

# Simultaneous analysis of alkylphenol ethoxylates using ultra-high speed LC-MS/MS

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Daisuke Kasai<sup>1</sup>, Jun Watanabe<sup>2</sup>, Keiko Matsumoto<sup>2</sup>,  
Koji Takinami<sup>1</sup>

<sup>1</sup>Nissenken Quality Evaluation Center, 2-16-11  
Kuramae Taito-ku, Tokyo 111-0051, Japan;

<sup>2</sup>Shimadzu Corporation. 1, Nishinokyo-Kuwabaracho  
Nakagyo-ku, Kyoto 604-8511, Japan

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### 1. Introduction

Alkylphenol ethoxylates (APEs), kinds of non-ion surfactants, are used in the manufacture of textiles as the detergent and the dispersant in industry. The quantity of production of nonylphenol ethoxylates (NPEs), in which the carbon number of an alkyl group is 9, has most as about 80% in all APEs, and most of remaining is octylphenol ethoxylates (OPEs), the carbon number 8.

It is known that APEs can break down to alkyl phenol form by biodegradation. On the other hand, it has been becoming an environmental problem that the surfactant contained in wash drainage makes water quality pollute so it is desired to measure these surfactants with sufficient accuracy. Here, we developed the simultaneous analysis method using LC-MS/MS of typical APEs.

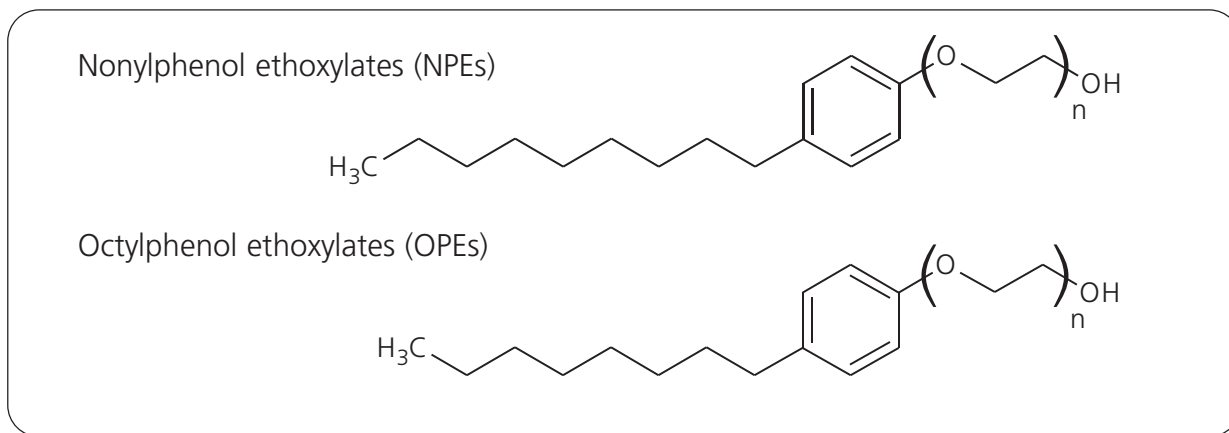


Fig. 1 Structure of NPEs and OPEs

### 2. Methods and Materials

The commercial reagents of APEs and the textile products were used for this experiment. Standards of APEs were diluted with methanol to suitable concentration and then loaded to LC-MS/MS. As an LC-MS/MS system, Nexera X2 UHPLC system was connected to LCMS-8030 triple quadrupole mass spectrometer. Separation occurred on a ODS column, which temperature was maintained at 40°C. Samples were eluted at flow rate 300 uL/min with a binary

gradient system; the mobile phase consisted of (A) 10 mM ammonium acetate buffer and (B) acetonitrile. LC-MS/MS with electrospray ionization (ESI) source was operated in multiple precursor ion scanning modes with ultra-high scanning speed in screening analysis for APEs and multiple-reaction-monitoring (MRM) mode for quantitative analysis.



#### High Speed Mass Spectrometer

- Ultra Fast Polarity Switching
  - 15 msec
- Ultra Fast MRM
  - Max. 555 transition /sec

Fig. 2 LCMS-8040 triple quadrupole mass spectrometer

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### 3. Results

#### 3-1. Quantitative method development for APEs

NPEs and OPEs as standard APEs reagents were analyzed in terms of being widely used. Flow injection analysis (FIA) with MS scanning mode was carried out for determination of the ionization polarity of compounds and subsequently FIA for MRM transition optimization were performed. As a result of that, all NPEs and OPEs were detected the precursor ion,  $[M+H]^+$ , in positive polarity and same fragment ion of  $m/z$  89 was similarly obtained as optimal

MRM transition for NPEs which number of added ethylene oxide (EO) is  $>2$ , same fragment of  $m/z$  89 for OPEs, EO  $>2$ . As a result of several examining LC mobile phase condition; the salt and the organic solvent, NPEs and OPEs were able to be retained on the following condition, but it was hard to separate each compound completely in short LC run time.

