOLD
Waters Spherisorb Phenyl	Waters	80	200	3	Hypersil GOLD Phenyl
Waters Spherisorb SAX	Waters	80	200	–	Hypersil SAX
Waters Spherisorb SCX	Waters	80	200	–	BioBasic SCX
Waters Spherisorb W (silica)	Waters	80	200	–	Hypersil GOLD Silica
SunFire™ C18	Waters	90	340	16	Syncronis C18
SunFire C8	Waters	90	340	16	Syncronis C8
SunShell™ C18	ChromaNik	90	150	7	Accucore C18
SunShell C8	ChromaNik	90	150	4.5	Accucore C8
SunShell PFP	ChromaNik	90	150	4.5	Accucore PFP
Supelcosil™ LC-1	Supelco	120	170	–	Hypersil SAS
Supelcosil LC-18	Supelco	120	170	11	Hypersil GOLD
Supelcosil LC-18DB	Supelco	120	170	11	Hypersil GOLD
Supelcosil LC-8	Supelco	120	170	–	Hypersil GOLD C8
Supelcosil LC-CN	Supelco	120	170	–	Hypersil GOLD CN
Supelcosil LC-NH_2	Supelco	120	170	–	Hypersil GOLD Amino
Supelcosil LC-Si	Supelco	120	170	–	Hypersil GOLD Silica
Symmetry™ C18	Waters	100	335	19	Syncronis C18
Symmetry C8	Waters	100	335	12	Syncronis C8
Synergi™ Hydro-RP	Phenomenex	80	475	19	Syncronis aQ
TSKgel™ G2000SW (incl XL)	Tosoh	125	–	–	BioBasic SEC 120
TSKgel Octyl-80TS	Tosoh	80	200	11	Hypersil GOLD C8
TSKgel ODS-120A	Tosoh	120	200	22	Hypersil GOLD
TSKgel ODS-120T	Tosoh	120	200	22	Syncronis C18
TSKgel ODS-80TM	Tosoh	80	200	15	Hypersil GOLD
TSKgel Super Octyl	Tosoh	110	–	5	Hypersil GOLD C8
TSKgel Super ODS	Tosoh	110	–	8	Hypersil GOLD
TSKgel Super Phenyl	Tosoh	110	–	3	Hypersil GOLD Phenyl
TSKgel SuperSW3000	Tosoh	250	–	–	BioBasic SEC 300

Phase	Manufacturer	Pore Size (Å)	Area (m^2/g)	% Carbon	Recommended Thermo Scientific Alternative
Vydac™ 201SP C18	Grace	90	–	–	Hypersil GOLD
Vydac 201SP Selectapore 90M C18	Grace	90	250	–	Hypersil GOLD
Vydac 201TP C18	Grace	300	–	–	BioBasic 18
Vydac 202TP C18	Grace	300	–	–	BioBasic 18
Vydac 208TP C8	Grace	300	–	–	BioBasic 8
Vydac 214TP	Grace	300	–	–	BioBasic 4
Vydac 218TP	Grace	300	–	–	BioBasic 18
Vydac 218WP Selectapore 300M C18	Grace	300	70	–	BioBasic 18
XBridge™ C18	Waters	–	–	–	Hypersil GOLD
XBridge C8	Waters	–	–	–	Hypersil GOLD C8
XBridge HILIC	Waters	130	185	–	Hypersil GOLD Silica
XBridge Phenyl	Waters	–	–	–	Hypersil GOLD Phenyl
XTerra™ MS C18	Waters	125	180	16	Hypersil GOLD
XTerra MS C8	Waters	125	180	12	Hypersil GOLD C8
YMCbasic™	YMC	–	–	–	Hypersil GOLD C8
YMC-Pack™ C4	YMC	120	300	7	HyPURITY C4
YMC-Pack C8	YMC	120	300	10	Acclaim C8
YMC-Pack CN	YMC	120	300	7	Hypersil GOLD CN
YMC-Pack Diol	YMC	120	300	–	BETASIL Diol
YMC-Pack NH_2	YMC	120	–	–	Hypersil GOLD Amino
YMC-Pack ODS AQ	YMC	120	300	16	Syncronis aQ
YMC-Pack ODS-A	YMC	120	300	17	Syncronis C18
YMC-Pack ODS-A	YMC	300	150	6	BioBasic 18
YMC-Pack Phenyl	YMC	120	300	9	Syncronis Phenyl
YMC-Pack Pro C18	YMC	120	350	16	Syncronis C18
YMC-Pack Silica	YMC	120	–	–	Syncronis Silica
YMC-Pack TMS (C1)	YMC	120	300	4	BETASIL C1
Zorbax™ Eclipse XDB C18	Agilent	80	180	10	Hypersil GOLD
Zorbax Eclipse XDB C8	Agilent	80	180	8	Hypersil GOLD C8
Zorbax Eclipse XDB Phenyl	Agilent	80	180	8	Hypersil GOLD Phenyl
Zorbax Eclipse Plus C18	Agilent	95	160	8	Hypersil GOLD
Zorbax Eclipse Plus C8	Agilent	95	160	6	Hypersil GOLD C8
Zorbax RRHT Eclipse Plus C18	Agilent	95	160	8	Hypersil GOLD (1.9µm)
Zorbax RRHT Eclipse Plus C8	Agilent	95	160	6	Hypersil GOLD C8 (1.9µm)
Zorbax RRHT Eclipse XDB-C18	Agilent	80	180	10	Hypersil GOLD (1.9µm)
Zorbax RRHT Eclipse XDB-C8	Agilent	80	180	7.5	Hypersil GOLD C8 (1.9µm)
Zorbax RRHT SB-CN	Agilent	80	180	4	Hypersil GOLD CN (1.9µm)
Zorbax SB Aq	Agilent	80	180	–	Hypersil GOLD aQ
Zorbax SB C18	Agilent	80	180	10	Hypersil GOLD
Zorbax SB C18	Agilent	300	45	3	BioBasic 18
Zorbax SB C8	Agilent	80	180	6	Hypersil GOLD C8
Zorbax SB C8	Agilent	300	45	2	BioBasic 8
Zorbax SB CN	Agilent	80	180	4	Hypersil GOLD CN
Zorbax SB Phenyl	Agilent	80	180	6	Hypersil GOLD Phenyl

Principles of Fast LC

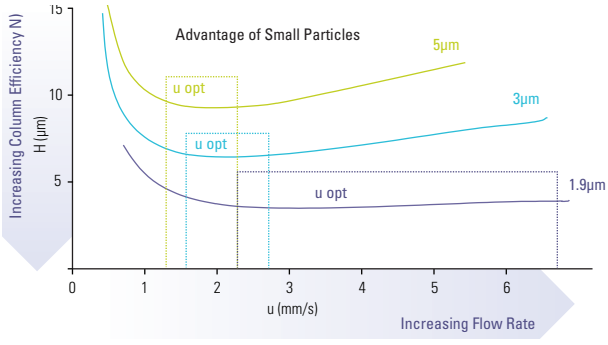
Effect of particle size and type

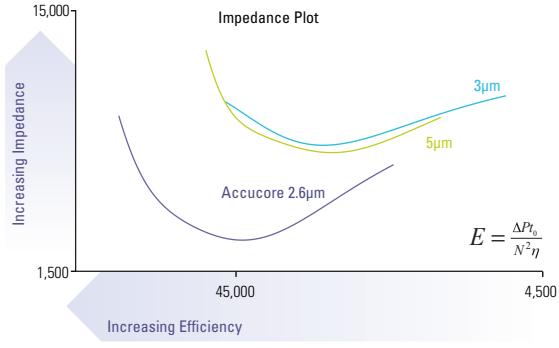
It is now well established that columns packed with smaller diameter particles generate higher efficiencies over a wider range of flow rates than larger particle columns – as shown in the plot below.

An alternative to small diameter particles is the Core Enhanced Technology used in Accucore HPLC columns. As shown in the impedance plot below, Accucore columns generate higher efficiencies in shorter times than columns packed with 5µm or 3µm particles and does so at low backpressures.

$$E = \frac{\Delta P t_0}{N^2 \eta}$$

E Impedance
ΔP Backpressure
t_0 Retention time of unretained peak
N Efficiency
η Mobile phase viscosity





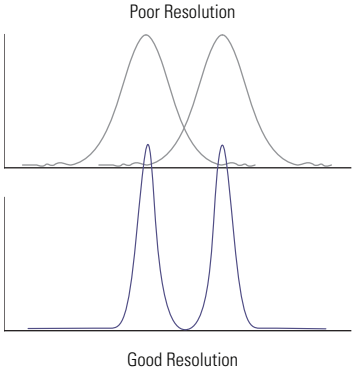
Speed and Resolution

The general chromatographic resolution equation shows that resolution is directly proportional to efficiency. High efficiencies across a wider range of linear velocities mean that shorter columns and/or faster flow rates can be used to increase the speed of separations without sacrificing resolution.

