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Sepax *HP*-Diol Column Manual

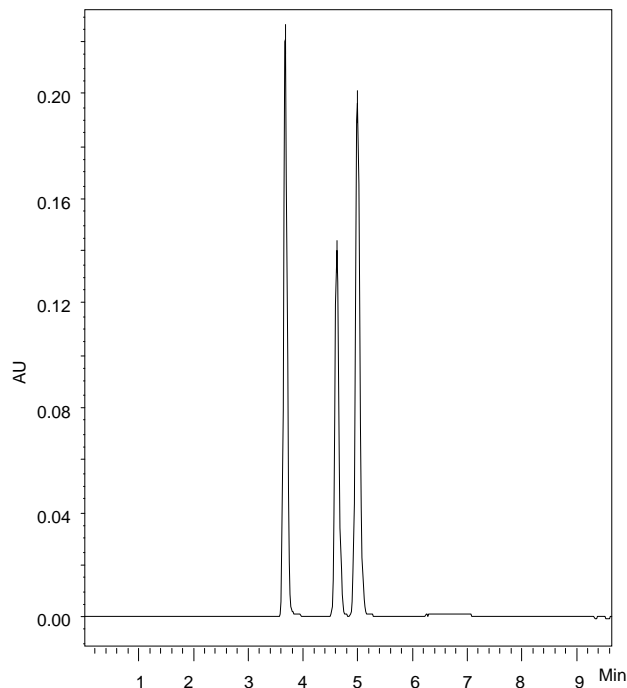
Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Sepax *HP*-Diol bonded phases have been innovatively and specially designed to ensure maximum mono-functional coverage and full end-capping, which leads to carbon content as high as 8.8%. The uniform, spherical Sepax *HP*-Diol particles have a nominal surface area of 300 m²/g with controlled pore sizes of 80 Å and 120 Å. The intrinsic polar characteristics of 1,2-Dihydroxypropylether phase offer much stronger interaction with polar compounds. Sepax *HP*-Diol columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Typical applications for *HP*-Diol are the separations of peptides, proteins, and polar drug molecules, etc. Besides, size exclusion mechanism of *HP*-Diol also plays important roles in bio-separation.

Column Stability and Performance

Sepax *HP*-Diol uses full coverage bonded silica packing, which allows exceptional high stability. Such high stability allows *HP*-Diol extremely suitable for validation of various analyzes. The chemistry of monolayer formation and end-capping is completely controlled that results in very reliable column-to-column reproducibility.

A typical test chromatogram for quality control is shown here for a Sepax *HP*-Diol 4.6x250 mm column.



Column: *HP*-Diol (5 μm, 120 Å, 4.6×250 mm)

Mobile phase: Hexane: Ethanol = 80:20 (v/v)

Flow rate: 1.0 mL/min

Column temperature: Ambient (23°C)

Detector (UV): 254 nm

Injection volume: 1.0 μL

Sample: 1. Anisole

2. Phenol

3. 4-Nitrophenol

Safety Precaution

Sepax *HP*-Diol columns are normally operated under high pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper protections should be used to avoid inhalation of the small silica particles.

Samples and Mobile Phases

New *HP*-Diol columns are shipped in the mobile phase used for QC test. During stocking and shipping, the silica packing could be dried out. It is recommended that 10-20 column volumes of 100% ethanol be purged to activate the column. Flush the column with your mobile phase with gradual increasing the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable. If the column backpressure and baseline fluctuate, this might be due to the air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 2 mL/min for a 4.6x150 mm column.

To avoid clogging the column, all samples and solvents including buffers should be filtered through 0.45 µm or 0.2 µm filters before use. *HP*-Diol bonded stationary phase has wide compatibility with wide range of solvents, including non-polar, such as isopropanol/hexane, polar organic solvents, such as water, a mixture of organic and water (e.g. methanol or acetonitrile and water), and aqueous buffer, such phosphate or borate. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Column Care

pH Avoid use of *HP*-Diol below pH 2 or above 9. Higher pH will dissolve silica, creating defects of 1,2-Dihydroxypropylether bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for longest lifetime are at pH 2 - 8.

Pressure Even though *HP*-Diol can operate at pressure up to 5,000 psi, the normal operation is usually under 3,000 psi. Continuous use at high pressure may eventually damage the column as well as the pump. Since the pressure is generated by the flow rate. The maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in

backpressure suggests that the column inlet frit might be plugged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent.

Temperature The maximum operating temperature is 60°C. Continuous use of the column at higher temperature (>75°C) can damage the column, especially under high pH (>8.5).

Storage When not in use for extended time, do not allow water or aqueous buffer to remain in the column. Remove any aqueous buffers by washing with at least 20-30 column volumes of 50% methanol or acetonitrile aqueous solution, followed by 20-30 column volumes of 100% ethanol. Each column is shipped with two removable end plugs. To prevent the drying of the column bed, seal both ends of the column with the end plugs provided.

Sepax *HP*-Diol Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	116423-2115
2.1x100mm	3 µm	120 Å	116423-2110
2.1x50mm	3 µm	120 Å	116423-2105
2.1x30mm	3 µm	120 Å	116423-2103
4.6x250mm	3 µm	120 Å	116423-4625
4.6x150mm	3 µm	120 Å	116423-4615
4.6x100mm	3 µm	120 Å	116423-4610
4.6x50mm	3 µm	120 Å	116423-4605
2.1x250mm	5 µm	120 Å	116425-2125
2.1x150mm	5 µm	120 Å	116425-2115
2.1x100mm	5 µm	120 Å	116425-2110
2.1x50mm	5 µm	120 Å	116425-2105
2.1x30mm	5 µm	120 Å	116425-2103
4.6x250mm	5 µm	120 Å	116425-4625
4.6x150mm	5 µm	120 Å	116425-4615
4.6x100mm	5 µm	120 Å	116425-4610
4.6x50mm	5 µm	120 Å	116425-4605
7.8x250mm	5 µm	120 Å	116425-7825
10.0x250mm	5 µm	120 Å	116425-10025
21.2x250mm	5 µm	120 Å	116425-21225
21.2x150mm	5 µm	120 Å	116425-21215
21.2x50mm	5 µm	120 Å	116425-21205