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HPLC-Charged Aerosol Detection Surfactants and Emulsifiers Applications Notebook

Complex Compounds, Universal Chromatographic Analysis

The Liquid Chromatography System

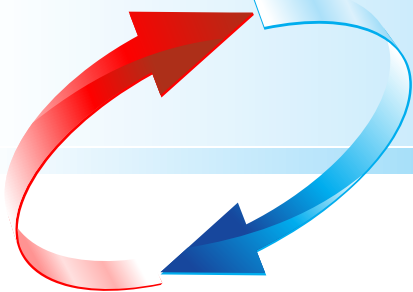


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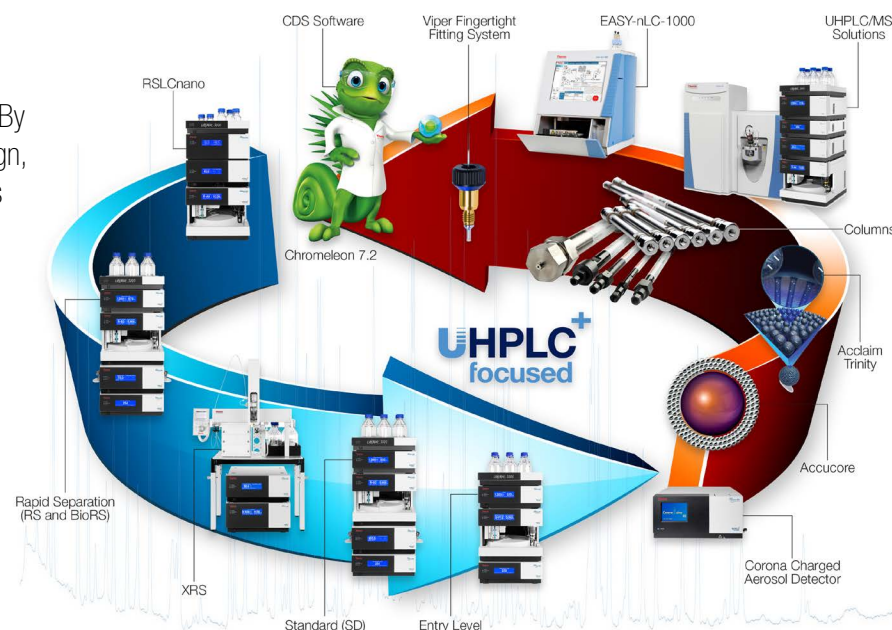
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Surfactants are a diverse group of chemicals whose structures vary widely but typically contain an oil-soluble hydrocarbon chain and a water-soluble hydrophilic group. Surfactants can be categorized based upon their structure and include nonionic, anionic, and cationic classes. They have widespread use as detergents in shampoos and cleaning products, ion pairing agents used in chromatography, and complex dispersants used to treat oil spills. Emulsifiers are used to maintain a uniform suspension of immiscible materials. These compounds are typically surfactants, and can be designed for use in specific applications and products in both the food and pharmaceutical industries. Many of these commercial surfactants are mixtures of members of a homologous series (often referred to as congeners), and such mixtures can be defined or characterized using LC. Chromatographic approaches can be used to separate the molecules on the basis of carbon chain length, chain branching, or positional isomer distribution. This is important, for example, when studying lot-to-lot variability, which can be important to the biopharma industry. Conversely, when trying to quantitate total amounts of surfactants or emulsifiers, the chromatographic conditions need to be changed so that all the congeners elute as a single peak, thus simplifying the determination.

Using our UHPLC-ready systems, highly sensitive and selective detectors, and state of the art column technologies, along with proven analytical methods, precise automation and advanced data handling will help you to:

- Characterize or quantify many typical classes of surfactants
- Analyze compounds in a broad range of samples
- Implement simplified methods with improved sensitivity and reproducibility



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No single liquid chromatography LC detector delivers ideal results. Often, one analyte responds more strongly than another, or may not respond at all. UV absorbance requires that the molecule contain a chromophore and inter-analyte response can vary greatly depending upon the nature of the chromophore present.

Refractive index cannot be used with gradient elution and is temperature sensitive.

Mass spectrometry can only measure compounds that will form gas-phase ions.

Evaporative light scattering shows varied inter-analyte response, often poor sensitivity and complex, non-linear calibration curves.

What is most desired in a universal detector is the ability to accurately measure a wide range of analytes with consistent response. Charged aerosol detection can measure any non-volatile and many semi-volatile analytes at sub-nanogram levels and does not require a compound to contain a chromophore or be able to ionize. Variance in inter-analyte relative response is minimal whether analyzing small molecules or proteins. The technique is fully gradient compatible.

Surfactants typically do not contain a UV chromophore, so they are measured directly with non-suppressed or suppressed mode conductivity, or indirectly using photometric detection. Furthermore, as response is similar for all compounds and independent of chemical structure, charged aerosol detection is ideal for measurement of surfactant species.

