High Resolution Analysis of Tween 20 by HPLC with ELSD

Application Note

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Introduction

Surfactants represent an important class of commercial chemicals used in a variety of processes and manufactured goods. Generally speaking, surfactants are water soluble, surface active agents comprising a hydrophobic portion, usually a long alkyl chain, attached to hydrophilic or water solubility-enhancing functional groups. Since variations in both the alkyl chain length and the polar functional group have a decisive influence on their physiochemical properties, it is necessary to analyze surfactants with respect to composition. This is commonly achieved using HPLC.

Tween 20, also known generically as Polysorbate 20, is used extensively in industry but is usually confined to pharmaceuticals and other high-end applications due to its relatively high cost. Tween 20, or polyoxyethylene sorbitan monolaurate, is a non-ionic surfactant that effectively suppresses unspecific reactions between antibodies, antigens and other molecules. It is also used as a solubilizer in membrane chemistry and for density centrifugation of viruses.

The absence of a usable UV chromophore and the requirement for gradient elution often mean that neither refractive index (RI) or UV detection can be used in HPLC methods developed for surfactant analysis. For non-UV absorbing compounds, the Agilent evaporative light scattering detector is the primary choice since the principle of detection does not rely on the optical properties of the solute. The Agilent ELSD offers significant benefits as it operates over very rapid changes in eluent composition and temperature with no effect on the baseline stability, and offers very high sensitivity.

PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight surfactants because the very small pore sizes have extremely high surface areas available to the solutes. An excellent demonstration of the high performance of the Agilent 380-ELSD with this type of column is provided by the analysis of Tween 20.

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Instrumentation

Column:	PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Detection:	Agilent 380-ELSD (neb=50 °C, evap=50 °C, gas=1.6 SLM)

Materials and Reagents

Eluent A:	Water
Eluent B:	ACN

Sample Preparation

1 mg Tween 20/mL

Conditions

Gradient:	10-100% B in 45 min
Flow Rate:	1.0 mL/min
Injection Volume:	10 µL

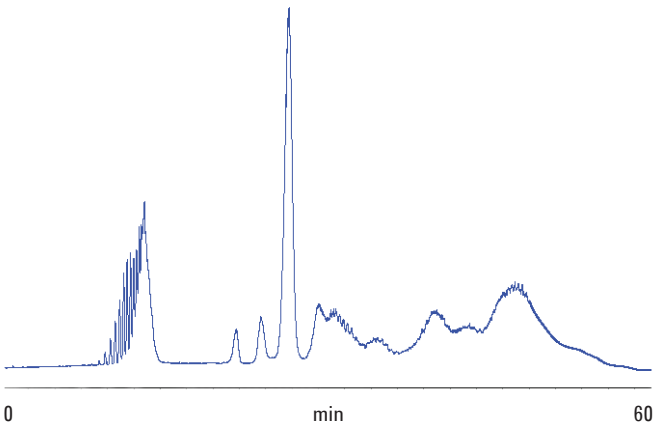

Figure 1. Separation of Tween 20 by HPLC with ELSD.

Results and Discussion

The chromatogram shows a separation of Tween 20 using a gradient HPLC method revealing that it is composed of a complex mixture of components.

Conclusion

The Agilent evaporative light scattering detector combined with a PLRP-S 100Å column successfully resolved all of the Tween 20 components and offered high sensitivity and low limits of detection, because of the optimum match of column, detector and sample.

PLRP-S columns are ideally suited to the analysis of many small molecules. The 100Å pore size has an exceptionally high surface area that is accessible to the solutes. It is more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and, therefore, even polar molecules, such as carboxylic acids, may be retained much longer, resulting in greater resolution.

The Agilent 380-ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Agilent 380-ELSDs unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100% water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the ELSD provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

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