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High throughput analysis of anion surfactant using ultra-high speed LC-MS/MS and 1 mm inside diameter column

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Introduction

High resolution separations with high speed MS/MS data acquisition have created new opportunities in academic and applied research. High resolution separations can be achieved by ultra-high pressure columns with a particle diameter > 2 um or as an alternative technology using particles with a diameter of 3 um and 1 mmID. Both technologies are designed for higher resolution but they approach the problem in a different way. In this paper we have applied a 1 mm inside diameter column phase to the analysis of linear alkylbenzene sulfonate anion surfactants using a high speed MS/MS detection system.

Methods and Materials

Nexera MP UHPLC system was connected to LCMS-8030 triple quadrupole mass spectrometer.

Autosampler SIL-30ACMP

- Utrafast injection performance exceeding that of current models
- Ultralow carryover
- 6 microtiter plates can be loaded, enabling a maximum of 2304 samples to be analyzed

LCMS-8030

High Speed Mass Spectrometer
Polarity Switching 15 msec
Scanning Speed Max. 15000 u/sec

Fig. 1 UHPLC Nexera MP &LCMS-8030

LAS surfactants (C10 to C14) were obtained from Wako Pure Chemical Ind., Ltd(Osaka,Japan). Several levels of calibrators were made from the stock solution. Commercial LAS products consist of more than 20 individual components. The ratio of the various homologues and isomers representing different alkyl chain lengths and aromatic ring positions along the linear alkyl chains is relatively constant across the various household applications. The analysis of LAS surfactants (C10 to C14) share a common fragment ion at *m/z* 183 independent of linear carbon chain length and a fragment ion at *m/z* 119.

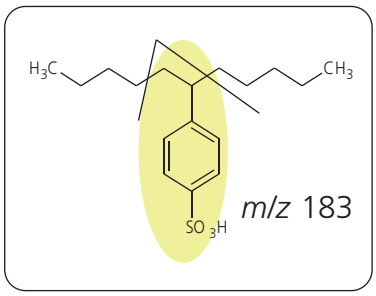

Fig. 2 Structure of LAS (C11)

Results

Conventional HPLC Analysis

As high resolution analysis, various isomers of LAS surfactant can be separated and be detected severally. Chromatographic separations were carried out using Unison UK-C18 HT (1 mmI.D. , 50 mmL., 3 um) , which has high pressure resistance and large sample capacity[1]. The column temperature was maintained at 40°C . Flow rate was 0.15 mL/min with a binary gradient system. Components were detected in electrospray negative MRM mode for quantitative analysis.

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UHPLC conditions (Nexera MP system)

Column:	Unison UK-C18 HT 1 mmI.D.x 50 mmL., 3 um
Mobile phase A:	10 mM Ammonium acetate, B: Acetonitrile
Flow rate:	0.15 mL/min
Time program:	B conc.40%(0 min) 60%(5 min) 95%(5.01-7 min) 40%(7.01-10 min)
Injection vol.:	5 µL
Column temperature:	40°C

MS conditions (LCMS-8030)

Ionization: ESI, Negative MRM mode
MRM transition are shown in Table 1.

Table 1 MRM transition of LAS

	C10	C11	C12	C13	C14
Quantitative ion Q1/Q3	297/183	311/183	325/183	339/183	353/183
Qualitative ion Q1/Q3	297/119	311/119	325/119	339/119	353/119

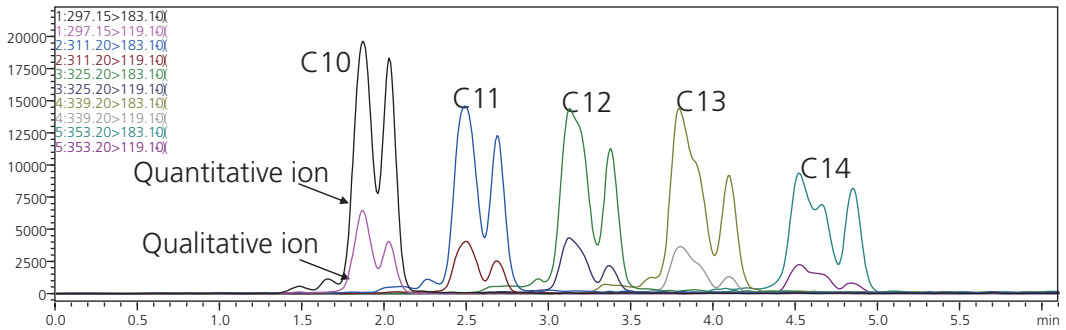

Fig. 3 Mass chromatograms of conventional conditions (LAS C10 - C14 each 20 ppb)

Several Peaks for each LAS carbon chain were detected. The result shows that excellent separation was achieved with these conditions in spite of comparatively high speed analysis. Spiked tap water sample (20ppb LAS) showed good recovery at almost 100% (Table 2). These results indicate that the LC-MS/MS method was not influenced by sample matrix in tap water.