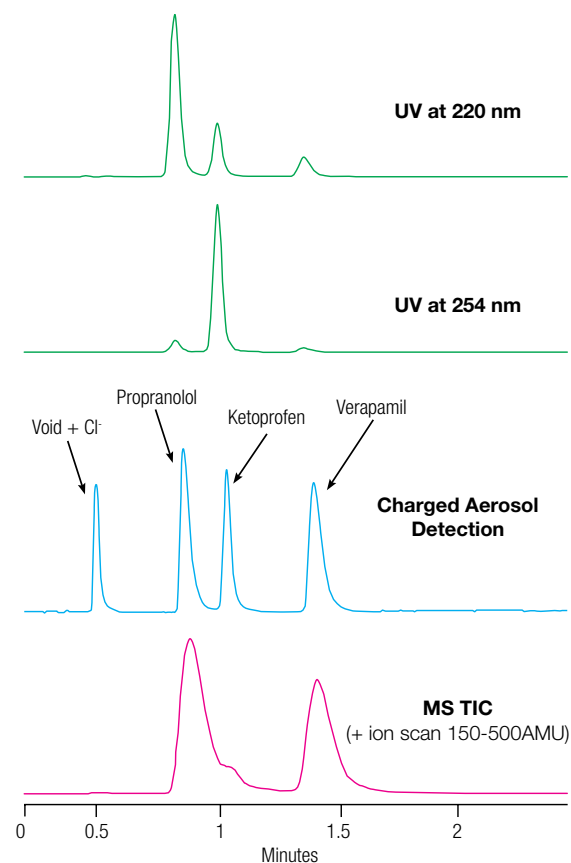


Figure 1. Comparison of Charged Aerosol Detection to UV and MS.

Charged Aerosol Detection

How the Technology Works

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Charged aerosol detection first nebulizes eluent from the column. Large droplets exit the detector to waste. Selected smaller droplets enter the drying tube and form particles once the solvent is evaporated. Particles then enter a reaction chamber where they collide with ionized gas formed when nitrogen is passed over a corona wire. Charge is transferred from the ionized gas to the analyte particles. Once unreacted ionized gas is removed by an ion trap, the charge on the particle is measured by a sensitive electrometer. The response of the detector is directly related to the mass of the analyte entering the detector. An increase in the amount of an analyte eluting from the column leads to an increase in the size of the particles being formed.

Larger particles can accommodate more charge resulting in a higher response from the detector. As long as the analyte will form a particle it will be measured by charged aerosol detection, independent of its chemical structure.

Charged aerosol detection delivers predictable results without the need for complex detector optimization and has the flexibility to measure a broad range of analytes in many different matrices. The Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector is HPLC/UHPLC compatible and with its extended flow rate range, can be used with microbore and analytical scale columns. The detector improves on all the benefits of charged aerosol detection in a design perfectly matched to your laboratory's needs.

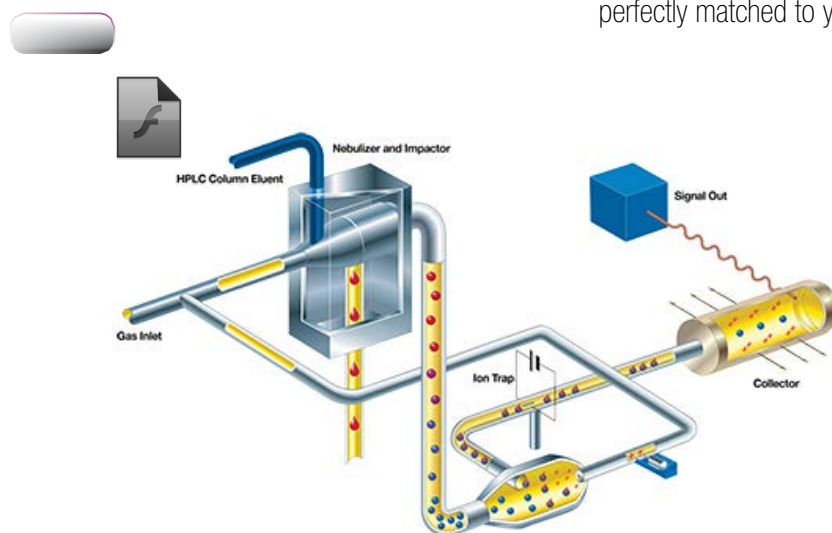




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For surfactant analysis using high-sensitivity detection, the column of choice is the Thermo Scientific™ Acclaim™ Surfactant Plus LC column. This new generation of high-efficiency silica-based columns features exceptionally low bleed for use with charged aerosol detectors and mass spectrometers. The columns provide excellent selectivity for sensitive separations of a wide variety of surfactants. The advanced surface chemistry provides both reversed-phase and anion-exchange retention mechanisms and significantly improves the resolution of surfactants.

