Use of Reversed Phase and HILIC in the 2D-LC Separation of Cationic, Anionic, and Hydrotropic Surfactants

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Introduction

- Surfactants are frequently found in pharmaceutical and biopharmaceutical drug applications as well as common household products.
- Surfactants can be polar, non-polar, or amphoteric.
- This structural diversity of the surfactants and complexity of the sample matrix can make the separation and identification of surfactants by HPLC challenging.
- Historically, this has been achieved using Reversed Phase Chromatography supplemented with Ion Exchange Chromatography.
- When performed individually, these techniques can be used to characterize the surfactant profile.
- We have evaluated multiple reversed phase columns and a HILIC column for the separation of polar and non-polar surfactants in a single injection using 2D-LC.

Material and Methods

Columns:	TSKgel® ODS-100Z (M0015-83M), TSKgel ODS-140HTP (P0031), TSKgel NH_2-100, TSKgel NH_2-100 DC
Instrument:	All analyses were carried out using an Agilent 1200 HPLC system run by Chemstation (ver B.04.02)
Mobile phase:	A: CH_3CN B: 100 mmol/L ammonium acetate, pH 5.4
Gradient:	as noted in chromatograms
Flow rate:	1.0 mL/min (4.6 mm ID columns) 0.4 - 0.5 mL/min (2.0 mm ID columns)
Detection:	UV @ 280 nm, 254 nm, and 210 nm FLD λ_{ex} 280 nm, λ_{em} 350 nm
Temperature:	30 °C
Injection vol.:	1 µL
Samples:	Triton™ X Triton N sodium xylenesulfonate sodium dodecylbenzene sulfonate benzyl dimethyldodecyl ammonium chloride benzalkonium chloride hexadecylpyridinium chloride All samples are 1 mg/mL