Table 2 Recovery data spiked in tap water sample at 20 ppb (n=5)

	C10	C11	C12	C13	C14
20 ppb Standard sample	263865	218485	240309	255308	205600
Spiked tap water sample	282372	227065	244975	265646	222209
Recovery (%)	107.0	103.9	101.9	104.0	108.1

The above counts are peak area

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High throughput analysis

Although several peaks were detected for each LAS as mentioned above, it does however make quantification more complex. In high throughput analysis, the condition of which each LAS is detected as one peak was re-examined

The Nexera UHPLC system has a unique wash process in which the needle and sample loop can be washed, separating it from the HPLC line after injection. Washing of the needle seal and shortened the HPLC line result from this function (Fig. 4). Using this function, chromatographic separations were carried out on LC-MS/MS condition as follows.

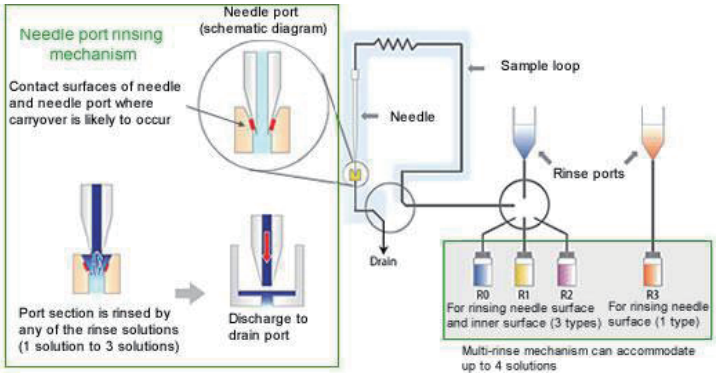

Fig. 4 HPLC path of SIL-30AC

Modified UHPLC conditions:

Flow rate: 0.3 mL/min(0-1 min) 0.5 mL/min(1.01-1.30 min) 0.3 mL/min(1.31-1.5 min)

Time program: B conc.55%(0 min) 90%(0.7 min) 95%(0.71-0.75 min) 55%(0.76-1.5 min)

MS conditions (LCMS-8030): same as previously used.

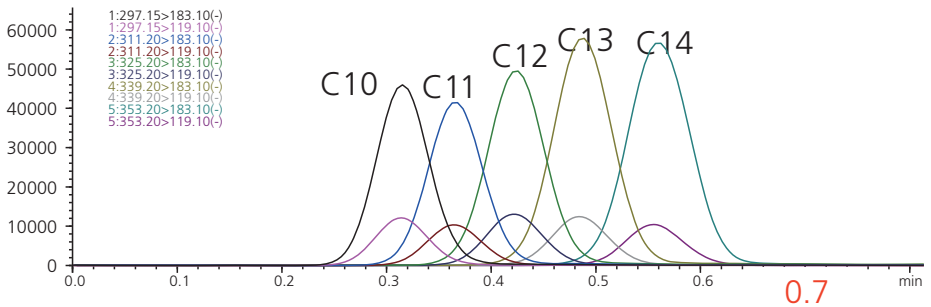

Fig. 5 Mass chromatograms of high throughput condition
(LAS C10 ～ C14 each 20 ppb)

Each LAS was separated as one peak within 1 minute.
Total analysis cycle was within 2 minutes.
In this condition, the linearity of calibration curve and repeatability for each LAS was excellent and all LAS can be detected from 0.1ppb (Fig. 6, Table 3). Spiked Tap water sample (200 ppb LAS) showed good recoveries with almost 100%. It indicates that this LC-MS/MS method was not influenced by sample matrix in tap water.

