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Sepax *HP*-Amino Column Manual

Column Information

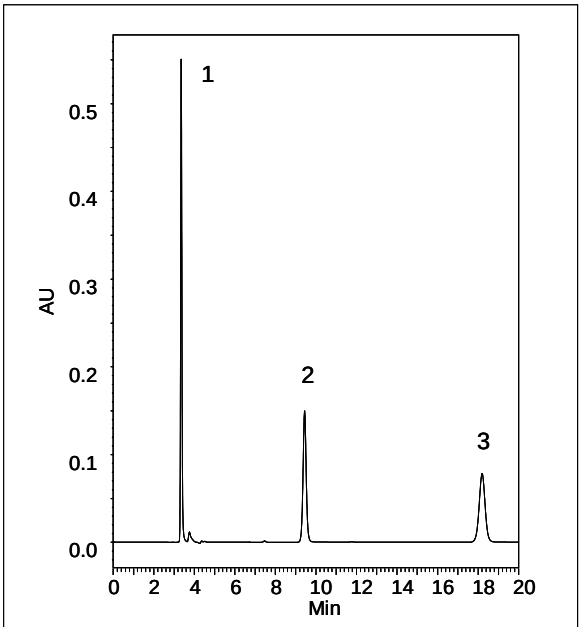
Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, *HP*-Amino bonded phases have been innovatively and specially designed to ensure maximum amino group coverage, which leads to carbon content as high as 4.0%. The chemistry of polymeric monolayer formation is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows *HP*-Amino to have exceptional stability. The uniform, spherical *HP*-Amino particles have a nominal surface area of 300 m^2/g with a controlled pore size of 120Å. *HP*-Amino columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. *HP*-Amino columns are specially designed to enable high selectivity for polar and hydrophilic compounds. The intrinsic polar characteristics of 3-aminopropyl phase offers much less retention for most non-polar compounds. *HP*-Amino columns can perform excellent separations in organic solvents, and a mixture of water and organic solvent, such as methanol, and acetonitrile. Typical applications for *HP*-Amino are the separations of both polar and non-polar compounds, such as pharmaceuticals, carbohydrates, amino acids, nucleotides, oligosaccharides, and organic acids.

Column Stability and Performance

Sepax *HP*-Amino uses full coverage bonded silica packing, which allows exceptional high stability. Such high stability allows *HP*-Amino extremely suitable for validation of various analytes. The unique mono-functional bonding chemistry for *HP*-Amino avoids the formation of multiple aminopropyl 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 *HP*-Amino 4.6x250mm column.

Safety Precaution

Sepax *HP*-Amino 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: HP-Amino (5µm, 120Å, 4.6x250mm)
Mobile phase: 14.7% EtOH/0.3% H_2O/85% Isooctane
Flow rate: 1.0 mL/min
Detector (UV): 254 nm
Injection volume: 3.0 µL
Temperature: Ambient (23 ºC)
Sample: 1. Toluene
2. Benzanilide
3. p-Nitroaniline

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 *HP*-Amino columns are shipped in the mobile phase used for QC test. During stocking and shipping, the silica packing could be dried out. It is recommended that 10-20 column volumes of pure organic solvents be purged to activate the column. You should pay attention to the compatibility with the solvent the column stored. 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.

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. *HP*-Amino 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 *HP*-Amino below pH 2 or above 9. Higher pH will dissolve silica, creating defects of aminopropyl bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for longest lifetime are at pH 2 - 7.5.

Pressure Even though *HP*-Amino 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 (>8).

Storage When not in use for extended time, do not allow water or aqueous buffer to remain in the column. Please store the column in the pure acetonitrile or the mobile phase for quality control of the column. 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 HP-Amino Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	115303-2115
2.1x100mm	3 µm	120 Å	115303-2110
2.1x50mm	3 µm	120 Å	115303-2105
2.1x30mm	3 µm	120 Å	115303-2103
4.6x250mm	3 µm	120 Å	115303-4625
4.6x150mm	3 µm	120 Å	115303-4615
4.6x100mm	3 µm	120 Å	115303-4610
4.6x50mm	3 µm	120 Å	115303-4605
2.1x250mm	5 µm	120 Å	115305-2125
2.1x150mm	5 µm	120 Å	115305-2115
2.1x100mm	5 µm	120 Å	115305-2110
2.1x50mm	5 µm	120 Å	115305-2105
2.1x30mm	5 µm	120 Å	115305-2103
4.6x250mm	5 µm	120 Å	115305-4625
4.6x150mm	5 µm	120 Å	115305-4615
4.6x100mm	5 µm	120 Å	115305-4610
4.6x50mm	5 µm	120 Å	115305-4605
7.8x250mm	5 µm	120 Å	115305-7825
10.0x250mm	5 µm	120 Å	115305-10025
21.2x250mm	5 µm	120 Å	115305-21225
21.2x150mm	5 µm	120 Å	115305-21215
21.2x50mm	5 µm	120 Å	115305-21205
7.8x250mm	7 µm	120 Å	115307-7825
10.0x250mm	7 µm	120 Å	115307-10025
21.2x250mm	7 µm	120 Å	115307-21225
21.2x150mm	7 µm	120 Å	115307-21215
21.2x50mm	7 µm	120 Å	115307-21205