Figure 1: Structures of Hydrotropic, Anionic, and Cationic Surfactants

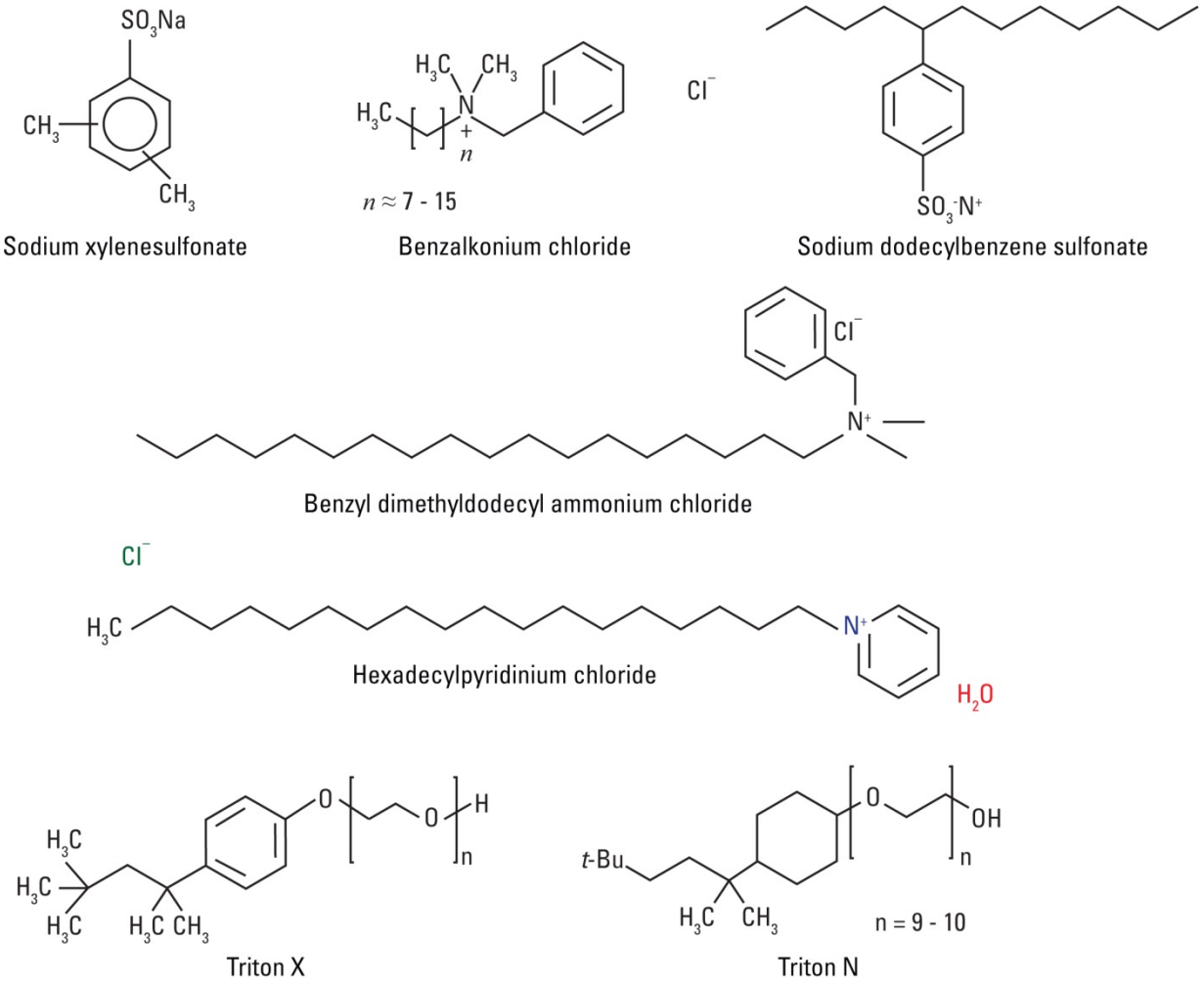
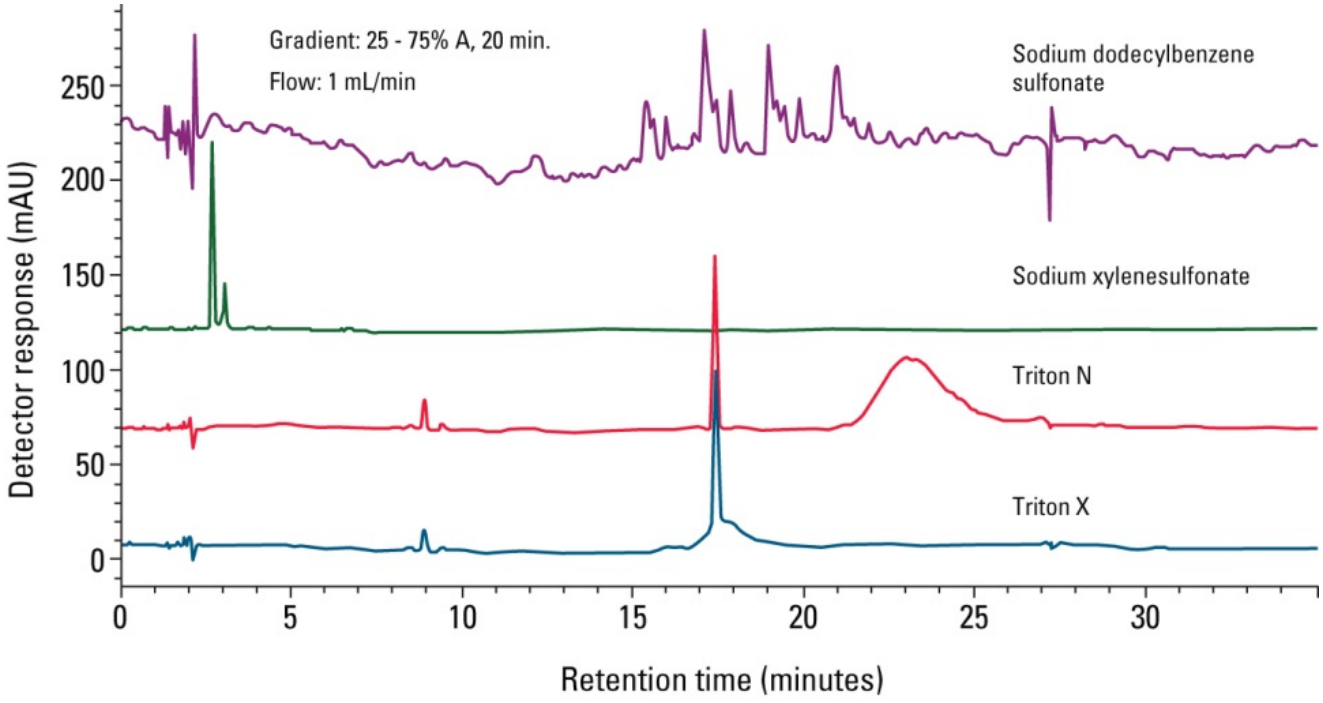
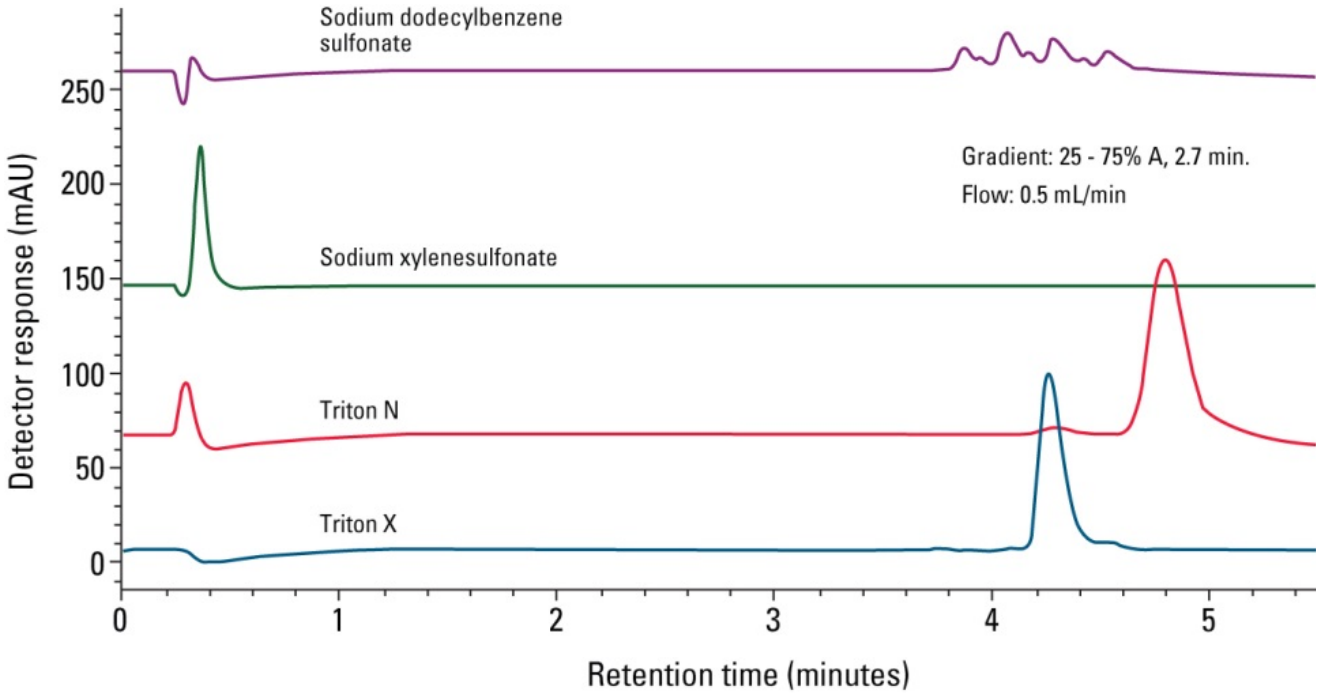