The Acclaim Surfactant Plus LC column provides improved performance, versatility and throughput for surfactant analysis

- Ideal selectivity for simultaneous separation of anionic, nonionic, cationic, and amphoteric surfactants
- Resistant to dewetting under highly aqueous mobile phase conditions
- Excellent resolution between strongly hydrophilic compounds
- Rugged separation under a variety of conditions



Simultaneous Separation of Different Surfactants



Surfactants are widely used in consumer products, agricultural, pharmaceutical, biopharmaceuticals, and chemical markets, in products as diverse as pesticides, detergent powders, petroleum products, cosmetics, and pharmaceuticals. Their separation and identification can be challenging due both to the diversity of surfactants and complexity of the sample matrix. Although many HPLC columns are available and have been

used for the analysis of surfactant formulations, none of these columns are capable of separating anionic, nonionic, cationic and amphoteric surfactants in a single analysis. In this example, the Acclaim Surfactant Plus column provides ideal selectivity for the simultaneous separation of anionic, nonionic, cationic and amphoteric surfactants, whereas the C18 column fails to resolve them under the same or other conditions.

Conditions

Column:	Acclaim Surfactant Plus, 3 µm, 3.0 x 150 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	0.1 M Ammonium acetate, pH 5
Flow Rate:	0.60 mL/min
Injection Volume:	5 µL
Temperature:	30 °C
Detection:	Charged Aerosol

Peaks: (100 - 400 µg/mL each)

1. Xylene sulfonate
2. Laurylpyridinium
3. Lauryldimethylbenzyl ammonium
4. Triton X-100
5. Cetyl betaine
6. Decyl sulfate
7. Dodecyl sulfate
8. Linear alkylbenzene sulfonate (LAS)

Gradient:

Time (min)	% A	% B	Curve
-8	25	75	5
25	78	75	5
10	80	20	5
15	80	20	5

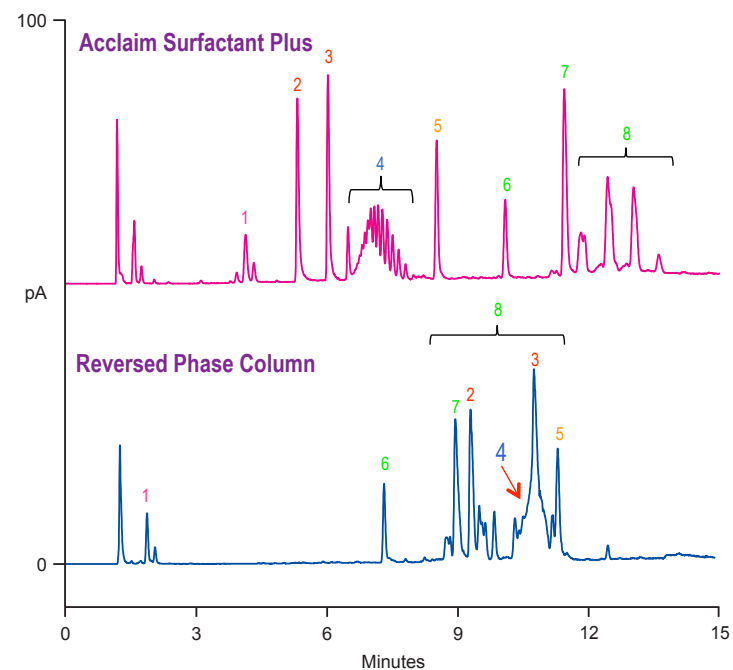


Figure 2. Simultaneous separation of different types of surfactants.

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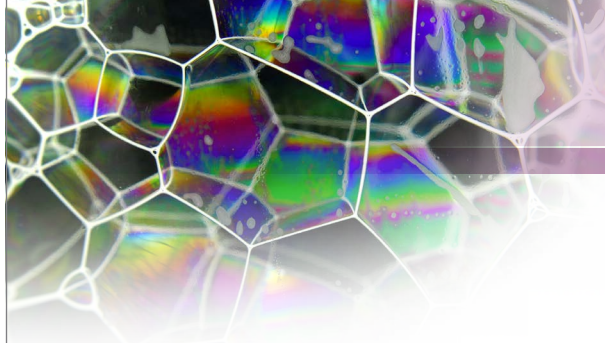


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Separation and identification of surfactants can be challenging due to both the diversity of the surfactants and the complexity of the sample matrix in which they are contained. The surface chemistry of the Acclaim Surfactant Plus column effectively deactivates surface silanol activity, thereby decreasing peak asymmetry by decreasing the degree of secondary analyte/silanol interaction with the stationary phase. This makes the column ideally suited for the analysis of cationic surfactants such as alkyl quaternary ammonium, benzylalkylammonium, and alkyl pyridinium salts. In this example, the Acclaim Surfactant Plus column was used for the successful separation of cationic surfactants. Under reversed-phased, gradient conditions, baseline resolution with excellent peak shape was achieved for all cationic surfactants in less than ten minutes.

