

GC and HPLC Phases and Packings for US Pharmacopoeia Methods

The official pharmaceutical analysis monographs in the United States Pharmacopoeia (USP) detail the methods used by pharmaceutical manufacturers for quality control of bulk drug substances and dosage form preparations. Each method specifies a particular gas chromatography or HPLC column or column type and the conditions under which the analysis is performed. This bulletin contains lists of the USP Codes for the supports, phases, and column types used in these methods, generic descriptions of the columns, and information about the Supelco products that conform to these descriptions.

Key Words:

- US Pharmacopoeia methods • USP methods
- pharmaceuticals analysis

In the *United States Pharmacopoeia 24, National Formulary 19*, on page 6 of *General Notices and Requirements*, under *Test and Assay/ Apparatus* the first sentence is: "A specification for a definite size or type of container or apparatus in a test or assay is given solely as a recommendation" (1). This statement allows a broad range of interpretation, and will be expanded in the year 2000 USP National Formulary (personal communication). This gives an analyst leverage for using an alternate phase, especially when the phase specified is no longer manufactured, or when a better phase has become available. USP also has made changes in requirements that give analysts opportunity to optimize their systems. USP realizes that columns are not the same from one manufacturer to another, and differences among instruments require optimization of parameters. For details, refer to *Pharmacopoeial Forum* (2). In addition, we recommend you consult the USP chemist in charge of the monograph with which you intend to work.

The following parameters are in proposed revision status.

System suitability tests are an integral part of gas and liquid chromatography methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. These tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such.

*Resolution, R_1 ** is a function of column efficiency, N , and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system and to ensure that internal standards are resolved from the drug. Column efficiency also may be specified as a system suitability requirement, especially if there is only one peak of interest in the chromatogram; however, relative to direct measurement, it is a less reliable means of ensuring resolution.

Column efficiency is a measure of peak sharpness, which is important for detecting trace components.

Replicate injections of a standard preparation used in the assay, or other standard solution, are compared to ascertain whether the requirements for *precision* are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analytes are used to calculate the relative standard deviation, S_{R_1} , if the requirement is 2.0% or less; data from six replicate injections are used if the relative standard deviation specification is greater than 2.0%.

The *tailing factor, T* , a measure of peak symmetry, is unity for perfectly symmetrical peaks, and increases as tailing becomes more pronounced (3). As peak asymmetry increases, integration, and hence precision, becomes less reliable. In some cases, values less than 1.0 may be observed.

These tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph. The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions (1). If operating conditions must be adjusted to meet system suitability requirements, each of the following is the maximum specification that can be considered, unless otherwise directed in the monograph. Adjustments are permitted only when reference standards are available for all analytes and are used to show that the adjustments have improved the quality of the chromatogram.

Column Length (GC, HPLC) – Column length can be adjusted by as much as 70%.

Column Inner Diameter (GC, HPLC) – Column ID can be adjusted by as much as $\pm 25\%$.

Detector Wavelength (HPLC) – Deviations from the wavelengths specified in the method are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is to be used to verify that error in the detector wavelength is, at most, $\pm 3\text{nm}$.

Flow Rate (GC, HPLC) – Flow rate can be adjusted by as much as $\pm 50\%$.

Injection Volume (GC, HPLC) – Injection volume can be reduced as far as is consistent with accepted precision and detection limits. Volume may be increased to as much as twice the volume specified, provided there are no adverse effects on factors such as baseline, peak shape, resolution, or retention time.

Mobile Phase: pH (HPLC) – The pH of the aqueous buffer used in preparing the mobile phase can be adjusted to within ± 0.2 units of the value or range specified.

Mobile Phase: Concentration of Salts in Buffer (HPLC) – The concentration of the salts used in preparing the aqueous buffer used in the mobile phase can be adjusted to within $\pm 10\%$, provided the permitted pH variation (see above) is met.

Mobile Phase: Ratio of Components (HPLC) – The amount of the minor component(s) can be adjusted by $\pm 30\%$ relative or $\pm 2\%$ absolute, whichever is larger. However, the change in any component cannot exceed $\pm 10\%$ absolute, nor can the final concentration of any component be reduced to zero.

Particle Size (HPLC) – Particle diameter can be reduced by as much as 50%.

Temperature, Column (HPLC) – Absolute temperature can be adjusted by as much as $\pm 2\%$.