The resolution equation also shows that a wide range of different bonded phases, each offering a different selectivity, is a useful way to improve resolution.

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

α Selectivity
N Efficiency
k' Capacity factor



Peak Capacity

As an alternative to speeding up analysis the excellent resolution offered by high efficiency columns can also be used to improve complex separations through an increase in peak capacity – the number of peaks that can be separated in a given gradient time.

$$n_c = 1 + \left(\frac{t_g}{W}\right)$$

n_c Peak capacity
t_g Gradient time
W Peak width (10% height)

Sensitivity

According to the formula shown below, sensitivity is increased in high efficiency separations by increasing the concentration of the peak and thus the detector signal to noise ratio.

$$c_{max} \propto \frac{\sqrt{N}\, V_i}{L\, d_c^2 (1 + k')}$$

c_{max} Concentration at peak apex
N Efficiency
V_i Injection volume
L Column length
d_c Column internal diameter
k' Capacity factor

Miniaturization

The sensitivity formula also shows that peak concentration can be increased through the use of shorter columns and more importantly, with narrower column internal diameters.

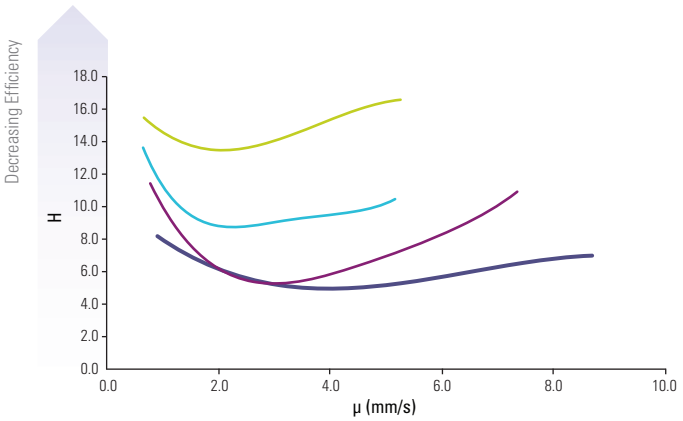
When transferring a method to a different column geometry adjustments must be made to the following parameters:

- Flow Rate
- Injection Volume
- Gradient Profile

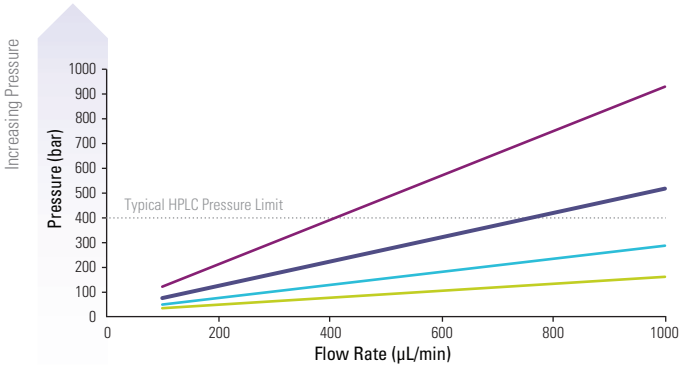
A convenient method transfer tool is available at the Chromatography Resource Center (www.thermoscientific.com/crc).

Optimization

In order to preserve high efficiency, and therefore resolution and sensitivity, the HPLC system in use should be optimized to reduce any potential causes of peak broadening.



Accucore HPLC columns are more efficient than 5μm and 3μm columns.



Column dimensions: 100 x 2.1mn

Accucore HPLC columns achieve this efficiency without excessive backpressure

Accucore 2.6μm HPLC Columns Optimum Conditions and Ratings

Column ID (mm)	Optimum Flow Rate	Maximum Inj. Volume	Backpressure Rating	Temperature Rating
2.1	400μL/min	1μL	1000 bar	70°C
3.0	800μL/min	3μL	1000 bar	70°C
4.6	1800μL/min	5μL	1000 bar	70°C

LC Technical Information

Method Transfer to Accucore 2.6 µm Columns

Containing solid core particles, which are engineered to a diameter of 2.6µm and a very narrow particle size distribution; Accucore HPLC columns allows high speed, high resolution separation, with back pressures significantly lower than those associated with sub-2µm fully porous particles.

When transferring methods from conventional HPLC to a solid core column several approaches can be taken, depending on the analytical needs. If column dimensions are maintained and the particle characteristics, design and diameter, are changed then an improvement in efficiency and, therefore, sensitivity, resolution and peak capacity is obtained. A second approach is to change not only particle characteristics but also column dimensions, which has the benefit of further reducing analysis time and increasing sensitivity.

An understanding of some practical calculations can help to achieve the correct scaling and maintain a consistent assay profile between the original and transferred method. An identical approach can be used when transferring a conventional HPLC analysis to a UHPLC method using columns packed with sub-2µm fully porous particles such as Hypersil GOLD 1.9µm and Syncronis 1.7µm.

There are three main considerations when transferring a method to a shorter column using different particles: Scaling the flow rate, adjusting the injection volume and adjusting the gradient profile. These are discussed in more detail below.*

1. Scale the Flow Rate

To maintain an equivalent separation when transferring a method it is important to keep the linear velocity constant between the original and new method. The linear velocity is related to the flow rate, internal diameter of the column and particle size. A simple equation can be derived to calculate the flow rate (F_2) required for the new method. This is shown below, normalized for particle size.

$$\mathbf{F_2 = F_1 \times (d_{c2}^2 / d_{c1}^2) \times (d_{p1} / d_{p2})}$$

F_1 – original flow rate (mL/min)
d_{c1} – original column internal diameter (mm)
d_{p1} – original column particle size (µm)
d_{c2} – new column internal diameter (mm)
d_{p2} – new column particle size (µm)

2. Adjust the Injection Volume

Because sub-3µm solid core based methods are most often transferred to smaller volume columns, the same injection volume will take up a larger proportion of the new column, possibly leading to band broadening or potentially overloading the column. It is therefore important to scale down the injection volume to match the change in column volume. Once again, a simple equation can be used to calculate the injection volume (V_{i2}) required for the new method.

$$\mathbf{V_{i2} = V_{i1} \times (d_{c2}^2 \times L_2 / d_{c1}^2 \times L_1)}$$

V_{i1} – original injection volume (µL)
d_{c1} – original column internal diameter (mm)
L_1 – original column length (mm)
V_{i2} – new injection volume (µL)
d_{c2} – new column internal diameter (mm)
L_2 – new column length (mm)

3. Adjust the Gradient Profile

Geometrical transfer of the gradient requires calculation of the number of column volumes of mobile phase in each segment (time interval) of the gradient in the original method to ensure that the new calculated gradient takes place over the same number of column volumes, for the new column.

The following calculation should be performed for each time segment of the gradient, including column re-equilibration. It takes into consideration the void volume of each column (V_c, calculation described below), the flow rate in the original method and the flow rate in the new method (calculated in step 1 above) and the time segment in the original method.

$$\mathbf{t_{g2} = t_{g1} \times (V_{c2}/V_{c1}) \times (F_1/F_2)}$$

t_{g1} – Time segment in original gradient (min)
t_{g2} – Time segment in new gradient (min)
V_{c1} – Original column void volume (mL)
V_{c2} – New column void volume (mL)
F_1 – Original flow rate (mL/min)
F_2 – New flow rate (mL/min)

The void volume of the column is the volume that is not taken up by the stationary phase (approximately 68% of the column volume):

$$\mathbf{V_c = 0.68 \times \pi \times r^2 \times L}$$

V_c – column volume (mL)
L – column length (cm)
r – column radius (cm)

An example of a method transferred following steps 1 to 3 above is illustrated in the following table:

Column: Fully porous 5 µm, 150 x 4.6mm		**Column:** Accucore RP-MS 2.6 µm, 100 x 2.1mm		**Column:** Accucore RP-MS 2.6µm, 50 x 2.1mm	
Flow rate (mL/min)	1.00	**Flow rate (mL/min)**	0.4	**Flow rate (mL/min)**	0.4
Inj. volume (µL)	1	**Inj. volume (µL)**	1.4	**Inj. volume (µL)**	0.7
Gradient Time (min)	%B	Gradient Time (min)	%B	Gradient Time (min)	%B
0.0	35.0	0.0	35.0	0.0	35.0
10.0	60.0	3.5	60.0	1.7	60.0
11.0	35.0	3.8	35.0	1.9	35.0
17.0	35.0	6.0	35.0	3.0	35.0
Backpressure	59	**Backpressure**	218	**Backpressure**	120
Resolution	2.6	**Resolution**	2.5	**Resolution**	1.5
Run Time (minutes)	17.0	**Run Time (minutes)**	6.0	**Run Time (minutes)**	3.0
Solvent Used (mL)	17	**Solvent Used (mL)**	2.4	**Solvent Used (mL)**	1.2

Method transfer conditions from HPLC (150 x 4.6mm, 5µm columns) to Accucore (100 x 2.1mm, 2.6µm and 50 x 2.1mm, 2.6µm columns).
*We offer a convenient method transfer calculator at the Chromatography Resource Center (www.thermoscientific.com/crc)

System Considerations

To obtain the best data using fast chromatography it is critical that the LC instrument system is optimized to operate under these conditions. All system components for the assay should be considered. System volume (connecting tubing ID and length, injection volume, flow cell volume in UV) must be minimized, detector time constant and sampling rate need to be carefully selected, and when running fast gradients pump dwell volume needs to be minimal.

