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Application News

No.L499B

Supercritical Fluid Extraction / Chromatography

Application of Online SFE-SFC-PDA for Cleaning Validation

Cleaning validation is a process step that is extremely important for ensuring high quality and safety at pharmaceutical manufacturing sites. Cloth used for surface wiping, called a swab, is used to wipe a given part of a piece of manufacturing equipment, and analysis of the wiped area of the swab is performed by using high-performance liquid chromatography (HPLC) or a total organic carbon analysis (TOC). Evaluations using HPLC have been increasingly used in recent years because HPLC enables determination of individual compounds. Prior to analysis, an extraction procedure must be performed on the swab. Using supercritical fluid extraction (SFE) as the pretreatment method allows for simple and quick target component extraction. Using supercritical fluid chromatography (SFC) after SFE also means that analysis results can be obtained simply by preparing the sample for SFE, which unifies the work flow from pretreatment to analysis. Please see Application News L496 for an overview of online SFE-SFC. This article describes the process of column selection using the Nexera-UC Chiral Screening System as the first step in analysis of the target compound alkylbenzenesulfonate.

■ **Analytical Column Selection**

For SFC analysis, selection of the optimal column for the sample has a substantial effect on analysis reliability. We performed SFC separation of alkylbenzenesulfonate in four different columns under the conditions shown in Table 1 and Fig. 1, and chose the Shim-pack UC-Sil analytical column as it had the best peak shape. Based on an investigation of gradient profiles, we also found a relatively steep gradient profile is suitable for quantitative analysis as the properties of alkylbenzenesulfonate, which have different length of carbon chains, mean the significant peak broadiening if the gradient slope is not steep. Based on this information, we optimized analytical conditions using the Shim-pack UC-Sil column and performed online SFE-SFC analysis of a sample from a swab.

Table 1 SFC Analytical Conditions for Column Selection

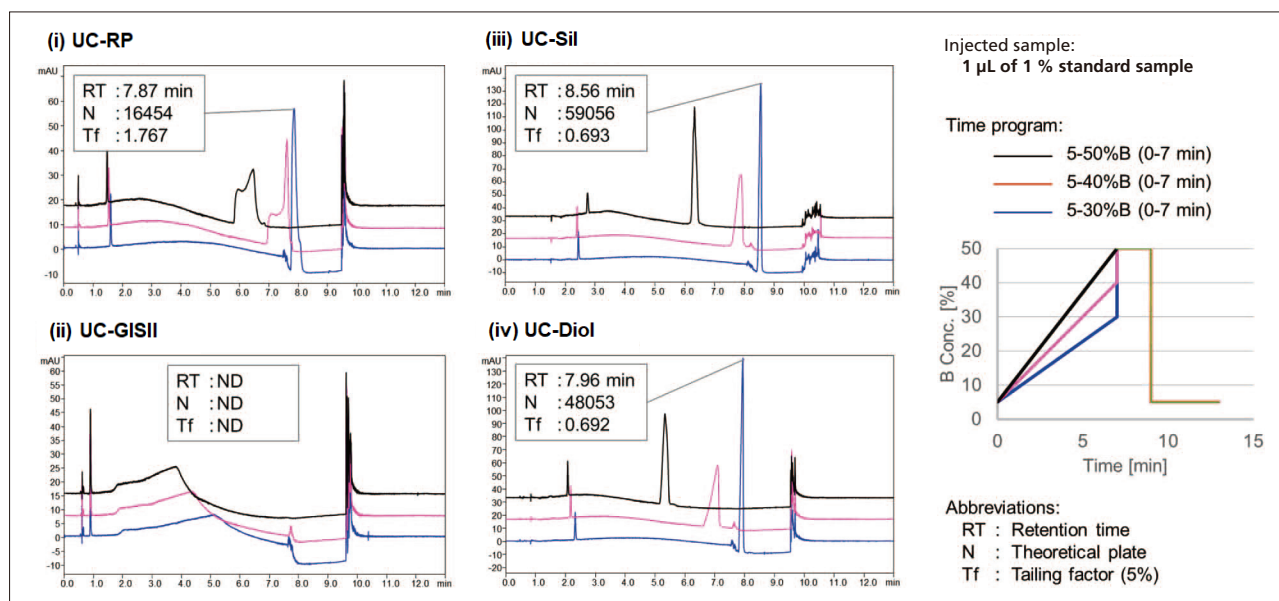|	
Column	: Shim-pack UC series columns (250 mm L. × 4.6 mm I.D., 5 μm)
	(i) UC-RP (ODS with polar group), (ii) UC-GISII (ODS), (iii) UC-Sil, (iv) UC-Diol
Mobile Phase	: A: CO_2; B: Methanol
Time Program	: Shown in the figure
Flowrate	: 3.0 mL/min
Column Temp.	: 40 °C
Back Pressure	: 15 MPa
Wavelength	: 220 nm
Injection Vol.	: Shown in figure