Temperature, Oven Program (GC) – Specified temperature hold times, or times for changing from one temperature to another, may be adjusted by up to 20%.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

To ascertain the effectiveness of the final operating system, the system should be subjected to suitability testing. Replicate injections of the standard preparation required to demonstrate adequate system precision may be made before the injection of samples, or standard injections may be interspersed among sample injections. System suitability must be demonstrated throughout the run by injection of an appropriate control preparation at appropriate intervals. The control preparation may be a standard preparation or a solution containing a known amount of analyte and any additional materials useful in the control of the analytical system, such as excipients or impurities. Whenever there is a significant change in equipment or in a critical reagent, suitability testing should be performed before samples are injected. No sample analysis is acceptable unless the requirements of the system suitability have been met. Sample analyses obtained while the system fails requirements are unacceptable (3).

* All terms and symbols are defined in the USP Glossary of Symbols.

USP Codes and Descriptions

Unless otherwise specified, mesh sizes of 80 to 100 or, alternatively, 100 to 120, are intended.

USP Code	Generic Description	Supelco Equivalent ¹
<i>Supports for Gas Chromatography</i>		
S1	Siliceous earth for gas chromatography. Unless otherwise specified, it has been flux-calcined by mixing diatomite with Na ₂ CO ₃ , flux and calcining above 900°C, then washed with water and acid and/or base (as needed) to neutrality and silanized by treating with an agent such as dimethyldichlorosilane to mask surface silanol groups. Alternative treatments, as defined below, are required where the letter indicated appears as a suffix in the designation (e.g., S1C).	SUPELCOPORT™
S1A	Siliceous earth from gas chromatography has been flux-calcined by mixing diatomite with Na ₂ CO ₃ flux and calcining above 900°C. The siliceous earth is acid-washed, then water-washed until neutral, but not base-washed. The siliceous earth may be silanized by treating with an agent such as dimethyldichlorosilane to mask surface silanol groups.	SUPELCOPORT Chromosorb® WAW-DMCS Chromosorb WHP
S1AB	The siliceous earth as described above is both acid and base washed.	Gas Chrom® Q No longer available. Substitute base-washed SUPELCOPORT (Not a direct equivalent. May need to increase % phase coating >1-2 fold.)
S1C	A support prepared from crushed firebrick and calcined or burned with a clay binder above 900 degs. with subsequent acid-wash. It may be silanized. Note: S1C was updated 4-1-98	Chromosorb PAW or Chromosorb PAW-DMCS/ stated in method ²
S1NS	The siliceous earth is untreated.	Chromosorb NAW
S2	Styrene-divinylbenzene copolymer having a nominal surface area of less than 50 m ² /g and an average pore diameter of 0.3 to 0.4 μm.	Chromosorb 101
S3	Copolymer of ethylvinylbenzene and divinylbenzene having a nominal surface area of 500 to 600 m ² /g and an average pore diameter of 0.0075 μm.	HayeSep® Q Porapak® Q
S4	Styrene-divinylbenzene copolymer with aromatic -O and -N groups, having a nominal surface area of 400 to 600 m ² /g and an average pore diameter of 0.0076 μm.	HayeSep R Porapak R
S5	40/60 mesh high molecular weight tetrafluoroethylene polymer.	Chromosorb T
S6	Styrene-divinylbenzene copolymer having a nominal surface area of 250 to 350 m ² /g and an average pore diameter of 0.0091 μm.	Chromosorb 102
S7	Graphitized carbon having a nominal surface area of 12 m ² /g.	Carbopack™ C
S8	Copolymer of 4-vinyl-pyridine and styrene-divinylbenzene.	HayeSep S Porapak S
S9	Porous polymer based on 2,6-diphenyl-p-phenylene oxide.	Tenax® TA
S10	Highly polar cross-linked copolymer of acrylonitrile and divinylbenzene.	HayeSep C
S11	Graphitized carbon having a nominal surface area of 100 m ² /g modified with small amounts of petrolatum and polyethylene glycol.	3% SP™-1500 on 80/120 Carbopack B
S12	Graphitized carbon having a nominal surface area of 100 m ² /g.	Carbopack B

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² Some methods designate silanized or non-silanized.