Minimizing System Volume

Excess system volume gives rise to band broadening, which has a detrimental effect on the chromatographic performance. This can arise from the column, the autosampler, the tubing connecting the column to injector and detector and in the detector flow cell. The extra column effects become more significant for scaled down separations because of the smaller column volumes and for less retained peaks which have a lower peak volume making it even more critical to minimize extra column dispersion.

Detector Sampling Rate

With 1.9µm particles, operating parameters can be optimized to give fast analysis. This results in narrow chromatographic peaks which may be of the order of 1-2 seconds or less in width. It is important to scan the detector (whether it is UV or MS) fast enough to achieve optimum peak definition, otherwise resolution, efficiency and analytical accuracy will be compromised.

Dwell Volume

The HPLC pump dwell volume is particularly important when running high speed applications using fast gradients, typical of high throughput separations on small particle packed columns. This is because the pump dwell volume affects the time it takes for the gradient to reach the head of the column. If we consider a method using a flow rate of 0.4mL/min and a fast gradient of 1 minute, the theoretical gradient reaches the column immediately. A pump with a 65µL dwell volume will get the gradient onto the column in 9.75 seconds. A traditional quaternary pump with a dwell volume of 800µL will take 2 minutes to get the gradient to the column. When running rapid gradients this is too slow and it may become necessary to introduce an isocratic hold at the end of the gradient to allow elution of the analytes.

Scaling Down a Method

Reasons to Scale Down a HPLC or LC-MS Method

There are applications where it is desirable to scale down a method without transferring the method to UHPLC. These reasons may be to:

- Maximize sensitivity when small amounts of sample are available
- Make flow rate compatible with ionization technique in MS detection
- Reduce costs by reducing solvent consumption

Transfer Method to a Narrower Column

Reducing the scale of a separation by reducing the column internal diameter may be necessary when transferring a method from UV to MS detection, or when only very small amounts of sample are available, such as in drug discovery or proteomics. In the first case ionization technique or source design determines the best flow rate range (see table above) and in the latter case, method sensitivity is maximized because solutes elute in more concentrated chromatographic bands.

If all other method parameters (column length and particle size, column chemistry, mobile phase composition, gradient range and time, separation temperature) are kept unchanged, the change to a narrower column only requires adjustment of the flow rate.

$$\mathbf{F_2 = F_1 \times (d_{c2}/d_{c1})^2}$$

F_1 – original flow rate (to be reduced)
F_2 – new flow rate
d_{c1} – original column internal diameter
d_{c2} – new column internal diameter

This is applicable to both isocratic and gradient methods. The new method should produce a chromatogram with identical resolution and identical run time. If small changes in retention times and resolution are observed this is generally caused by system dwell volume (discussed below).

Typical Flow Rates for Analytical, Narrowbore, Capillary and Nanobore Columns (5µm Particles)

Column ID (mm)	Flow Rate Range (µL/min)	Optimum Flow Rate[1] (µL/min)	Recommended Injection Volume[2] (µL)	API Source
4.6	1000 – 1500	1250	30	APCI or High flow ESI
3.0	400 – 600	500	10	APCI or High flow ESI
2.1	200 – 300	250	5	APCI or Micro-ESI
1.0	40 – 60	50	1	Micro-ESI
0.5	10 – 25	12	0.35	Micro-ESI
0.32	4 – 10	5	0.15	Micro-ESI
0.18	1 – 3	2	0.05	Micro-ESI
0.1	0.4 – 1	0.5	0.015	Nanospray
0.075	0.2 – 0.5	0.3	0.01	Nanospray

1. Recommended for good efficiency and moderate pressure. Higher flow rates may lead to column voids. Lower flow rates are recommended for washing column bed or changing solvents.
2. Estimates based on negligible loss of efficiency and isocratic elution with sample solvent identical to mobile phase. Larger volumes can be introduced under gradient conditions or using weaker sample solvent.

Transfer Method to a Shorter Column

In gradient elution, the simplest way to reduce the method cycle time is to reduce the column length. If all other method parameters (column ID and particle size, column chemistry, mobile phase composition, gradient range, flow rate, separation temperature) are kept unchanged the only requirement is to change the gradient time using the equation below, where gradient time is reduced by the same factor as the reduction in column volume.

$$\mathbf{t_{g1}/V_{c1} = t_{g2}/V_{c2}}$$

t_{g1} – gradient time in original method (min)
t_{g2} – gradient time in new method (min)
V_{c1} – original column volume (mL)
V_{c2} – new column volume (mL)

Column volume V_c (mL) can be estimated using:

$$\mathbf{V_c = 0.68 \times \pi \times r^2 \times L}$$

V_c – column volume (mL)
L – column length (cm)
r – column radius (cm)

Dwell Volume

Dwell volume is just as important when scaling down a method as for method transfer to UHPLC. The effect of dwell volume on the separation is more significant when narrow columns are used at low flow rates. For instance, if the system has a dwell volume of 2.0mL and a 4.6mm ID column is run at 1mL/min, it takes 2 minutes for the gradient to reach the head of the column; however, if a 2.1mm ID column is used with a 0.4mL/min flow rate it will take 5 minutes for the gradient to reach the column. In high throughput gradient separations using small volume columns, dwell volume causes an increase in run times and longer re-equilibration time between runs.

Several approaches can be taken to minimize these effects:

- Select a pump with a small gradient delay volume (e.g., Thermo Scientific Accela high speed LC system has a delay volume of only 65µL);
- Delay sample injection until gradient has reached the head of the column;
- Set the pump at a higher flow rate and split the flow before the column.

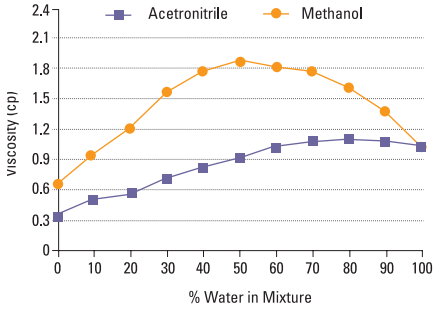
Scaling Up a Method

Reasons to Scale Up an HPLC Method

- Increase method capacity
- Isolation and purification of target compounds
- Increase sample throughput

Analytical methods may require scale up to preparative sizes to isolate and purify compounds from mixtures. In choosing the best column and packing material for your preparative application, consider the selectivity and loadability of the media as well as column dimensions, to give the results you need most quickly or economically. We have established a strong reputation for the manufacture and supply of high quality preparative silicas and bonded phases, designed to give the same levels of performance and reproducibility as our popular analytical silica ranges such as the Thermo Scientific Hypersil phases.

Scale up is easiest when starting from an analytical column packed with smaller particle size media offering the same selectivity as the larger particle size preparative media. The leading families of Thermo Scientific phases are offered in various sizes to complement lab scale operations and facilitate the scale up to preparative chromatography. Scout columns, typically 250 x 4.6mm packed with the media of interest can also be used to develop the separation. Once the method is finalized on the smaller column, a scaling factor can be applied.



Mobile phase viscosity changes with composition

Scaling Up to a Preparative Column

Flow rate and column load scaling are only required when changing the internal diameter of the column. The scaling of flow rates allows peak retention times to remain relatively constant between columns with different internal diameters. The typical solvent flow rate through a column is dependent on its internal diameter and the particle size of the column packing material. This scaling factor can also be used to estimate the loading capacity of a given column. Assuming column length is a constant, the scale factor can be calculated using the following formula:

Scale Factor = d_{c2}^2/d_{c1}^2

d_{c1} – original column internal diameter (mm)
d_{c2} – new column internal diameter (mm)

The column loading capacity and flow rate required for the new larger ID columns can be calculated using this factor.

