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HP-C18 and Bio-C18 Column Manual

Column Information

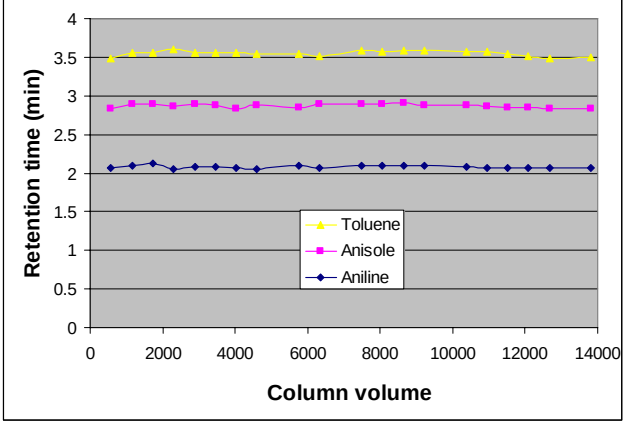
Utilizing highest purity and enhanced mechanical stability silica and pure bonding reagents, Sepax HP-C18 and Bio-C18 bonded phases have been innovatively and specially designed to ensure maximum mono-functional coverage and full end-capping, which leads to carbon content as high as 15% and 10% for HP-C18 and Bio-C18, respectively. The chemistry of monolayer formation and end-capping is completely controlled that results in very reliable column-to-column reproducibility. The maximum surface coverage allows HP-C18 and Bio-C18 to have exceptional stability. The uniform, spherical HP-C18 particles have a nominal surface area of 300 m^2/g or 200 m^2/g with a controlled pore size of 120 Å, or 200 Å respectively. The uniform, spherical Bio-C18 particles have a nominal surface area of 200 m^2/g or 100 m^2/g with a controlled pore size of 200 Å, or 300 Å respectively. Their proprietary surface modification with polar groups embedded in the stationary phases allows performing excellent separations even in pure water, resulting in no collapse of C18 phase. HP-C18 and Bio-C18 columns are specially designed to enable extended retention and selectivity for polar and hydrophilic compounds. HP-C18 and Bio-C18 columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Typical applications for HP-C18 are the separations of both polar and non-polar compounds, such as pharmaceuticals, peptides, amino acids, nucleotides, and organic acids. Typical applications for Bio-C18 are the separations of biological compounds, such as proteins, peptides, amino acids, nucleotides, and oligosaccharides.

Column Stability and Performance

HP-C18 and Bio-C18 uses full coverage bonded silica packing, which allows exceptional high stability. Figure 1 shows extremely reproducible retention time for three standard compounds: aniline, anisole and toluene after 13,000 column volume runs in a mobile phase of 85% methanol and 15% water.

Such high stability allows HP-C18 and Bio-C18 extremely suitable for validation of various analytes. The unique mono-functional bonding chemistry for HP-C18 and Bio-C18 avoids the formation of multiple C18 layers. Such uniform stationary phase allows the separation to achieve high selectivity and high efficiency. A typical test chromatogram for quality control is shown in Figure 2 for a 4.6x150mm HP-C18 column.

