## Allowable Adjustments to United States Pharmacopeia (USP) Methods for ISOCRATIC SEPARATIONS

Component	United States Pharmacopeia (USP)
Mobile phase minor component ( $\leq$ 50%)	$\pm$ 30 % Relative; Cannot exceed $\pm$ 10 % Absolute change; Cannot be reduced to zero
Mobile phase pH	± 0.2 pH units
Buffer concentration	± 10 %
Column temperature	± 10 °C
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection reqs.
Detector wavelength	Cannot be modified
Flow rate	$\pm$ 50% (at given ID)
Column inner diameter	Can be adjusted so long as linear velocity is maintained
Column length	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25 % and +50 $\%^{\star}$
Particle size	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25 % and +50 $\%^{\star}$
Stationary phase	No change of the identity of the substituent permitted
Guards	Same stationary phase as column; guard ID $\leq$ column ID; guard length $\leq$ 15 % column length

\*Alternatively (as for the application of particle size adjustment to superficially porous particles), other L/dp combinations can be used provided that the number of theoretical plates (N) is within -25 % to +50 %

## Allowable Adjustments to United States Pharmacopeia (USP) Methods for **GRADIENT SEPARATIONS**

Component	United States Pharmacopeia (USP)	
Mobile phase minor component (≤ 50 %)	Changes to gradient composition are not recommended	
Mobile phase pH	$\pm$ 0.2 pH units	
Buffer concentration	± 10%	
Column temperature	± 10 °C	
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection reqs.	
Detector wavelength	Cannot be modified	
Flow rate	Changes to flow rate are not allowed	
Column inner diameter	Changes to column length, particle size, or inner diameter are not allowed	
Column length	Changes to column length, particle size, or inner diameter are not allowed	
Particle size	Changes to column length, particle size, or inner diameter are not allowed	
Stationary phase	No change of the identity of the substituent permitted	
Guards	Same stationary phase as column; guard ID $\leq$ column ID; guard length $\leq$ 15 % column length	



## **HPLC Column Selection by USP Listing**

IICD	Column Classification	Recommended Phenomenex	Particle
USP			Shape
L1	Octadecyl silane chemically bonded to porous or non-porous silica or ceramic microparticles, 1.5 to 10 µm in diameter,	Gemini <sup>®</sup> NX-C18	Spherical
	or a monolithic rod.	Kinetex <sup>®</sup> C18	Core-Shell
		Kinetex EVO C18	Core-Shell
		Kinetex Polar C18	Core-Shell
		Kinetex XB-C18	Core-Shell
		Luna® C18(2)	Spherical
		Luna Omega C18	Spherical
		Luna Omega PS C18	Spherical
		Luna Omega Polar C18	Spherical
		Gemini C18	Spherical
		Synergi <sup>™</sup> Hydro-RP	Spherical
		Synergi Fusion-RP	Spherical
		Onvx <sup>™</sup> C18	Monolith
		Jupiter <sup>®</sup> C18	Spherical
		Clarity <sup>®</sup> Oligo-BP	Spherical
		Clarity Oligo-MS	Core-Shell
		Clarity Oligo XT	Core-Shell
		Aeris™ WIDEPORE XB-C18	Core-Shell