Analyte	Analyte Name	Retention Time		Resolution	
		Mean (min)	% RSD	Mean	% RSD
1	ddTMABr	5.18	0.07	N/A	N/A
2	DdPyCl	5.46	0.05	2.56	0.76
3	BzACI (n-C ₁₂)	6.32	0.04	8.61	0.69
4	BzACI (n-C ₁₄)	7.50	0.05	11.34	0.83
5	ATmABr	7.93	0.06	3.32	0.86
6	CPyCl	8.20	0.10	1.71	1.99

Table 1. Chromatographic performance of the Acclaim Surfactant Plus column. Statistical assessments based on data derived from 10 replicate injections.

Conditions

Column:	Acclaim Surfactant Plus, 3 μ m, 2.1 x 100 mm
Mobile Phase A:	Ammonium acetate, 100 mM, pH 5 / propan-2-ol (95:5 v/v)
Mobile Phase B:	Acetonitrile / propan-2-ol (95:5 v/v)
Flow Rate:	0.40 mL/min
Gradient:	Acetonitrile: 0—0.5 min, 20%; 0.5—4 min, 20—40%; 4—7 min, 40—20% ; 7—10 min, 20%
Injection Volume:	2 μ L
Temperature:	30 °C
Detection:	Charged Aerosol

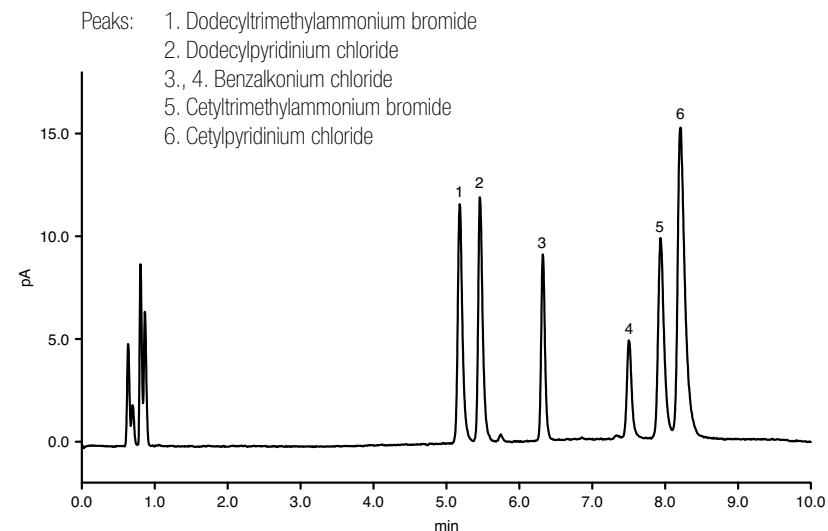


Figure 3. Separation of various cationic surfactants on the Acclaim Surfactant Plus column.



The novel column chemistry of Acclaim Surfactant Plus column offers excellent resolution for individual oligomers of ethoxylated surfactants. Nonionic ethoxylated surfactants account for about 40% of surfactants consumption worldwide. Most nonionic surfactants are considered low-foaming products, have good cold water solubility, and low critical micelle

concentration. Their compatibility with cationic fabric softeners makes them preferable in certain formulations. In this example, the Acclaim Surfactant Plus column provides excellent resolution between individual oligomers in an ethoxylated surfactant.

Conditions

Column:	Acclaim Surfactant Plus, 3 μ m, 3.0 x 150 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	0.1 M Ammonium Acetate, pH 5
Flow Rate:	0.60 mL/min
Injection Volume:	5 μ L
Temperature:	30 $^{\circ}$ C
Detection:	Charged Aerosol

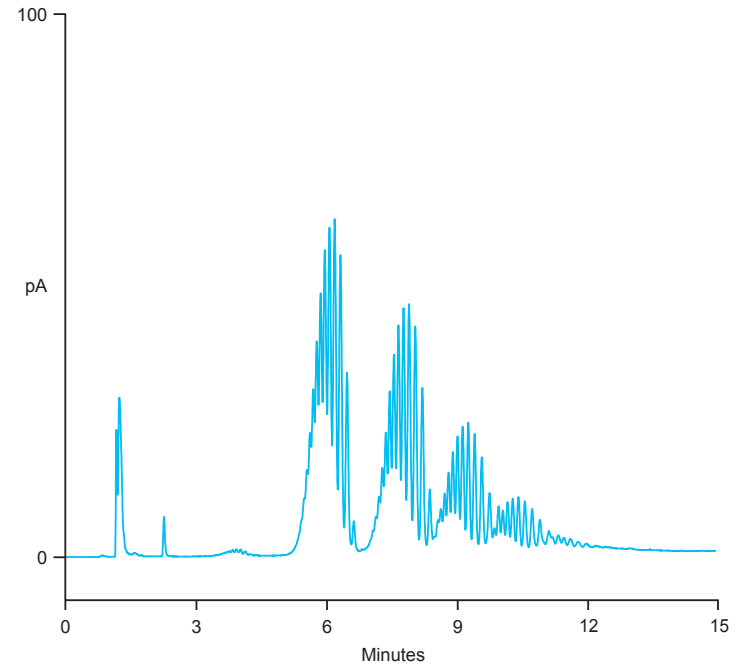


Figure 4. Analysis of zonyl FSO fluorosurfactant.

