

Bioseparation HPLC Columns

Sepax Technologies, US based HPLC column manufacturer, specialized in bioseparation SEC and IEX HPLC columns for large molecule protein and MAb separation and most comprehensive small molecule separation phases including HILIC, Supercritical Fluid Chromatography (SFC), RP, sub2 micron UHPLC silica and PS/DVB based phases from analytical to prep. scale.



Zenix and SRT

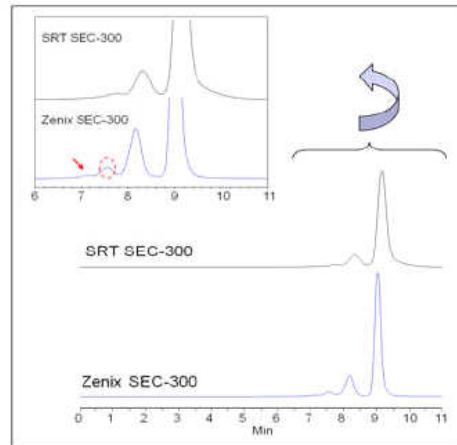
SEC, Spherical, high purity Silica; highest resolution and efficiency SEC in the

market (High Resolution and lot-to-lot consistent alternative to TSK Super and SW series), made of proprietary uniform, hydrophilic, and neutral nanometer surfaced layered silica results in highly reproducible separation power for large molecule separation.

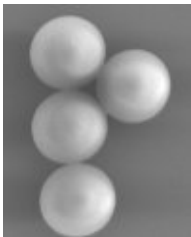
Zenix-C and SRT-C:

laydown monolayer on porous silica.

High Resolution Difference – 3µm vs. 5µm SEC



Columns:
SRT 5µm, Zenix 3µm,
7.8x300mm
Mobile phase:
150mM Phosphate Buffer, p
Flowrate: 1 mL/min
Temperature: ambient
Detection: UV 214 nm
Sample: BSA (5mg/ml)
Injection: 10 µL

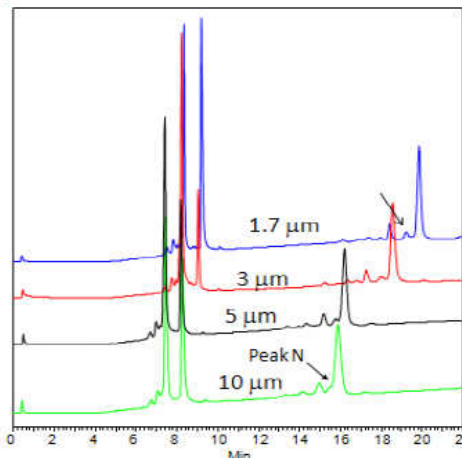


Proteomix IEX

PS/DVB Ion-exchange; NP 1.7, 3, 5, 10 with lot-to-lot consistent high resolution and high capacity for Protein, Oligo, Peptide, Carbohydrate,

and etc application.

Separation of Proteins vs. Ion-exchange Resin Size



Proteomix WXC-NP (4.6x50mm)

A: 20 mM PBS
B: A+1.0 M NaCl
0-100%B (20 min)
Flow rate:
1.0 mL/min
0.75 mL/min (WXC-NP1.7)
Proteins (1.0 mg/mL):
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
UV 280 nm



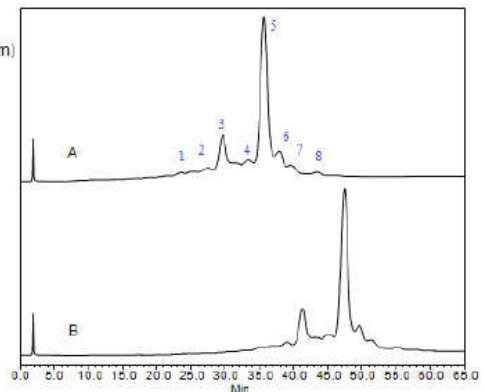
Antibodix WXC

PS/DVB; available in NP1.7, 3, 5, 10 for MAb separation (High Resolution

alternative to Dionex Propac)

