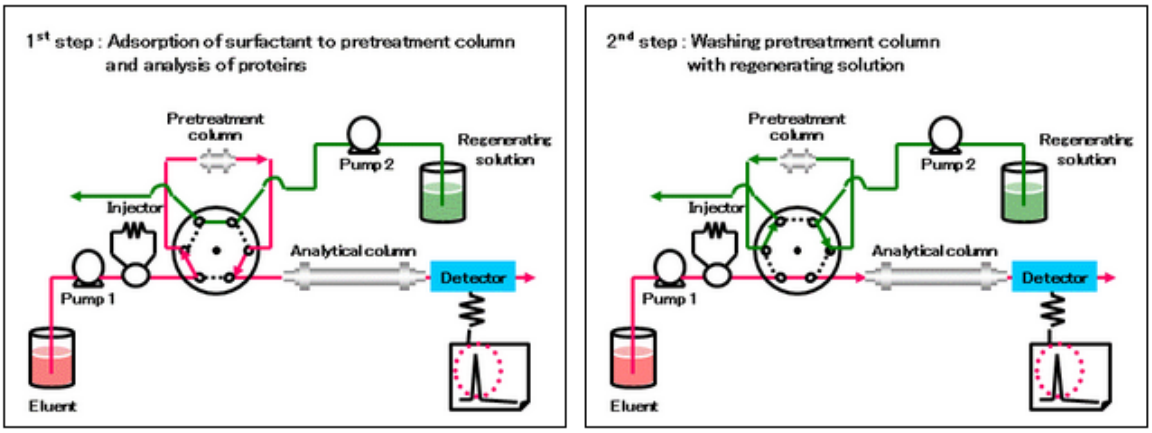
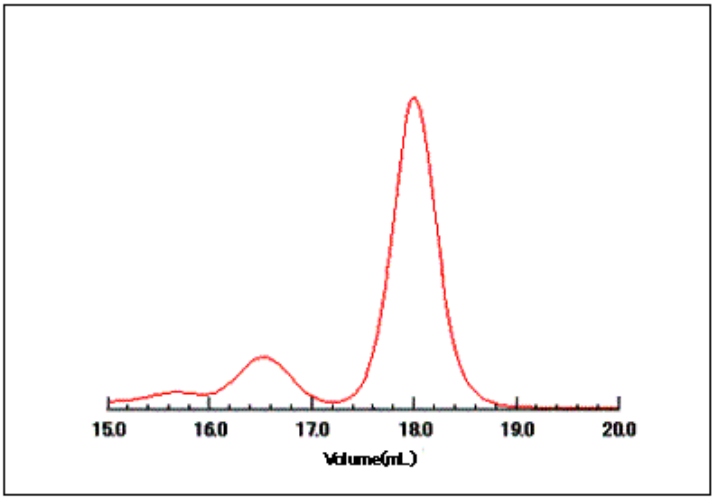
BSA Analysis in Surfactant (On-line Surfactant Removal)

Surfactants are often used to solubilize cell membrane and/or to prevent peptide/protein to be adsorbed on the container surface. However, the presence of surfactant may deteriorate LC column and LC/MS system, resulting in poor repeatability. By using a column-switching sample preparation column, MSpak GF-4A, surfactants can be removed prior to the sample being introduced to the analytical column. Therefore, good repeatable analysis can be achieved and the time required for sample preparation can be reduced. In the following system diagram, in the initial step, the surfactant in the sample is adsorbed to GF-4A by hydrophobic interactions and the protein sample is introduced into the analytical column and separated. The next step involves switching the hexagonal valve such that a regenerating solution (such as 10mM CH_3COONa aq. (pH 7.0)/CH_3CN=30/70) flows only to GF-4A and washes out the adsorbed surfactant, regenerating GF-4A.





Sample: BSA

Pretreatment

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Column          : Shodex MSpak GF-4A (4.6 mm I.D. x 10 mm)
Eluent          : 50 mM Sodium phosphate buffer (pH 7.0) + 150 mM NaCl
Flow rate       : 1.0 mL/min
Column temp.    : Room temp.
Switching time  : 5 min
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Analysis

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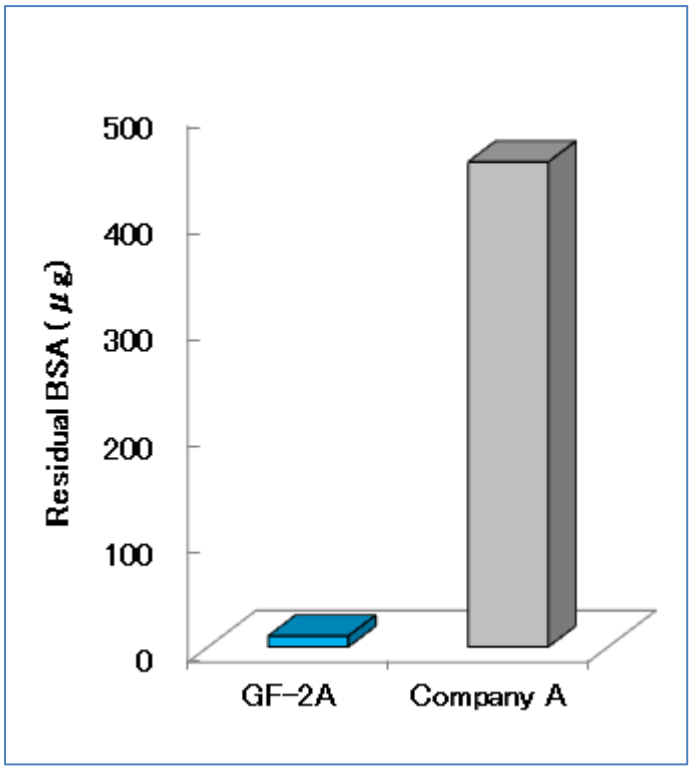
Column          : Shodex PROTEIN KW-804 + KW-802.5 (8.0 mm I.D. x 300 mm each)
Eluent          : 50 mM Sodium phosphate buffer (pH 7.0) + 150 mM NaCl
Flow rate       : 1.0 mL/min
Detector        : UV (280 nm)
Column temp.    : Room temp.
```

Source : https://www.shodex.com/en/dc/10/08.html

Ratio of Deproteinization

The ratio of deproteinization with MSpak GF-4A (a column for Column switching) and with a corresponding column of other company were compared. Since the ratio of deproteinazation is high, GF-2A can suppress the adsorption of protein to analytical columns or LC/MS as much as possible, and keep the reproducibility of analysis. (GF-2A (2.0mmI.D. x 10mm) is a custom-made column of GF-4A.)



Sample: 200mg/mL BSA 100µL

Ratio of Deproteinization	
GF-2A	Company A
99.80%	97.68%

Pretreatment

Eluent : 10mM CH_3COONH_4(pH7.0)
Flow rate : 0.5mL/min
Column temp. : Room temp.
Switching time : 5min

Analysis

Column : Shodex PROTEIN *KW-604S (6.0mmI.D. x 50mm) *KW-604S is phase-out product.
Eluent : 0.1% TFA in (H_2O/CH_3CN=50/50)
Flow rate : 0.5mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Source : https://www.shodex.com/en/dc/10/09.html