Figure 2: Surfactant Separation using TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm Reversed Phase Column



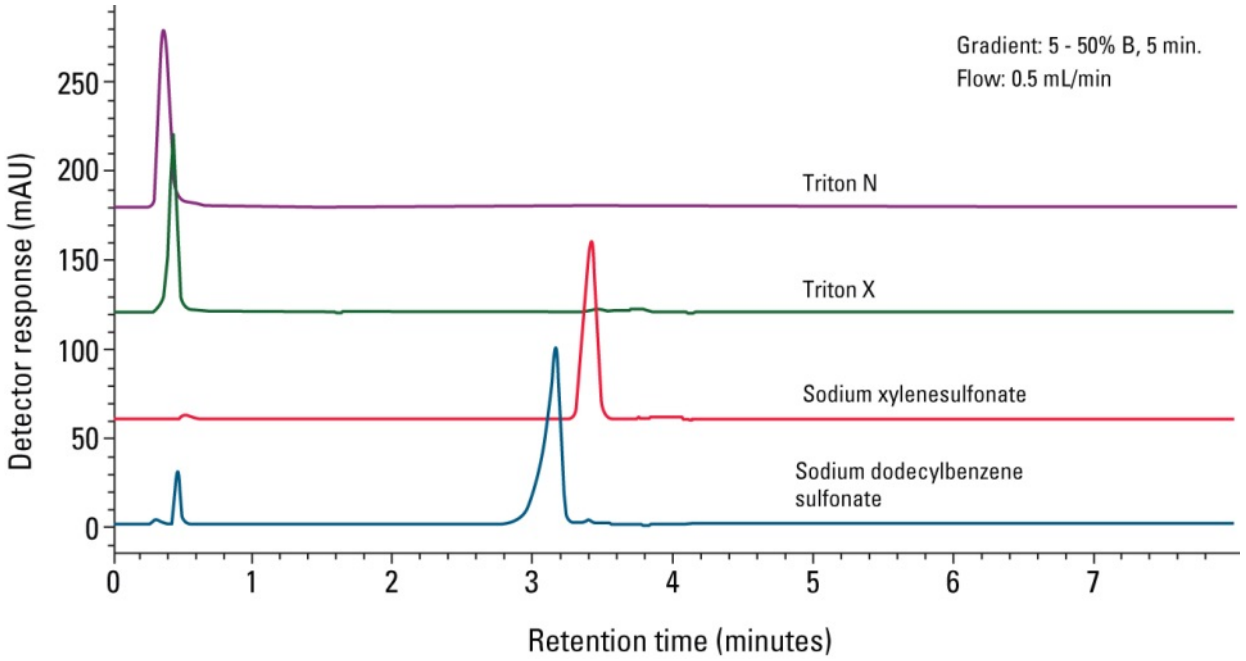
- The 20% carbon C18 phase yields very sharp peaks and relatively high retention of non-polar, hydrotropic surfactants.
- The highly polar sodium xylenesulfonate elutes in the void volume of the column.