Table 1 MRM transitions of NPEs and OPEs

Compound	Q1 (u)	Q3 (u)	CE (V)	Compound	Q1 (u)	Q3 (u)	CE (V)
OPE 16	928.6	89.0	52	NPE 18	1030.7	89.0	56
OPE 15	884.6	89.0	50	NPE 17	986.6	89.0	54
OPE 14	840.6	89.0	48	NPE 16	942.6	89.0	52
OPE 13	796.6	89.0	46	NPE 15	898.6	89.0	50
OPE 12	752.5	89.0	44	NPE 14	854.6	89.0	48
OPE 11	708.5	89.0	42	NPE 13	810.6	89.0	46
OPE 10	664.5	89.0	40	NPE 12	766.6	89.0	44
OPE 9	620.5	89.0	38	NPE 11	722.5	89.0	42
OPE 8	576.4	89.0	36	NPE 10	678.5	89.0	40
OPE 7	532.4	89.0	35	NPE 9	634.5	89.0	38
OPE 6	488.4	89.0	34	NPE 8	590.4	89.0	36
OPE 5	444.3	89.0	33	NPE 7	546.4	89.0	35
OPE 4	400.3	89.0	32	NPE 6	502.4	89.0	34
OPE 3	356.3	89.0	31	NPE 5	458.3	89.0	33
				NPE 4	414.3	89.0	32
				NPE 3	370.3	89.0	31

#### Analytical Conditions

##### HPLC : UHPLC Nexera X2 system

Column	: Zorbax Eclipse XDB-C18 (150 mm L. × 2.1 mm I.D., 5 μm) or Cadenza CD-C18 (150 mm L. × 2 mm I.D., 3 μm)
Mobile phase	: A ; 10 mM ammonium acetate - water B ; Acetonitrile
Time program	: B conc.60% (0-1 min) - 98% (5-10 min) - 60% (10-15 min)
Flow rate	: 0.3 mL/min
Column temperature	: 40 °C
Injection volume	: 5 μL

##### MS : LCMS-8030 Triple quadrupole mass spectrometer

Ionization	: ESI Positive
Ion spray voltage	: +4.5 kV
MRM	: MRM transitions shown in Table 1

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OPEs were eluted first in the order as OPE 16, 15, 14, ..., 3 and then NPEs were eluted in the order as NPE 18, 17, 16, ..., 3. The dilution series of these compound standards were analyzed and all compounds were detected with excellent sensitivity and good linearity was obtained in the calibration curves of all. In addition, the quantitative

analysis of the textile products was carried out and then some APEs could be detected. It turned out that this method is effective in real samples. Typical chromatograms of APEs; standards and real sample were shown in Fig. 3.

