Blossmate® SAX User Manual

Overview

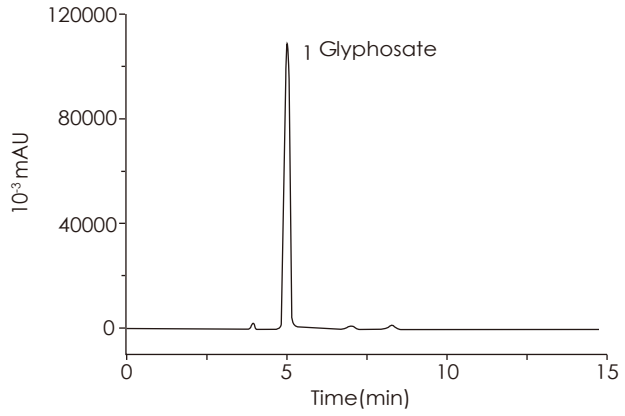
Welch Blossmate® SAX column is a high-performance anion exchange column designed for efficient separation. It utilizes ultra-high purity spherical silica gel with densely bonded quaternary ammonium functional groups, ensuring mechanical stability. The column operates effectively under high flow rates and pressures, accommodating rapid ionic strength changes for swift re-equilibration. The pH range of this column is 2.0-8.0, pore size is 120Å and surface area is 300 m^2/g. The packing material's stability is remarkable, maintaining baseline and sensitivity integrity even at neutral pH. Blossmate® SAX excels in polar small molecule analysis like glyphosate and nucleotides, as well as the separation and purification of large biomolecules like proteins and peptides.

Chromatographic condition and chromatogram

The testing was conducted following the method for determining the content of glyphosate in technical material outlined in 'GB 12686-2004 Glyphosate Technical Material'. The concentration of glyphosate was set at 2.0 mg/ml for the analysis.

Chromatographic conditions

Column: Blossmate® SAX, 4.6×250mm, 5μm
Mobile Phase: 100mM Potassium Dihydrogen Phosphate: Methanol = 85:15 (pH adjusted to 2.0 after thorough mixing)
Column Temperature: 25℃
Flow Rate: 1.0mL/min
Detection Wavelength: 195nm
Injection Volume: 20μL



No.	Retention time	Number of theoretical plates	Tailing Factor	Resolution(USP)
1	5.006	9552	0.98	-

Conclusion:

This chromatography column complies with the determination requirements for glyphosate content outlined in 'GB 12686-2004 Glyphosate Technical Material'.

Precautions

Blossmate® SAX chromatography columns are typically operated under high pressure. Inadequate connections in the tubing can result in leaks of organic solvents and injected samples, potentially affecting the health of operators. In case of leaks, proper gloves should be worn for handling. Furthermore, when opening the chromatography column, suitable protective measures must be taken to prevent tiny silica gel particles from entering the respiratory system.

Installation and operation

When transporting the chromatography column or when it's not in use, both ends are always sealed with plugs. Upon connecting the column to the chromatographic instrument system, remove the plugs from both ends first. Ensure that the direction of the mobile phase flow matches the direction marked on the column. Unless specific circumstances require otherwise, such as for flushing to remove dirt clogging the column inlet, it is advised to adhere to the direction indicated on the column when connecting it. As the column connection is an integral part of the chromatographic process, improper ferrule, incorrect installation, or mismatched screw type may lead to solution leaks. Follow the steps below to connect the chromatography column correctly:

a) For the first use of the connection tube, please pack the tube fitting and the ferrule on the outside of 1/16” tube in order. The wide end of the ferrule should face the tube fitting.

b) Insert the tubing firmly into the column interface and slide the tube fitting and the ferrule forward, ensuring the threads of the tubing fitting align with the threads of the column port. Tighten the tubing fitting securely. If the tubing is made of polymer material, proceed to step (d); if using metal tubing, continue with step (c).

c) After firmly pressing the tubing into the column end interface, use a 1/4" wrench to further tighten the already secured nut.

d) Apply the same procedure to the other end of the chromatography column.

The new Blossmate® SAX column is stored in pure methanol upon delivery. When using, start by washing the column successively with 50% methanol and pure water, using 20 column volumes for each step to activate the chromatography column. Subsequently, users can equilibrate the chromatography column with their chosen mobile

phase. Note that if the mobile phase consists of an organic solvent and a buffer salt, it's essential to transition with pure water as the mobile phase to prevent salt precipitation. Gradually increase the flow rate from 0.1 mL/min to the desired operating conditions until the baseline stabilizes. If there are significant fluctuations in column pressure and baseline, this might be due to air bubbles trapped in the column. In this case, a higher flow rate can be used to flush the column for 2-5 minutes. For example, a 4.6×150mm column can be flushed at a flow rate of 2.0 mL/min.

Sample and mobile phase

To prevent column blockage, all samples and solvents, including buffer salts, must be filtered using a 0.45μm or 0.22μm filter membrane before use. The Blossmate® SAX bonded phase is compatible with many solvents, including organic solvents, water, and buffer salts, but not compatible with anionic detergents. Prior to usage, it is recommended to degas the mobile phase.

Increasing the proportion of organic solvents in the mobile phase will reduce the retention of solutes. The separation mode of the Blossmate® SAX column is ion exchange separation. The mobile phase can consist of pure aqueous phase or a mixture of organic solvent and water, but using pure organic solvent as the mobile phase should be avoided whenever possible. When using a mobile phase with a high proportion of organic solvent, it is recommended to exercise caution or avoid using poorly soluble phosphate salts or other non-volatile buffer salts to prevent salt precipitation that could potentially damage the chromatography column.

Maintenance

pH

Avoid using the Blossmate® SAX column under conditions where the pH is below 2 or above 8. Higher pH levels can dissolve the silica gel, causing partial or complete detachment of the bonded phase from the silica gel surface, leading to decreased separation efficiency and altered retention times. For optimal separation performance and extended column lifespan, it's recommended to use mobile phases with pH values within the range of 2.0 to 8.0.

Pressure

While the Blossmate® SAX column can be operated under pressures as high as 5000 psi, the normal operating pressure should remain below 3000 psi. Prolonged operation under high pressure can damage the column and the fluid delivery pump. Since pressure is influenced by flow rate, the maximum flow rate will be limited by the pressure capacity of the system. Typically, the column pressure will gradually increase with prolonged use of the chromatography column. A sudden increase in pressure indicates clogging of the inlet frit. In such cases, it's advised to reverse the column and wash it with an appropriate solvent.

Temperature

The maximum operating temperature is 60°C. Prolonged operation at high temperatures (>75°C) can also damage the chromatography column, especially under high pH conditions (>8.5).

Cleaning of column

After multiple uses, impurities from certain samples may accumulate on the inlet frit or packing material. When this accumulation reaches a certain level, it can lead to increased pressure and broadening of peaks. In such cases, after flushing the column with buffer salts to clean it thoroughly, it is recommended to wash the column with 10% methanol at a low flow rate for 10-20 column volumes. Following this, the column should be stored in 10% pure methanol.

Storage

When the column is not in use for an extended period, flush it with at least 20-30 column volumes of 10% methanol and store it. Each column is shipped with two removable plugs. To prevent drying out of the column bed, securely seal both ends of the column with the plugs.

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