Figure 3: Surfactant Separation using the TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm Reversed Phase Column



- The 2.3 µm particle size and small geometry of the TSKgel ODS-140 HTP column allows for good separation of the surfactants and their impurities in under 6 minutes.
- Similar to the TSKgel ODS-100Z, the highly polar sodium xylenesulfonate elutes in the void volume of the column.

Figure 4: Surfactant Separation using the TSKgel NH_2-100, 3 µm, 2.1 mm ID × 15 cm HILIC Column

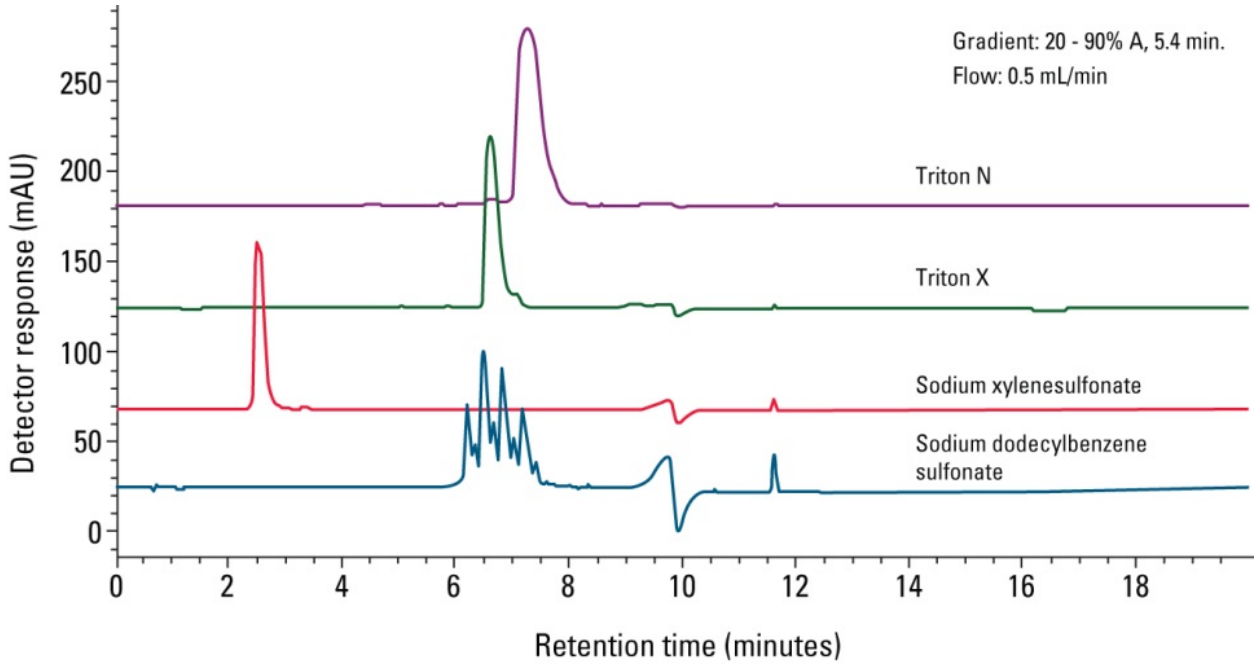


- The more polar surfactants (sodium xylenesulfonate and sodium dodecylbenzene sulfonate) are well retained using the amino-functionalized TSKgel NH_2-100 column in HILIC mode.
- The non-polar Triton surfactants both elute in the void under HILIC conditions.
- An overall orthogonal separation relative to the TSKgel ODS-140HTP is observed with the TSKgel NH_2-100.

2D-LC Separation of Surfactants using the TSKgel ODS-140HTP and the TSKgel NH_2-100 Columns