Column Backpressure

Column operating backpressure is affected by column length, internal diameter, media particle size, temperature, solvent properties and solvent flow rate. It can also be affected by the use of gradients, where the pressure may vary with solvent composition. Typical operating backpressure for columns or cartridges can be calculated using the following equation:

$$\textbf{Pressure (atm)} = \frac{2.1 \times \Phi \times L \times \eta}{d_p^2 \times d^2}$$

Φ = column impedance
(1000 for 4.6mm ID columns)
L = column length (mm)
d_p = particle diameter (µm)
d = column diameter (mm)
η = mobile phase viscosity (centipoises)

The mobile phase viscosity varies with composition. As an example, the figure above shows how water viscosity varies with the addition of methanol or acetonitrile. This variability is a critical component in maximizing throughput with respect to the chromatography instrumentation being used.

Selecting the Media

Media selection for your preparative separation is important. Choose media that has a narrow particle size distribution which will provide high efficiency columns with a low back pressure, since there are no 'fines' to block frits or impede flow. The uniformly spherical particles, with narrow pore size distribution, apparent in Thermo Scientific preparative columns, provide reproducible performance and a longer column life. Media that is available in a range of particle sizes offers choice for scale up applications with controlled selectivity. We offers a range of choices for preparative media in several particle sizes to tailor the media to your application.

High Load and High Retention – HyperPrep HS

Materials with higher surface area can offer increased loadability. This drive to maximize surface area must be undertaken in a considered manner particularly with regard to particle pore diameter and pore volume. Too high a pore volume will compromise stability and robustness of the bed and too small a pore diameter will influence mass transfer at the expense of efficiency. The high surface area provides enhanced retention of polar compounds. A high carbon loading gives a robust, stable phase. Please contact Technical Support for more information on Thermo Scientific HyperPrep columns and media.

Peak Shape – Hypersil GOLD Media

In analytical HPLC, the use of packings based on highly pure silica has been shown to improve peak shape. Our highly developed and reproducible manufacturing processes ensure that our leading analytical brand of Hypersil GOLD media is also available in a range of particle sizes suitable for preparative LC without compromise on performance.

Polar Compounds and Isomers – Hypercarb Columns, Hypersil GOLD aQ

Often when dealing with very polar compounds, achieving sufficient retention can be a challenge. We are able to offer a variety of choices to overcome this common problem: The polar endcapping on Hypersil GOLD aQ provides a controlled interaction mechanism by which moderately polar compounds can be retained. Hypersil GOLD AX can be used in HILIC mode to provide retention of polar compounds. Hypercarb columns offer truly orthogonal selectivity to C18 in reversed phase LC and can be used to retain highly polar compounds. Hypercarb columns can also be used to differentiate between very closely related compounds including geometric and positional isomers.

Peptides and Proteins – BioBasic and Hypersil GOLD Media

When it comes to the analysis of peptides, the correct selection of packing material becomes ever more important. When deciding on which pore size of packing material to use in the analysis of a polypeptide mix, molecular weight and hydrophobicity of the peptides must be taken into account. Our breadth of silica offerings allow the chromatographer to obtain the best resolution using materials with pore diameters in the 100 to 300Å range. A general rule is that hydrophilic peptides with a molecular weight of less than 2000 daltons can be analyzed using a lower pore volume media, such as Hypersil GOLD media. Above this molecular weight, access to small pores is restricted, and separations tend to be inefficient. For hydrophobic peptides with a molecular weight greater than 2000, a 300Å media such as Thermo Scientific BioBasic is recommended. For the separation of small or hydrophilic peptides, a 100Å material such as HyperPrep HS may give better resolution.

HPLC Troubleshooting

Before you start any troubleshooting, it is essential to observe safe laboratory practices. Know the chemical and physical properties of any solvents used and have the appropriate Material Safety Data Sheets (MSDSs) readily available. All electrically powered instruments should be shut down and unplugged before starting. Eye protection should also be worn.

The following table lists common HPLC problems encountered, the possible causes and solutions for your quick reference.

Symptom	Cause	Action
Pressure Related Problems		
Low Pressure	Low viscosity mobile phase.	Confirm expected pressure using the Kozeny-Carmen or similar equation.
	Piston seals leaking.	Check for evidence of leaking or wear and replace where necessary.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Air in solvent lines or pump.	Ensure that the reservoirs and solvent lines are fully primed and the purge valve is fully closed.
High Pressure	High viscosity mobile phase.	Confirm expected pressure using the Kozeny-Carmen or similar equation.
	Pump flow-rate malfunction.	Contact manufacturer.
	Tubing blocked.	Working backwards from detector outlet, check source of blockage and replace item as necessary.
	Guard blocked.	Replace guard cartridge.
	Sample precipitation.	Consider sample clarification steps such as filtration or SPE.
	Detector blockage.	Clean the flow cell according to the manufacturer's instructions.
Baseline Related Problems		
Fluctuating Baseline	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Bubbles in flow cell.	Degas the mobile phase and pass degassed solvent through the flow-cell. Do not exceed the cell's pressure limit.
	Contaminated guard.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Detector contamination.	Clean the flow cell according to the manufacturer's instructions.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Sloping Baseline	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Gradient mobile phase.	Consider purer solvents or higher wavelengths. Otherwise, this is normal.
	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Temperature fluctuations.	Use a thermostatically controlled column oven.
	Contaminated column.	Wash the column using an appropriate solvent. Ensure that a gradient method has a wash period at the end.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Blocked solvent reservoir frits.	Ultrasonicate the reservoir frits in water and then methanol.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Peak Shape Problems		
Broad Peaks	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Injection solvent too strong.	Ensure that the injection solvent is the same or weaker strength than the mobile phase.
	Injection volume too high.	Reduce the injection volume to avoid overload. Typically injection volumes of < 40% of the expected peak width should be used.
	Injected mass too high.	Reduce the sample concentration to avoid mass overload.
	Extra column volume too high.	Reduce diameter and length of connecting tubing. Reduce the volume of the flow cell where possible.
	Temperature fluctuations.	Use a thermostatically controlled column oven. Higher temperatures will produce sharper peaks.
	Old guard cartridge.	Replace the guard cartridge.
	Old column.	Do not use columns that have been used with ion-pair reagents for reverse-phase methods. Old columns give much lower efficiencies than new columns. Replace the column if necessary.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Double Peaks	Old guard cartridge.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Negative Peaks	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Wrongly wired detector.	Check the signal polarity from the detector to the recording device.
	Unbalanced RI detector optics.	Refer to manufacturer's instructions.
	Ion pair method.	Inject the sample in the mobile phase.