Fig. 1 Comparison of SFC Separation of Standard Alkylbenzenesulfonate in Four Different Columns

Online SFE-SFC Analysis of a Swab Containing Alkylbenzenesulfonate

We investigated column selection by the scouting system, chose the Shim-pack UC-Sil analytical column, optimized each analytical condition for online SFE-SFC analysis, then performed analysis using the conditions shown in Table 2 below.

Table 2 Analytical Conditions for Online SFE-SFC

[Sample Preparation]
A total of 10 to 500 µg standard samples in methanol were dropped onto swabs.
The swabs were enclosed into an extraction vessel and set to the SFE unit.
[Static Extraction]
Extraction Time : 3 min
Mobile Phase   : A: CO_2; B: 0.1 % (w/v) Ammonium Formate in Methanol
B Conc.   : 10 %
Flowrate   : 3.0 mL/min
Back Pressure : 15 MPa
[Dynamic Extraction]
Extraction Time : 3 min
Mobile Phase   : A: CO_2; B: Methanol
B Conc.   : 10 %
Flowrate   : 3.0 mL/min
Back Pressure : 15 MPa
[SFC]
Column   : Shim-pack UC-Sil (250 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase : A: CO_2; B: Methanol
Time Program : 10 %B (0-2 min), 10-60 %B (2-7 min),
                60 %B (7-9 min), 10 %B (9-13 min)
Flowrate   : 3.0 mL/min
Column Temp. : 40 °C
Back Pressure : 15 MPa
Wavelength   : 220 nm

The peak for the surfactant alkylbenzenesulfonate was well-separated and detected as shown in Fig. 2 below. Fig. 3 shows the results of performing repeated SFE-SFC analyses from the same swab to which had been added an equivalent of 100 ng of alkylbenzenesulfonate. Since there was almost no alkylbenzenesulfonate peak evident from the second and later sample extractions, the extraction procedure was almost entirely complete after the first SFE. Fig. 4 shows the results of adding amounts of alkylbenzenesulfonate to swabs in the range of 10 to 500 µg, and checking linearity. Within this range, the coefficient of determination that represents linearity was 0.996. Fig. 5 shows the result of five consecutive analyses of separate swabs to which were added 100 µg of alkylbenzenesulfonate. Considering the process including extraction, the repeatability of retention times was 0.19 %RSD, and repeatability of peak area was 5.76 %RSD. Based on these results, we confirmed the usefulness of the Nexera-UC Online SFE-SFC System in this application.
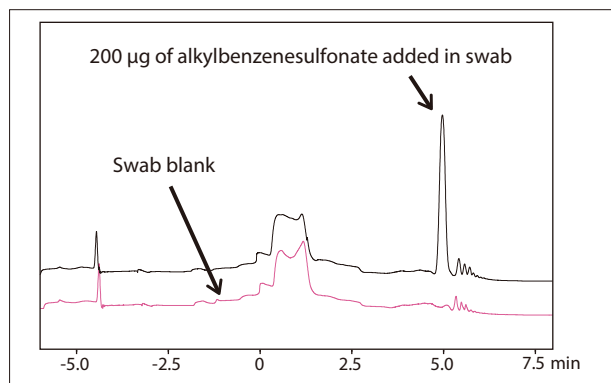