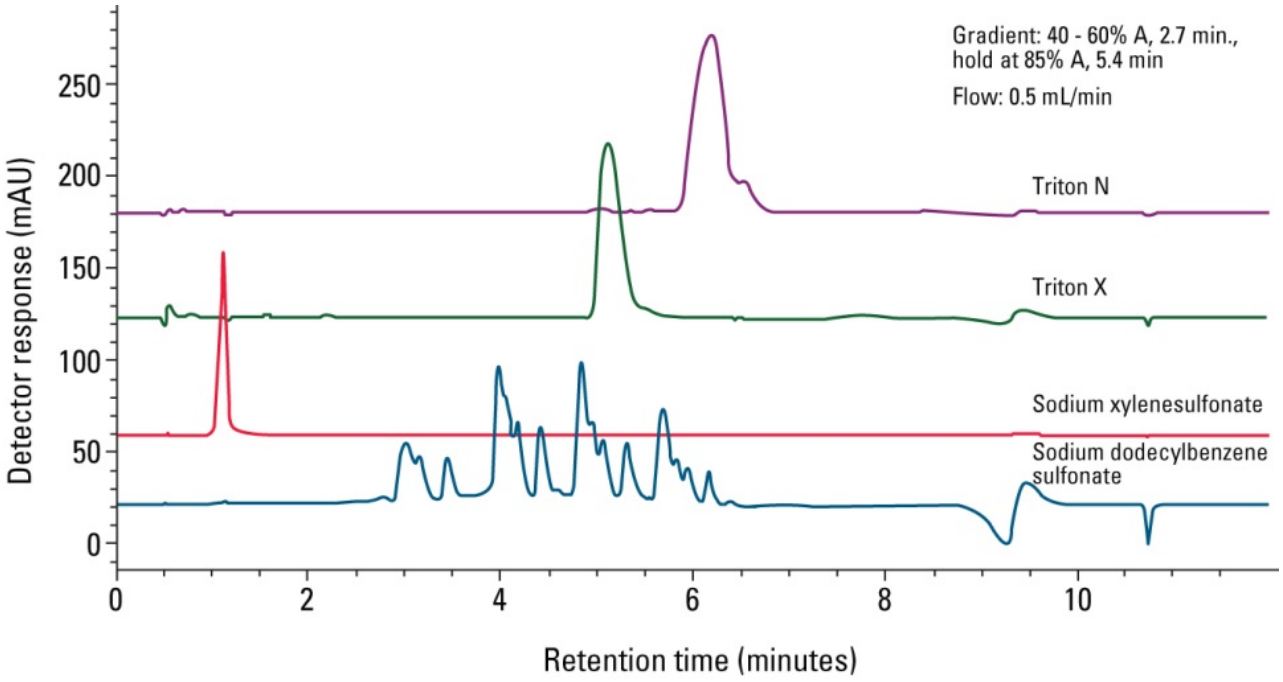
- Combining the TSKgel ODS-140HTP and the TSKgel NH_2-100 will allow for the simultaneous separation of polar and non-polar surfactants in a single run.
- The gradient slope plays a large role in the extent of reversed phase and HILIC retention on the analytes.

Figure 5A: 2D-LC Separation of Surfactants using the TSKgel ODS-140HTP and the TSKgel NH_2-100 Columns



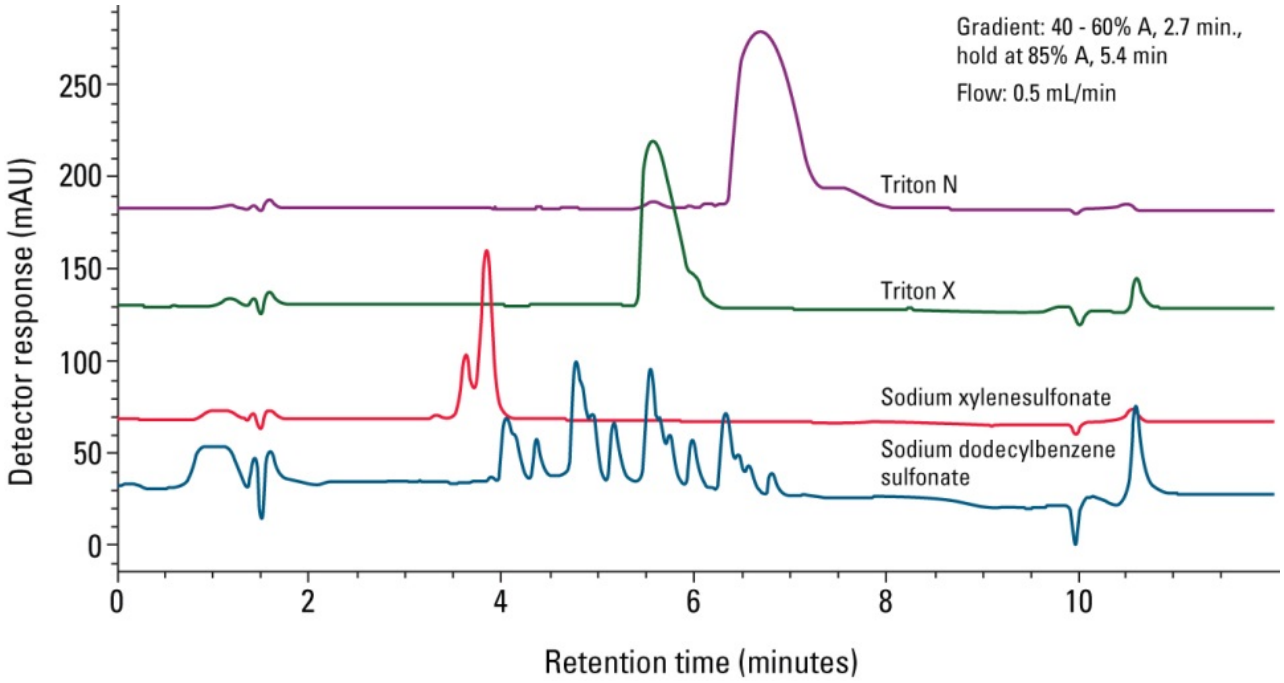
- The wide gradient provides strong retention of all analytes.
- Peak shape and resolution are satisfactory under these conditions.
- It should be noted that sodium xylenesulfonate is well retained due to the incorporation of the TSKgel NH_2-100 column.

