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Sepax *HP-SAX* Column Manual

Column Information

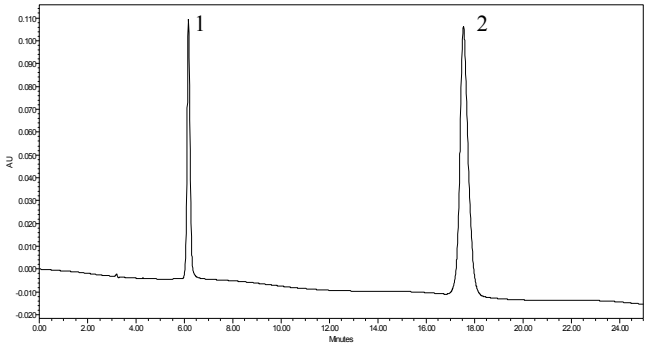
Sepax *HP-SAX* is a strong anion exchanger with quaternary ammonium functional groups chemically bonded to the silica. Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, *HP-SAX* phase is synthesized with a mixed chemical structure of quaternary ammonium functional group and phenyl group. *HP-SAX* bonded phase has been innovatively and specially designed to ensure maximum surface coverage, which leads to carbon content as high as 16.0%. The maximum surface coverage allows *HP-SAX* to have exceptional stability. The uniform, spherical *HP-SAX* particles have a nominal surface area of 300 m^2/g with a controlled pore size of 120Å. The chemistry of the phase synthesis is completely controlled that results in very reliable column-to-column reproducibility. *HP-SAX* columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. With a mixed mode of strong anion-exchange and hydrophobicity, *HP-SAX* phase offers high selectivity and high resolution separation for a variety of organic acids such as aromatic and aliphatic carboxylic acids and sulfonic acids. Specific application areas include pesticides, herbicides, pharmaceuticals, inorganic anions, and biological species such as nucleotides and carbohydrates. *HP-SAX* columns can be compatible with a variety of mobile phases including organic solvents, mixture of water and organic solvent, such as methanol or acetonitrile, and aqueous buffers, such as phosphate.

Column Stability and Performance

Sepax *HP-SAX* uses full coverage bonded silica packing, which allows exceptional high stability. The *HP-SAX* packing material is stable in the mobile phases with pH range 2-8.5. Each lot of packing has passed rigorous performance requirements, including stability, carbon loading, capacity and selectivity. Each column is tested to control the quality by meeting its specifications. A typical test chromatogram for quality control is shown here for a *HP-SAX* 4.6x250mm column.

Safety Precaution

Sepax *HP-SAX* 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 and solvent vapor.



Column: HP-SAX (5 µm, 4.6×250mm)
Mobile Phase: 150 mM Phosphate buffer, pH 3.0
Flow Rate: 1.0 mL/min
Detection: UV 254 nm
Temperature: Ambient
Injection volume: 3 µL
Samples: 1. p-Amino Phenol, 2. p-Amino Benzoic 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 *HP-SAX* 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. *HP-SAX* bonded stationary phase has wide compatibility with wide range of organic solvents, a mixture of organic solvent (e.g. methanol or acetonitrile) and water, and aqueous buffer, such as phosphate or borate. HP-SAX columns are compatible with nonionic and zwitterionic detergents. ***HP-SAX columns are incompatible with anionic detergents.*** Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under aspirator vacuum.

Column Care

PH Avoid use of *HP-SAX* below pH 2 or above 8.5. Higher pH will dissolve silica, creating defects of bonded phase 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-SAX* 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 (>8.5).

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 HP-SAX Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 µm	120 Å	122663-2115
2.1x100mm	3 µm	120 Å	122663-2110
2.1x50mm	3 µm	120 Å	122663-2105
2.1x30mm	3 µm	120 Å	122663-2103
4.6x250mm	3 µm	120 Å	122663-4625
4.6x150mm	3 µm	120 Å	122663-4615
4.6x100mm	3 µm	120 Å	122663-4610
4.6x50mm	3 µm	120 Å	122663-4605
2.1x250mm	5 µm	120 Å	122665-2125
2.1x150mm	5 µm	120 Å	122665-2115
2.1x100mm	5 µm	120 Å	122665-2110
2.1x50mm	5 µm	120 Å	122665-2105
2.1x30mm	5 µm	120 Å	122665-2103
4.6x250mm	5 µm	120 Å	122665-4625
4.6x150mm	5 µm	120 Å	122665-4615
4.6x100mm	5 µm	120 Å	122665-4610
4.6x50mm	5 µm	120 Å	122665-4605
7.8x250mm	5 µm	120 Å	122665-7825
10.0x250mm	5 µm	120 Å	122665-10025
21.2x250mm	5 µm	120 Å	122665-21225
21.2x150mm	5 µm	120 Å	122665-21215
21.2x50mm	5 µm	120 Å	122665-21205
7.8x250mm	10 µm	120 Å	122669-7825
10.0x250mm	10 µm	120 Å	122669-10025
21.2x250mm	10 µm	120 Å	122669-21225
21.2x150mm	10 µm	120 Å	122669-21215
21.2x50mm	10 µm	120 Å	122669-21205

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Sepax 硅胶基质离子交换色谱柱使用和维护注意事项

请在色谱柱使用前仔细阅读本说明，并按要求进行操作，以保证色谱柱良好的重现性和耐用性。

色谱柱安装：

1. 色谱柱安装时，确认液路流向与色谱柱标签所示箭头方向一致。
2. 色谱柱接入仪器系统，接头松紧适中，系统开启后，请注意压力变化，确认与管路接头处无液体渗漏。

色谱柱使用和维护：

1. 请首先按照色谱柱出厂 QC 方法对色谱柱进行检测，理论塔板数和拖尾因子等应与 QC 报告相符。(因为仪器和实验条件的差异，实际检测结果与 QC 报告可能存在偏差，如偏差超过±20%请及时与厂家或色谱柱供应商联系)。
2. 请务必在说明书要求的柱温、压力和 pH 值范围内使用色谱柱，任何超出范围的色谱条件都可能导致色谱柱不可修复的损伤。
3. 色谱柱出厂时保存在 150 mM 磷酸盐缓冲液（pH 7.0）中。若客户流动相中含有机溶剂（如甲醇、乙腈），需先用 5-10 倍柱体积的纯水过渡，再使用色谱柱；实验完毕，仍需先用 5-10 倍柱体积的纯水冲洗，最后请用至少 10-20 倍柱体积的中性磷酸盐缓冲液冲洗并保存色谱柱。若客户流动相中不含有机溶剂，可直接使用，使用完毕后，请用至少 10-20 倍柱体积的中性磷酸盐缓冲液冲洗并保存色谱柱。
4. 建议采用流动相溶解样品，以避免溶剂效应的产生。此外，要保证待测样品与流动相有很好的溶解性，以免样品在流动相中析出而导致柱压升高和系统污染，若出现此情况，可对色谱柱进行低流速反向冲洗，以除去堵塞柱头的杂质。

色谱柱保存：

1. 如无特殊说明，每支色谱柱出厂时均保存在该色谱柱 QC 测试报告所述的溶剂中（报告底部）。建议的保存方法是该色谱柱存放的最佳方法；并建议每月用保存溶剂对色谱柱进行一次活化。
2. 如长期不用，请将色谱柱从仪器系统中卸下，塞上堵头，以免柱头干涸，影响下次使用。一段时间后使用色谱柱如出现峰形异常，可用保存溶剂低流速冲洗色谱柱活化过夜。