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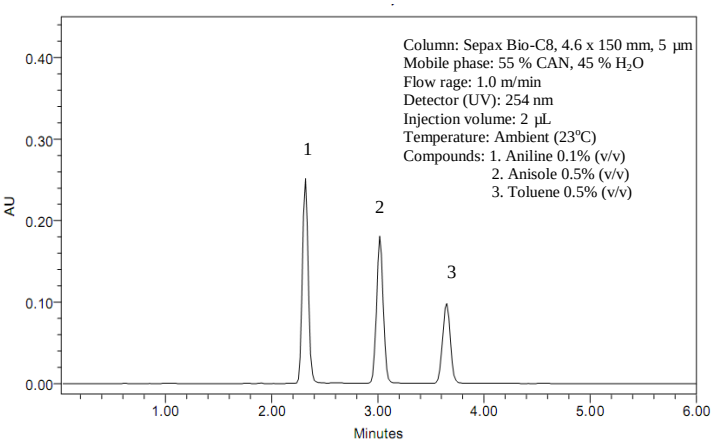
Sepax *Bio*-C8 Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Sepax *Bio*-C8 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 4.0%. 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 *Bio*-C8 to have exceptional stability. The uniform, spherical Sepax *Bio*-C8 particles have a nominal surface area of 105 m^2/g with a controlled pore size of 300 Å. Sepax *Bio*-C8 columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Sepax *Bio*-C8 packing materials are bonded with octyl groups that lead to fairly high hydrophobicity. Sepax *Bio*-C8 columns have great selectivity and peak symmetry with fairly high retention for separations of acidic, neutral and basic organic compounds, such as drugs, peptides, organic acids. Sepax *Bio*-C8 columns are especially designed for separation of various organic compounds, peptide fragments which are difficult to elute due to the strong interaction with C18 phase.

Column Stability and Performance

Sepax *Bio*-C8 uses full coverage bonded silica packing, which allows exceptional high stability. Such high stability allows Sepax *Bio*-C8 extremely suitable for validation of various analytes. The unique mono-functional bonding chemistry for Sepax *Bio*-C8 avoids the formation of multiple octyl layers. Such uniform stationary phase allows the separation to achieve high selectivity and high efficiency. A typical test chromatogram for quality control is shown here for a 4.6x150 mm Sepax *Bio*-C8 column. Compared with Sepax *Bio*-C18 phase, Sepax Bio-C8 has relatively lower hydrophobicity. The high efficiency and less hydrophobic Sepax *Bio*-C8 phase make it very suitable for separating compounds with a wide range of hydrophobicity. It is highly recommended for separating the compounds which are too strongly retained on C18 phases.



Safety Precaution

Sepax *Bio*-C8 columns are normally operated under high pressure. Loose connections will cause leaking of mobile phase and injected samples, all of which should be considered as 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 mobile phases.

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. Follow the flow

direction as marked on the column. 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 *Bio*-C8 columns are shipped in a mixture of 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 or acetonitrile be purged to activate the column. Flush the column with starting mobile phase with gradually increasing the flow rate from 0.1 mL/min to operation condition, until the baseline is stable. If the column backpressure and baseline fluctuate, this might be due to air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 2 mL/min for 4.6x150 mm.

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 *Bio*-C8 bonded stationary phase is nonpolar in nature. It is recommended that the mobile phase be a mixture of organic solvent, such as methanol or acetonitrile and water, even though Sepax *Bio*-C8 can tolerate aqueous buffers as mobile phases. 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 *Bio*-C8 columns often begin with 5% methanol or acetonitrile as the initial mobile phase.

Column Care

PH: Avoid use of Sepax *Bio*-C8 below pH 2 or above 9. Higher pH will dissolve silica, creating defects of C8 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 *Bio*C8 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 usage. 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 ^{o}C. Continuous use of the column at higher temperature (>75 ^{o}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 provided end plugs.

Sepax Bio-C8 Products

Part Number	Particle Size	Pore Size	ID×Length
108083-4605	3 µm	300 Å	4.6×50 mm
108083-4615	3 µm	300 Å	4.6×150 mm
108083-4625	3 µm	300 Å	4.6×250 mm
108085-4605	5 µm	300 Å	4.6×50 mm
108085-4615	5 µm	300 Å	4.6×150 mm
108085-4625	5 µm	300 Å	4.6×250 mm
108085-10005	5 µm	300 Å	10.0×50 mm
108085-10015	5 µm	300 Å	10.0×150 mm
108085-10025	5 µm	300 Å	10.0×250 mm
108085-2120	5 µm	300 Å	21.2×10 mm
108085-21205	5 µm	300 Å	21.2×50 mm
108085-21215	5 µm	300 Å	21.2×150 mm
108085-21225	5 µm	300 Å	21.2×250 mm
108085-30005	5 µm	300 Å	30.0×50 mm
108085-30015	5 µm	300 Å	30.0×150 mm
108085-30025	5 µm	300 Å	30.0×250 mm
108089-10005	10 µm	300 Å	10.0×50 mm
108089-10015	10 µm	300 Å	10.0×150 mm
108089-10025	10 µm	300 Å	10.0×250 mm
108089-21205	10 µm	300 Å	21.2×50 mm
108089-21215	10 µm	300 Å	21.2×150 mm
108089-21225	10 µm	300 Å	21.2×250 mm
108089-30005	10 µm	300 Å	30.0×50 mm
108089-30015	10 µm	300 Å	30.0×150 mm
108089-30025	10 µm	300 Å	30.0×250 mm