Figure 1. Sepax*HP* -C18 (4.6x150mm) Stability Test



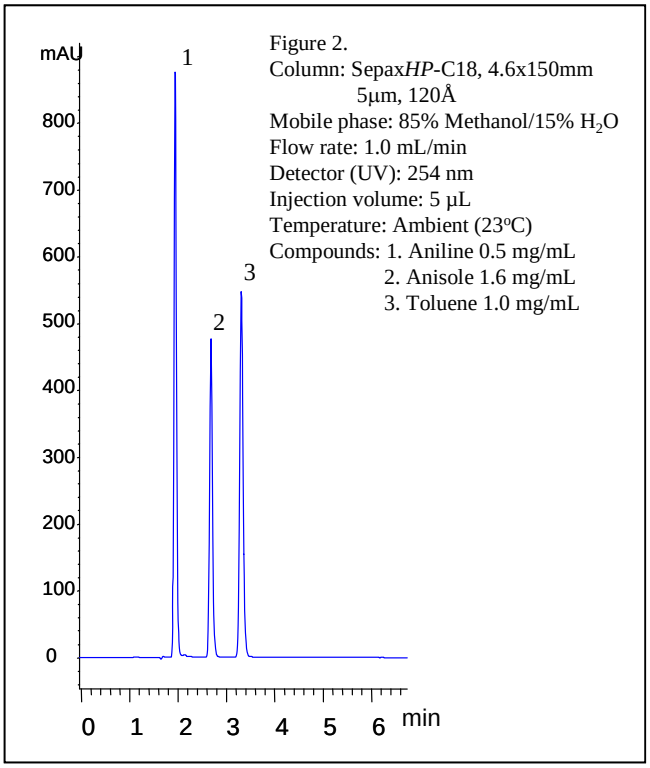


Figure 2.
Column: Sepax*HP*-C18, 4.6x150mm
5µm, 120Å
Mobile phase: 85% Methanol/15% H_2O
Flow rate: 1.0 mL/min
Detector (UV): 254 nm
Injection volume: 5 µL
Temperature: Ambient (23°C)
Compounds: 1. Aniline 0.5 mg/mL
2. Anisole 1.6 mg/mL
3. Toluene 1.0 mg/mL

Safety Precaution

HP-C18 and Bio-C18 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 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-C18 and Bio-C18 bonded stationary phase are specially designed to be compatible with aqueous mobile phase. HP-C18 and Bio-C18 can tolerate the separations in pure water. Its choice for mobile phase is broad, including organic solvent, aqueous buffer or a mixture of organic and water, such as methanol or acetonitrile and water. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum. Gradient elution methods for HP-C18 or Bio-C18 columns often begin with 5% methanol or acetonitrile as the initial mobile phase.

Column Care

PH Avoid use of HP-C18 or Bio-C18 below pH 2 or above 9. Higher pH will dissolve silica, creating defects of C18 bonding that causes separation efficiency loss and retention time change. The optimum performance and operation for longest lifetime are at pH 3 - 7.5.

Pressure Even though HP-C18 or Bio-C18 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. 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.

HP-C18 Products

ID x Length	Particle size	Pore size	P/N
2.1x150mm	3 μm	120 Å	103183-2115
4.6x250mm	3 μm	120 Å	103183-4625
4.6x150mm	3 μm	120 Å	103183-4615
4.6x50mm	3 μm	120 Å	103183-4605
2.1x150mm	5 μm	120 Å	103185-2115
2.1x100mm	5 μm	120 Å	103185-2110
2.1x50mm	5 μm	120 Å	103185-2105
2.1x30mm	5 μm	120 Å	103185-2103
4.6x250mm	5 μm	120 Å	103185-4625
4.6x150mm	5 μm	120 Å	103185-4615
21.2x250mm	5 μm	120 Å	103185-21225
21.2x150mm	5 μm	120 Å	103185-21215

Bio-C18 Products

ID x Length	Particle size	Pore size	P/N
4.6x250mm	3 μm	200 Å	105183-4625
4.6x50mm	3 μm	200 Å	105183-4605
4.6x250mm	5 μm	200 Å	105185-4625
4.6x150mm	5 μm	200 Å	105185-4615
21.2x250mm	5 μm	200 Å	105185-21225
4.6x250mm	5 μm	300 Å	106185-4625
4.6x150mm	5 μm	300 Å	106185-4615
4.6x50mm	5 μm	300 Å	106185-4605
21.2x250mm	5 μm	300 Å	106185-21225

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Sepax C18 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. Sepax C18 柱最大耐受的水相比例不超过 95%，HP-C18 可耐受 100%纯水相。
4. 流动相中有缓冲盐时，为避免盐析出，先用低比例的有机溶剂（如 10%乙腈）冲洗色谱柱 10 倍柱体积，再用流动相平衡；使用完毕后，用低比例的有机溶剂（如 10%乙腈）冲洗 10 倍柱体积，再用纯溶剂（如纯乙腈）冲洗 10-20 倍柱体积，保存色谱柱。
5. 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。
6. 当流动相使用三相混合，或离子对试剂等复杂体系时，要保证色谱柱平衡足够长的时间，以减小保留时间的漂移。添加离子对试剂的流动相在使用完毕后，可用洗脱强度较大的有机溶剂（如异丙醇、四氢呋喃）与水混合相对色谱柱进行再生活化（流动相替换及活化过程中，请关注柱压变化并保证溶剂间的兼容性）。

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。
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。