Symptom	Cause	Action
Peak Shape Problems		
Flat topped Peaks	Detector overload.	Reduce the sample concentration.
	Detector set-up.	Check the detector attenuation and re-zero.
Tailing Peaks	Old guard cartridge.	Replace the guard cartridge.
	Injection solvent too strong.	Ensure that the injection solvent is the same or weaker strength than the mobile phase.
	Injection volume too high.	Reduce the injection volume to avoid overload. Typically injection volumes of < 40% of the expected peak width should be used.
	Injected mass too high.	Reduce the sample concentration to avoid mass overload.
	Old column.	Do not use columns that have been used with ion-pair reagents for reversed phase methods. Old columns give much lower efficiencies than new columns. Replace the column if necessary.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Voided column.	Replace the column. Do not use outside the recommended pH range.
Fronting Peaks	Old or damaged column.	Replace the column.
Peak Size and Retention Problems		
Small Peaks	Degraded sample.	Inject a fresh sample.
	Low analyte concentration.	Increase the analyte concentration.
	Detector set-up.	Check the detector attenuation and re-zero.
	No wash solvent.	Check that the solvent wash reservoir is filled with a miscible solvent and that the injector is set to wash between injections.
	Damaged or blocked syringe.	Replace the syringe.
	Incorrect amount injected.	Check injector loop size and that no more than 50% of this volume is used for partial loop injections.
	Viscous injection solvent.	Reduce syringe draw-time.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
No Peaks	Sample vial empty.	Fill sample vial.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Damaged or blocked syringe.	Replace the syringe.
	Different dwell volume.	For gradient methods, check that the dwell volume of any new system is not very different from any previous system. Apply a final hold time if necessary.
	Old detector lamp.	Replace the lamp, particularly when this has been in use for > 2000 hours.
Missing Peaks	Degraded sample.	Inject a fresh sample.
	Immiscible mobile phase.	Check that any solvent already in the column is miscible with the mobile phase. Flush with propan-2-ol or ethanol where necessary.
	Fluctuations in pH.	Buffer the mobile phase so that retention of ionizable compounds is controlled.
Extra Peaks	Degraded sample.	Inject a fresh sample.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade. Gradient methods often show 'ghost-peaks'.
	Immiscible mobile phase.	Check that any solvent already in the column is miscible with the mobile phase. Flush with propan-2-ol or ethanol where necessary.
	Fluctuations in pH.	Buffer the mobile phase so that retention of ionizable compounds is controlled.
	Contaminated guard cartridge.	Replace the guard cartridge.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem , replace the column.
Varying Retention	System not equilibrated.	Equilibrate the column with 10 volumes of mobile phase.
	Leak in system.	Check for and replace any leaking tubing or fittings.
	Temperature fluctuations.	Use a thermostatically controlled column oven.
	Contaminated column.	Wash the column using an appropriate solvent. If this does not resolve the problem, replace the column.
	Blocked solvent reservoir frits.	Ultrasonicate the reservoir frits in water and then methanol.
	Pump not mixing solvents properly.	Where being used, ensure that the proportioning valve is mixing the solvents correctly. If the method is isocratic, blend the solvents manually.
	Contaminated solvents.	Use freshly prepared solvents of HPLC grade.
	Different dwell volume.	For gradient methods, check that the dwell volume of any new system is not very different from any previous system. Apply a final hold time if necessary.
	Piston seals leaking.	Check for evidence of leaking or wear and replace where necessary.
	Air in solvent lines or pump.	Ensure that the reservoirs and solvent lines are fully primed and that the purge valve is fully closed.

For more information, please request Successful HPLC Operation – A Troubleshooting Guide, TG20094.

HPLC Definitions and Equations

Backpressure

The pressure required to pump the mobile phase through the column. It is related to mobile phase viscosity (η), flow rate (F), column length (L) and diameter (d_c), and particle size (d_p) by the following equation:

$$\text{Pressure Drop (psi)} = \frac{250\, L \eta F}{d_p^2 d_c^2}$$

L = column length (cm)
η = viscosity
F = flow rate (mL/min)
d_p = particle diameter (µm)
d_c = column internal diameter (cm)

Capacity Factor (k)

Expression that measures the degree of retention of an analyte relative to an unretained peak, where t_R is the retention time for the sample peak and t_0 is the retention time for an unretained peak. A measurement of capacity will help determine whether retention shifts are due to the column (capacity factor is changing with retention time changes) or the system (capacity factor remains constant with retention time changes).

$$k = \frac{t_R - t_0}{t_0}$$

Efficiency *(N)*

Also number of theoretical plates. A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. The most common are shown here, with the ones most sensitive to peak shape shown first:

5-Sigma $N = 25(t_R/W)^2$
W = peak width at 4.4% peak height

4-Sigma or Tangential $N = 16(t_R/W)^2$
W = tangential peak width or 13.4% peak height

Half-Height $N = 5.54(t_R/W)^2$
W = peak width at 50% peak height

Elution Volume *(V_R)*

Refers to the volume of mobile phase required to elute a solute from the column at maximum concentration (apex).

$$V_R = F \cdot t_R$$

where F is flow rate in volume/time and t_R is the retention time for the peak of interest.

HETP

Height equivalent to a theoretical plate. A carryover from distillation theory: a measure of a column's efficiency. For a typical well-packed HPLC column with 5µm particles, HETP (or *H*) values are usually between 0.01 and 0.03mm.

$$\text{HETP} = L/N$$

where L is column length in millimeters and *N* is the number of theoretical plates.

Linear Velocity

The flow rate normalized by the column cross section. This effects column performance and is directly related to column pressure. Linear velocity is given by the following equation where L is column length and t_0 is the breakthrough time of an unretained peak:

$$\mu = \frac{L}{t_0}$$

Resolution *(R_s)*

The ability of a column to separate chromatographic peaks. Resolution can be improved by increasing column length, decreasing particle size, changing temperature, changing the eluent or stationary phase.

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

It can also be expressed in terms of the separation of the apex of two peaks divided by the tangential width average of the peaks:

$$R_s = \frac{(t_2 - t_1)}{0.5(W_1 + W_2)}$$

Selectivity (α)

A thermodynamic factor that is a measure of relative retention of two substances, fixed by a certain stationary phase and mobile phase composition. Where k_1 and k_2 are the respective capacity factors.

$$\alpha = \frac{k_2}{k_1}$$

Tailing Factor (T)

A measure of the symmetry of a peak, given by the following equation where $W_{0.05}$ is the peak width at 5% height and f is the distance from peak front to apex point at 5% height. Ideally, peaks should be Gaussian in shape or totally symmetrical.

$$T = W_{0.05}/2f$$

van Deemter Equation

An equation used to explain band broadening in chromatography. The equation represents the height equivalent of a theoretical plate (H) and has three terms. The A term is used to describe eddy diffusion, which allows for the different paths a solute may follow when passing over particles of different sizes.

The B term is for the contribution caused by molecular diffusion or longitudinal diffusion of the solute while passing through the column. The C term is the contribution of mass transfer and allows for the finite rate of transfer of the solute between the stationary phase and mobile phase. u is the linear velocity of the mobile phase as it passes through the column.

$$H = A + \frac{B}{u} + Cu$$

Selecting the Right Buffer

A partial list of common buffers and their corresponding pH values is shown in the Common Buffer Systems table. Perhaps the most common HPLC buffer is some form of phosphoric acid. Remember that a true buffer should have the ability to resist pH change when a sample is introduced at a different pH, and that buffer capacity is only 100% at the pK value of the acid or base. At pH 4, phosphate is a poor buffer and would change rapidly toward one of its pK_a values if a more acidic or basic sample were introduced.

As a rule, one should work within ±1pH unit of the buffer pK_a value for good pH control of the mobile phase. Adequate buffer concentrations for HPLC tend to be in the 10-100 millimolar level depending on the size and nature of the sample, as well as the column packing material. Phases based on highly pure silica with robust bondings such as the Hypersil GOLD range, are often more compatible with dilute buffers than traditional packings.

When control at a lower pH (2-3) is desired, phosphate, or stronger organic acids such as TFA or acetic acid, are commonly used. If control at pH 4-5 is desired, an organic acid buffer such as acetate or citrate should be considered in place of phosphate.

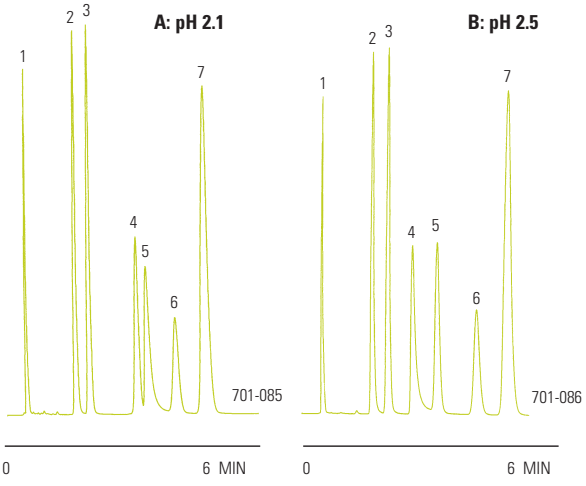
The figure to the right shows the importance of choosing the correct pH for a separation. Even slight changes in pH, either from measuring errors, mixing complications with the pump, or atmospheric water adsorption into the mobile phase, can alter any method if not properly buffered.

Care should be taken when choosing a buffer and organic modifier mixture to ensure that a solution of the two does not produce a solid salt which could cause blockages and system contamination.

Buffers should always be flushed from the analytical column and instrument after use to avoid salts being deposited on delicate frits etc.