Å = angstrom; μm = micron

USP Code	Generic Description	Supelco Equivalent ¹
<i>Phases for Gas Chromatography</i>		
G1	dimethylpolysiloxane oil	OV [®] -101 SP-2100
G2	dimethylpolysiloxane gum	OV-1
G3	50% phenyl - 50% methylpolysiloxane	OV-17 SP-2250
G4	diethylene glycol succinate polyester	DEGS
G5	2-cyanopropylpolysiloxane	SP-2340 Silar [®] -10 CP
G6	trifluoropropylmethylpolysiloxane	SP-2401 OV-210
G7	50% 3-cyanopropyl - 50% phenylmethylsilicone	SP-2300 Silar-5 CP
G8	90% 3-cyanopropyl - 10% phenylmethylsilicone	SP-2330
G9	methylvinylpolysiloxane	UCW [®] 982
G10	polyamide	Poly A-103
G11	bis(2-ethylhexyl) sebacate polyester	di-octyl sebacate
G12	phenyldiethanolamine succinate polyester	
G13	sorbitol	
G14	polyethylene glycol (av. mol. wt. of 950 to 1050)	Carbowax 1000
G15	polyethylene glycol (av. mol. wt. of 3000 to 3700)	Carbowax 4000
G16	polyethylene glycol compound (av. mol. wt. about 15,000 to 20,000). A high molecular weight compound of polyethylene glycol and a diepoxide.	Carbowax 20M
G17	75% phenyl - 25% methylpolysiloxane	OV-25
G18	polyalkylene glycol	UCON [®] LB 550X UCON LB 1800X Both are acceptable/USP
G19	25% phenyl - 25% cyanopropyl - 50% methylsilicone	OV-225
G20	polyethylene glycol (av. mol. wt. 380 - 420)	Carbowax 400
G21	neopentyl glycol succinate	
G22	bis(2-ethylhexyl) phthalate	
G23	polyethylene glycol adipate	
G24	diisodecyl phthalate	
G25	polyethylene glycol compound TPA. A high molecular weight compound of a polyethylene glycol and a diepoxide that is esterified with terephthalic acid.	Carbowax 20M-TPA
G26	25% 2-cyanoethyl - 75% methylpolysiloxane	XE [®] -60
G27	5% phenyl - 95% methylpolysiloxane	SE [®] -52, SPB [™] -5
G28	25% phenyl - 75% methylpolysiloxane	DC-550
G29	β-β-thiodipropionitrile	
G30	tetraethylene glycol dimethyl ether	
G31	nonylphenoxy poly(ethyleneoxy) ethanol (av. ethyleneoxy chain length is 30); Nonoxynol 30	Igepal [®] CO-880
G32	20% phenylmethyl - 80% dimethylpolysiloxane	OV-7
G33	20% carborane - 80% methylsilicone	Dexsil [®] 300
G34	diethylene glycol succinate polyester stabilized with phosphoric acid	DEGS-PS
G35	A high molecular weight compound of a polyethylene glycol and a diepoxide that is esterified with nitro-terephthalic acid.	SP-1000 FFAP
G36	1% vinyl - 5% phenylmethylpolysiloxane	SE-54
G37	polyimide	PolyI-110
G38	Phase G1 containing a small percentage of a tailing inhibitor.	SP-2100/ 0.2% Carbowax 1500
G39	polyethylene glycol (av. mol. wt. about 1500)	Carbowax 1500
G40	ethylene glycol adipate	
G41	phenylmethyldimethylsilicone (10% phenyl substituted)	OV-3
G42	35% phenyl - 65% dimethylvinylsiloxane (percentages refer to molar substitution)	SPB-35
G43	6% cyanopropylphenyl - 94% dimethylpolysiloxane (percentages refer to molar substitution)	OV-1301 OVI-G43

