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Application News

No.L487

High Performance Liquid Chromatography

Analysis of Polysorbate 80 in IgG Aqueous Solution by Online SPE Using a Shim-pack MAYI Column – Part 2

Polysorbates play a role in maintaining the stability of proteins to prevent their denaturation, so they are often added to protein formulations. Therefore, evaluation of the quality and stability of these formulations also requires evaluation of the quality of the polysorbate. A polysorbate is not just a mixture of polyoxyethylene sorbitan fatty acid esters with polyoxyethylene chains of different degrees of polymerization. It has a complex composition which includes a variety of by-products as impurities, and those are said to affect the stability of protein formulations. Thus, it is believed that utilizing the high resolution offered with an HPLC coupled with a mass spectrometer can provide effective monitoring of quality.

In Application News No. L486, a method in which automated deproteinization can be conducted followed by quantitative analysis of polysorbate 80 in an antibody model sample was presented. Here, using this system for higher resolution analysis, we conducted detection and mass spectral measurement of possible by-product components of polysorbate 80.

Analysis of Antibody Model Sample

In accordance with the analytical conditions of Table 1, a model sample (20 mg/mL IgG) spiked with 100 µg/mL of polysorbate 80 was injected.

Table 1 Analytical Conditions (Sample Loading)

Column	: Shim-pack MAYI-ODS (5 mm L. × 2.0 mm I.D., 50 µm)
Mobile Phase	: A: 10 mmol/L Ammonium Formate in Water B: 2-Propanol
Time Program	: Solvent switching A (0 - 3.5 min) → B (3.5 - 5 min) → A (5 - 110 min)
Flowrate	: 0.6 mL/min (0 - 5 min, 95.01 - 110 min) 0.1 mL/min (5.01 - 95 min)
Extraction Time	: 3 min
Injection Vol.	: 5 µL
Column Temp.	: 40 ˚C
Detection	: UV280 nm (Semi-micro cell)

After about 3 minutes, switching was made to include the pretreatment column in the analytical flow line, and polysorbate 80 began to elute from that column. In this case, separation with a more gradual gradient elution was achieved using an analytical column longer than that used in quantitative analysis (Application News No. L486). For detection, the LCMS-8050 was used (Fig. 1, Table 2). Fig. 2 shows the TIC chromatogram in which elution is achieved with an especially gradual slope and retention times up to 35 minutes.

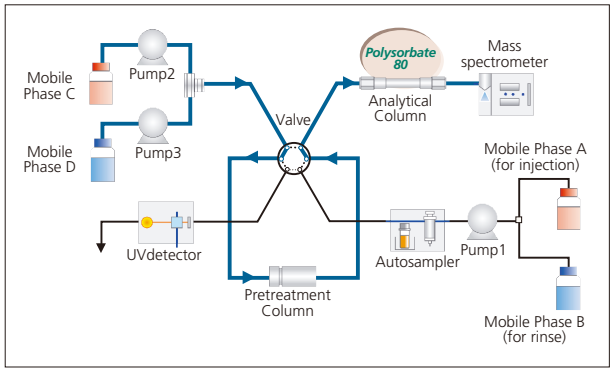

Fig. 1 Flow Diagram

Table 2 Analytical Conditions

Column	: Kinetex 5 µm C18 (100 mm L. × 2.1 mm I.D., 5 µm)
Mobile Phase	: C: 10 mmol/L Ammonium Formate in Water D: 2-Propanol
Time Program	: D.Conc. 3 % (0 - 3 min) → 15 % (35 min) → 100 % (100 min) → 3 % (100.01 - 110 min)
Flowrate	: 0.2 mL/min
Column Temp.	: 40 ˚C
Detection	: LCMS-8050
Ionization Mode	: ESI Positive
Applied Voltage	: 4.5 kV
Nebulizer Gas Flow	: 2 mL/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
Interface Temp.	: 300 ˚C
DL Temp.	: 250 ˚C
Block Heater Temp.	: 400 ˚C
Scan Range	: *m/z* 300 - 2000