Common Buffer Systems

Buffer		pK_a	Useful pH Range	MS-Compatible
TFA		0.30		Yes
Phosphate	pK_1	2.1	1.1 – 3.1	No
	pK_2	7.2	6.2 – 8.2	No
	pK_3	12.3	11.3 – 13.3	No
Citrate	pK_1	3.1	2.1 – 4.1	No
	pK_2	4.7	3.7 – 5.7	No
	pK_3	5.4	4.4 – 6.4	No
Formate		3.8	2.8 – 4.8	Yes
Acetate		4.8	3.8 – 5.8	Yes
Tris Base (Trizma, THAM)		8.3	7.3 – 9.3	Yes
Ammonia		9.2	8.2 – 10.2	Yes
Borate		9.2	8.2 – 10.2	No
Diethylamine		10.5	9.5 – 11.5	Yes
Carbonate	pK_1	6.4	5.4 – 7.4	Yes
	pK_2	10.3	9.3 – 11.3	Yes
Triethanolamine		7.80		Yes



BETASIL C18, 5µm, 50 x 4.6mm

Part Number:	70105-054630
Eluent:	50% ACN / 50% 25mM KH_2PO_4 at pH indicated
Flow Rate:	0.8mL/min
Detector:	UV @ 220nm
Sample:	1. Uracil 2. Tolmetin 3. Naproxin 4. Fenoprofen 5. Diflunisal 6. Indometacin 7. Ibruprofen

Effect of small changes in pH on the separation of mildly ionizable compounds

Buffer Selection for LC-MS

Buffer choice will be very dependent on the analyte and the instrumentation used. Ideally, LC-MS applications should use a volatile buffer as this will not form a contaminating deposit on the source. Inorganic acids, involatile buffers and ion-pair reagents should all be avoided. Typical LC/MS buffers include:

- Ammonium acetate/formate/hydrogen carbonate (< 50mM)
- Formic/acetic acid (0.01 – 1% v/v)
- Trifluoroacetic acid (< 0.1% v/v)
- Trialkylamine (< 0.1% v/v) and aqueous ammonia type bases
- TRIS
- BIS-TRIS propane

Note: There are LC-MS instruments available, for example the Thermo Scientific Surveyor MSQ LC-MS, which incorporate a self-cleaning mechanism to reduce the build up of inorganic buffers on the source during routine use. Care should still be taken not to purposefully over-contaminate the instrument source as this will lead to operating difficulties.

Preparation of Mobile Phases

Correct solvent preparation is very important. It can save vast amounts of time spent troubleshooting spurious peaks, baseline noise etc.

Quality

All reagents and solvents should be of the highest quality. HPLC grade reagents may cost slightly more than lower grade reagents, but the difference in purity is marked. HPLC grade reagents contain no impurities to produce spurious peaks in a chromatogram baseline whereas lower grade reagents do contain trace levels of impurities, which may produce spurious baseline peaks.

Ensure that any water used in buffer preparation is of the highest purity. Deionized water often contains trace levels of organic compounds and therefore is not recommended for HPLC use. Ultra pure HPLC water (18mΩ resistivity) is generated by passing deionized water through an ion exchange bed. Modern water purification instruments use this mechanism to produce water of suitable quality in high volumes. Preferably, HPLC grade water can be purchased from solvent suppliers.

Important: Do not store HPLC grade water in plastic containers. Additives in the plastic may leach into the water and contaminate it. Always store HPLC grade water in glass containers.

Buffers

All buffers should be prepared freshly on the day required. This practice ensures that the buffer pH is unaffected by prolonged storage and that there is no microbial growth present. Changes in pH and microbial growth will affect chromatography.

If buffer solutions are stored, be aware that they have a finite lifetime. Refer to pharmacopoeia monographs or similar for further guidance on buffer shelf life.

Buffer reagents can contain a stabilizing agent, for example, sodium metabisulphite. These stabilizing agents often affect the optical and chromatographic behavior of buffer solutions, so it is often worth buying reagents that contain no stabilizer. Containers of solid reagent are easily contaminated by repeated use. For this reason, we recommend that reagents be purchased in low container weights.

Filtration

Ideally, all HPLC solvents should be filtered through a 0.45µm filter before use This removes any particulate matter that may cause blockages. After filtration, the solvents should be stored in a covered reservoir to prevent re-contamination with dust etc.

Filtering HPLC solvents will benefit both your chromatography and the wear and tear of the HPLC system. Pump plungers, seals and check valves will perform better and lifetimes will be maximized.

Degassing

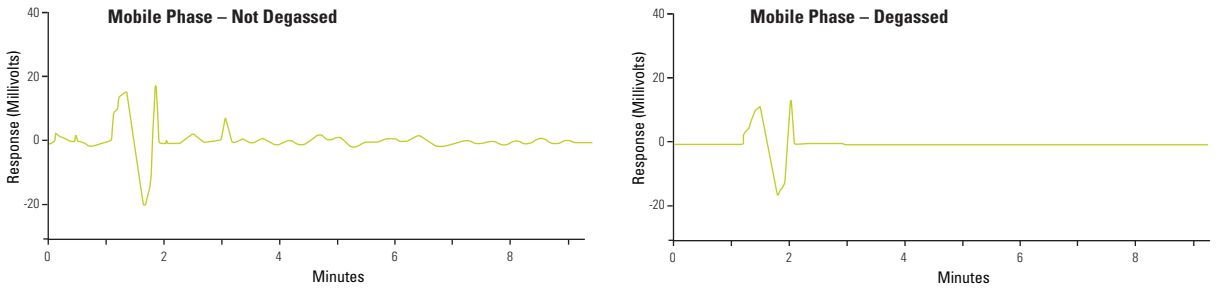
Before the freshly prepared mobile phase is pumped around the HPLC system, it should be thoroughly degassed to remove all dissolved gasses. Dissolved gas can be removed from solution by:

- Bubbling with helium
- Sonication
- Vacuum filtration

If the mobile phase is not degassed, air bubbles can form in the low pressure of the detector cell resulting in problems with system instability, spurious baseline peaks etc.

The most efficient form of degassing is bubbling with helium or another low solubility gas. If this method is available, we recommend that the mobile phase is continually degassed at very low levels throughout the analysis. This will inhibit the re-adsorption of gases over the analysis time.

Note: Ensure that the solvent reservoir has a vent to the atmosphere to prevent the build up of pressure inside the reservoir.