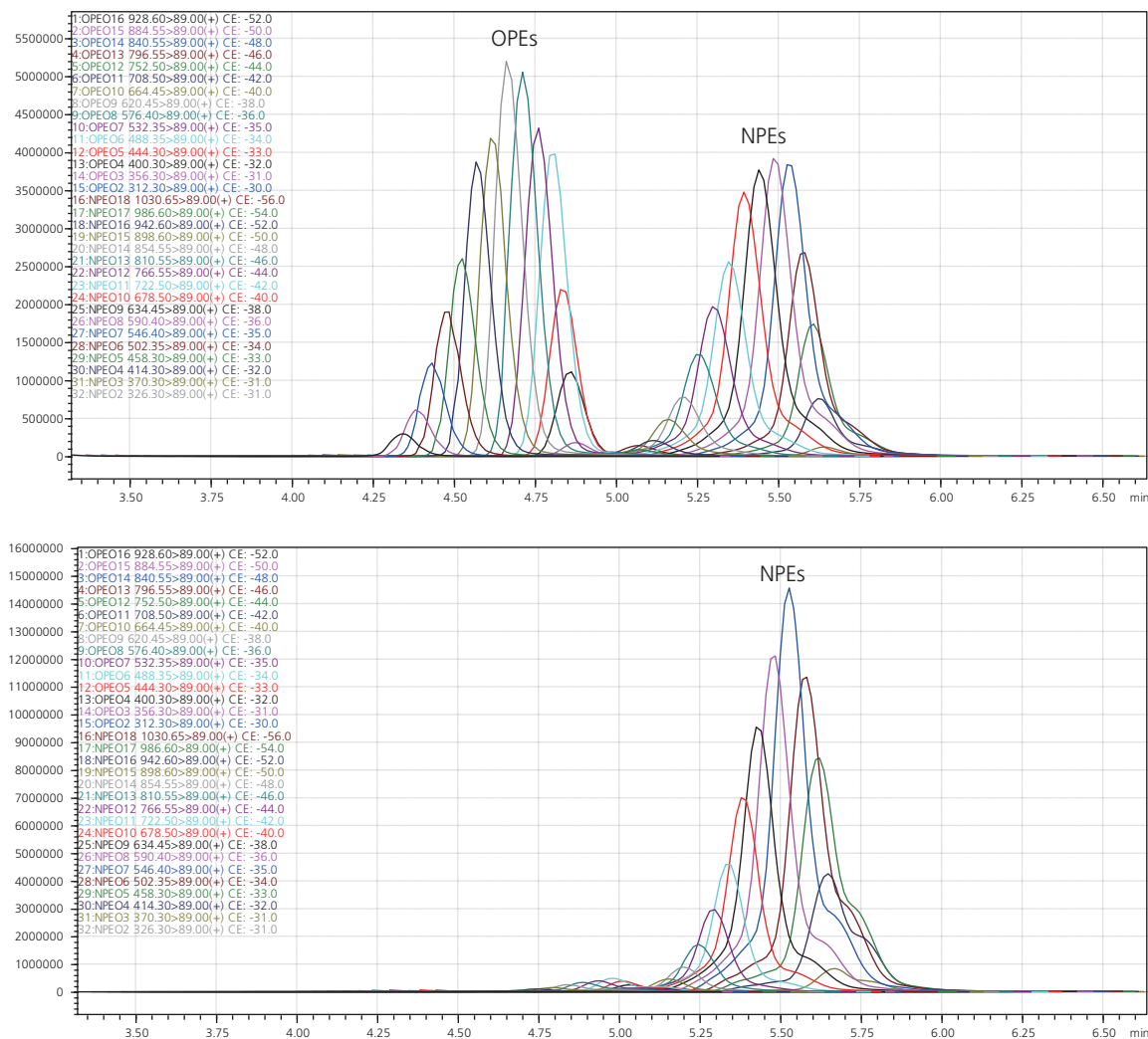


Fig. 3 Typical chromatograms of APEs; (a) chromatograms of APEs standards (b) chromatograms of a textile product

### 3-2. Screening method development for APEs

Furthermore, in order to conduct screening analysis of how many carbon numbers of an alkyl group in APEs were detected, the method which performed the high-speed precursor ion scans (scan rate >3000 u/sec) of  $m/z$  89 for OPEs and NPEs detection with multiple collision energy settings was built (Fig. 4). It is known that a precursor ion

scan detected with high scanning speed using triple quadrupole mass spectrometer will occur mass shift to the high  $m/z$  side, however, in this analysis, the mass shift was not seen because of high-speed-correspond mass spectrometer.

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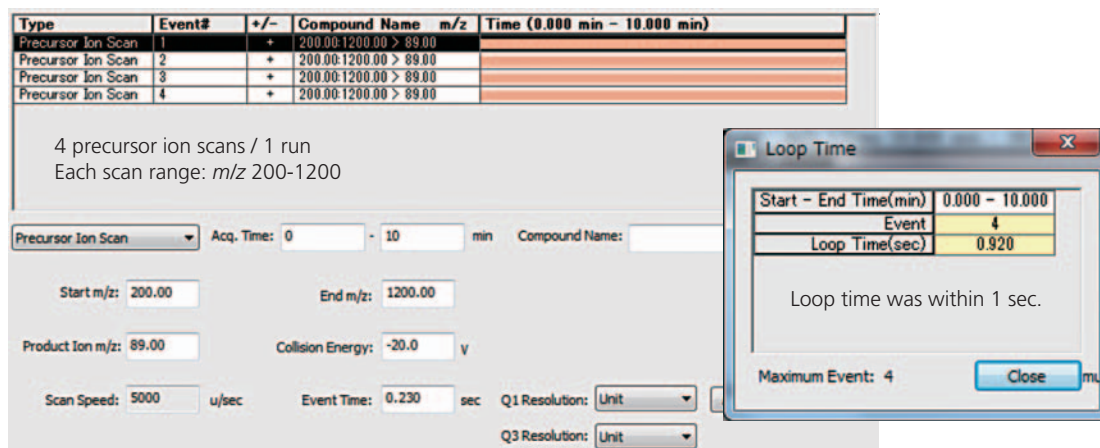


