



Sepax Technologies, Inc.

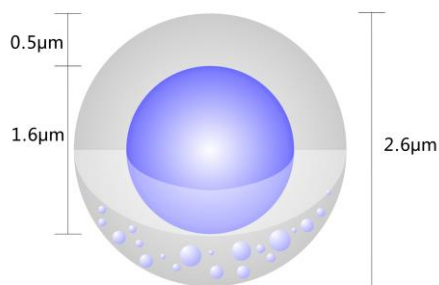
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Opalshell-C18 Column Manual

Column Information

Sepax Opalshell-C18 columns use a core-shell bonded silica stationary phase that has a solid silica core of 1.6 μm in diameter with a porous outer layer 0.5 μm thick and a total particle size of 2.6 μm . The uniform, spherical particles have a nominal surface area of 150 m^2/g with a controlled pore size of 90 \AA . The size distribution of the Opalshell particle is much narrower than that of conventional totally porous particles, which leads to reduced inter particle spacing in the column and higher efficiency and performance by less Eddy Diffusion.

Sepax Opalshell-C18 columns have great selectivity and peak symmetry with moderate retention for separations of acidic, neutral and basic organic compounds.



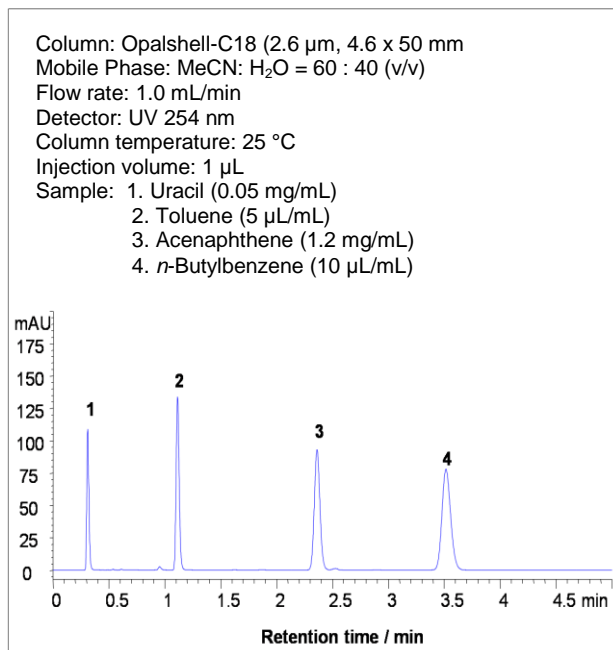
Column Stability and Performance

The surface modification is completely controlled, which results in very reliable column-to-column reproducibility. Sepax Opalshell-C18 columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. A typical test chromatogram for quality control is shown in Figure 1 for a 4.6 x 50 mm Sepax Opalshell-C18 column.

Safety Precaution

Sepax Opalshell-C18 columns are normally operated under high pressure. Loose connections will cause leaking of solvents and/or samples that should be considered hazardous. In case of leakage, proper gloves should be worn for handling the leaking column. When opening the column, proper protection should be used to avoid inhalation of the small particles.

Figure 1: QC standard substance on Opalshell-C18 column



Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When installing 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:

- 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.
- Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.
- While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to further tighten if necessary.
- Repeat this coupling procedure for the other end of the column.

Sepax Opalshell-C18 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, acetonitrile be purged to activate the column. Flush the column with your mobile phase by gradually increasing the flow rate from 0.3 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 4.6 x 150 mm.

Samples and Mobile Phases

To avoid clogging the columns, all samples and solvents including buffers should be filtered through 0.45 μm or 0.2 μm filters before use. Sepax Opalshell-C18 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. Care should be taken not to pass any mobile phase through the column that might cause precipitation. Use only HPLC grade solvents and freshly prepared aqueous buffer solutions; degas and filter all mobile phases prior to use. A simple way for degassing is to sonicate it for 5 minutes under vacuum.

Column Care

pH Avoid use of Sepax Opalshell-C18 columns below pH 1.5 or above 10. Higher pH will dissolve silica, creating defects of C18 bonding that causes separation efficiency loss and retention time change.

Pressure Even though Sepax Opalshell-C18 columns can operate at pressure up to 8,000 psi, the normal operation is usually under 5,000 psi. Continuous use at high pressure may eventually damage the column as well as the pump. Since the pressure is related to the flow rate, the maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with service. A sudden increase in backpressure suggests that the column inlet frit might be clogged. 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.

Storage Sepax Opalshell-C18 columns are shipped in a mixture of acetonitrile and water. When not in use for extended period of 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% aqueous acetonitrile, followed by 20-30 column volumes of a pure solvent such as acetonitrile. Each column is shipped with two removable end plugs. To prevent drying of the column bed, seal both ends of the column with the end plugs provided.

Sepax Opalshell-C18 Column

ID x Length	Particle Size	Pore Size	P/N
2.1 x 100mm	2.6 μm	90 Å	104182-2110
2.1 x 150mm	2.6 μm	90 Å	104182-2115
4.6 x 50mm	2.6 μm	90 Å	104182-4605
4.6 x 100mm	2.6 μm	90 Å	104182-4610

Sepax Opalshell-C18 Guard

Description	P/N
Guard Cartridge with Holder	104182-4001C
Guard Refill Cartridge (5 pcs/pk)	104182-4001F