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Ethoxylated lauryl sulfates, also called laureth sulfates, are prepared by adding oxyethylene groups to an alcohol that is then sulfated. Ethoxylation enhances water solubility and foaming, making these surfactants ideal

components in shampoos and detergents. The figure below shows the profiles of laureth sulfates obtained on an Acclaim Surfactant Plus column using a charged aerosol detector.

Conditions	
Column:	Acclaim Surfactant Plus, 3 μ m, 3.0 x 150 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	0.1 M Ammonium Acetate, pH 5
Flow Rate:	0.60 mL/min
Injection Volume:	2 μ L
Temperature:	30 $^{\circ}$ C
Gradient:	Acetonitrile: -10—0 min, 45%; 0—15 min, 45—75%; 15—20 min, 75%
Detection:	Charged Aerosol

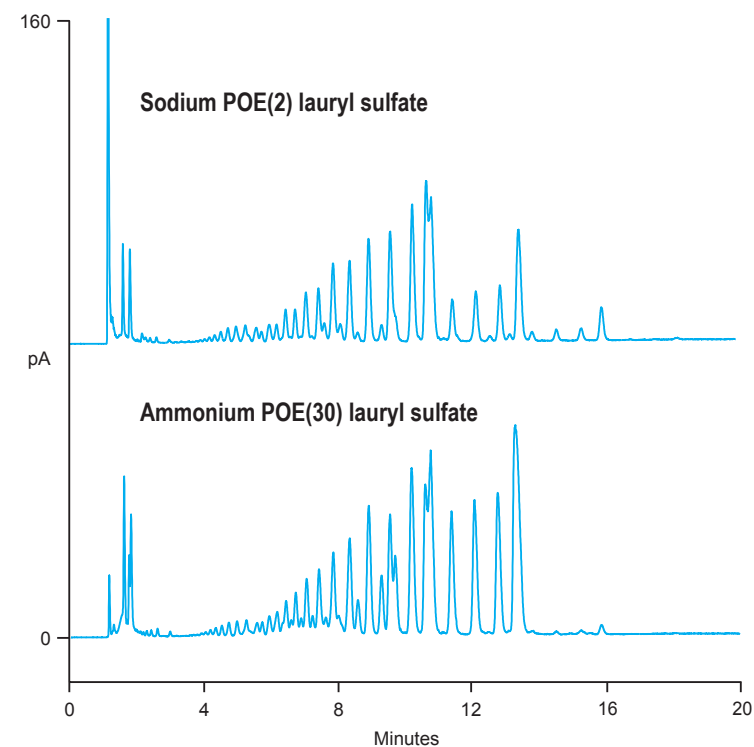


Figure 5. Profile of ethoxylated lauryl sulfates.

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Polyethylene glycols (PEGs) are often non surfactant impurities found in ethoxylated surfactants, typically in the range of 1–10%. The oligomer distribution is similar to, but broader than that of the surfactant. The figure below illustrates the exceptional resolution of the Acclaim Surfactant Plus

Conditions	
Column:	Acclaim Surfactant Plus, 3 μ m, 3.0 x 150 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	0.1 M Ammonium Acetate, pH 5
Flow Rate:	0.60 mL/min
Injection Volume:	2 μ L
Temperature:	30 $^{\circ}$ C
Gradient:	Acetonitrile: -8—0 min, 2%; 0—20 min, 2—20%; 20 min, 20%
Detection:	Charged Aerosol
Sample:	2.5 mg/mL each of: PEG (MW ~300) PEG (MW ~400) PEG (MW ~600) PEG (MW ~1000)

column for individual oligomers in various PEGs. PEGs have uses in their own right and are commonly found in various products including skin creams and toothpastes.

