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Sepax Polar Imidazole Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, *Polar-Imidazole* bonded phases have been innovatively and specially designed to ensure maximum imidazole group coverage, which leads to organic content as high as 8%. The chemistry of polymeric monolayer formation is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows *Polar-Imidazole* to have exceptional stability. The uniform, spherical *Polar-Imidazole* particles have a nominal surface area of 300 m^2/g with a controlled pore size of 120Å. *Polar-Imidazole* columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. *Polar-Imidazole* columns are specially designed to enable high selectivity and can perform excellent separations in organic solvents, and a mixture of water and organic solvent, such as methanol, and acetonitrile. Typical applications for *Polar-Imidazole* are the separations of both polar and non-polar compounds, such as pharmaceuticals, carbohydrates, amino acids, nucleotides, and organic acids.

Column Stability and Performance

Polar-Imidazole uses full coverage bonded silica packing, which allows exceptionally high stability. Such high stability allows *Polar-Imidazole* to be extremely suitable for validation of various analytes. The unique polymeric bonding chemistry for *Polar-Imidazole* avoids the formation of multiple imidazole 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 a mixture of acetonitrile and water. A typical test chromatogram for quality control is shown in Figure 1 for a 4.6x250mm column.

Safety Precaution

Polar-Imidazole 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 hazards. In case of leaking, proper gloves should be worn for handling the leaked columns. When opening the columns, proper protection should be used to avoid inhalation of the small silica particles.

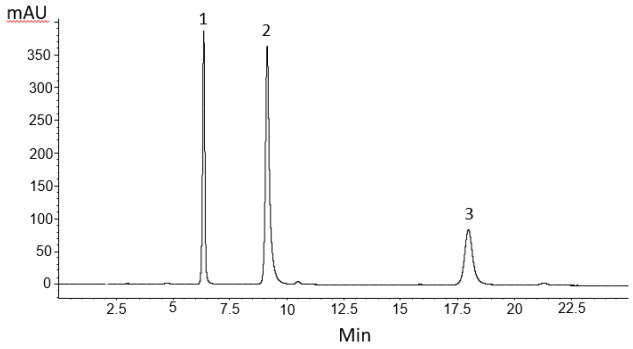

Figure 1. Elution profiles of a standard mixture by *Polar Imidazole* (5µm, 120Å) column

Column:	4.6x250 mm
Mobile phase:	90% acetonitrile and 10% ammonium acetate (10 mM)
Flow rate:	1.0 mL/min
Temperature:	Ambient (~23° C)
Detection:	UV 214nm
Injection volume:	5 µL
Sample:	1) Uracil, 2) Adenosine, and 3) Cytidine.

Column Characteristics

Silica: *Spherical, high purity (<10 ppm metals)*
Particle size: *1.8, 2.2, 3, and 5µm*
Column dimensions (mm): *2.1x100, 2.1x150, 3.0x150, 4.6x50, 4.6x100, 4.6x150, and 4.6x250*

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 a 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 *Polar-Imidazole* columns are shipped in a mixture of 90% acetonitrile and 10% ammonium acetate (10 mM). It is recommended to activate or condition new columns and columns that have not been used for a long time. To activate, flush the column with 10-20 column volumes of the running mobile phase starting at a low flow rate and gradually increasing until desired operating flow rate is met, and 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 a 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. *Polar-Imidazole* bonded stationary phase has wide 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 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 *Polar-Imidazole* below pH 3 or above 9. Higher pH will dissolve silica, creating defects of imidazole bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for the longest lifetime are at pH 3 - 8.5.

Pressure Even though *Polar-Imidazole* 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 back pressure. 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 (>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.

Technical Support: *If you have any other additional questions, please contact technical support @ techsupport@sepax-tech.com or call (302) 366-1101*