Figure 5B: 2D-LC Separation of Surfactants using the TSKgel ODS-140HTP and the TSKgel NH_2-100 Columns



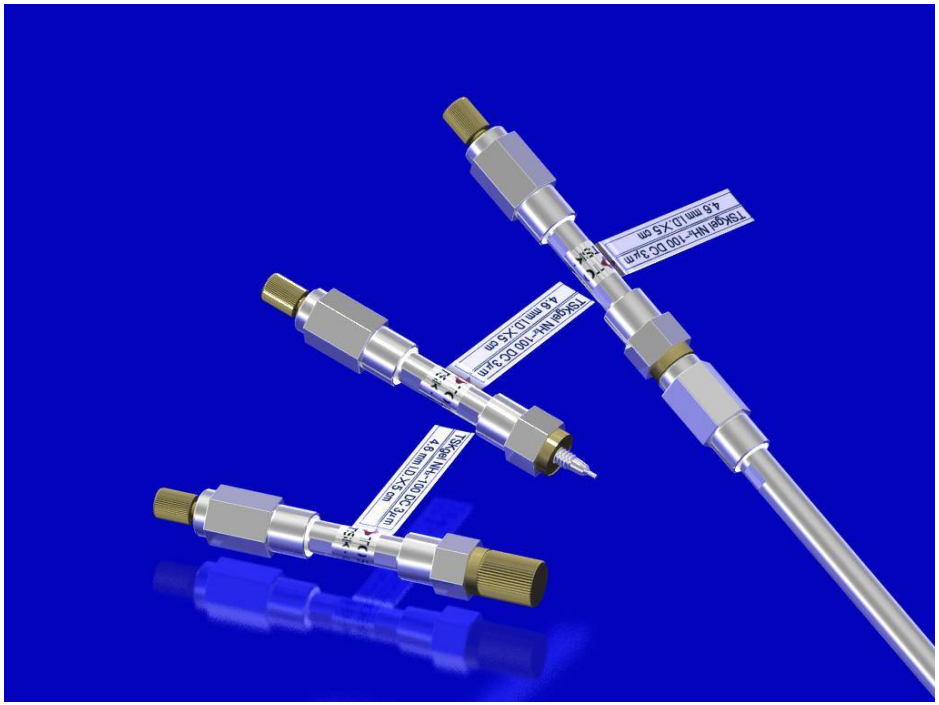
- Further shallowing of the gradient increases resolution of sodium dodecylbenzene sulfonate impurities.
- The use of 85% CH_3CN during the isocratic hold leads to a moderately later retention time of Triton N.

Figure 6: 2D-LC Separation of Surfactants using the TSKgel ODS-140HTP and the TSKgel NH_2-100 DC Columns – Order Reversed



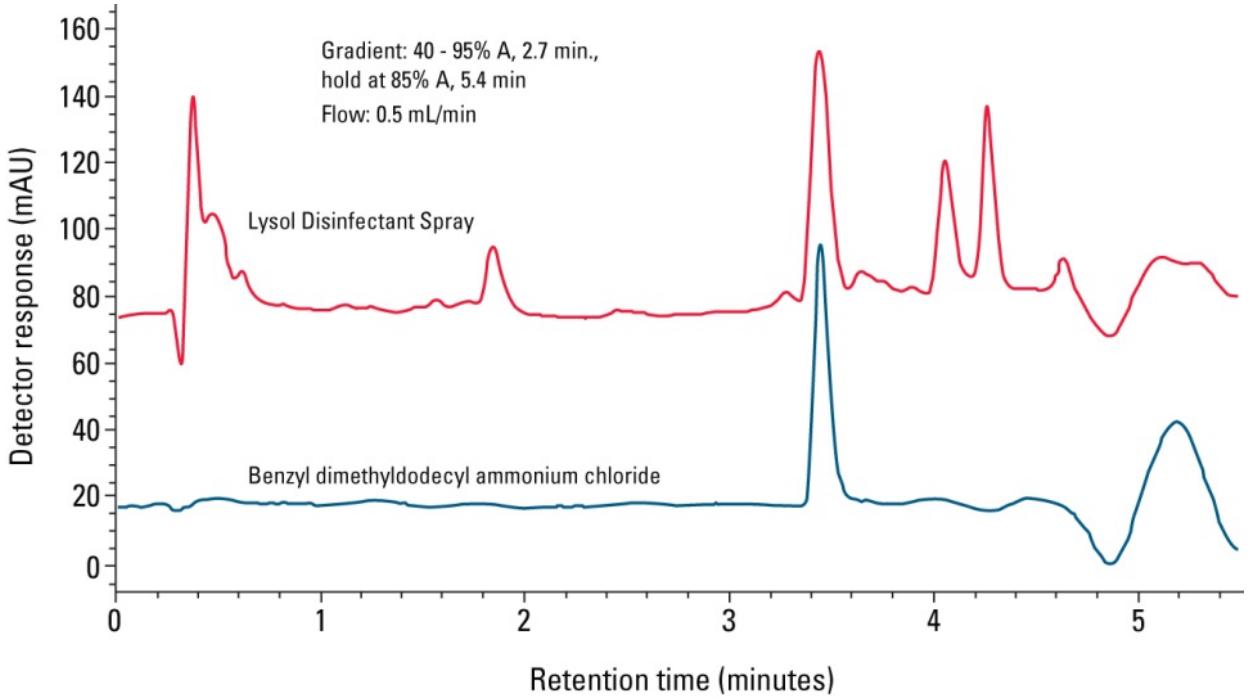
- The TSKgel NH_2-100 DC HILIC column allows for a direct connection to the TSKgel ODS-140HTP column, eliminating the need of additional tubing (see following slide).
- By placing the TSKgel NH_2-100 DC before the TSKgel ODS-140HTP, a significant increase in retention of the highly polar sodium xylenesulfonate is observed.
- Peak widths of the non-polar Triton N and Triton X increase relative to the previous figure.

