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Sepax *SFC-Pyridine* Column Manual

Column Information

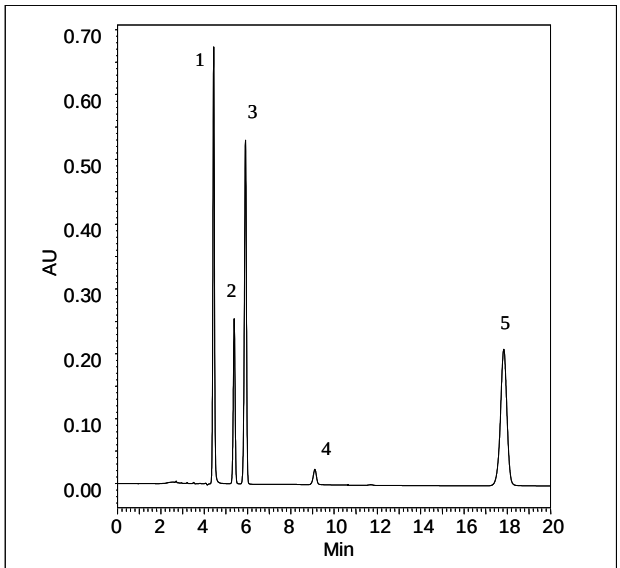
Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, *SFC-Pyridine* bonded phases have been innovatively and specially designed to ensure maximum pyridyl group coverage, which leads to carbon content as high as 8.0%. The chemistry of polymeric monolayer formation is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows *SFC-Pyridine* to have exceptional stability. The uniform, spherical *SFC-Pyridine* particles have a nominal surface area of 300 m^2/g with a controlled pore size of 120Å. *SFC-Pyridine* columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. *SFC-Pyridine* columns are specially designed to enable high selectivity for polar and hydrophilic compounds. The intrinsic polar pyridine phase offers much less retention for most non-polar compounds. *SFC-Pyridine* columns can perform excellent separations in organic solvents, and a mixture of water and organic solvent, such as methanol, and acetonitrile. Typical applications for *SFC-Pyridine* are the separations of both polar and non-polar compounds, such as pharmaceuticals, carbohydrates, amino acids, nucleotides, and organic acids.

Column Stability and Performance

SFC-Pyridine uses full coverage bonded silica packing, which allows exceptional high stability. Such high stability allows *SFC-Pyridine* extremely suitable for validation of various analytes. The unique polymeric bonding chemistry for *SFC-Pyridine* avoids the formation of multiple pyridine 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 here for a *SFC-Pyridine* 4.6x250mm column.

Safety Precaution

SFC-Pyridine 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: SFC-Pyridine (5µm, 120Å, 4.6x250mm)
Mobile phase: 80% Hexane/20% Ethanol
Flow rate: 1.0 mL/min
Detector (UV): 254 nm
Injection volume: 3.0 µL
Temperature: Ambient (23 ºC)
Sample: 1. Pyridine
2. Aniline
3. Phenol
4. Uracil
5. p-Aminobenzoic acid

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 *SFC-Pyridine* 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 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. *SFC-Pyridine* 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 *SFC-Pyridine* 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 *SFC-Pyridine* 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.

SFC-Pyridine Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	5 µm	120 Å	619255-2115
2.1x100mm	5 µm	120 Å	619255-2110
2.1x50mm	5 µm	120 Å	619255-2105
4.6x250mm	5 µm	120 Å	619255-4625
4.6x150mm	5 µm	120 Å	619255-4615
4.6x100mm	5 µm	120 Å	619255-4610
4.6x50mm	5 µm	120 Å	619255-4605
10x250mm	5 µm	120 Å	619255-10025
10x150mm	5 µm	120 Å	619255-10015
10x100mm	5 µm	120 Å	619255-10010
10x50mm	5 µm	120 Å	619255-10005
21.2x250mm	5 µm	120 Å	619255-21225
21.2x150mm	5 µm	120 Å	619255-21215
21.2x100mm	5 µm	120 Å	619255-21210
21.2x50mm	5 µm	120 Å	619255-21205
30x250mm	5 µm	120 Å	619255-30025
30x150mm	5 µm	120 Å	619255-30015
30x100mm	5 µm	120 Å	619255-30010
30x50mm	5 µm	120 Å	619255-30005