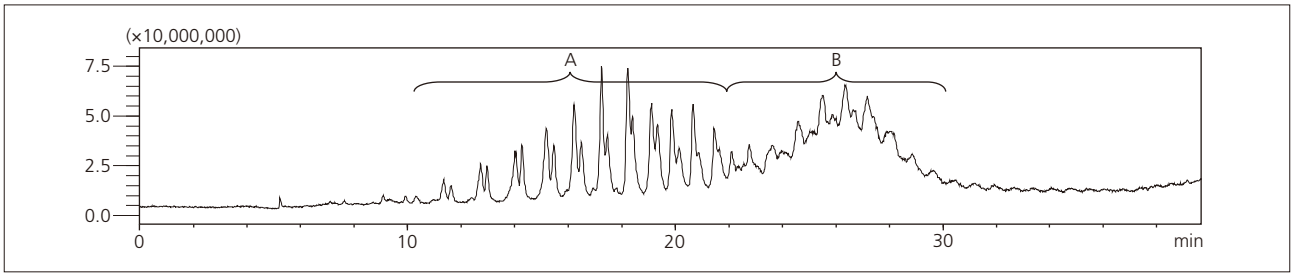

Fig. 2 TIC Chromatogram of Model Sample (0 – 35 min)

Confirmation of Mass Spectra

The TIC chromatogram is shown in Fig. 5, and the mass spectra are shown in Figs. 3, 4 and 6. Peak D, observed as the principle component in the TIC chromatogram, matched the quantitation target substance discussed in Application News No. L486, as well as the spectrum.
Regarding peak A, peaks with mass differences of 44 and 22 are singly and doubly charged ions, and from the literature, it is presumed that they are derived from polyoxyethylene isosorbide and polyoxyethylene. Peak E eluting in the second half showed a similar spectrum, and is assumed to be an ester of this substance.

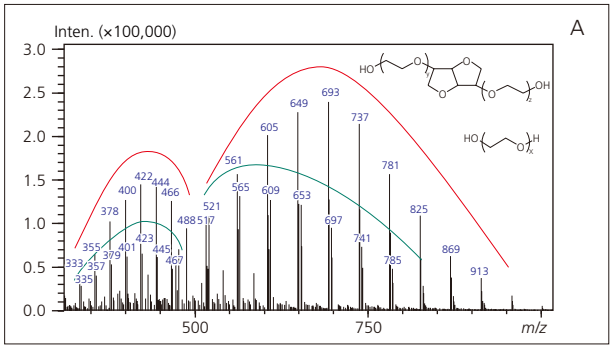

Fig. 3 Mass Spectrum of the Peaks (A)

As for peak B, it is detected as a divalent and trivalent peak similar to the principal component peak D, and is presumed to be polyoxyethylene sorbitan derived from hydrolysis of the ester according to the literature.
Regarding the mass spectra of peaks C, F, and G associated with components eluted in the latter half, this series has the same features, in which the oleic acid bond number and fatty acid type are different from those of the principal component.

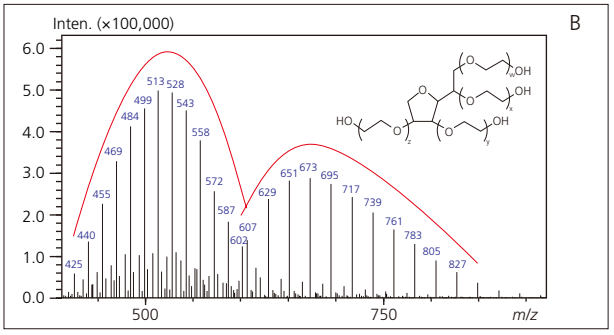

Fig. 4 Mass Spectrum of the Peaks (B)