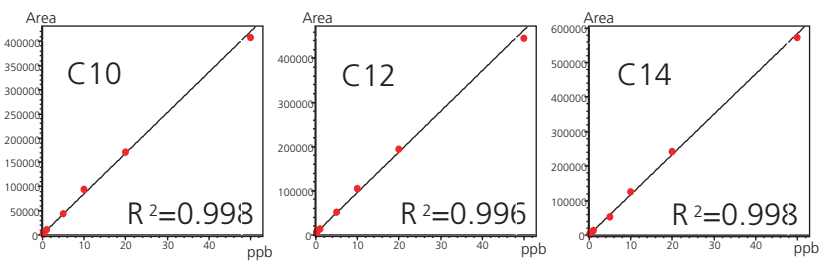

Fig. 6 Representative calibration curve in high thoughput conditions (LAS C10, C12, C14)

Table 3
Area repeatability of 0.5 ppb
(n=5)

	%RSD
C10	3.09
C11	4.11
C12	5.77
C13	7.50
C14	3.43

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Simultaneous screening analysis of LAS using precursor ion scanning

Simultaneous screening analysis of LAS using high speed precursor ion scanning was also conducted. M/z 183 was applied as a common fragment ion of LAS. It is commonly known that increasing the scanning speed in a conventional triple-quadrupole mass spectrometer results in mass errors. Increasing a scan speed, the detected *m/z* of LAS was examined using LCMS-8030.

UHPLC conditions: same as previous high thoughput analysis condition

MS conditions (LCMS-8030)

Ionization: ESI, Negative, precursor ion scan mode Prec of 183 (Scan range: *m/z* 180-500)
Scan time: 1 sec (326 µ/sec), 0.33 sec (1000 µ/sec), 0.1 sec (3750 µ/sec).

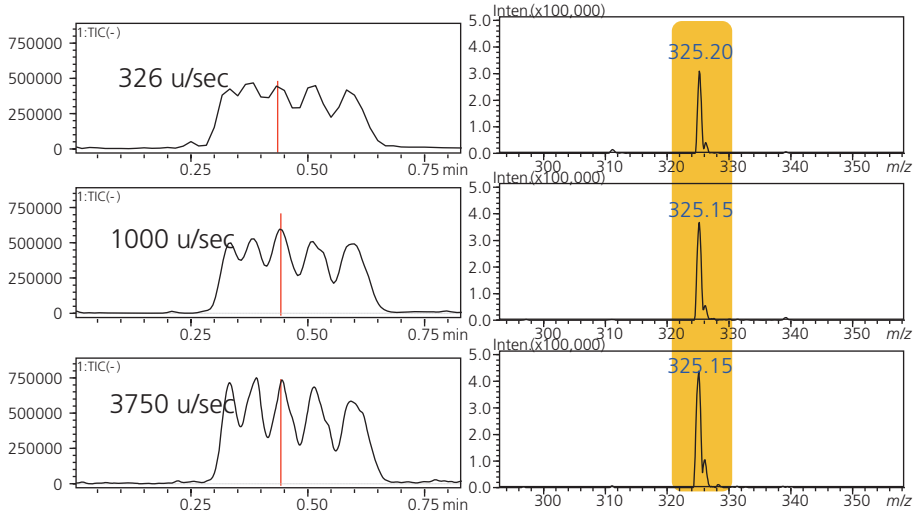

Fig. 7 Measurement results of precursor ion scans; LAS standard solution
left : TIC chromatogram, right : C12 (third peak) peaktop mass spectrum

LAS was analyzed at various scan speeds. The TICs and mass spectra are shown above for measurements at 326, 1000 and 3750 u/sec. At 326 u/sec, a proper peak shape was not obtained due to insufficient data points. The mass chromatogram at 3750 u/sec shows sharper peaks. The results indicate that sufficient data points are obtained at a scan rate of over 3000 u/s.

In addition, no precursor ion mass error was apparent at any scanning speed in the results.

Conclusions

- Using 1 mm inside diameter column, 5 LAS were separated with high resolution within 1 minute and were detected with high sensitivity. The excellent linearity was obtained in the calibration curves of all LAS.
- LCMS-8030 ultra fast precursor ion scanning is useful and reliable even for such a high thoughput analysis where extremely narrow peaks were obtained.

References

1) J. Watanabe et al., 56th ASMS Conference, LCMS I (WP)-295 (2008).

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