



Sepax Technologies, Inc.

Delaware Technology Park

5 Innovation Way, Suite 100 Newark DE 19711 USA

Phone: (302) 366-1101; Fax: (302) 366-1151

Toll Free: (877) SEPAX-US; www.sepax-tech.com

Amethyst C18-P Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Amethyst C18-P 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 20%. The chemistry of monolayer formation and end-capping is completely controlled that results in very reliable column-to-column reproducibility. The uniform, spherical Amethyst C18-P particles have a nominal surface area of 300 m²/g with a controlled pore size of 120Å. The maximum surface coverage allows Amethyst C18-P to have exceptional stability in pH of 1.5 to 9.0. The column can be tolerated by washing with an alkali mobile phase at extreme pH as low as pH 1.0, such as 1.0% TFA in the mobile phase, and as high as 13, such as dilute NaOH aqueous solution in MeOH or EtOH. Amethyst C18-P is especially designed for separation and purification of pharmaceuticals, peptides, and natural products.

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 Amethyst C18-P 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. Amethyst C18-P bonded stationary phases are specially designed to be compatible with aqueous mobile phase. Amethyst C18-P can 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 Amethyst C18-P columns often begin with 5% methanol or acetonitrile as the initial mobile phase.

Column Care

pH Avoid use of Amethyst C18-P below pH 1.5 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 1.5 -9.

Pressure Even though Amethyst C18-P 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 (>9).

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.

Amethyst C18-P Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	321183-2115
2.1x100mm	3 µm	120 Å	321183-2110
2.1x50mm	3 µm	120 Å	321183-2105
2.1x30mm	3 µm	120 Å	321183-2103
4.6x250mm	3 µm	120 Å	321183-4625
4.6x150mm	3 µm	120 Å	321183-4615
4.6x100mm	3 µm	120 Å	321183-4610
4.6x50mm	3 µm	120 Å	321183-4605
2.1x250mm	5 µm	120 Å	321185-2125
2.1x150mm	5 µm	120 Å	321185-2115
2.1x100mm	5 µm	120 Å	321185-2110
2.1x50mm	5 µm	120 Å	321185-2105
2.1x30mm	5 µm	120 Å	321185-2103
4.6x250mm	5 µm	120 Å	321185-4625
4.6x150mm	5 µm	120 Å	321185-4615
4.6x100mm	5 µm	120 Å	321185-4610
4.6x50mm	5 µm	120 Å	321185-4605
4.6x30mm	5 µm	120 Å	321185-4603
7.8x250mm	5 µm	120 Å	321185-7825
10.0x250mm	5 µm	120 Å	321185-10025
21.2x150mm	5 µm	120 Å	321185-21215
21.2x100mm	5 µm	120 Å	321185-21210
21.2x50mm	5 µm	120 Å	321185-21205