MAb separation - as many as eight peaks separated

Columns:
Antibodix-NP10 (10µm, 4.6x250 mm)
Mobile phase:
A, 10 mM phosphate, pH 7.0
B, A + 0.1M NaCl
Gradient:
A) 25-75%B in 60 min
B) 15-65%B in 60 min
Flowrate: 0.8 mL/min
Temperature: 25 °C
Detection: UV 214 nm
Sample: MAb-X22
Injection: 10 µL (1.5 mg/mL)

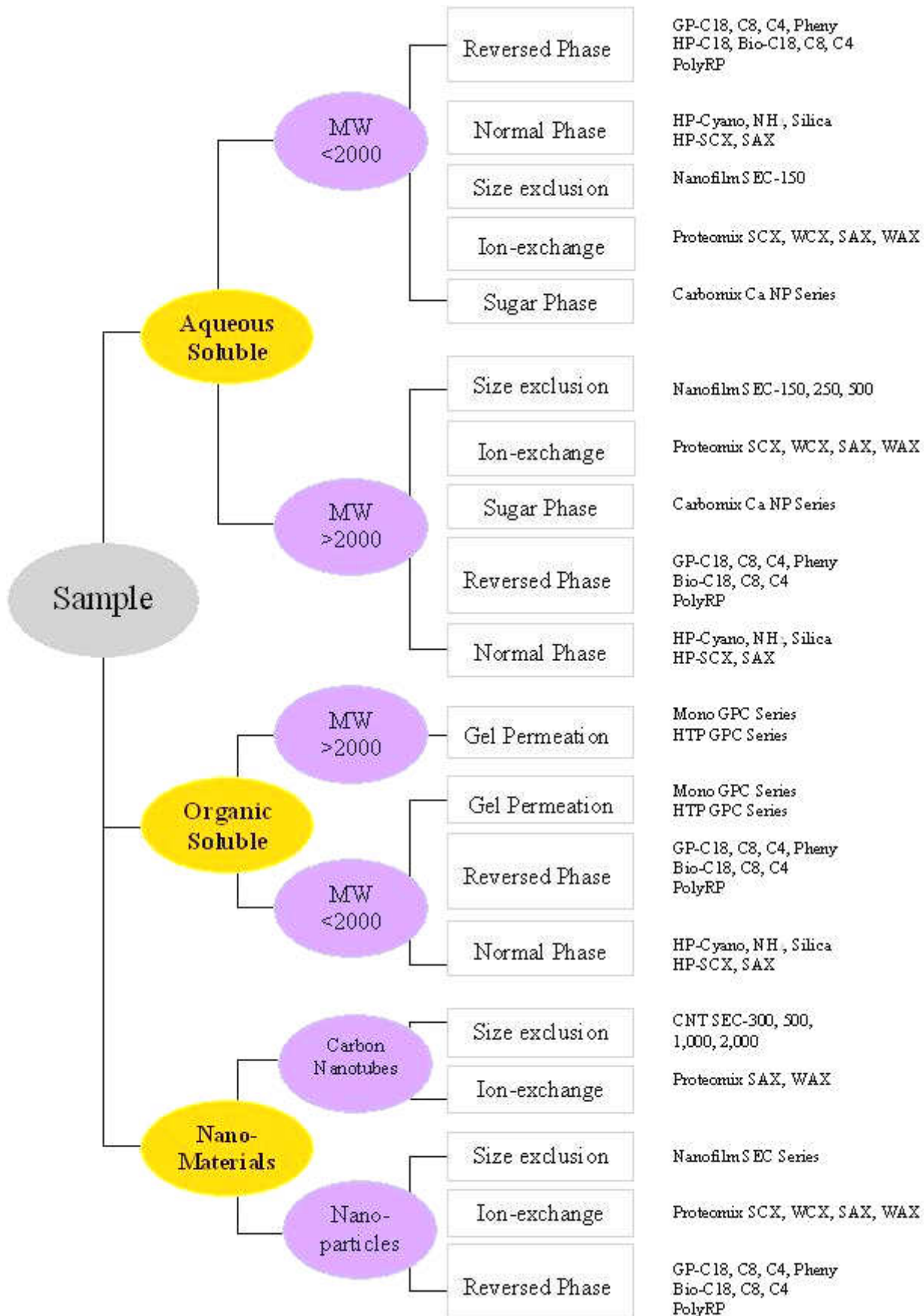


Carbomix

PS/DVB; surfaces functional groups are sulfonic acid (-SO3H), or metal ions, such as calcium (Ca+2).