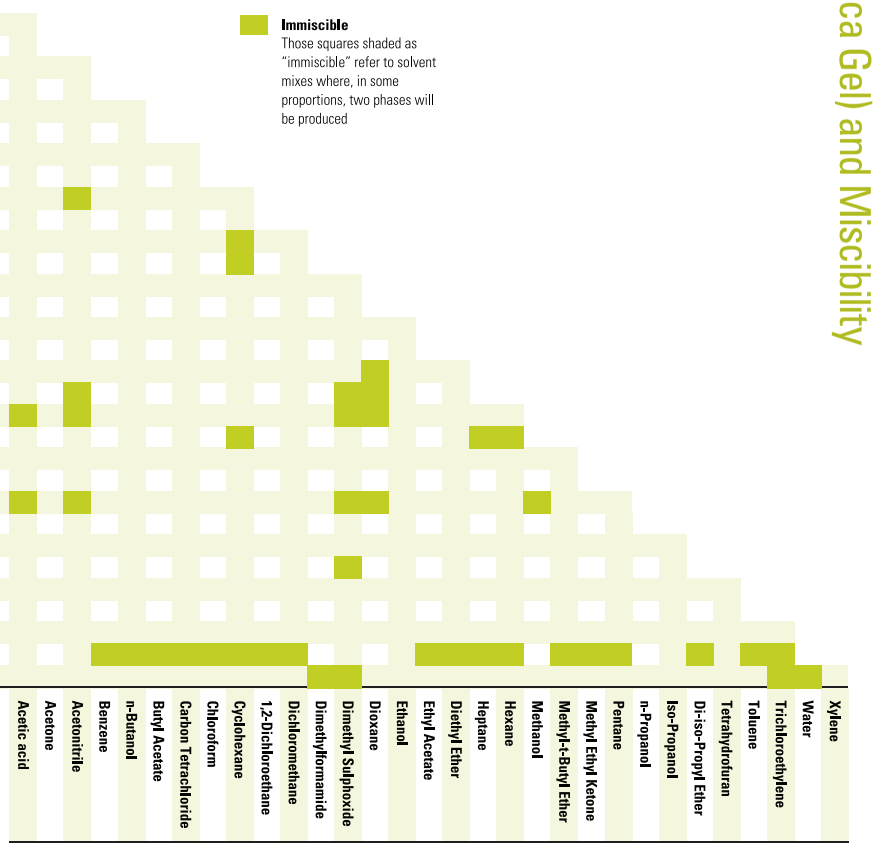
Baseline noise from gas in mobile phase

Solvent Properties and Miscibility

Solvent Strength	Polarity Index	UV Cutoff (nm)	Refractive index	Viscosity (cP, 20°C)	Boiling point (°C)	Water solubility (W/W%)	Solvent
0.01	0.1	215	1.391	0.50	99	0.0002	**Isooctane**
0.04	0.0	200	1.410	0.92	174	0.01	**n-Decane**
0.05	0.1	200	1.407	0.44	49	0.01	**Cyclopentane**
0.1	1.0	220	1.402	0.45	78	0.11	**1-Chlorobutane**
0.21	2.1	220	1.397	0.64	142	0.19	**n-Butyl Ether**
0.28	2.4	220	1.368	0.37	68	0.62	**Isopropyl Ether**
0.42	3.1	233	1.424	0.44	40	1.6	**Methylene Chloride**
0.43	4.2	334	1.396	0.51	117	-	**Methyl Butyl Ketone**
0.47	4.7	320	1.451	2.00	156	-	**Cyclohexanone**
0.55	5.5	210	1.402	1.72	125	Miscible	**Methoxyethanol**
0.6	4.5	260	1.362	0.37	57	-	**Methyl Acetate**
0.64	6.0	380	1.344	0.67	101	2.1	**Nitromethane**
0.65	6.5	268	1.438	0.84	166	Miscible	**N,N'-Dimethylacetamide**
0.69	6.0	265	1.447	1.65	182	-	**N-Methylformamide**
1.11	6.9	210	1.432	19.9	198	Miscible	**Ethylene Glycol**
2	6.0	260	1.372	1.26	118	Miscible	**Acetic acid**
0.56	5.1	330	1.359	0.36	56	Miscible	**Acetone**
0.65	5.8	190	1.344	0.38	82	Miscible	**Acetonitrile**
-	2.7	238	1.501	0.65	80	0.18	**Benzene**
0.39	3.9	215	1.399	2.98	117	7.8	**n-Butanol**
-	4.0	254	1.394	0.73	126	0.43	**Butyl Acetate**
-	1.6	263	1.460	0.97	77	0.08	**Carbon Tetrachloride**
0.4	4.1	245	1.446	0.57	61	0.815	**Chloroform**
0.04	0.2	200	1.427	1.00	81	0.01	**Cyclohexane**
-	3.5	228	1.445	0.79	83	0.81	**1,2-Dichloroethane**
-	3.1	233	1.424	0.44	40	1.3	**Dichloromethane**
0.64	6.4	268	1.431	0.92	153	Miscible	**N-N' Dimethylformamide**
0.62	7.2	268	1.478	2.24	189	Miscible	**Dimethyl Sulphoxide**
0.56	4.8	215	1.422	1.37	101	Miscible	**Dioxane**
0.88	4.3	210	1.361	1.20	79	Miscible	**Ethanol**
0.58	4.4	256	1.372	0.45	77	8.7	**Ethyl Acetate**
-	2.8	218	1.352	0.23	35	6.89	**Diethyl Ether**
0.01	0.1	200	1.388	0.40	98	0.0004	**n-Heptane**
0.01	0.1	200	1.375	0.31	69	0.0012	**n-Hexane**
0.95	5.1	205	1.329	0.55	65	Miscible	**Methanol**
0.35	2.5	210	1.369	0.27	55	4.8	**Methyl-t-Butyl Ether**
0.51	4.7	329	1.379	0.43	80	24	**Methyl Ethyl Ketone**
-	0.0	190	1.358	0.23	36	0.004	**Pentane**
0.82	4.0	210	1.385	2.30	97	Miscible	**n-Propanol**
0.82	3.9	205	1.378	2.40	82	Miscible	**Iso-Propanol**
-	2.2	220	1.368	0.37	68	-	**Di-iso-Propyl Ether**
0.45	4.0	212	1.407	0.55	66	Miscible	**Tetrahydrofuran**
0.29	2.4	284	1.496	0.59	111	0.05	**Toluene**
-	1.0	273	1.477	0.57	87	0.11	**Trichloroethylene**
2	10.2	190	1.000	1.00	100	-	**Water**
0.26	2.5	288	1.506	0.81	144	0.018	**o-Xylene**

Data Sourced from: CRC Handbook of Chemistry and Physics - 73rd Edition
The Merck Index - 12th Edition
High Purity Solvent Guide, Burdick & Jackson Laboratories, Inc.
The HPLC Solvent Guide, 2nd Edition, Paul C Sadek
HPLC Columns, Theory, Technology & Practice, Uwe D Neue
Fisher Solvent Table

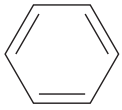
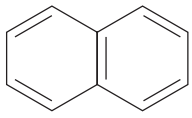
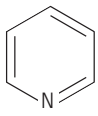
Immiscible
Those squares shaded as "immiscible" refer to solvent mixes where, in some proportions, two phases will be produced



Solvent Properties (vs Silica Gel) and Miscibility

Chromophore Detection Wavelengths

Chromophores are light absorbing groups. Their behavior is used to allow the detection of analytes. They have one or more detection wavelengths, each of which has a molar adsorbtivity associated with it. The information contained in the following table is intended as a guide to common chromophores. It is not an exhaustive list.

Chromophore		λmax (nm)	εmax (L/m/cm)
Acetylide	-C==C-	175 – 180	6,000
Aldehyde	-CHO	210	Strong
		280 – 300	11 – 18
Amine	$-NH_2$	195	2,800
Azidin	> C=N-	190	5,000
Azo	-N=N-	285 – 400	3 – 25
Benzene		184	46,700
		202	6,900
		255	170
Carboxyl	-COOH	200 – 210	50 – 70
Ester	-COOR	205	50
Ether	-O-	185	1,000
Ethylene	-C=C-	190	8,000
Ketone	> C=O	195	1,000
		270 – 285	18 – 30
Napthalene		220	112,000
		275	175
		312	5,600
Nitrate	$-ONO_2$	270	12
	$-(C=C)-_2$ acyclic	210 – 230	21,000
	$-(C=C)_3$	260	35,000
	C=C-C=C	219	6,500
	C=C-C=N	220	23,000
	C=C-C=O	210 – 250	10,000 – 20,000
	$C=C-NO_2$	300 – 350	Weak
Nitrile	-C==N	160	–
	-ONO	220 – 230	1,000 – 2,000
		300 – 400	10
Nitro	$-NO_2$	210	Strong
Nitroso	-N=O	302	100
Oxime	-NOH	190	5,000
Pyridine		174	80,000
		195	6,000
		251	1,700
Sulfone	$-SO_2-$	180	Very strong
Sulfoxide	> S-O	210	1,500
Thioether	-S-	194	4,600
	215	1,600	
Thiol	-SH	195	1,400

Column Cleaning and Regeneration

Testing of column performance can be undertaken using the experimental conditions in the test certificate provided with the column. The column efficiency, capacity factor, etc. should be measured at the start and end of the clean-up procedure to ensure that it has been performed successfully and has improved the column performance.

In all instances, the volume of solvent used is 40 – 60 column volumes unless otherwise stated. Ensure that no buffers or samples are present on the column and that the solvent used prior to the clean up is miscible with the first wash solvent. After the clean up, ensure that the test mobile phase is miscible with the last solvent in the column.

Normal Phase Media

1. Flush with tetrahydrofuran
2. Flush with methanol
3. Flush with tetrahydrofuran
4. Flush with methylene chloride
5. Flush with benzene-free n-hexane

Reversed Phase Media

1. Flush with HPLC grade water; inject 4 aliquots of 200µL DMSO during this flush
2. Flush with methanol
3. Flush with chloroform
4. Flush with methanol

Anion Exchange Media

1. Flush with HPLC grade water
2. Flush with gradient of 50mM to 1M appropriate buffer solution
3. Flush with HPLC grade water
4. Flush with methanol
5. Flush with chloroform

Cation Exchange Media

1. Flush with HPLC grade water; inject 4 aliquots of 200µL DMSO during this flush
2. Flush with tetrahydrofuran

Protein Size Exclusion Media

There are two wash/regeneration procedures associated with the removal of contaminants from protein size exclusion media.

Weakly Retained Proteins

1. Flush with 30mL 0.1M pH 3.0 phosphate buffer

Strongly Retained Proteins

1. Flush for 60 minutes using a 100% water to 100% acetonitrile gradient

Porous Graphitic Carbon

There are four wash or regeneration procedures associated with porous graphitic carbon. The one(s) used will depend on the analytes and solvents that have been used with the column

Acid/Base Regeneration

Suitable for ionized species analyzed in strongly aqueous mobile phases.

