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Application News

No.L477

High Performance Liquid Chromatography

Analysis of Anionic Surfactants by Prominence-i and RF-20Axs Fluorescence Detector

According to the Ministerial Ordinance on Water Quality Standards[1], an HPLC method using a fluorescence detector has been adopted as the test method for anionic surfactants[2]. (Please refer to Application News No. L303.)
Since either the RF-20A or RF-20Axs fluorescence detector can be connected to the new Prominence-i integrated high-performance liquid chromatograph, the combination of integrated operability and high-sensitivity fluorescence detection is possible.
Here we present an example of the analysis of five anionic surfactants using the Prominence-i integrated high-performance liquid chromatograph with the RF-20Axs high-sensitivity fluorescence detector.

Analysis of Standard Anionic Surfactants

Fig. 1 shows the basic structural formula of the five anionic surfactants which differ by the length of the hydrocarbon chain. Quantitative analysis of anionic surfactants in water samples is conducted by classifying the approximately twenty peaks obtained from the analysis of a standard solution containing the C10 – C14 branched- chain surfactants, and then summing the respective area values.
Depending on the type of column used for separation, there is a type that can resolve branched chains for each carbon number and will produce multiple peaks, and there is a type which cannot resolve branched chains, so only a single peak appears for each carbon number.
Fig. 2 shows the chromatograms of a standard solution of anionic surfactants in accordance with the water quality inspection method (total of 50 mg/L for 5 substances, each at 10 mg/L), and Table 1 shows the analytical conditions used. This concentration is based on the standard concentration in accordance with the indicated pretreatment procedure (test water at 250-fold concentration).
Fig. 3 shows an example of high speed analysis using a commercially available column (which cannot resolve branched chains).

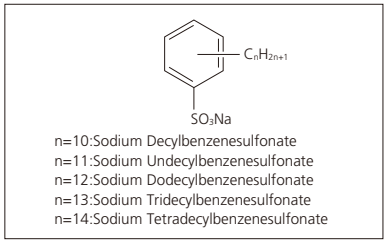

Fig. 1 Structure of Anionic Surfactants

Table 1 Analytical Conditions

Column (1)	: Shim-pack VP-ODS (250 mm L × 4.6 mm I.D., 5 µm)
Flowrate (1)	: 1.0 mL/min
Column (2)	: Wakosil AS-Aqua (250 mm L × 4.6 mm I.D., 5 µm)
Flowrate (2)	: 0.7 mL/min
Mobile Phase	: A) Water B) Acetonitrile containing 0.1 M Sodium Perchlorate B. Conc. 65 %
Column Temp.	: 40 °C
Injection Volume	: 20 µL
Detection	: RF-20Axs, Ex at 221 nm, Em at 284 nm

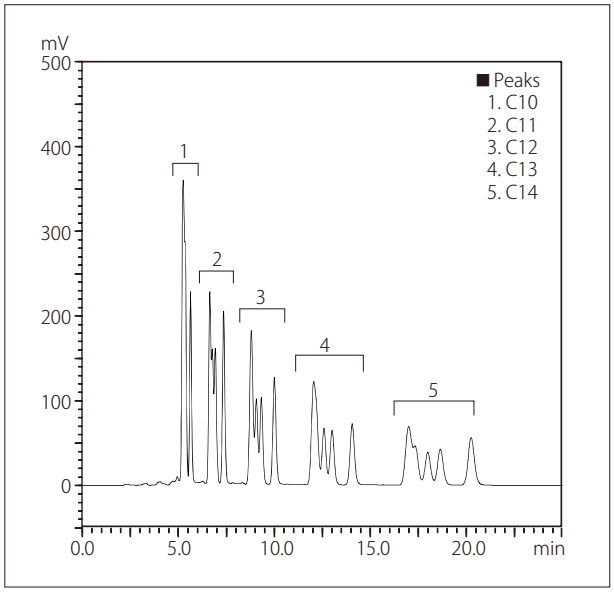

Fig. 2 Chromatogram of Standard Mixture of 5 Anionic Surfactants (Using Column 1) (10 mg/L each, total of 50 mg/L, 20 µL Inj.)

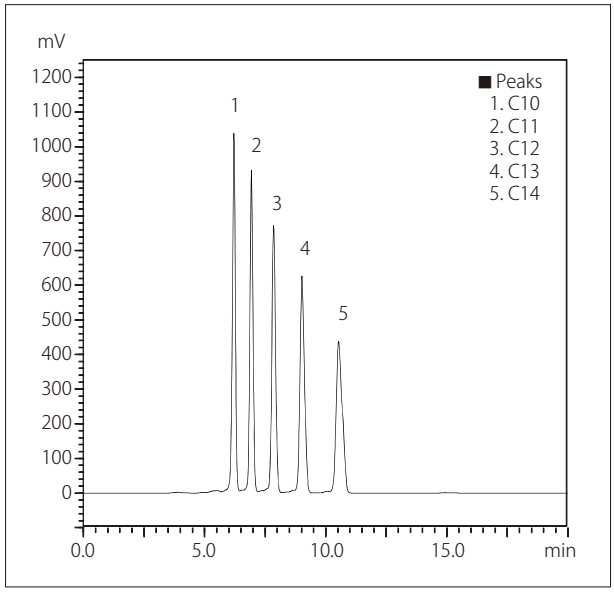

Fig. 3 Chromatogram of a Standard Mixture of 5 Anionic Surfactants (Using Column 2) (10 mg/L each, total of 50 mg/L, 20 µL Inj.)

