Sepax Technologies, Inc.

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Monomix H2P-SAX column

Column Information

Monomix H2P-SAX columns are designed to effectively quantify neutral amphiphilic surfactants, such as Tween 80, Tween 20, and Poloxamers, which are commonly present in formulated biological samples,such as Monoclonal Antibodies. The rigid and spherical solid support of the resin is composed of highly cross-linked poly(styrene/divinylbenzene/modifier) (PS/DVB/M). The average particle size is 40 µm, with a narrow size distribution (D_{90}/D_{10} < 1.3), and ideal porous structure and robust chemical/physical stability. The polymeric porous beads are further covalently modified with mixed-mode ligands so that the beads are water wettable, and at the same time can absorb amphiphilic surfactants.

The column is compatible with commonly used aqueous buffers containing acetate, phosphate, tris, and others, as well as organic solvents such as isopropanol, ethanol, methanol, and acetonitrile. The Monomix H2P-SAX column can be used as standalone operation for surfactant quantitation or used in 2D chromatography operation coupled with 2nd reversed phase column for surfactant degradation study.

Technical Specifications

Application	Surfactant quantification
Brand	Monomix H2P-SAX
Chemistry	Mixed-Mode
Ion exchange capacity	0.5 meq/gram
Mass spec. compatibility	Yes
Particle size	40 µm
Water wettable	Yes
pH stability	1-13
Operating temperature limit	80 ℃
Operating pressure limit	3,000 psi
Mobile phase compatibility	Aqueous solvents, organic solvents, or their mixtures
Flow rate	0.1-1 mL/min

Safety Precaution

Monomix H2P-SAX columns are normally operated under high pressure. Loose connections will cause leaking of buffers 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.

Column Installation and Operation

When a Monomix H2P-SAX column is shipped or not in use, it is always capped at both ends. When installing the

column to a system, first remove the end caps and connect with reference to the flow direction as marked on the column. Unless a user has a specific need to reverse the column flow direction (such as removal of inlet blockage) 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 tubing with suitable size. 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 finger tighten the nut.

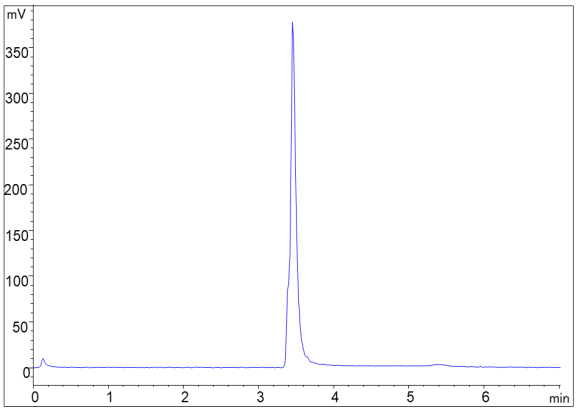
(c) While continuing to press the tube firmly into the end fitting, use a suitable size wrench to further tighten.

(d) Repeat this coupling procedure for the other end of the 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. It is also strongly recommended to use a pre-column filter (0.5 µm frit) or a guard column to protect the column. Always use an inline degassor or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Typical Chromatogram and Condition on Tween 20



Column: Monomix H2P-SAX, 2.1 x 20 mm, SS (PN: 282640990-2102); Instrument: HPLC; Detector: ELSD; Mobile phase A: 2% formic acid in water; B: 2% formic acid in IPA; Column temperature: 23 ℃; Flow low rate:

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1.00 mL/min; Sample: 0.0125% Tween 20; Injection amount: 50 µL

Column Care

Shipping solvent Monomix H2P-SAX columns are usually shipped in 0.1 M Ammonium Acetate (pH=6.75) with 0.02% NaN_3.

First-time use During stocking and shipping, the packing could be dried out. It is recommended that 10-20 column volumes (CV) of the running buffer 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 mobile phase or pH is quite different from the stock buffer in the column, it is recommended that the column is washed first with the new mobile phases for 10 column volumes.

pH The column can be used at a pH range of 1 - 13.

Pressure Monomix H2P-SAX columns can sustain at a pressure up to 3,000 psi. Continuous use at high pressure may eventually damage the column. 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. It is recommended to wait until the pressure drops to zero to safely disconnect the column from testing apparatus at the end of the test.

Temperature The maximum operating temperature is 80 ℃. The optimum temperature operation for the longest lifetime is 10-50 ℃. Continuous use of the column at higher temperature (>80 ℃) can damage the column, especially under extremely pH (>13 or < 1.0).

Flow rate range Normal operational flow rate is 0.1-1.0 mL/min for 2.1 mm I.D. columns.

Storage When not in use for an extended period of time, store Monomix H2P-SAX columns in 0.1 M Ammonium Acetate at pH 6.75 with 0.02% NaN_3 or with 10% Methanol. Flush the column with the storage buffer for at least 15 column volumes. Then seal both ends with the removable end plugs provided with the column, to prevent the drying of the column bed.

Column cleaning Column can be cleaned with organic solvents. If aqueous buffers such as sodium phosphate is being used in the method, make sure to wash the Monomix H2P-SAX column for at least 10 CVs, before switching to organic solvent for column cleaning, to remove any residual buffer salt to prevent precipitation.

1) Organic solvents, such as 15-100% IPA. Wash the Monomix H2P-SAX column in reverse flow direction at 0.35 mL/min for 15 minutes. Equilibrate the column with running buffer before resuming the desired application.

Or

2) 0.5 M NaOH. A solution of 0.5 M NaOH can be applied to remove the deposits. The Monomix H2P-SAX column should be washed in a reverse flow direction at 0.35 mL/min for 15 minutes followed by a 30 min wash with water. Equilibrate the column with running buffer before resuming the desired application.

Ordering Information

PN	Product
282640990-2102	Monomix H2P-SAX, 2.1 x 20 mm, SS
282640990-2105	Monomix H2P-SAX, 2.1 x 50 mm, SS