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Sepax BR-C18 Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, BR-C18 bonded phases have been innovatively and specially designed to ensure maximum surface coverage and full end-capping, which leads to carbon content as high as 19.5%. The bonding chemistry is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows BR-C18 to have exceptional stability, resulting in high pH stability in the range of 1.5 to 10.5. The uniform, spherical BR-C18 particles have a nominal surface area of 350 m^2/g with a controlled pore size of 120 Å. BR-C18 columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. BR-C18 columns have great selectivity and peak symmetry with moderate retention for separations of acidic, neutral and basic organic compounds, such as drugs, peptides, organic acids. BR-18 is especially designed for separation of various basic compounds.

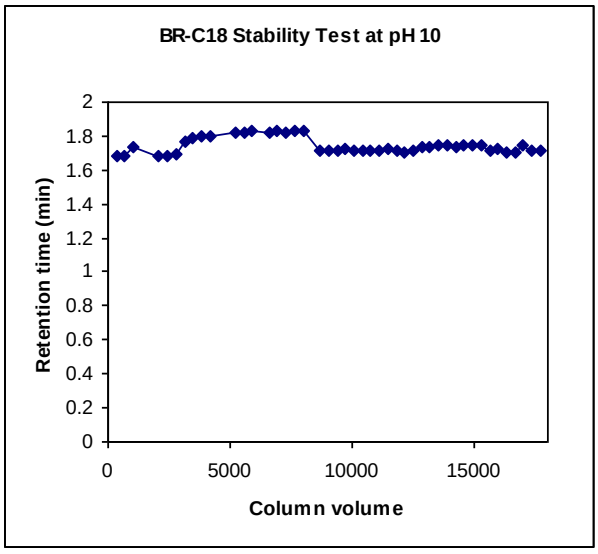

Figure 1. A BR-C18 column (3 µm, 4.6x50 mm) was operated at pH 10 under the conditions: mobile phase, 10 mM ammonium bicarbonate buffer in 55%ACN/45%H2O; flow rate, 0.5 mL/min; room temperature; detection, UV 254 nm; sample, toluene.

Column Stability and Performance

BR-C18 uses full coverage bonded silica packing, which allows exceptional high stability at high pH. Figure 1 shows extremely reproducible retention time for a test compound: toluene after 18,000 column volume runs in a mobile phase of 55% acetonitrile and 45% water at pH 10. Such high stability allows BR-C18 extremely suitable for validation of various analytes. The proprietary bonding chemistry for BR-C18 allows achieving high selectivity and high efficiency separation. A typical test chromatogram for quality control is shown in Figure 2 for a 4.6x250mm BR-C18 column.

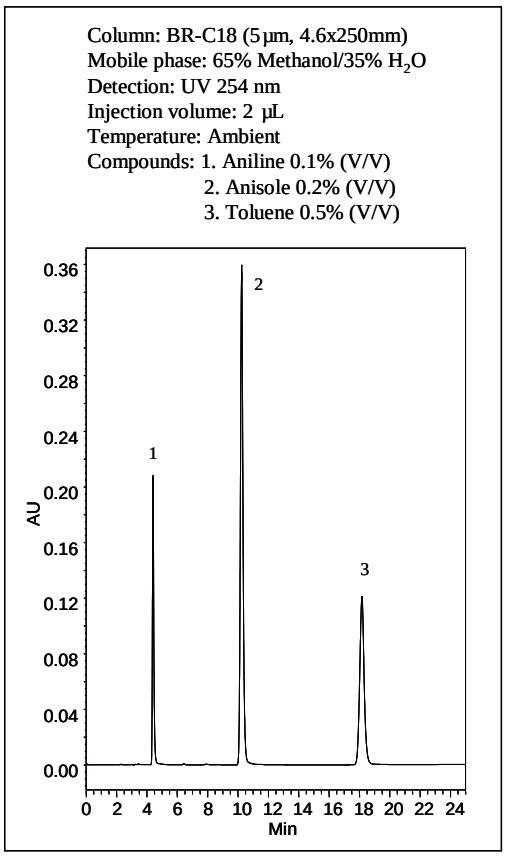

Figure 2. A typical QC chromatogram for BR-C18 column.

Safety Precaution

BR-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 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 4.6x150mm.

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. BR-C18 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 BR-C18 can tolerate aqueous buffers as mobile phases, pure aqueous mobile phase might reduce their high performance. 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 BR-C18 columns often begin with 5% methanol or acetonitrile as the initial mobile phase.

Column Care

PH Avoid use of BR-C18 below pH 1.5 or above 11. 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 1.5 - 10.5.

Pressure Even though BR-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 (>11).

Storage When not in use for extended time, do not allow water or aqueous buffer or high pH (>10) mobile phase 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.

BR-C18 Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	102183-2115
2.1x50mm	3 µm	120 Å	102183-2105
2.1x30mm	3 µm	120 Å	102183-2103
4.6x250mm	3 µm	120 Å	102183-4625
4.6x150mm	3 µm	120 Å	102183-4615
4.6x50mm	3 µm	120 Å	102183-4605
2.1x150mm	5 µm	120 Å	102185-2115
2.1x50mm	5 µm	120 Å	102185-2105
2.1x30mm	5 µm	120 Å	102185-2103
4.6x250mm	5 µm	120 Å	102185-4625
4.6x150mm	5 µm	120 Å	102185-4615
4.6x100mm	5 µm	120 Å	102185-4610
4.6x50mm	5 µm	120 Å	102185-4605
10.0x250mm	5 µm	120 Å	102185-10025
21.2x250mm	5 µm	120 Å	102185-21225
21.2x150mm	5 µm	120 Å	102185-21215
21.2x50mm	5 µm	120 Å	102185-21205
10.0x250mm	10 µm	120 Å	102189-10025
21.2x250mm	10 µm	120 Å	102189-21225
21.2x150mm	10 µm	120 Å	102189-21215
21.2x50mm	10 µm	120 Å	102189-21205