Screening method for Nonylphenol (NP) in River Water by Stir Bar Sorptive Extraction (SBSE)-Thermal Desorption(TD)-Comprehensive two dimensional GC (GCxGC) coupled to quadrupole mass (qMS)

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Introduction

Nonylphenol (NP) is widely used as a plastic additive and an antioxidant; a derivative of NP, nonylphenol polyethoxylate (NPE), is commonly used as a nonionic surfactant. NPE degrade in wastewater treatment systems to form a variety of degradation products including NP by sequential deethoxylation. NP, as found in most environmental samples, including sediments, is a mixture of several isomers due to the branching of the C-9 group. Theoretically, NP has about 170 isomers and isomer specific analysis of NP in environmental matrixes is a subject of interest for several reasons. Recently, it has been shown that NP elicits estrogenic responses in a variety of aquatic organisms and in vitro assays (Kim et al., 2004). Structural characterization of NP isomers is of great interest because only a few isomers elicit such estrogenic potential. To date, toxicity of individual NP isomers has not been reported due to the inability to separate individual isomers from such complex mixtures. Thus, it is important to analyze NP isomers more separately and to know their characteristics and distribution in the environment. In addition, highly selective and sensitive instrumental method is essential because individual NP isomer in water sample is ultra trace level.

Furthermore, preparation of water sample for the analysis of NP involves several steps (e.g. extraction, clean up, and concentration). These steps are tedious, time consuming, and labor intensive. A stir bar sorptive extraction (SBSE) method was developed in 1999 (Baltussen et al., 1999), which uses similar principles as that of solid phase micro-extraction (SPME), but the sample enrichment factor is up to hundred times higher than SPME. The SBSE has been successfully applied to the analysis of trace amount of NP in water samples (Kawaguchi et al., 2004).

Comprehensive two-dimensional gas chromatography (GCxGC) has been shown to be useful for the analysis of complex samples and environmental pollutants (Marriott et al., 2003). GCxGC offers a significantly greater peak capacity than conventional GC and heart-cutting two-dimensional GC (GC-GC). Therefore, the opportunity for improved resolution of target compounds in a single analysis will be provided. In GCxGC, second dimensional GC employs a fast GC with short and narrow bore capillary column. Typical peak widths of GCxGC analysis are very narrow (about 40-200 msec). Consequently, the detector for GCxGC is requires very fast data acquisition capabilities. Previous studies have indicated that the data acquisition capabilities needed for the GCxGC detector is about 20-100 Hz. Thus, time-of-flight mass spectrometry (TOFMS) has been selected for the proper monitoring of GCxGC chromatograms when structural information is needed. Recently, quadruple mass spectrometer (qMS) operating with a limited scan range, has been successfully applied to a detector of GCxGC (Shellie et al., 2003, Mondello et al., 2005, Adahchour et al., 2005). The limited scan range made it possible for qMS to perform approximately 20-33 Hz acquisition rate.

The aim of this study was to develop a method for screening NP in river water sample by using a SBSE extraction and GCxGC-qMS analysis.

Material and Methods

Materials

All solvents used were high purity, pesticide grade (Wako Pure Chemical Industries, Tokyo, Japan). 4-NP standard (97.4% purity, Tokyo Kasei Kogyo, Tokyo, Japan), 4-nonylphenol-d_4 (4-NP-d_4, Kanto Chemical Company, Japan), and 4-nonylphenol-$^{13}C_6$ (n-NP-$^{13}C_6$, Cambridge Isotope Laboratories Inc, USA)) were used. Two NP isomers, 4-

(1,1,4-trimethyl-hexyl)-phenol (NP5) and 4-(1,1-dimethyl-2-ethyl-pentyl) - phenol (NP7) were synthesized.

Instrumentation

An Agilent 6890A GC interfaced with 5973A mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with a Zoex KT2004 system (Zoex Inc., Lincon, NE, USA) was used. The first dimension column (1st column) was a 30 m length x 0.25 mm i.d. DB-5 (95% dimethyl, 5% diphenyl polysiloxane; Agilent Technologies) with a film thickness of 1 µm. Optimization of separation for NP isomers was carried out by use of five different second-dimension columns (2ndcolumn), DB-WAX (polyethylene glycol; Agilent), DB-17 (50% methyl and 50% phenyl polysiloxane; Agilent), DB-1701 (14% cyanopropyl phenyl, 86% dimethyl polysiloxane; Agilent), DB-225 (50% cyanopropylmethyl, 50% phenylmethyl polysiloxane; Agilent) and Rt-bDEX (permethylated b cyclodextrin; Restek Corporation), while using DB-5 as the first-dimensional column. The 2nd dimension columns were 2 m length x 0.1 mm i.d. x 0.1 µm film thickness except for Rt-bDEX (2.0m length x 0.18mm i.d. x 0.18µm film thickness). All these columns were connected by using a deactivated glass connecter with polyamide resin.