Figure 7: TSKgel NH_2-100 DC HILIC Column



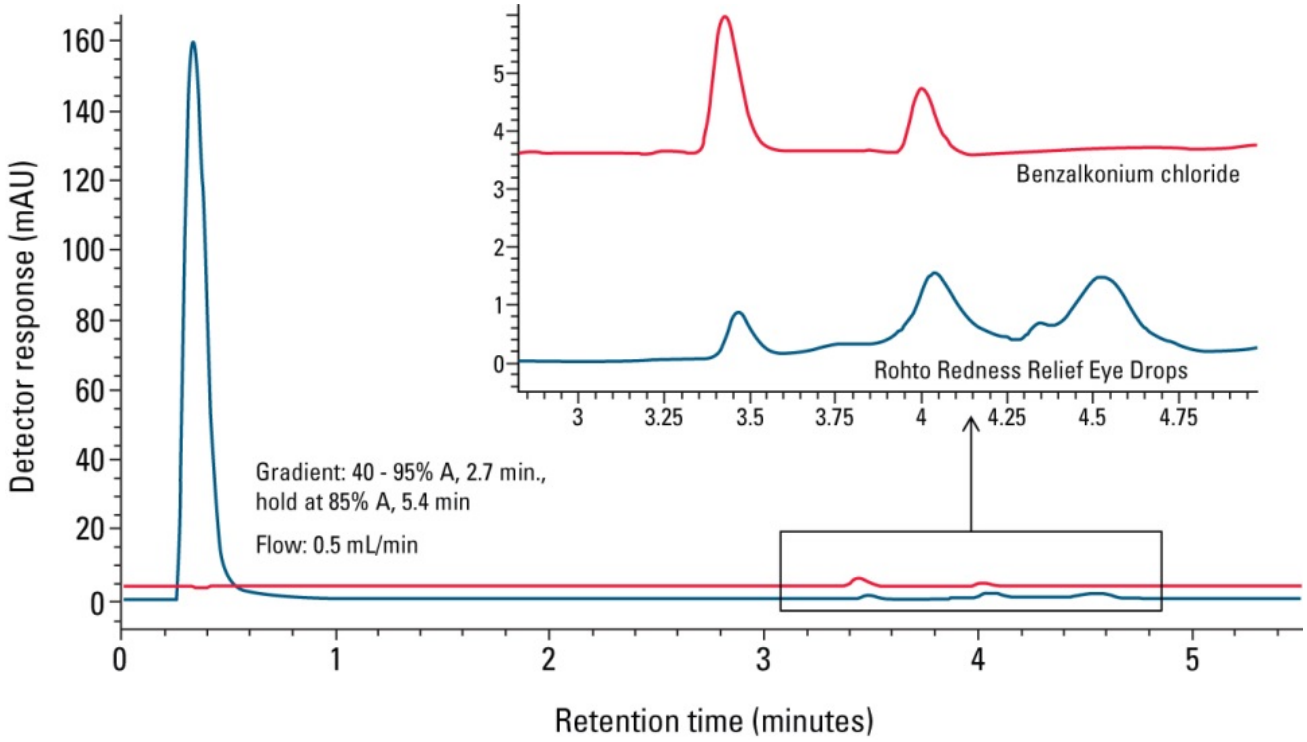
- The male fitting on the outlet of the TSKgel NH_2-100 DC HILIC column allows for a direct connection with any conventional HPLC column.
- This eliminates the need for additional tubing when connecting columns without producing the commonly observed drop in column efficiency due to band broadening

Figure 8: Characterization of Lysol® Disinfectant Spray using the TSKgel ODS-140HTP Reversed Phase Column