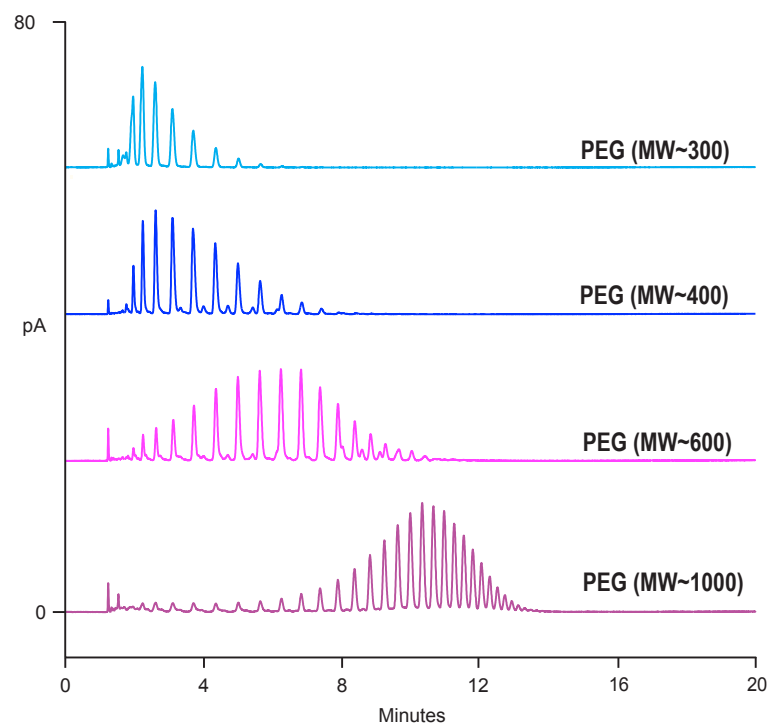


Figure 6. Separation of 4 different polyethylene glycols.

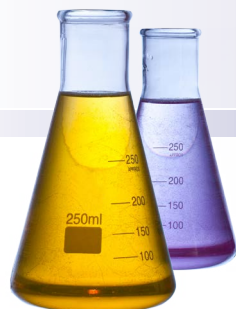


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Polysorbates (e.g. Tween® 20, 60, 80) are used in large quantities throughout the food and pharmaceutical industries. Because these products have a significant demand across industries they are often produced in large lots with varying limits of impurities (peroxides, carbonyls, and metals) reported. The characterization and quantification of polysorbates is difficult because these compounds are heterogeneous mixtures with no chromophore. As a result, physical tests and testing for impurities are typically used for release criteria. While this testing is

Conditions

Column:	Acclaim 300 C18, 3 µm, 4.6 x 150 mm
Mobile Phase A:	Acetonitrile/methanol/DI water/trifluoroacetic acid (8/2/90/0.1)
Mobile Phase B:	Acetonitrile /methanol/DI water/trifluoroacetic acid (72/18/10/0.1)
Flow Rate:	0.40 mL/min
Injection Volume:	2 µL
Temperature:	30 °C
Detection:	Charged Aerosol

sufficient for the manufacturers to release quality material, it may not be sufficient for the end user. At the point of use, physical characterization such as color change may indicate chemical composition changes which could impact the final product, but the inability to test for composition along with the varying levels of impurities, make lot-to-lot consistency difficult to quantify. The example below demonstrates a reliable approach to measure impurities and lot-to-lot variability in Polysorbate 80 products.

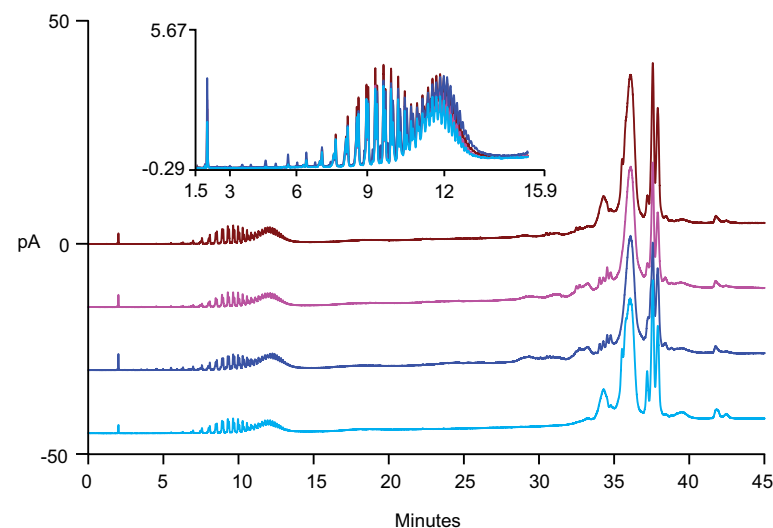


Figure 7. Stacked plot of four commercially available Polysorbate 80 products using a full gradient method with charged aerosol detection. Low molecular weight components are seen between 2.5 and 15 min and the major components elute between 30 to 45 min. Inset: Low molecular weight components overlaid.