The PS/DVB support has cross-linking degree of 5%, 8% and 10%. for analytical and preparative separations of carbohydrates, organic acids, peptides, and nucleic acids.

Sepax HPLC Column Selection



Sepax Proteomix[®] SAX IEX for Glycan and Carbohydrate separation

– part of your MAb workflow

Feature Highlights:

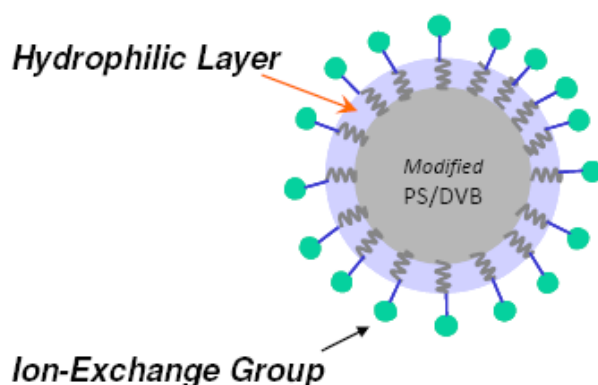
- Unprecedented high resolution
- Particle size choice of 1.7, 3, 5, 10 μm
- Non-porous particles with mono-dispersity and high capacity

What it means to you:

- More glycan isoforms separated
- Consistently performing columns from lot to lot

Sepax Proteomix[®] Ion-exchange Stationary Phases

New Technology for non-porous ion-exchange resins



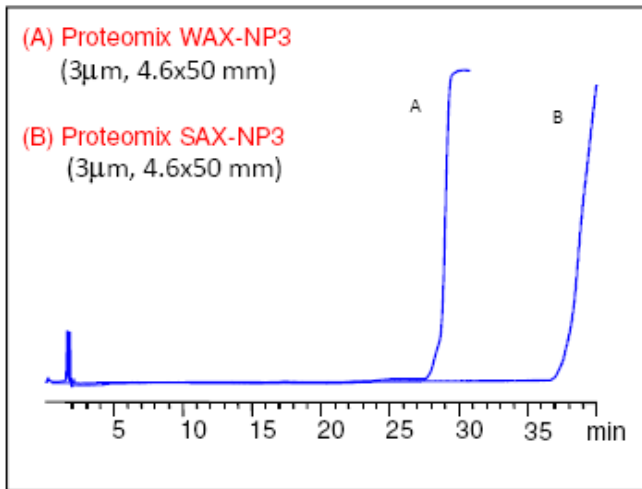
Multiple ion-exchange groups on one anchoring site

Results from the Surface Chemistry

- Uniform coating
- Elimination of non-specific interaction
- Controlled and Increased capacity

High Capacity of Non-porous Ion-exchange Resins

Dynamic Binding Capacity of Proteomix[®] SAX and WAX



| Packings | Particle (µm) | SA (m ²) | Capacity (mg/mL) |
|------------------|---------------|----------------------|------------------|
| <i>Proteomix</i> | | | |
| SAX-NP1.7 | 1.7 | NP | 43 |
| SAX-NP3 | 3 | NP | 35 |
| SAX-NP5 | 5 | NP | 28 |
| SAX-NP10 | 10 | NP | 17.5 |
| WAX-NP1.7 | 1.7 | NP | 35 |
| WAX-NP3 | 3 | NP | 26 |

Test conditions:

10 mM Tris/HCl pH 8.0

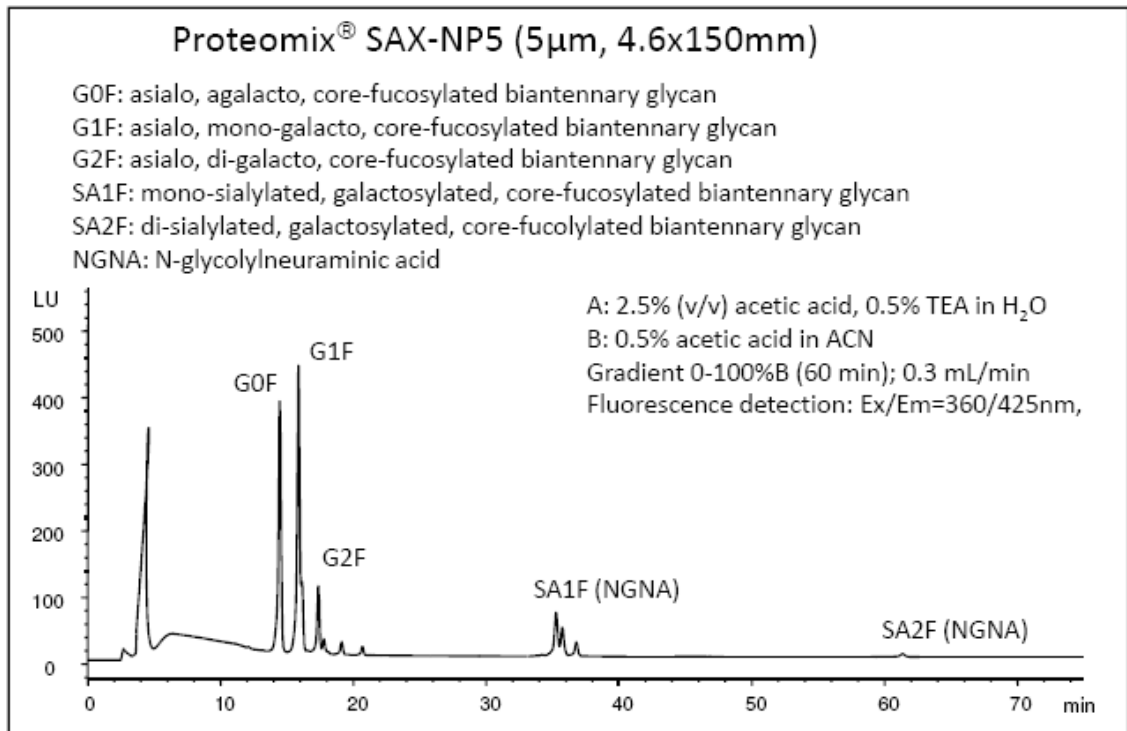
3 mg/mL BSA

Flow rate: 0.25 mL/min

Detection: 280 nm

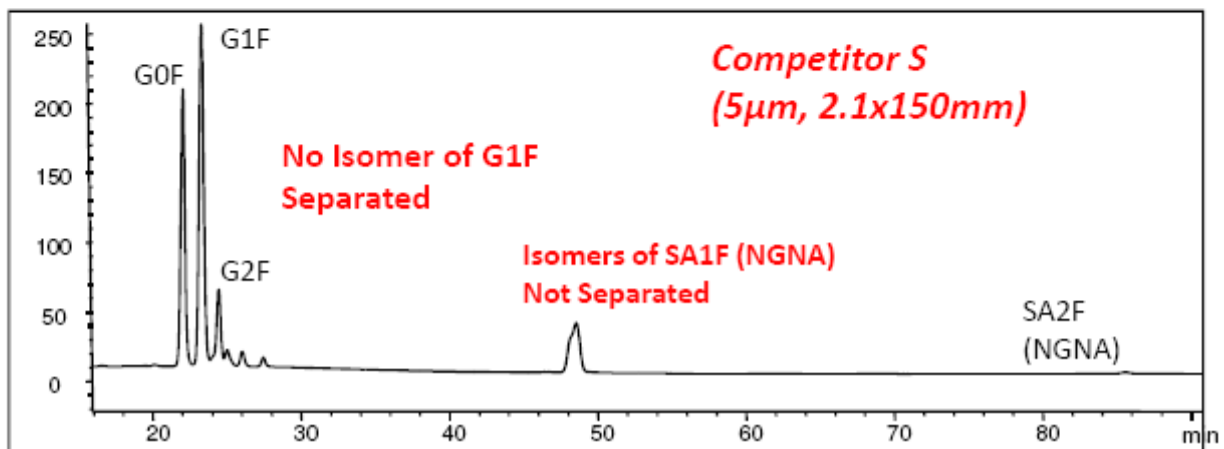
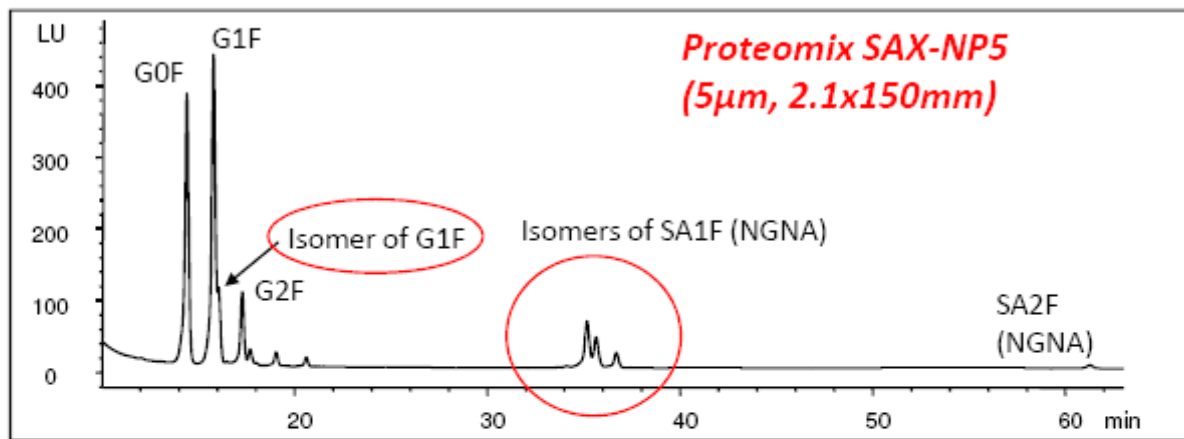
High Resolution Separation of Glycan and Isomers

2-AA (anthranilic acid) labeled N-linked oligosaccharide profiling of an IgG1 sample



Comparison Separation of Glycan and Isomers

2-AA labeled N-linked oligosaccharides profiling of an IgG1 sample



von CP-ANALYTICA



Tween 80 & Tween 20

Analysis of MAb 221 with Tween 80 and Tween 20 by Sepax SEC Column

THE CHALLENGE:

Tween 80 gives disturbing peak just where your protein elutes?

