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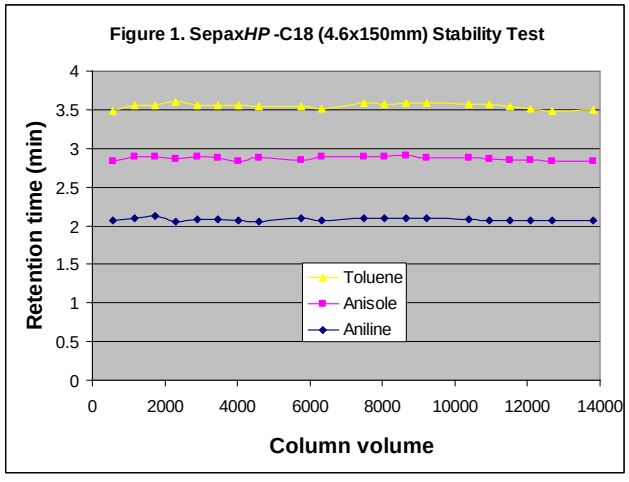
Sepax*HP*-C18 Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Sepax*HP*-C18 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 15%. The chemistry of monolayer formation and end-capping is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows Sepax*HP*-C18 to have exceptional stability. The uniform, spherical Sepax*HP*-C18 particles have a nominal surface area of 300 m^2/g or 200 m^2/g with a controlled pore size of 120 Å, or 200 Å respectively. Sepax*HP*-C18 columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Sepax*HP*-C18 columns are specially designed to enable extended retention and selectivity for polar and hydrophilic compounds. The proprietary surface modification can perform excellent separations even in pure water, resulting in no collapse of C18 phase. Typical applications for Sepax*HP*-C18 are the separations of both polar and non-polar compounds, such as pharmaceuticals, peptides, amino acids, nucleotides, oligosaccharides, and organic acids.

Column Stability and Performance

Sepax*HP*-C18 uses full coverage bonded silica packing, which allows exceptional high stability. Figure 1 shows extremely reproducible retention time for three standard compounds: aniline, anisole and toluene after 13,000 column volume runs in a mobile phase of 85% methanol and 15% water.



Such high stability allows Sepax*HP*-C18 extremely suitable for validation of various analytes. The unique mono-functional bonding chemistry for Sepax*HP*-C18 avoids the formation of multiple C18 layers. Such uniform stationary phase allows the separation to achieve high selectivity and high efficiency. A typical test chromatogram for quality control is shown in Figure 2 for a 4.6x150mm Sepax*HP*-C18 column.

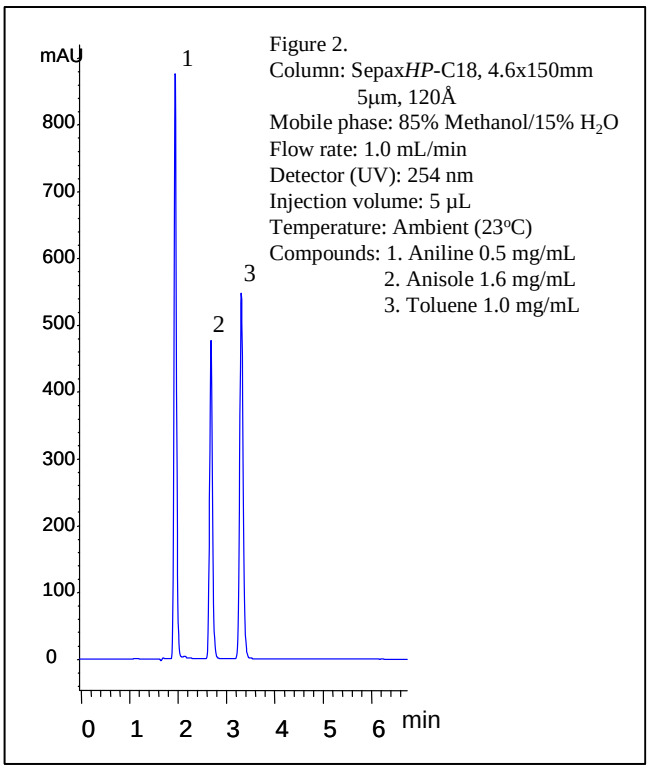


Figure 2.
Column: Sepax*HP*-C18, 4.6x150mm 5µm, 120Å
Mobile phase: 85% Methanol/15% H_2O
Flow rate: 1.0 mL/min
Detector (UV): 254 nm
Injection volume: 5 µL
Temperature: Ambient (23ºC)
Compounds: 1. Aniline 0.5 mg/mL
2. Anisole 1.6 mg/mL
3. Toluene 1.0 mg/mL

Safety Precaution

Sepax*HP*-C18 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.

Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for

example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to tighten the nut 90 degrees past fingertightness.

(d) Repeat this coupling procedure for the other end of the column.

New Sepax*HP*-C18 columns are shipped in a mixture of methanol or acetonitrile and water. During stocking and shipping, the silica packing could be dried out. It is recommended that 10-20 column volumes of pure organic solvents, such as methanol, acetonitrile 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.

Samples and Mobile Phases

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. Sepax*HP*-C18 bonded stationary phase is specially designed to be compatible with aqueous mobile phase. Sepax*HP*-C18 can even tolerate the separations in pure water. Its choice for mobile phase is broad, including organic solvent, aqueous buffer or a mixture of organic and water, such as methanol or acetonitrile and water. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum. Gradient elution methods for Sepax*HP*-C18 columns often begin with 5% methanol or acetonitrile as the initial mobile phase.

Column Care

PH Avoid use of Sepax*HP*-C18 below pH 2 or above 9. Higher pH will dissolve silica, creating defects of C18 bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for longest lifetime are at pH 3 - 7.5.

Pressure Even though Sepax*HP*-C18 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 recommend 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).

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 the pure solvent such as acetonitrile. 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.

SepaxHP-C18 Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	103183-2115
2.1x100mm	3 µm	120 Å	103183-2110
2.1x50mm	3 µm	120 Å	103183-2105
2.1x30mm	3 µm	120 Å	103183-2103
4.6x250mm	3 µm	120 Å	103183-4625
4.6x150mm	3 µm	120 Å	103183-4615
4.6x100mm	3 µm	120 Å	103183-4610
4.6x50mm	3 µm	120 Å	103183-4605
2.1x250mm	5 µm	120 Å	103185-2125
2.1x150mm	5 µm	120 Å	103185-2115
2.1x100mm	5 µm	120 Å	103185-2110
2.1x50mm	5 µm	120 Å	103185-2105
2.1x30mm	5 µm	120 Å	103185-2103
4.6x250mm	5 µm	120 Å	103185-4625
4.6x150mm	5 µm	120 Å	103185-4615
4.6x100mm	5 µm	120 Å	103185-4610
4.6x50mm	5 µm	120 Å	103185-4605
7.8x250mm	5 µm	120 Å	103185-7825
10.0x250mm	5 µm	120 Å	103185-10025
21.2x250mm	5 µm	120 Å	103185-21225
21.2x150mm	5 µm	120 Å	103185-21215
21.2x50mm	5 µm	120 Å	103185-21205
7.8x250mm	7 µm	120 Å	103187-7825
10.0x250mm	7 µm	120 Å	103187-10025
21.2x250mm	7 µm	120 Å	103187-21225
21.2x150mm	7 µm	120 Å	103187-21215
21.2x50mm	7 µm	120 Å	103187-21205