The modulation period was 4 sec and hot gas duration time was 250 msec. The optimized oven temperature (for 1st column: DB-5, and 2nd column: DB-WAX) was as follows: initial temperature 40 ºC increased at 30 ºC /min to 205 ºC, and then at 3 ºC/min to 250 ºC (held for 10min). Helium was used as a carrier gas at 2.3 ml/min (constant pressure mode). The scan range was set from *m/z* 105 to 170 (approximately 25Hz). 4-nonylphenol mixture (NP, 97.4% purity, working solution; 248 ppm) was used.

Sample preparation

River water samples were collected from the Ayase and Arakawa Rivers in Japan. Fifty four ml of water sample and 6ml of methanol were poured into a 60-ml glass vial, and stir bars coated with 100% polydimethylsiloxane (PDMS; Twister, 0.5 mm film thickness, 20 mm length, 48µl PDMS from GERSTEL, Mülheim an der Ruhr, Germany) were placed in the vials. The vials were stirred for 120 min at 1000 rpm. After extraction, stir bars were removed from sample vials with tweezers and dried briefly with a lint-free tissue paper.

SBSE-GCxGC-qMS

After sample preparation, stir bars were transferred into an empty glass thermal desorption tube and desorbed using a thermal desorption system (TDS, GERSTEL) equipped with an auto sampler (TDSA, GERSTEL). Stir bars were thermally desorbed by programming the TDS from 20 ºC (held for 1 min) to 250 ºC (held for 3 min) at a rate of 60 ºC/min. Desorbed compounds were cryo-focused into a programmable temperature vaporization (PTV) inlet at -100 ºC for subsequent GCxGC-qMS analysis. After desorption, PTV was programmed from -100 ºC to 280 ºC (held for 10 min) at a rate of 12 ºC/sec. Injection was performed in the split-less mode. For water samples, separations were performed using DB-5 (30 m length x 0.25 mm i.d. x 1µm film thickness, Agilent Technologies) as the 1st column and SP-WAX (1m length x 0.1mm i.d. x 0.1µm film thickness, Supelco) as the 2nd column . SP-WAX was used instead of DB-WAX after being confirmed for the performance, because sample analysis needed higher temperature. The oven temperature was programmed from 40 ºC (held for 2 min) to 250 ºC at a rate of 10 ºC/min (held for 15 min), and then to 280 ºC at a rate of 10 ºC/min (held for 24 min). Helium was used as a carrier gas at 2.3 ml/min. Inlet pressure was kept at 472.6 kpa in the constant pressure mode.

Results and Discussion

Optimization of column combination for GCxGC

Five different columns were evaluated as the 2nd column in GCxGC analysis of NP mixture. Figure 1 and 2 shows contour plots of NP isomers. DB-WAX (Fig. 2) was found to be the best for NP isomers separation, because it could separate more than 100 isomers in the technical NP mixture. Also, this column combination showed a unique pattern of NP in the contour plots. This will be useful for comparing different kinds of environmental samples to elucidate sources and distributions of NP, in future studies.

Figure 2 shows contour plots and 3D view of 4-NP standards and the position of NP isomers. We have synthesized

NP isomers (NP5 and NP7), so we could chromatographically compare these isomer standards along with technical 4-NP standards. These two isomers in 4-NP standard were identified on the contour plots based on the retention time and mass spectrum of each isomer, and we calculated the NP isomer concentrations using these standards.