		Aeris PEPTIDE XB-C18	Core-Shell
L3	Porous silica particles, 1.5 to 10 μm in diameter, or a monolithic silica rod.	Kinetex HILIC	Core-Shell
		Luna Silica(2)	Spherical
		Onyx Silica	Monolith
L7	Octyl silane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a	Kinetex C8	Core-Shell
	monolithic silica rod.	Luna C8(2)	Spherical
		Onvx C8	Monolith
		Aeris WIDEPORE XB-C8	Core-Shell
L8	An essentially monomolecular layer of aminopropyl-silane chemically bonded to totally porous silica gel support, 1.5 to 10 um in diameter, or a monolithic silica rod.	Luna NH <sub>2</sub>	Spherical
L9	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation exchange coation. 3 to 10 um in diameter	Luna SCX	Spherical
L10	Nitrile groups chemically bonded to porous silica particles. 1.5 to 10 µm in diameter, or a monolithic silica rod.	Luna CN	Spherical
L11	Phenyl groups chemically bonded to porous silica particles 1.5 to 10 µm in diameter, or a monolithic silica rod	Kinetex Biphenyl	Core-Shell
		Kinetex Phenyl-Hexyl	Core-Shell
		Synergi Polar-BP	Spherical
		Luna Phenyl-Hexyl	Spherical
		Gemini C6-Phenyl	Spherical
		Prodiav™ PH-3	Spherical
113	Trimethylsilane chemically honded to norous silica particles 3 to 10 µm in diameter	Develosil <sup>®</sup> TMS-UG (C1) 130 Å	Spherical
114	Timenyoliane onomically bonded strongly based and an analysis of the management of the strong strong strong the strong the strong stron	PhenoSnhere™ SAX	Spherical
514	5 to 10μm in diameter.		ophonour
L15	Hexyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.	PhenoSphere C6	Spherical
L17	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12 µm in diameter.	Rezex <sup>™</sup> RHM-Monosaccharide Rezex ROA-Organic Acid	Spherical Spherical
L19	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer	Rezex RCM-Monosaccharide	Spherical
	in the calcium form, 5 to 15µm in diameter.	Rezex RCU-Sugar Alcohols	Spherical
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to $10\mu m$ in diameter,	Luna HILIC	Spherical
	or a monolithic silica rod.	BioSep <sup>™</sup> -SEC-S	Spherical
		Yarra <sup>™</sup> SEC	Spherical
L21	A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30 μm in diameter.	PolymerX <sup>™</sup> RP-1	Spherical
		Phenogel <sup>™</sup> 100 Å	Spherical
122	A cation-exchange resin made of norous polystyrene gel with sulfonic acid groups 5 to 15 µm in diameter	Bezex BOA-Organic Acid	Spherical
1 22	A gaine sector sector production of persons polymethese generation of a gaine groups, or to primin diameter.	Shodoy® IEC 0A 825	Sphorical
LZJ	ammonium groups, 7-12 µm in size.	SHOULS IEP MA-050	Spriencal
L25	Packing having the capacity to separate compounds with a MW range from 100 to 5000 daltons	PolySep <sup>™</sup> -GFC-P2000	Spherical
	(as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers. A polymethacrylate resin base, crosslinked with poly-hydroxylated ether (surface contained some residual	Shodex OHpak SB-802.5HQ	Spherical