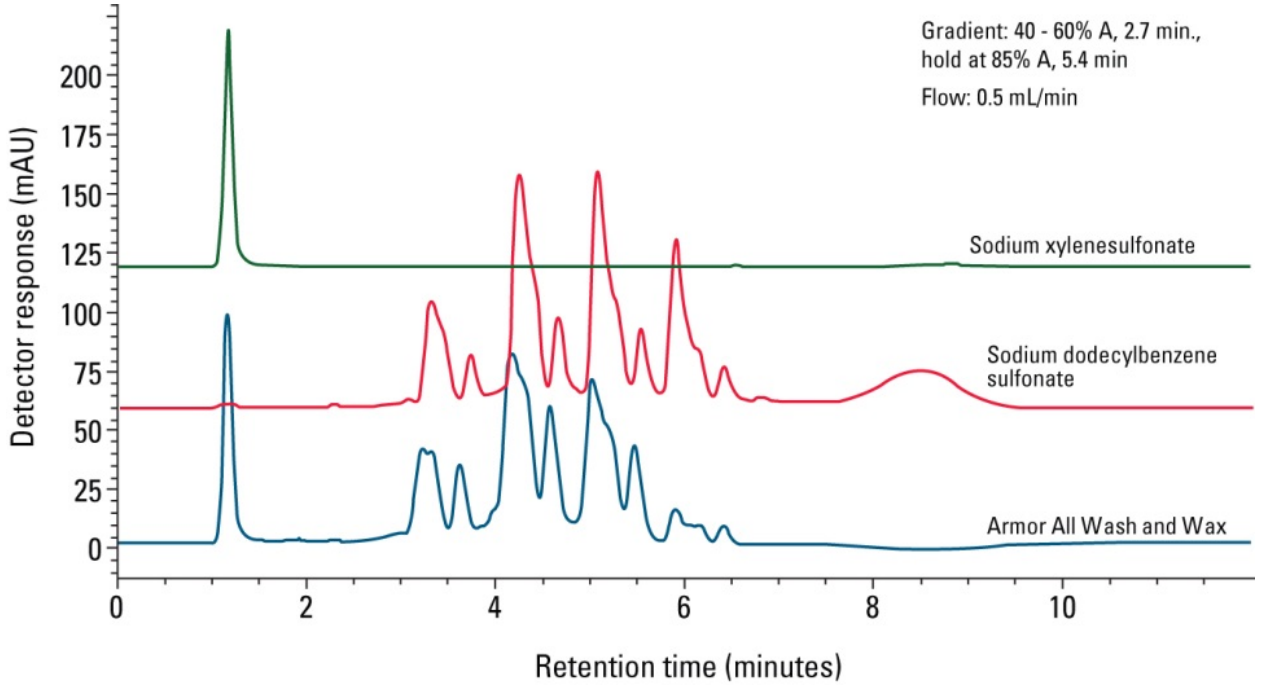
- One of the active ingredients in Lysol Disinfectant Spray is the relatively non-polar benzyl dimethyldodecyl ammonium chloride.
- The high hydrophobic character of this surfactant does not lend itself to adequate separation by 2D methodology, therefore the use of the TSKgel ODS-140HTP column proves to be a useful tool in separating it from the other components found in Lysol.

Figure 9: Characterization of Rohto® Eye Drops using the TSKgel ODS-140HTP Reversed Phase Column



- The cationic surfactant, benzalkonium chloride is an extremely effective biocide for ophthalmic solutions at extremely low concentrations.
- As shown, separation of the highly non-polar surfactant from the active ingredient in commercial eye drops is achieved using the TSKgel ODS-140HTP column.

Figure 10: Characterization of Armor All® Wash and Wax using 2D-LC with the TSKgel ODS-140HTP and TSKgel NH_2-100 Columns



- The use of 2D-LC methodology yields great separation and retention of the 2 surfactants, sodium dodecylbenzene sulfonate and sodium xylenesulfonate found in Armor All Wash and Wax.
- Fluorescence detection was used to increase sensitivity of the low level surfactants present in the formulation.

Conclusions

- The purpose of this study was to illustrate the effectiveness of 2D RP/HILIC for the separation of anionic, cationic, and hydrotropic surfactants present in common household products and OTC medicines.
- The use of the TSKgel ODS-140HTP column in series with the TSKgel NH_2-100 column allows for simultaneous retention and separation of both polar and non-polar hydrotropic, nonionic, and anionic surfactants in a single injection.
- When compared to other TSKgel reversed phase columns, the TSKgel ODS-140HTP column provides a high level of separation in short run times and with minimal solvent waste.
- The TSKgel ODS-140HTP and the TSKgel NH_2-100 columns yield strong retention and good peak shape of complex surfactant mixtures found in common household products and OTC medicine without interference from matrix components in the product formulation.
- This work illustrates the effectiveness of 2D RPC-HILIC using the TSKgel ODS-140HTP and TSKgel NH_2-100 columns in series for the separation of diverse surfactants in solution.