1. Invert the column
2. Flush with 50mL tetrahydrofuran:water (1:1) containing 0.1% trifluoroacetic acid
3. Flush with 50mL tetrahydrofuran:water (1:1) containing 0.1% triethylamine or sodium hydroxide
4. Flush with 50mL tetrahydrofuran:water (1:1) containing 0.1% trifluoroacetic acid
5. Flush column with 70 column volumes of THF
6. Flush with methanol/water (95:5) to re-equilibrate
7. Re-invert the column

Author: R. Plumb – Glaxo, UK

Strong Organic Regeneration

Suitable for applications involving polar and/ or ionized species analyzed in aqueous mobile phases.

1. Flush with 50mL acetone
2. Flush with 120mL dibutylether
3. Flush with 50mL acetone
4. Flush with aqueous mobile phase until equilibrated

Normal Phase Regeneration

Suitable for applications running predominantly in normal phase mobile phases.

1. Flush with 50mL dichloromethane
2. Flush with 50mL methanol
3. Flush with 50mL water
4. Flush with 50mL 0.1M hydrochloric acid
5. Flush with 50mL water
6. Flush with 50mL methanol
7. Flush with 50mL dichloromethane
8. Flush with mobile phase until equilibrated

Author: A. Karlsson – Uppsala, Sweden

Removal of TFA and DEA

TFA and DEA have the potential to adsorb to the surface of porous graphitic carbon; after using these additives in the mobile phase, regeneration of the column should be undertaken to ensure the original Hypercarb selectivity and optimum performance will always be achieved. The regeneration is as follows:

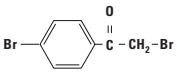
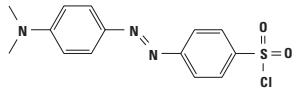
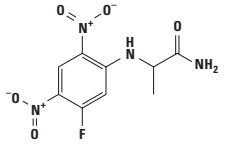
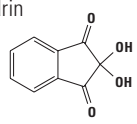
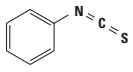
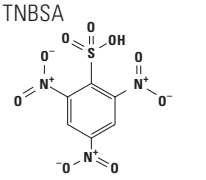
1. Removal of TFA: Flush column with 70 column volumes of THF.
2. Removal of DEA: Set column oven to 75°C and flush column with 120 column volumes of ACN.

Polymeric Media with Metallic Counter Ions

There are three types of regeneration available for polymeric columns with metal counter ion. Details of each procedure are listed in the following table.

Column Type	Metal Contamination	Organic Contamination	Column Cleaning
Hydrogen Counter Ion	Pump in reverse flow mode at 0.1mL/min with 0.1M H_2SO_4 @ 25°C for 4 to 16 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN: H_2O @ 25°C for 4 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN: 0.01M H_2SO_4 @ 65°C for 4 hr
Calcium Counter Ion	Pump in reverse flow mode at 0.1mL/min with 0.1M $Ca(NO_3)_2$ @ pH 6.3 and 85°C for 4 to 16 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN:H_2O @ 25°C for 4 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN:H_2O @ 25°C for 4 hr
Sodium Counter Ion	Pump in reverse flow mode at 0.1mL/min with 0.1M $NaNO_3$ @ 85°C for 4 to 16 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN:H_2O @ 25°C for 4 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN:H_2O @ 25°C for 4 hr
Lead Counter Ion	Pump in reverse flow mode at 0.1mL/min with 0.1M $Pb(NO_3)_2$ @ pH 5.3 and 85°C for 4 to 16 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN: H_2O @ 25°C for 4 hr	Pump in reverse flow mode at 0.1mL/min with 20:80 ACN: H_2O @ 25°C for 4 hr

Derivatization Reagents for HPLC

Functional Group	Description	Detection*	Comments
Carboxylic Acid 	*p*-Bromophenacylate 	UV	Formulation of 1.0mmol/ml p-bromophenacyl bromide and 0.005mmol/ml crown ether in acetonitrile; pre-column; nanomole detection levels: λ_{max} = 260nm[1-7]
Primary Amine 	Dabsyl Chloride 	Vis	4-N, N-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride); pre-column; nanomole detection levels: λ_{max} = 436nm[8-14]
	FDAA, Marfey's Reagent 	UV	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA); pre-column; nanomole detection levels: λ_{max} = 340nm. For chiral separations of amino acids.[15, 28-29]
	Ninhydrin 	Vis	Post-column; nanomole detection levels: λ_{max} = 570nm[22]
	PITC 	UV	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: λ_{max} = 254nm[23-24]
	TNBSA  TNBSA MW 293.17	EC, UV	2,4,6-Trinitrobenzene-sulfonic acid (TNBSA); pre- or post-column; nanomole detection levels with EC and UV, GC - 0.85V; λ_{max} = 250nm[25-26]
Secondary Amine	Ninhydrin (see structure above)	Vis	Post-column; nanomole detection levels: λ_{max} = 440nm[22]
	PITC (see structure above)	UV	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: λ_{max} = 254nm[23-24]

*EC = electrochemical; F = fluorescence; UV = ultraviolet; Vis = visible.

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Developments in Amino Acid Analysis

Improvements in amino acid analysis by ion exchange chromatography have involved the analytical system, as well as the instrumentation. Systems have been developed (by varying buffer pH or ionic strength) that work to displace the amino acids into discrete bands. The buffer systems are compatible with single- or two-column analysis of amino acids found in protein hydrolyzates or physiological fluids. Buffer systems are determined by the counter ion used (sodium or lithium) and by the method of buffer changes introduced to the resin (step changes or gradient elution).[9-15] The most commonly used buffering component, citrate, is suitable for solutions below pH7.[16] Buffers are prepared either with citric acid or an alkali salt.

Unfortunately, for high-sensitivity work, citric acid is a significant contributor to amino acid contamination. Therefore, to achieve consistent analyses, it is essential to use high-purity reagents for buffer preparation.

Alternatives to ion exchange are available for the separation of amino acids. Because amino acid analysis is one of the basic protein chemistry tools available, more rapid and sensitive methods for separation and quantitation are desirable.[17] Several pre-column derivatization methods using reverse-phase HPLC have been developed.

Two of the most widely used of these methods involve the formation of dansyl[18-19] or o-phthalaldehyde (OPA)[20-24] derivatives of amino acids prior to HPLC analysis. Both methods offer greater sensitivity and shorter analysis time than post-column derivatization techniques. Other methods include the quantitative derivatization of amino acids with phenylisothiocyanate (PITC) and the separation and quantitation of the resulting phenylthiocarbonyl derivatives via HPLC. These derivatives are stable enough to eliminate in-line derivatization.

Sample Preparation and Hydrolysis

The extraction and purification of proteins play an important role in determining amino acid content. These methods are based on one or more of their physical characteristics (e.g., solubility, molecular size, charge, polarity and specific covalent or noncovalent interactions).

The techniques commonly used to separate proteins and peptides include:

- Reverse-phase HPLC
- Polyacrylamide gel electrophoresis
- Gel filtration
- Ion exchange chromatography
- Affinity chromatography
- The table below provides a more detailed list of methods for fractionating peptide mixtures.[25]

Hydrolysis

Most protein samples require some form of chemical treatment before their component amino acids are suitable for analysis. Protein and peptide samples must be hydrolyzed to free amino acids from peptide linkages. Acids (usually HCl) are the most widely used agents for hydrolyzing proteins.

A simplified hydrolysis procedure involves refluxing the protein with excess HCl, then removing the excess acid in vacuum.[26] The lyophilized protein then is suspended in constant boiling 6 N HCl and introduced into the hydrolysis tube. The sample is frozen by immersing the tube in dry ice and acetone. Before sealing, the tube is evacuated to avoid formation of cysteic acid, methionine sulfoxide and chlorotyrosine.[27] This procedure minimizes decomposition of reduced S-carboxymethylcysteine and analyzes S-carboxymethylated proteins. Hydrolysis is conducted at 110°C (with the temperature accurately controlled) for 20-70 hours by Moore and Stein's method.[28] After hydrolysis, residual HCl is removed in a rotary evaporator. The residue is dissolved in water and brought to the appropriate pH for addition to the analyzer column.[28]

Methods for the fractionation of peptide mixtures.

Technique	Properties of Peptide Molecules Exploited
Centrifugation	Solubility
Size exclusion chromatography	Size
Ion exchange chromatography	Charge, with some influence of polarity
Paper electrophoresis	Charge and size
Paper chromatography	Polarity
Thin layer electrophoresis	Charge and size
Thin layer chromatography	Polarity
Polyacrylamide gel electrophoresis	Charge and size
High-performance liquid chromatography (HPLC)	Polarity
Gas chromatography	Volatility of derivatives
Counter-current extraction	Polarity; sometimes specific interactions
Affinity chromatography	Specific interactions
Covalent chromatography or irreversible binding	Disulfide bond formation; reactivity of homoserine lactone

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