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Surfactants are generally used to control or affect the consistency of mixtures, to alter surface tension, and as an aid in mixing materials that normally would not mix. These surface-active agents exist in four different categories, amphoteric (zwitterionic), cationic, anionic, and non-ionic, each with a specific form of activity and use. Within each category hundreds of different compounds exist providing nearly any range of property needed for a specific application or use. Pluronic polymers, or poloxamers, are a special class of non-ionic surfactants, consisting of a triblock copolymer

Conditions

Column:	Acclaim Surfactant Plus, 3 μ m, 3.0 x 150 mm
Mobile Phase A:	Acetonitrile/methanol/DI water/trifluoroacetic acid (8/2/90/0.1)
Mobile Phase B:	Acetonitrile /methanol/DI water/trifluoroacetic acid (72/18/10/0.1)
Flow Rate:	0.60 mL/min
Injection Volume:	40 μ L
Temperature:	40 $^{\circ}$ C
Detection:	Charged Aerosol
Sample:	Pluronic F127 in isopropyl alcohol/water (1:1)

of one polypropylene oxide molecule connected to two polyethylene blocks. In this example, five different pluronics were analyzed, including L64, F68, F127, P85 and P123, using a buffered mobile phase and a fast organic gradient. The pluronics eluted within a retention time window of approximately one minute, with the gradient adjusted to provide best resolution and peak shape for these compounds while maintaining a single peak for best sensitivity for quantitation.

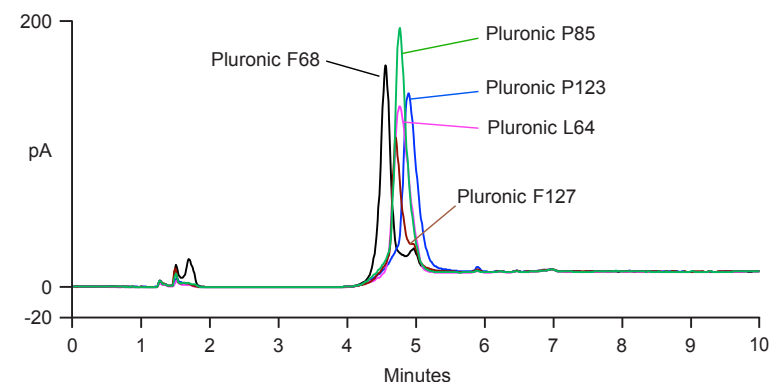


Figure 8. Overlay of five different Pluronic compounds (10 mg/mL in isopropanol/water (1:1)) measured by HPLC with Charged Aerosol Detection.

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Sorbitan esters (also known as Spans) are lipophilic nonionic surfactants that are frequently used with a polysorbate in varying proportions to produce water-in-oil or oil-in-water emulsions or creams with a variety of different textures and consistencies. Sorbitan esters are used as emulsifiers and stabilizers in food products. In the US Span 60 and 80 are approved for use as food additives while in the EU the range is

Conditions

Column:	Acclaim Surfactant Plus, 3 μ m, 3.0 x 150 mm
Mobile Phase A:	100 mM Ammonium acetate, pH 5.4
Mobile Phase B:	Acetonitrile / methanol / tetrahydrofuran / acetic acid (500:375:125:4)
Flow Rate:	0.8 mL/min
Injection Volume:	10 μ L
Temperature:	40 $^{\circ}$ C
Detection:	Charged Aerosol

increased to include Span 20, 40, 60, 65, and 80. In the example below, Span-80 (sorbitan monooleate), -83 (sorbitan sesquioleate), and -85 (sorbitan trioleate) were dissolved in isopropanol at a concentration of 20 mg/mL. The example in Figure 9 represents 3 concentrations of Span 80 (sorbitan monooleate).

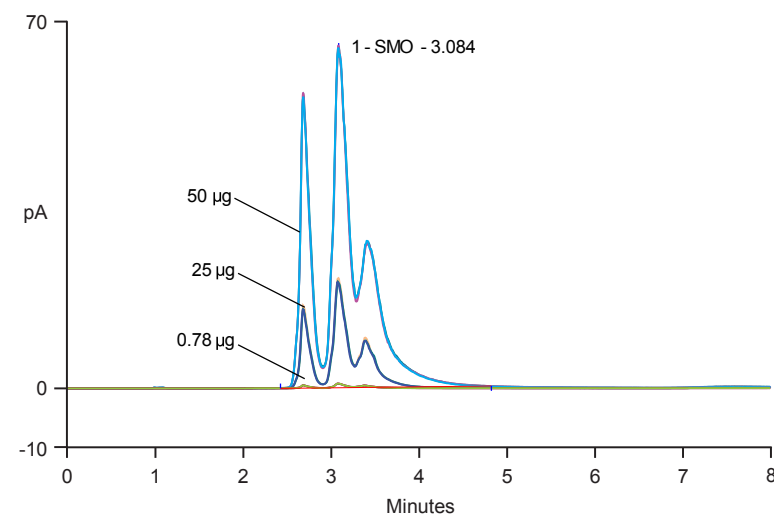


Figure 9. HPLC-Charged Aerosol Detection chromatogram overlays of Span 80 in 50, 25, 0.78 μ g on column, each in triplicate.



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Lecithin is a fatty substance occurring in animal and plant tissues. It is commonly used as an emulsifier in chocolate and spray oils to prevent sticking. Using this method detailed below, three samples were dissolved, clarified, and analyzed, including lecithin, a granola bar (Figure 10), and krill oil. This method was able to determine the amount of phosphatidylcholine found in a sample food, in the ingredient itself, as well as in a natural nutraceutical product, with results matching the official American Oil Chemists' Society (AOCS) method for phospholipids. Sensitivity was 20 ng on column LOQ.

