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## Application Note

# USE OF RPC AND HILIC IN THE 2D-LC SEPARATION OF CATIONIC, ANIONIC & HYDROTROPIC SURFACTANTS

Surfactants are frequently found in pharmaceutical and biopharmaceutical drug applications as well as in common household products. Because they can be polar, non-polar, or amphoteric, the structural diversity of the surfactants and complexity of the sample matrix can make their separation and identification by HPLC challenging. Historically, this has been achieved using Reversed Phase Chromatography supplemented with Ion Exchange Chromatography. This Application Note illustrates the effectiveness of a reversed phase high throughput column, TSKgel® ODS-140HTP, in series with a TSKgel NH2-100 HILIC column, for the separation of polar and non-polar surfactants in a single injection using 2D-LC.

MATERIALS AND METHODS

| | |
|---|---|
| Columns: | TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm |
| | TSKgel NH$_2$-100, 3 µm, 2 mm ID × 15 cm |
| Instrument: | Agilent 1200 HPLC system run by Chemstation |
| Mobile phase: | A: CH$_3$CN |
| | B: 100 mmol/L ammonium acetate, pH 5.4 |
| Gradient: | 40-60% A, 2.7 minutes, hold at 85% A, 5.4 min. |
| Flow rate: | 0.5 mL/min |
| Detection: | UV @ 280 nm, 254 nm, and 210 nm |
| | FLD ex 280 nm, em 350 nm |
| Temperature: | 30 °C |
| Injection vol.: | 1 µL |
| Samples: | Triton™ X, Triton N, sodium xylenesulfonate, sodium dodecylbenzene sulfonate |

RESULTS AND DISCUSSION

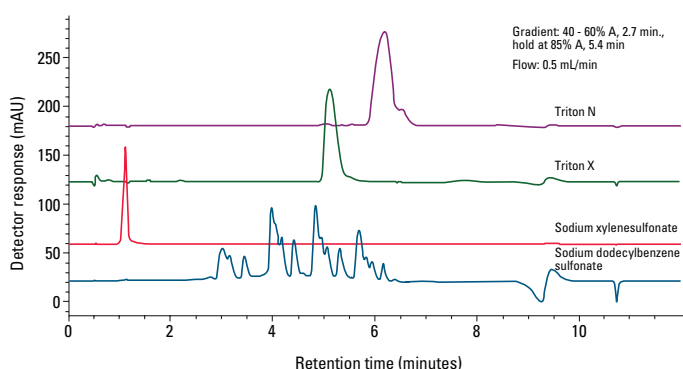Figure 1 depicts the separation of four surfactants using the TSKgel ODS-140HTP and TSKgel NH2-100 columns in

series. The use of a shallow, non-linear gradient of 40-60% CH$_3$CN, followed by an isocratic hold at 85% CH$_3$CN, allowed for high resolution of the sodium dodecylbenzene sulfonate impurities and good retention of sodium xylene sulfonate as well. Due to the success in resolving the surfactant standards in a single 2D-LC run as explained above, the usefulness of this technique in detecting the corresponding UV-absorbing surfactant ingredients in an actual commercial household product was evaluated. Figure 2 illustrates the characterization of the surfactant profile of Armor All™ Wash and Wax using the 2D-LC methodology described above. The use of the TSKgel ODS-140HTP and TSKgel NH2-100 columns in series yielded excellent separation and retention of the anionic surfactant sodium dodecylbenzene sulfonate and the hydrotropic surfactant sodium xylene sulfonate present in the Armor All formulation. Additionally, the use of fluorescence detection (ex: 225 nm, em: 300-400 nm) allowed for increased sensitivity of the low level surfactants found in the product.

CONCLUSIONS

The purpose of this study was to illustrate the effectiveness of 2D RP/HILIC for the separation of anionic, cationic, and hydrotropic surfactants. The use of a TSKgel ODS-140HTP column in series with a TSKgel NH$_2$-100 column allows for the simultaneous retention and separation of both polar an non-polar hydrotropic, nonionic, and anionic surfactants in a single injection. The characterization of surfactants in a common household product is also successfully performed with TSKgel ODS-140HTP and TSKgel NH2-100 columns, allowing for strong retention and good peak shape of various surfactants with no interference from other matrix compounds.

2D-LC SEPARATION OF SURFACTANTS USING THE TSKgel ODS-140HTP AND THE TSKgel NH2-100 COLUMNS



Figure 1

CHARACTERIZATION OF SURFACTANT PROFILE IN ARMOR ALL WASH AND WAX USING 2D-LC WITH THE TSKgel ODS-140HTP AND TSKgel NH$_2$-100 COLUMNS
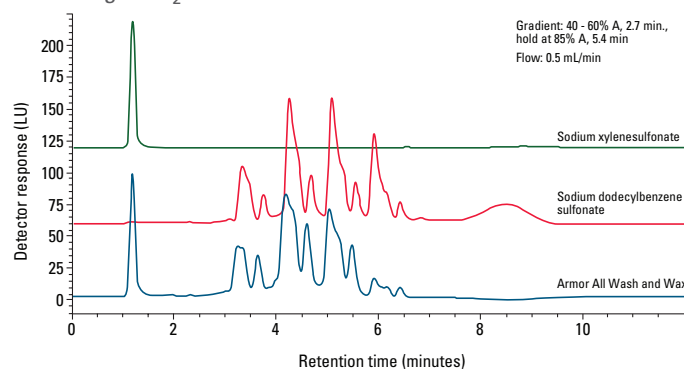


Figure 2

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