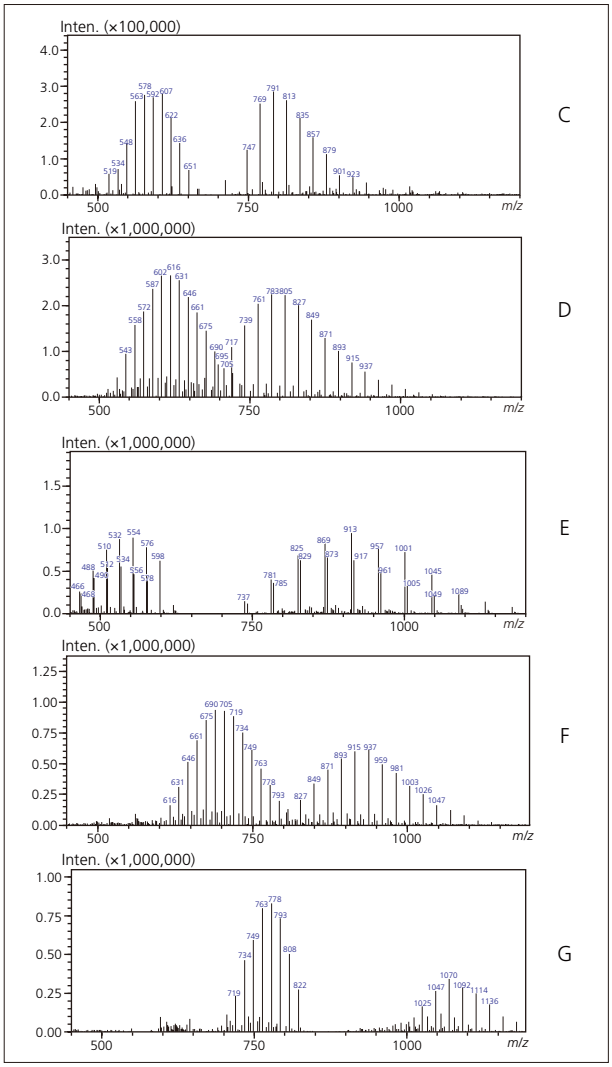

Fig. 6 Mass Spectra of the Peaks (C - G) in Fig. 5

The results showed that the online SPE system combined with high-resolution analysis allows identification and quantitation of each by-product, and that this system can be further applied to monitor degradation due to oxidization or hydrolysis.

[Reference]

E. Hvattum, W.L. Yip, D. Grace, K. Dyrstad, Characterization of polysorbate 80 with liquid chromatography mass spectrometry and nuclear magnetic resonance spectroscopy: Specific determination of oxidation products of thermally oxidized polysorbate 80, J Pharm Biomed Anal 62, (2012) 7-16

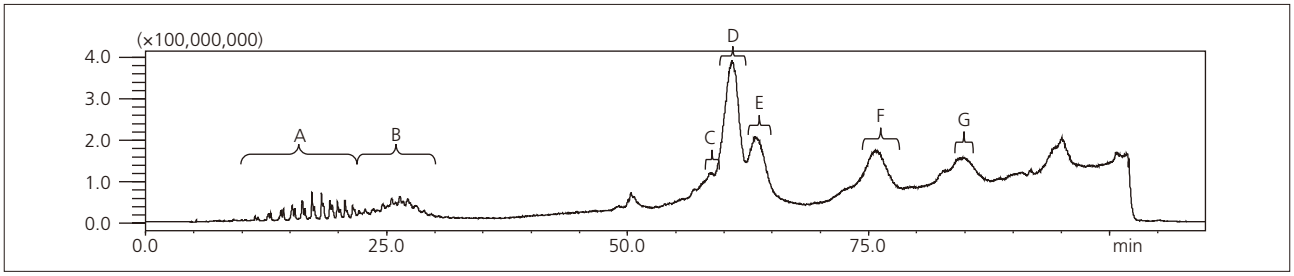

Fig. 5 TIC Chromatogram of Model Sample

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