Repeatability of Peak Area Values

Tables 2 and 3 show the peak area %RSD values obtained for each carbon number based on 6 repeat measurements of standard solutions of three anionic surfactants, each at a concentration of 1 mg/L and 5 mg/L. For all samples, the area %RSD was 0.6 % or less for both the 1 mg/L and 5 mg/L concentrations.

Table 2 Reproducibility of Peak Area (%RSD) from Repeat Injections (Using Column 1)
Upper: Standard Solution Containing 1 mg/L of Each Substance
Lower: Standard Solution Containing 5 mg/L of Each Substance

Each at 1 mg/L (n=6)

	C10	C11	C12	C13	C14
%RSD	0.36	0.36	0.47	0.39	0.43

Each at 5 mg/L (n=6)

	C10	C11	C12	C13	C14
%RSD	0.31	0.31	0.31	0.26	0.30

Table 3 Reproducibility of Peak Area (%RSD) from Repeat Injections (Using Column 2)
Upper: Standard Solution Containing 1 mg/L of Each Substance
Lower: Standard Solution Containing 5 mg/L of Each Substance

Each at 1 mg/L (n=6)

	C10	C11	C12	C13	C14
%RSD	0.39	0.41	0.38	0.60	0.34

Each at 5 mg/L (n=6)

	C10	C11	C12	C13	C14
%RSD	0.18	0.16	0.16	0.14	0.15

Analysis of Tap Water

The RF-20Axs high-sensitivity fluorescence detector features a temperature-controlled cell with a cooling function and is ideal for the direct injection of neat test water into the HPLC. The cell temperature control minimizes any fluorescence quenching at elevated or changing ambient temperature conditions.

Figs. 4 and 5 show examples of tap water analysis in which the undiluted samples were injected directly into the HPLC. In each pair of analysis results, the sample associated with the upper chromatogram consisted of tap water spiked with the five anionic surfactants subject to water quality standard limits (each 0.04 mg/L, total of 0.2 mg/L)[1)]. The lower trace is a water sample with no surfactants added. The analytical conditions are the same as those shown in Table 1.

In the Fig. 4 results from the column which resolves branched chains, a signal-to-noise ratio S/N=6 was obtained for the peak with the lowest intensity (third isomer of C14).

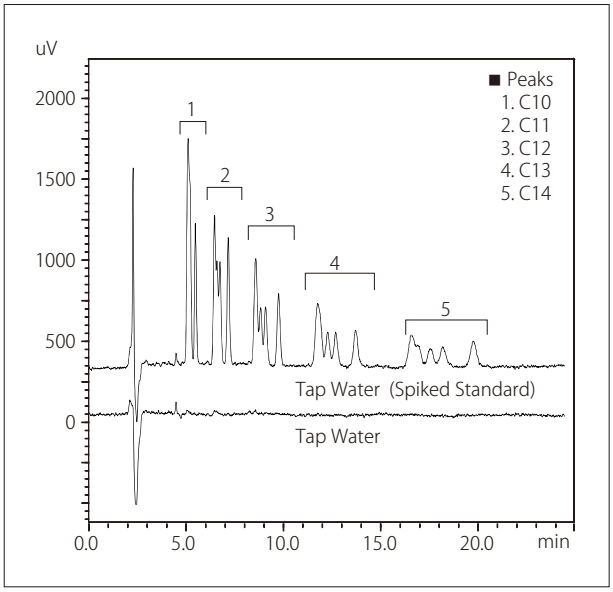

Fig. 4 Chromatograms of Tap Water (20 µL Inj.) (Using Column 1)
Upper: Water, 0.04 mg/L each, total 0.2 mg/L spiked
Lower: Water

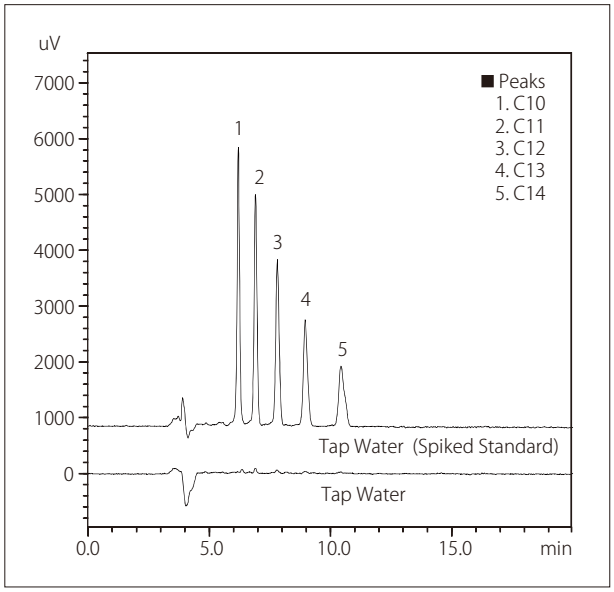

Fig. 5 Chromatograms of Tap Water (20 µL Inj.) (Using Column 2)
Upper: Water, 0.04 mg/L each, total 0.2 mg/L spiked
Lower: Water

[References]
1) Ordinance No. 101 of Japan's Ministry of Health, Labour and Welfare, May 30, 2003, (Partially revised by Ordinance No. 11 of Ministry of Health, Labour and Welfare, January 28, 2011)
2) Ordinance No. 261 of Japan's Ministry of Health, Labour and Welfare, July 22, 2003, (Partially revised by Ordinance No. 290 of Ministry of Health, Labour and Welfare, March 30, 2012)

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