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USP Code	Generic Description	Supelco Equivalent ¹
<i>Phases for Gas Chromatography (cont'd.)</i>		
G44	2% low molecular weight petrolatum hydrogrease and 1% solution of potassium hydroxide	2% Apiezon® L/1% KOH
G45	divinylbenzene - ethylene glycol - dimethacrylate	HayeSep N Porapak N HayeSep A (high purity HayeSep N)
G46	14% cyanopropylphenyl / 86% polysiloxane	SPB-1701
Supelco's 5A Molecular Sieve GC material meets USP/NF criteria for analysis of nitrogen purity: "...a molecular sieve prepared from a synthetic alkali-metal aluminosilicate capable of absorbing molecules having diameters of up to 0.5 nm, which permit complete separation of oxygen from nitrogen." Source: USP/NF XVII.		
<i>Packings for High Pressure Liquid Chromatography</i>		
L1 ²	Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter.	Discovery™ C18 SUPELCOSIL LC-18 SUPELCOSIL LC-18-DB SUPELCOSIL LC-318 (300Å) Spherisorb® ODS-2 Hypersil® ODS Nucleosil® C18
L2	Octadecyl silane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50 µm in diameter.	Pelliguard™ LC-18
L3	Porous silica particles, 5 to 10 µm in diameter.	SUPELCOSIL LC-Si Spherisorb S5W Spherisorb Silica Nucleosil Hypersil
L4	Silica gel of controlled surface porosity bonded to a solid spherical core, 30 - 50 µm in diameter.	Pelliguard LC-Si
L5	Alumina of controlled surface porosity bonded to a solid spherical core, 30 - 50µm in diameter.	No equivalent.
L6	Strong cation-exchange packing, sulfonated coated on a solid spherical core, 30 - 50 µm in diameter.	No equivalent.
L7	Octylsilane chemically bonded to totally porous silica particles, 3 - 10 µm in diameter	Discovery C8 SUPELCOSIL LC-8 SUPELCOSIL LC-8-DB Nucleosil C8 Spherisorb Octyl Hypersil MOS SUPELCOSIL LC-308
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 10 µm in diameter.	SUPELCOSIL LC-NH ₂ SUPELCOSIL LC-NH ₂ NP
L9	10 µm irregular, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating.	No equivalent.
L10	Nitrile groups chemically bonded to porous silica particles 3 to 10 µm in diameter.	SUPELCOSIL LC-CN Hypersil CPS Nucleosil CN Spherisorb CN
L11	Phenyl groups chemically bonded to porous silica particles 5 to 10 µm in diameter.	SUPELCOSIL LC-DP Hypersil Phenyl Nucleosil Phenyl (5µm) SUPELCOSIL LC-3DP
L12	A strong anion-exchange packing made by chemically bonding a quaternary amine to a silica spherical core, 30 to 50 µm in diameter.	No equivalent.
L13	Trimethylsilane chemically bonded to porous silica particles 3 to 10 µm in diameter.	SUPELCOSIL LC-1 Hypersil SAS
L14	Silica gel 10 µm in diameter having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating.	SUPELCOSIL LC-SAX (5µm) Spherisorb SAX
L15	Hexylsilane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.	No equivalent. Suggested substitute: Discovery C8 SUPELCOSIL LC-8 SUPELCOSIL LC-8-DB Nucleosil C8 Spherisorb Octyl Hypersil MOS SUPELCOSIL LC-308

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² Some monographs call for an L1 with 30% carbon loading. The column can be obtained from Phenomenex: Ultracarb 5µm ODS.