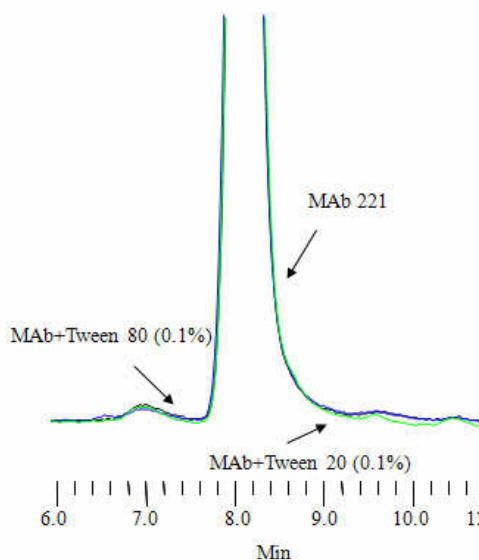
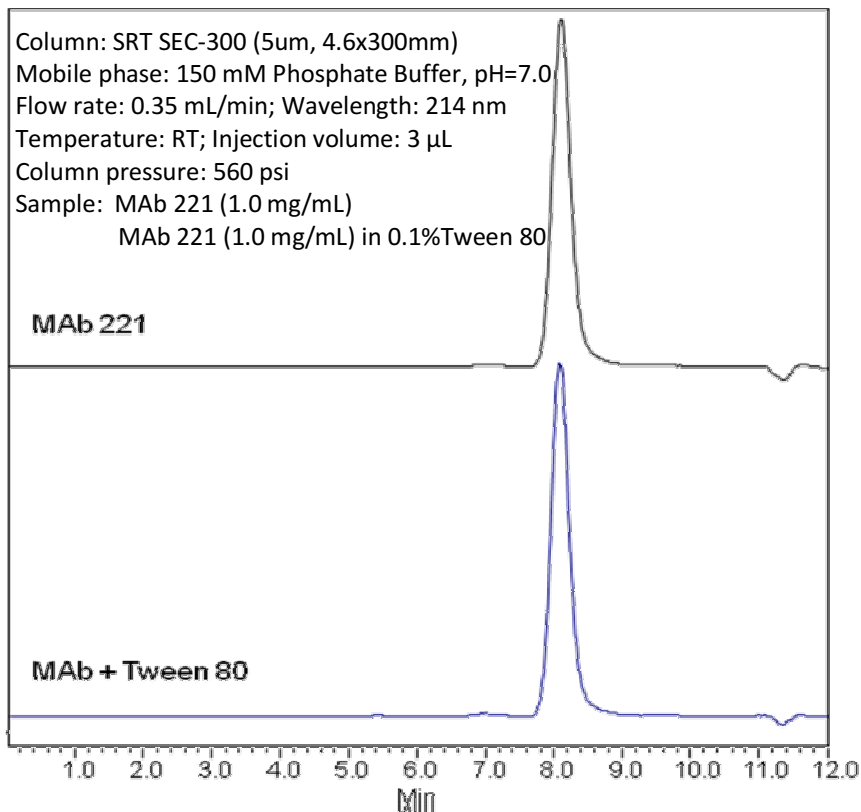
Commonly used surfactant, such as Tween 80 (Polysorbate 80) or Tween 20 (Polysorbate 20) in drug formulation buffer, coeluting with main protein peak.

THE SOLUTION:

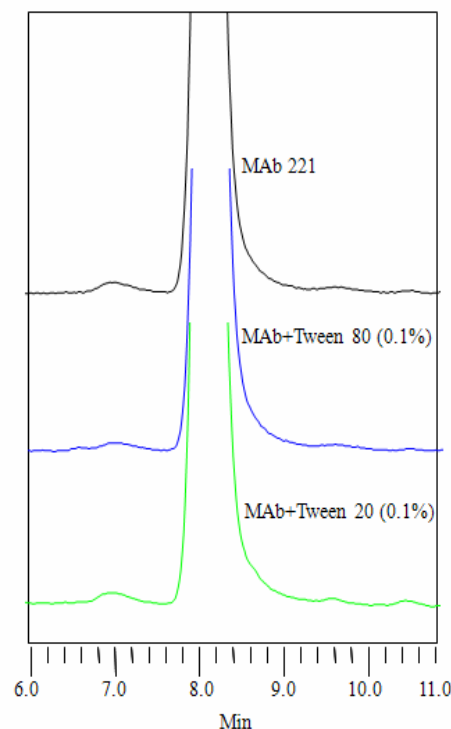
Sepax SEC (SRT 5um or Zenix 3um)

Delivers unrivaled high resolution separation with virtually no Tween 80 or Tween 20 interference due to unique proprietary surface coating technology. Sepax SEC allows you to focus on peaks of interest.

SRT SEC-300, 5um silica based SEC offers high resolution and non-interfering separation of MAb 221 with and without the presence of 0.1%



Column: SRT SEC-300 (5um, 300A, 4.6x300mm)
 Mobile phase: 150 mM Phosphate Buffer, pH=7.0;
 Flow rate: 0.35 mL/min; Detector: Refractive Index;
 Temperature: RT; Sample: MAb 221 (1.4 mg/mL);
 Injection Volume: 3 µL