Fig. 4 Screening method for APEs; 4 precursor ion scans; CE = 20, 30, 40, 50 V

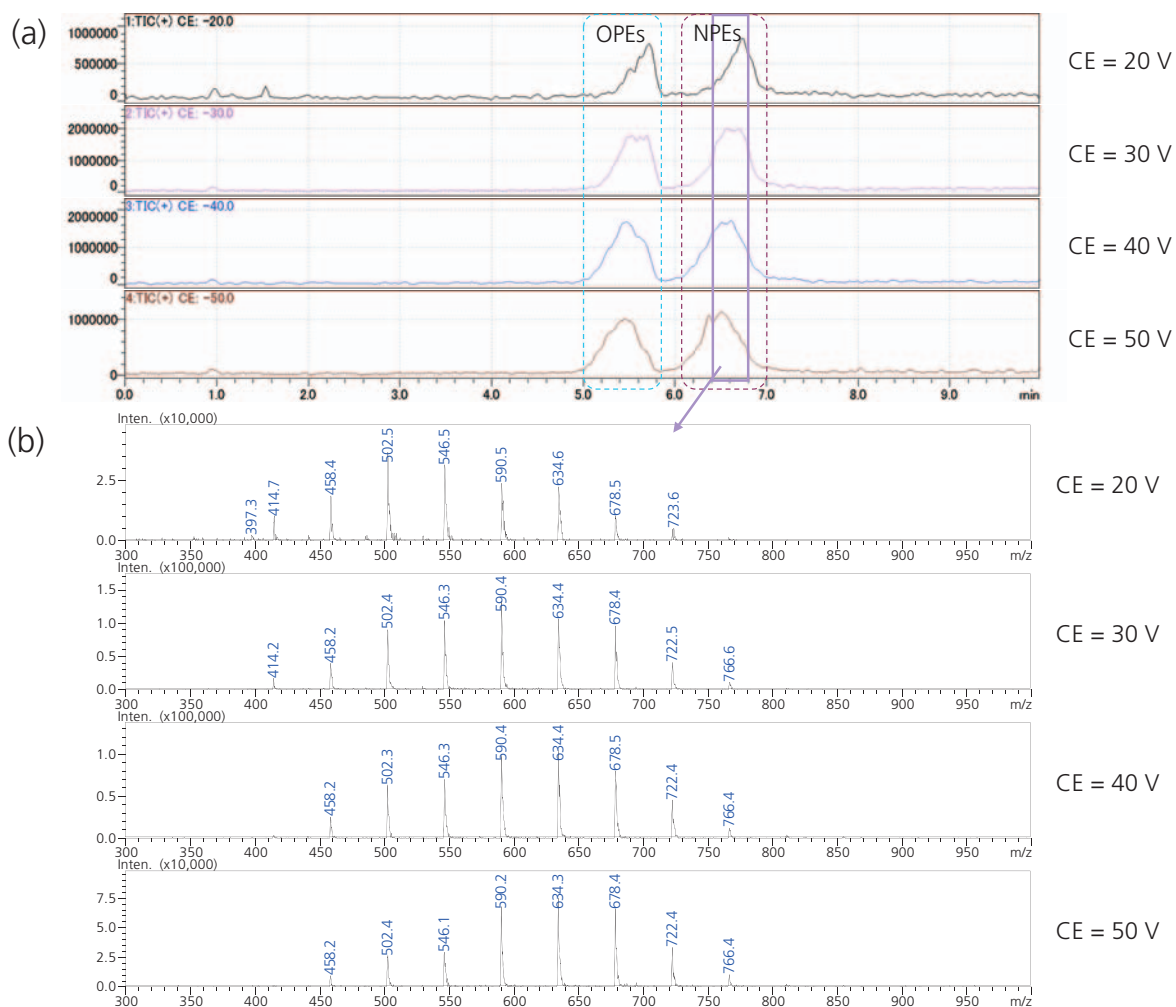


Fig. 5 Chromatograms (a) and spectra (b) of APEs

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### 4. Conclusions

- Simultaneous analysis method of APEs using ultra-high speed scanning technique of LC-MS/MS was developed.
- All APEs MRM transitions had common fragment ions ( $m/z$  89).
- The LC-MS/MS method consisting of multiple precursor ion scan ( $m/z$  89) with high scan speed was useful for screening of APEs.