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TweenTrap Columns

Column Information

The Sepax TweenTrap column is specifically designed for trapping surfactants, such as Tweens, in aqueous samples. The packing support is composed of rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) beads with a particle size of 5 µm. A proprietary surface coating is covalently bonded onto the PS/DVB support, which allows for exceptionally high stability as well as minimal non-specific binding of target compounds. The covalently bound amphophilic functional group allows for both hydrophilic and lipophilic adsorptions of detergents, such as Tween. The column is compatible with most aqueous buffers, including, acetate, phosphate, tris, and others.

TweenTrap columns can be run in tandem with SEC columns in aqueous samples, and are specifically designed to trap and remove surfactants, such as Polysorbate 80/20 (PS 80/20, Tween 80/20), which can interfere with quantitative protein SEC analysis. To achieve the best results, it is recommended to run the Sepax TweenTrap in aqueous based mobile phases, without the addition of organic solvents. An example is shown in Figure **1 & 2**. As illustrated, the Sepax TweenTrap column helps to minimize the interference of Tween in the reporting of dimer versus monomer distribution of Erbitux, a commercially available recombinant, monoclonal antibody (mAb).

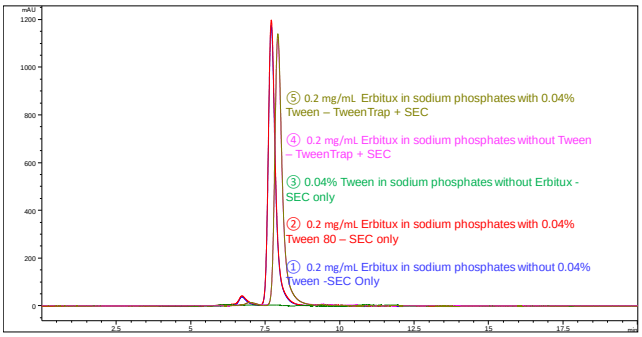

Figure 1. The effect of a TweenTrap Column on the SEC analysis of Erbitux. Mobile phase: 150 mM sodium phosphate, pH 7.0, Detection: 214 nm, Flow rate: 1 mL/min, Column temperature: ambient. Samples: as indicated. Injection amount: 100 µL

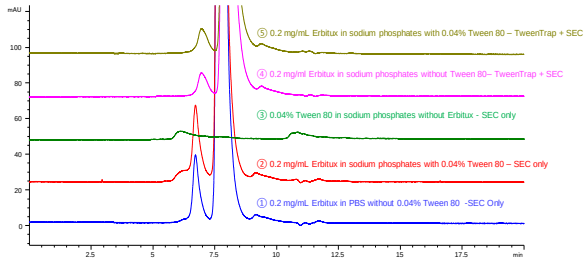

Figure 2. Zoomed-in staggered view of **Figure 1**.

Trace 1 shows the SEC analysis of Erbitux alone without tween. Clear aggregate peaks are seen in front of the monomer.

Trace 2 shows the SEC analysis of Erbitux in 0.04% Tween 80, without a TweenTrap column. Attention should be drawn to the front shoulder of the aggregate region, which can be attributed to Tween 80, based on the profile of an injection of Tween 80 alone (**Trace 3).** Indicating that there is possible Tween 80 interference in the quantitation of Erbitux (**Trace 2)**.

With the addition of a Sepax TweenTrap column, **Trace 5** illustrates a profile where Tween 80 interference is minimized. This is further confirmed by an injection just Erbitux, *with no Tween 80 formulation,* onto the same column setup (**Trace 4**).

Therefore, the chromatographic overlays in **Figures 1 and 2**, help to illustrate how Tween 80 in the formulation of Erbitux can be trapped onto the Sepax TweenTrap column, while still allowing for effective aggregate peak detection and integration. It can be noted that the peak shape of the Erbitux aggregate is slightly different across the traces. This can be potentially attributed to the interaction of Erbitux aggregates on the TweenTrap column, or perhaps just due to an increase in column dead volume. Such that users may need take precaution about this effect.

Safety Precaution

TweenTrap 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 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 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 finger tighten the nut.
(c) While continuing to press the tube firmly into the end fitting, use a 1/4" wrench to further tighten.
(d) Repeat this coupling procedure for the other end of the column.

Shipping solvent New TweenTrap columns are usually shipped in 100 mM phosphate buffer at pH 7.0.

Technical Specifications

Packing	Highly cross-linked PS/DVB resin support grafted with a densely packed, nanometer thick amphiphilic coating.
Particle size	5 µm
Pore structure	Non-porous
pH stability	2-13
Operating temperature limit	80 °C
Operating pressure limit	5,000 psi
Mobile phase compatibility	Typical buffers: phosphate, tris, and acetate. Organic additives will wash off Tween and other surfactants.
Flow rate	Typical 0.1-1.0 mL/min for a 4.6 mm I.D. column

Capacity on a default 4.6 x 35 mm column

Tween 80 Concentration	Injection Volume	# of Injections before reaching column capacity
0.01%	100 µL	20
0.04%	100 µL	5

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 (0.5µm frit) or a guard column to protect the column. The TweenTrap columns are compatible with aqueous mobile phases as they are intended to be run inline with Size Exclusion Chromatography (SEC) columns. Please note that aqueous SEC mobile phases with organic additives are not recommended to run on TweenTrap columns. 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.

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 volume.

PH The optimum performance and operation for the longest lifetime of a Sepax TweenTrap column is pH 2 - 13.

Pressure Even though the TweenTrap columns can operate at pressure up to 5,000 psi for 5 µm particles, the normal operation is usually under 3,500 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°C. The optimum temperature operation for the longest lifetime is 10 - 50°C. Continuous use of the column at higher temperature (>80°C) can damage the column, especially under extremely pH (>13 or <2.0).

Flow rate range Normal operation is 0.1-1.0 mL/min for 4.6 mm I.D. columns.

Storage When not in use for extended time, store TweenTrap columns in 100 mM phosphate buffer at pH 7.0/0.1% NaN_3. Flush the column with the storage buffer for at least 15 column volumes. And then seal both ends with the removable end plugs provided with the column, to prevent the drying of the column bed.

Column clean-up Disconnect the Sepax TweenTrap column from the SEC column before a CIP wash. If organic solvents are used to clean the column, make sure that the TweenTrap column has been washed by water for at least 10 CVs, to remove any residual buffer salt.

Trapped surfactants can be washed away with one of two methods:

- Organic solvents, such as 15% IPA. After disconnecting from the SEC column, simply wash the TweenTrap column in reverse flow direction at 0.35 mL/min for 15 minutes followed by 10 min wash with water. Equilibrate the column with running buffer before resuming the desired application.

-OR-

- 0.5 M NaOH. After disconnecting from the SEC column, a caustic 0.5 M NaOH solution can be applied to remove the surfactant. The TweenTrap 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:

Part Number	Description
010054-4603	Tween Trap-NP, 5µm, NP, 4.6×35mm
102002-COUPLER	One-piece PEEK coupler, recommended to connect columns in series.