Tween 80 & Tween 20

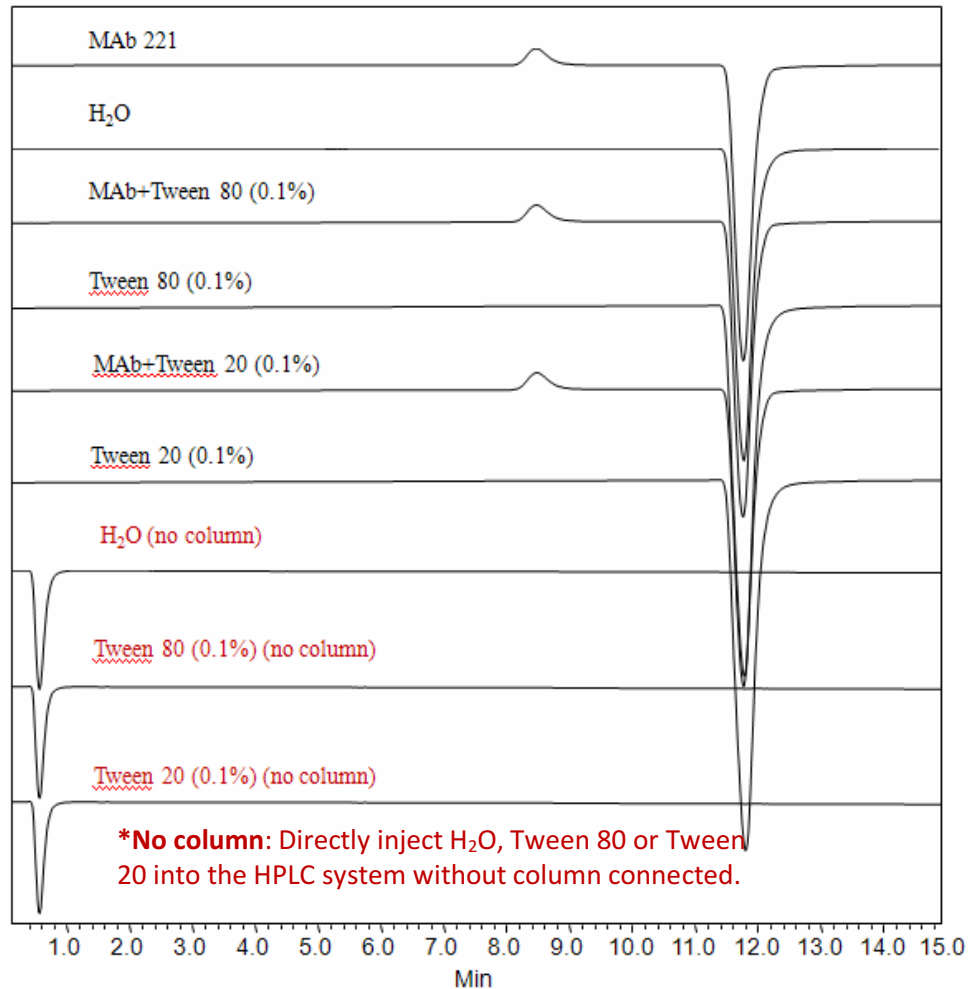
Tween 80 and Tween 20 have no impacts on the MAb separation

Data Supported Facts:

As shown in right figure, mixtures of MAb with Tween 80 (0.1%) and MAb with Tween 20 (0.1%) were subject to size-exclusion chromatography on SRT SEC-300 (5 μ m, 300A, 4.6x30mm) and detected using Refractive Index.

Further, water, Tween 80 and Tween 20 samples were individually injected to HPLC system without the use of SEC column.

The consistency in the three separation profiles suggests Tween 80 and Tween 20 samples pass through the column together with water which explains why when using Sepax SEC and in presence of Tween, the separation exhibits no MAb and Tween coelution interference.



Column Condition:

Column: SRT SEC-300 (5 μ m, 300A, 4.6x300mm); Mobile phase: 150 mM Phosphate Buffer, pH=7.0; Flow rate: 0.35 mL/min; Detector: Refractive Index; Temperature: RT; Sample: MAb 221 (1.4 mg/mL); Injection Volume: 3 μ L

Order Information

| | |
|-------------|---|
| 215300-7830 | SRT SEC-300, 5 μ m, 300A, 7.8x300mm |
| 215300-4630 | SRT SEC-300, 5 μ m, 300A, 4.6x300mm |
| 213300-7830 | Zenix SEC-300, 3 μ m, 300A, 7.8x300mm |