carboxyl functional groups) was found suitable.

## **HPLC Column Selection by USP Listing**

lied	Polumn Classification	Recommended Phenomenex	Particle
LOC	Journin Grassingation Districtions charging the based of a table common superficielly common silico and interaction in discussion	Goluliili Iurita ® 200 04	Snape
L20	Butyl shane chemically bonded to totally porous or supericially porous silica particles, 1.5 to 10 µm in diameter.	Aeris <sup>™</sup> WIDEPORE C4	Core-Shell
L27	Porous silica particles, 30 to 50 µm in diameter.	Sepra <sup>™</sup> Silica	Irregular
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	Yarra <sup>™</sup> SEC-2000	Spherical
	and processed to provide pH stability.	BioSep <sup>™</sup> -SEC-S2000	Spherical
		Yarra SEC-3000	Spherical
		BIOSEP-SEC-S3000	Spherical
		Yarra SEC-X300	Spherical
L34	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, 7 to 9 µm in diameter.	Rezex <sup>™</sup> RPM-Monosaccharide	Spherical
L35	A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular monolayer	(BioSep-SEC-S2000	Spherical
	bonded phase having a pore size of 150Å.	or Yarra SEC-2000 may be used)	Spherical
L37	Polymethacrylate gel packing having the capacity to separate proteins by molecular size over a	PolySep <sup>™</sup> -GFC-P3000	Spherical
	range of 2,000 to 40,000 daltons.	Shodex <sup>®</sup> OHpak SB-803HQ	Spherical
L38	Methacrylate-based size-exclusion packing for water-soluble samples.	PolySep-GFC-P series Shodex OHpak SB-800HQ	Spherical Spherical
L39	Hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.	PolySep-GFC-P series	Spherical
		Shodex OHpak SB-800HQ series	Spherical
1.40	Colluione tria 2.5 dimethylabanylaarbameta aastad paraya ailiga partiglaa 2 to 20 ym in diametar		Spherical
1.40	Centrolise (hs-c,c)-dimetriyiphetrykaraamate Coaled porous sinca particles (hs-c,c) to 20 jiii in indimeter.	Lux Cellulose-1	Coro Sholl
L43	rentandorophenyr groups chennicany bondeu to sinca parucies by a propyr spacer, 1.5 to 10 phr in diameter.	Luna <sup>®</sup> PFP(2)	Spherical
L45	Beta cyclodextrin, R, S-hydroxypropyl ether derivative, bonded to porous silica particles, 3 to 10 $\mu m$ in diameter	Shiseido® Chiral CD-Ph	Spherical
L51	Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 3 to 10 µm in diameter.	Lux Amylose-1	Spherical
L57	A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about $5\mu m$ in diameter, with a pore size of 120 Å.	Ultron <sup>®</sup> ES-0VM	Spherical
L58	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to $30\mu\text{m}$ in diameter.	Rezex RNM-Carbohydrate	Spherical
L59	Size-exclusion separations of proteins (separation by molecular weight) over the range of 5 to 7000 kDa.	Yarra SEC-2000	Spherical
	Spherical (1.5 to $10 \mu$ m), silica or hybrid packing with a hydrophilic coating.	BioSep-SEC-S2000	Spherical
		Yarra SEC-3000	Spherical
		BIOSEP-SEC-S3000 Varra SEC-X150	Spherical
		Yarra SEC-X300	Spherical
L62	C30 silane bonded phase on a fully porous spherical silica. 3 to 15 um in diameter.	Develosil <sup>®</sup> Combi-RP	Spherical
		Develosil RP-Aqueous	Spherical
		Develosil RP-Aqueous-AR	Spherical
L67	Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10 μm in diameter.	Asahipak <sup>®</sup> ODP-50	Spherical
L71	A rigid, spherical polymethacrylate 4 to $6\mu$ m in diameter.	Shodex RSpak DE-413	Spherical
1.70	(A) shandahaina and A.F. dinitaranilian una linkana analantu kandada a silian	Shodex RSpak DE-613	Spherical
L/2	(5)-prieriyiyiyone anu 5,5-unnuoannine urea inikage covalenuy bonded to sinca.		Spherical
100	oonuuooo unaje-mounyuonzuale)-tudateu, punuus, spinemaa, siinta parutites, si tu zu piin in diameter. Delvamine chamically hendad te cross linkad palvuinyi aleebal pelvmer 4 to 5 um in diameter.		Spherical
1.87	n organimo commeany pomoco to cross-mineo poryvinyi accomo porymel, 4 to 5 µm in diameter	Asanipak NHZE-30 Svpergi™ May PD	Spherical
LOI	Douecy shahe chemically boliced to porous sinca particles, 1.5 to 10 pm in diameter.	Jupiter Proteo	Spherical
L93	Cellulose tris (3,5-dimethylphenylcarbamate) reversed phase chiral stationary phase coated on 3 or 5 µm silica gel particles.	Lux Cellulose-1	Spherical
L96	Alkyl chain, reversed phase bonded totally or superficially porous silica designed to retain hydrophilic and other polar compounds when	Kinetex Polar C18	Spherical
	using nignly aqueous mobile phases, including 100 % aqueous, 1.5 to 10 µm in diameter.	KINCLEX EVU UIX	Spherical
		Luna Omega POlat 616	Spherical
		Synergi Hydro-RP	Spherical
L99	Amylose tris-(3,5-dimethylphenylcarbamate), immobilized on porous, spherical, silica particles, 3 to 5 µm in diameter.	Lux i-Amylose-1	Spherical
L107	Cellulose tris(4-methylbenzoate)-coated porous spherical particles, 3 to 5 µm in diameter, for use with reversed phase mobile phases.	Lux Cellulose-3	Spherical

Trademarks

Gemini, Kinetex, Luna, Jupiter, Clarity, and Lux are registered trademarks and Aeris, Yarra, Synergi, Onyx, Prodigy, PhenoSphere, Rezex, BioSep, PolymerX, PolySep, Sepra, and Phenogel are trademarks of Phenomenex. Develosil is a registered trademark of Nomura Chemical Co. Shodex and Asahipak are registered trademarks of Showa Denko K.K. Ultron is a registered trademark of Shinwa Chemical Industries. Chirex is a trademark of Chirex, Inc., licensed to Phenomenex. Shiseido is a registered trademark of Shiseido Company, Ltd.

Clarity Oligo-XT, Gemini, and Kinetex EVO are patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.

© 2017 Phenomenex, Inc. All rights reserved.