Sample	Phosphatidylcholine Found (mass-%)	Claim Amount	Percent of Target
Lecithin, Laboratory Grade	47.7	N/A	N/A
Granola Bar	0.05	< 2%	N/A
Krill Oil	34.1	34.9*	97.7

*AOCS Official Method Ja 7c-07

Table 2. Phosphatidylcholine found in food samples.

Conditions

Column:	Thermo Scientific™ Hypersil™ Silica column, 5 μm, 3.0 x 150 mm
Mobile Phase A:	Water, 18.2 MΩ cm
Mobile Phase B:	2-Propanol; C: iso-Octane
Flow Rate:	0.20—1.50 mL/min
Injection Volume:	2—10 μL
Temperature:	50 °C
Detection:	Charged Aerosol

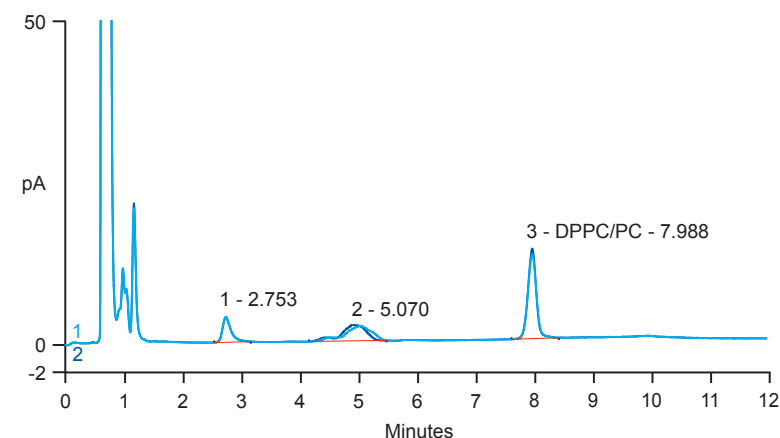


Figure 10. Analysis of lecithin (DCCP/PC) in an extracted granola bar using normal phase HPLC with Charged Aerosol Detection.

Hydroxypropylmethyl Cellulose (HPMC)



HPMC, sometimes referred to as hypromellose or modified cellulose, is often used to thicken dairy products and help improve flavor characteristics. HPMC is also an important emulsifier used in the pharmaceutical industry. Using the method described below, two samples were prepared and analyzed: a popsicle and a more complex frozen milk product (shown in Figure 9), each containing less than 1% HPMC. HPMC was calibrated over a wide range of concentrations, and the method was able to determine HPMC in two food products, including a spike-recovery of 83.5% and sensitivity to 10 ng on column LOQ.

Sample	HPMC Found (mass-%)	Claim Amount	Recovery
Popsicle	0.05	< 1%	N/A
Dairy Product	0.21	< 1%	N/A
Spiked Dairy Product	835 ng o.c. (spiked)	1000 ng o.c. spiked	83.5

Table 3. HPMC found in food samples.

Conditions

Column:	C18, 2.0 μm , 150 x 4.6 mm
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Mobile Phase C:	2-Propanol
Flow Rate:	0.40 mL/min
Injection Volume:	2—10 μL
Temperature:	40 $^{\circ}\text{C}$
Detection:	Charged Aerosol

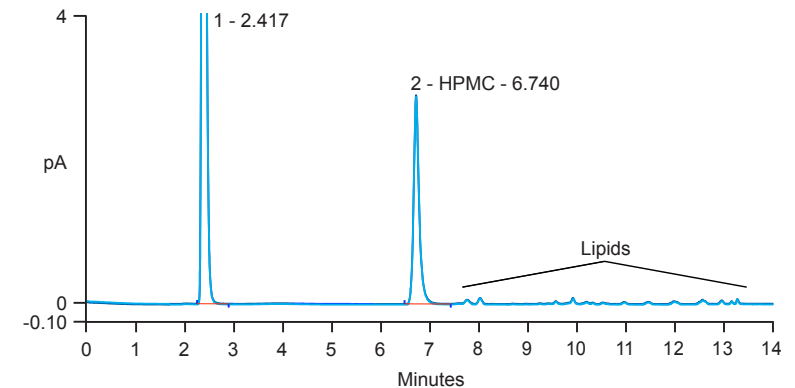


Figure 10. Overlaid HPLC-Charged Aerosol Detection chromatograms of HPMC in a frozen dairy product (analyzed in duplicate).

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Charged Aerosol Detection Bibliography

Since its introduction in 2005, charged aerosol detection has become the preferred universal LC detector for both routine and complex analyses. This bibliography is designed to readily show the analytical capabilities of charged aerosol detection.

See what other universal detectors are missing!

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