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Sepax Polar Phases - Hydrophilic Interaction Chromatography (HILIC)

Column Information

To solve the challenges of more and more highly polar pharmaceuticals and small biological molecules, Sepax developed Polar phases - a series of chemistries of weak acidic, neutral, and basic stationary phases for separating basic, neutral and acidic compounds of high polarity by the separation mode of HILIC. *Polar-100, -Diol, -Pyridine and –Imidazole are chemically bonded phases and Polar-Silica is an activated silica phase.* Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Sepax Polar phases have been innovatively and specially designed to ensure maximum surface coverage, resulting in high stability of the stationary phases. The chemistry of monolayer formation is completely controlled that results in very reliable lot-to-lot and column-to-column reproducibility. The uniform, spherical silica particles have a nominal surface area of 300 m^2/g with a controlled pore size of 120 Å. Sepax Polar columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency.

Structure of HILIC Stationary Phases

With the chemical structures shown in Figure 1, Polar-100 and Polar-Diol are neutral, polar phases, while Polar-Diol is more polar than Polar-100. Polar-Silica is a weak acidic phase. Polar-Pyridine and Polar-Imidazole are basic phases, while Imidazole phase is more basic than Pyridine phase.

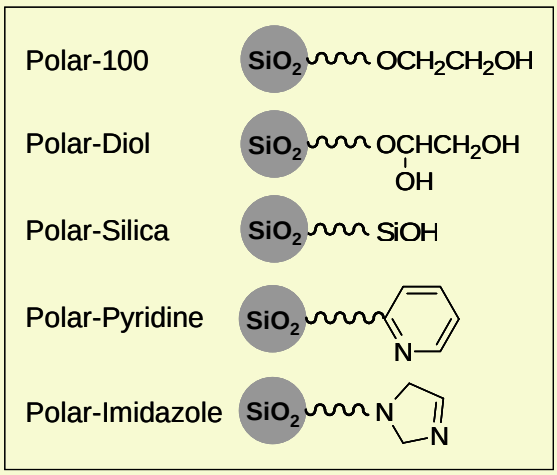

Figure 1. Chemical structures of HILIC phases.

Column Stability and Performance

Sepax Polar phases use full coverage bonded silica packing, which allows exceptional high stability. Such high stability allows Sepax Polar phases suitable for analysis and validation of various analytes. The unique bonding chemistry of Sepax Polar phases avoids the formation of multiple layers. Such uniform stationary phase allows achieving high selectivity and high efficiency separations. Separations could be in the non-polar solvents, such as hexane, or polar solvents, such as methanol and acetonitrile, and a mixture of organic solvent and water, such as ACN/H_2O.

Safety Precaution

Sepax Polar 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 Polar 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 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 Polar phases have compatibility with wide range of solvents, including non-polar, such as isopropanol/hexane, polar organic solvents, such as water, a mixture of organic and water (e.g. methanol or acetonitrile and water), and aqueous buffer, such as ammonium acetate, phosphate or borate. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Column Care

PH Avoid use of Sepax Polar phases below pH 2 or above 9. Higher pH will dissolve silica, creating defects of pyridine bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for longest lifetime are at pH 2 - 8.5.

Pressure Even though *Sepax Polar phases* 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^{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 end plugs provided.

Sepax Polar Phase Products

	1.8 μm, 120 Å Analytical Columns		
Phases	2.1x100mm	4.6x100mm	4.6x150mm
Polar-Silica	130001-2110	130001-4610	130001-4615
Polar-100	131581-2110	131581-4610	131581-4615
Polar-Diol	133331-2110	133331-4610	133331-4615
Polar-Pyridine	134251-2110	134251-4610	134251-4615
Polar-Imidazole	135331-2110	135331-4610	135331-4615

	2.2 μm, 120 Å Analytical Columns		
Phases	2.1x150mm	4.6x150mm	4.6x250mm
Polar-Silica	130002-2115	130002-4615	130002-4625
Polar-100	131582-2115	131582-4615	131582-4625
Polar-Diol	133332-2115	133332-4615	133332-4625
Polar-Pyridine	134252-2115	134252-4615	134252-4625
Polar-Imidazole	135332-2115	135332-4615	135332-4625

	3 μm, 120 Å Analytical Columns		
Phases	2.1x150mm	4.6x150mm	4.6x250mm
Polar-Silica	130003-2115	130003-4615	130003-4625
Polar-100	131583-2115	131583-4615	131583-4625
Polar-Diol	133333-2115	133333-4615	133333-4625
Polar-Pyridine	134253-2115	134253-4615	134253-4625
Polar-Imidazole	135333-2115	135333-4615	135333-4625

	5 μm, 120 Å Analytical Columns		
Phases	2.1x150mm	4.6x150mm	4.6x250mm
Polar-Silica	130005-2115	130005-4615	130005-4625
Polar-100	131585-2115	131585-4615	131585-4625
Polar-Diol	133335-2115	133335-4615	133335-4625
Polar-Pyridine	134255-2115	134255-4615	134255-4625
Polar-Imidazole	135335-2115	135335-4615	135335-4625

	5 μm, 120 Å Preparative Columns	
Phases	21.2x150mm	21.2x250mm
Polar-Silica	130005-21215	130005-21225
Polar-100	131585-21215	131585-21225
Polar-Diol	133335-21215	133335-21225
Polar-Pyridine	134255-21215	134255-21225
Polar-Imidazole	135335-21215	135335-21225