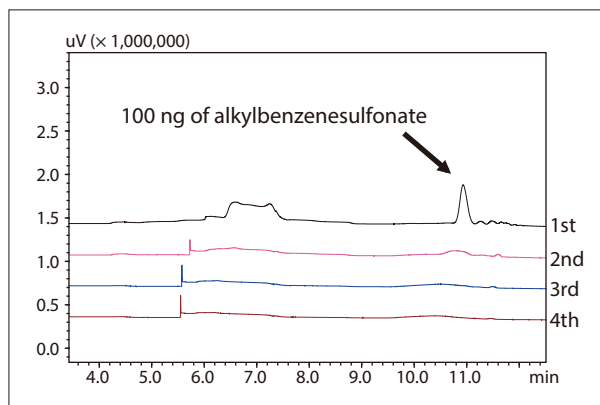**Fig. 2 Online SFE-SFC Analysis of Alkylbenzenesulfonate**

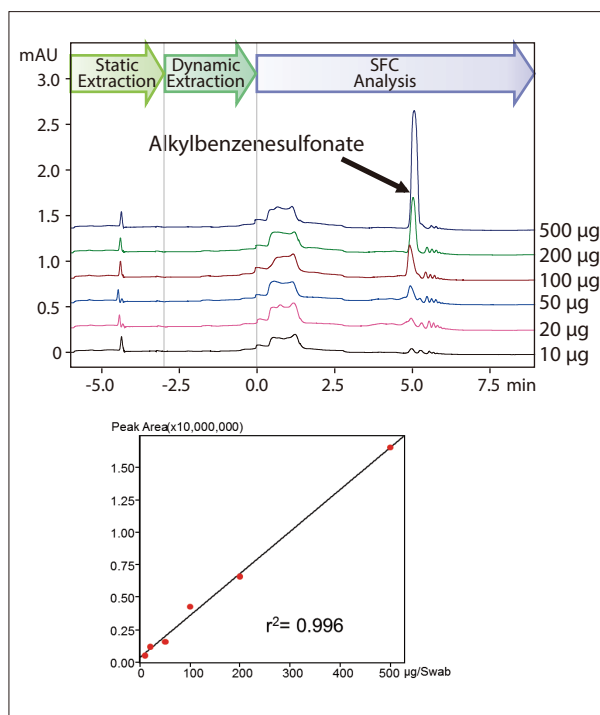**Fig. 3 Confirmation of Online SFE Extraction Efficiency**

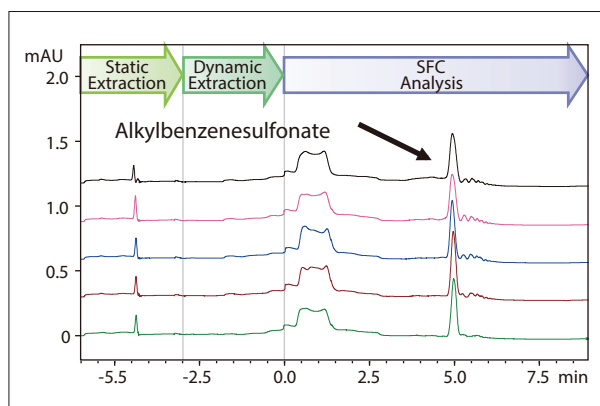**Fig. 4 Linearity of Online SFE-SFC Analysis Using a Swab**

Fig. 5 Repeatability of Online SFE-SFC Analysis Using a Swab

Note: Swab samples were provided by DAIICHI SANKYO COMPANY, LIMITED.

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