USP Code	Generic Description	Supelco Equivalent ¹
<i>Packings for High Pressure Liquid Chromatography (contd.)</i>		
L16	Dimethylsilane chemically bonded to totally porous silica particles, 5 to 10 µm in diameter.	No equivalent. Suggested substitute: SUPELCOSIL LC-1 Hypersil SAS
L17	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 - 11 µm in diameter.	SUPELCOGEL C-610H SUPELCOGEL H
L18	Amino and cyano groups chemically bonded to porous silica particles, 3 to 10 µm in diameter.	No equivalent.
L19	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, approx. 9 µm in diameter.	SUPELCOGEL Ca
L20	Dihydroxypropane groups chemically bonded to porous silica particles 5 to 10 µm in diameter.	SUPELCOSIL LC-Diol SUPELCOSIL LC-3Diol (300Å)
L21	A rigid, spherical styrene-divinylbenzene copolymer 5 to 10 µm in diameter.	Hamilton RPR-1 (custom)
L22	A cation exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in diameter. (polymethacrylate)	TSK-GEL SP-5P MCI-GEL ProtEx®-SP
L23	An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, about 10 µm in size.	TSK-GEL® DEAE 5PW
L24	A semi-rigid hydrophilic gel consisting of vinyl polymers with numerous hydroxyl groups on the matrix surface, 32 to 63 µm in diameter.	Toyopearl® HW, F-grade
L25	Packing having the capacity to separate compounds with a molecular weight range from 100 - 5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether (surface contained some residual carboxyl functional groups) was found suitable.	TSK-GEL G-Oligo PW
L26	Butyl silane chemically bonded to totally porous silica particles, 5 to 10 µm in diameter.	SUPELCOSIL LC-304 (300Å)
L27	Porous silica particles, 30 to 50 µm in diameter.	Sigma-Aldrich™ silica gel E. Merck® silica gel Davison silica gel
L28	Multifunctional support consisting of high purity 100 Å spherical silica substrate bonded with anionic exchanger (amine) functionality in addition to a conventional reversed phase C-8 functionality.	No equivalent.
L29	Gamma alumina reversed phase low carbon % by weight, alumina-based polybutadiene, spherical, 5 µm, 80 Å unit pore volume.	Aluspher® RP-Select-B (custom)
L30	Ethyl silane chemically bonded to totally porous silica particles, 3 - 10 µm in diameter.	LiChrosorb® RP-2
L31	Strong anion exchange quaternary amine on latex particle attached to a core of 8.5 µm macroporous particles with 2000 Å pore size, consisting of ethylvinyl benzene cross-linked with 55% divinyl benzene.	No equivalent. (Dionex® AS-10)
L32	A chiral ligand exchange packing consisting of a L-proline copper complex, covalently bonded to a 5 - 10 µm irregularly shaped silica particle.	No equivalent.
L33	Packing having the capacity to separate proteins by molecular size over a range of 4,000 - 400,000 daltons. It is spherical, silica-based, and processed to provide pH stability.	TSK-GEL-2000 plus TSK-GEL G3000 SW/SW _{XL}
L34	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinyl benzene copolymer in the lead form, about 9 µm in diameter.	SUPELCOGEL Pb
L35	A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) monolayer bonded phase having a pore size of 150 Å.	No equivalent.
L36	A 3,5-dinitrobenzoyl derivative of L-phenylglycine bonded to 5 µm aminopropyl silica.	No equivalent.
L37	Packing having the capacity to separate proteins by molecular size over a range of 2,000 - 40,000 daltons. It is a polymethacrylate gel.	Toyopearl HW 40F
L38	A methacrylate-based size-exclusion packing for water soluble samples.	TSK-GEL PW/PW _{XL}
L39	A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.	Toyopearl HW Series
L40	Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5 µm to 20 µm in diameter.	No equivalent.
<i>In the Pharmacopoeial Forum (In-process-revisions, additions, etc. to be published in future updates.)</i>		
L41	Immobilized alpha, acid glycoprotein on spherical silica particles, 5 µm in diameter.	No equivalent.
L42	Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5 µm in diameter.	No equivalent.

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USP Code	Generic Description	Supelco Equivalent ¹
L43	Pentafluorophenyl groups chemically bonded to silica particles, 5 - 10 µm in diameter.	SUPELCO SIL LC-F
L44	A multifunctional support, which consists of a high purity, 60 Å, spherical silica substrate that has been bonded with a cationic exchanger, sulfonic acid functionality in addition to a conventional reversed-phase C8 functionality.	No equivalent.
L45	Beta-cyclodextrin bonded to porous silica particles, 2 to 10 µm in diameter.	No equivalent.
L46	Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, 10 µm.	No equivalent.

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Additional Information

For descriptions and prices for packed GC columns, capillary GC columns, and HPLC columns, refer to the current Supelco catalog.

If you have any questions about our columns or their application in USP methods, just call our Technical Service chemists. Each of them has years of lab experience, and any of them can help solve your separation problems, recommend products for specific applications, clarify analytical methods, and suggest free technical literature.

References

1. *United States Pharmacopoeia 24, National Formulary 19*
Request from United States Pharmacopoeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD USA 20852 (tel. 800-227-8772).
2. *Pharmacopoeial Forum* 25 (3): 8229-8230 (May-June 1999).
3. *Pharmacopoeial Forum* 25 (3): 8230 (Figure 2) (May-June 1999).

Trademarks

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 HayeSep – Hayes Separations Inc.
 Hypersil – Hypersil, Life Sciences International Co.
 Igepal – Rhone-Poulenc Surfactants and Specialties, L.P.
 LiChrosorb – EM Science, Associate of Merck KGaA
 MCI GEL – Mitsubishi Chemical
 Nucleosil – Macherey Nagel GmbH & Co.
 OV – Ohio Valley Specialty Chemical Co.
 Porapak – Waters Associates, Inc.
 ProtEx-SP – Mitsubishi Chemical Corp.
 Spherisorb – Waters Associates, Inc.
 Tenax – Enka Research Institute Arnhem
 Toyopearl, TSK-GEL – Tosoh Corp.

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