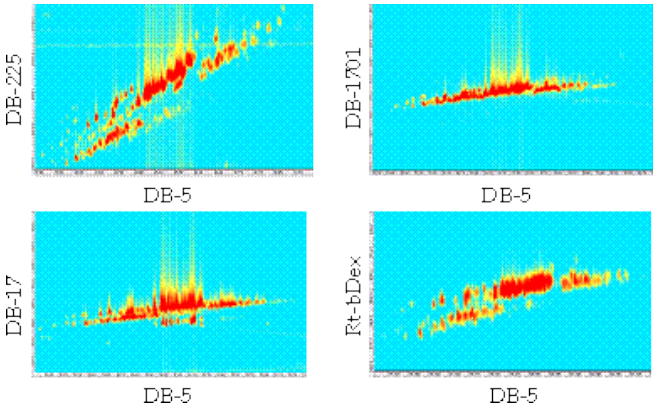

Fig.1 Contour plotsof 4-NP standard (results of 2nd column optimization)

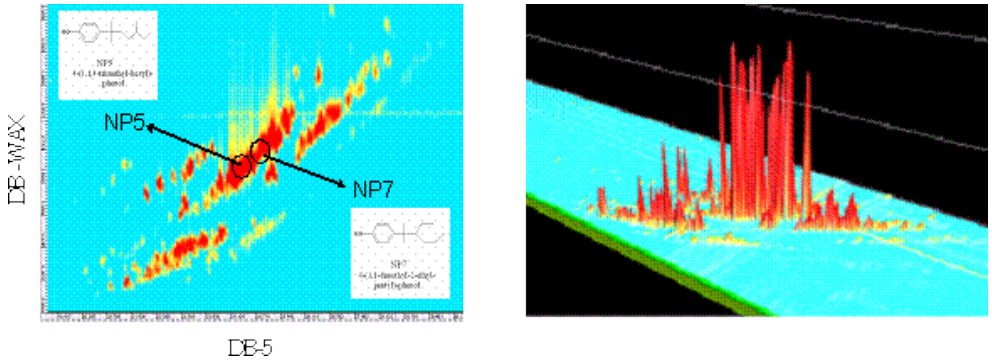

Fig.2 Contour plots and 3D view of 4-NP standard and the position of identified NP isomers

Optimization of SBSE condition

SBSE condition was evaluated as a function of extraction time, sample volume, PDMS volume and matrix effect by using Milli-Q water and river water spiked with NP at 25 pg/ml. Two hours of extraction time, 60 ml of sample volume and 48 µl of PDMS volume was selected for obtaining a maximum sensitivity. For the effect of sample matrix, 10% methanol addition compensated adsorption of NP to sample matrix (e.g. surface substances) and to glass wall of extraction vessel.

Method validation and determination of NPs in river water samples

To validate the method, a spiked Milli-Q water and river water having 5 concentration levels ranging from 5 to 100 pg/ml was analyzed The linearity was good and repeatability at 25 pg/ml was acceptable (r^2>0.995, R.S.D: less than 10%). The limit of detection (LOD) was calculated to be 0.5 pg/ml (S/N=3).

Finally, the method was applied to river water samples (Ayase and Arakawa Rivers). The contour plots of river water samples are shown in Fig.3. NP was clearly detected in both samples, and NP isomers were clearly separable. Determination of NP5 and NP7 was carried out by 5 point internal standard calibration. Table 1 shows the results of the concentration of NP in the Ayase River water. These results were compared with the results of JIS (Japan Industrial Standard) method. A good correlation was found between the results of the two methods.

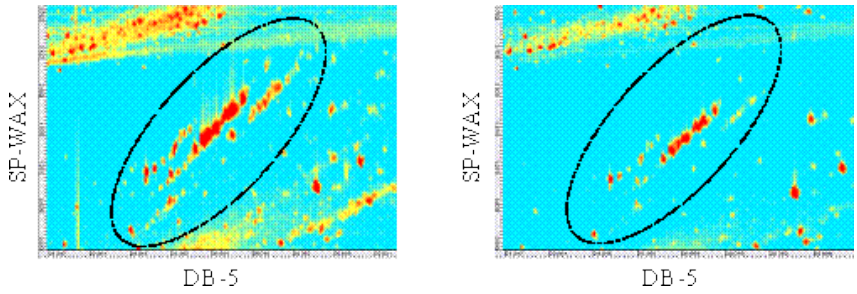

Fig.3 Contour plots of NP analysis in river water samples (left:Ayase, right:Arakawa)

Table 1 Concentrations of NP isomers in the Ayase River Water

	Internal standard ($^{13}C_4$)	Internal standard ($^{13}C_6$)
NP5	67	68
NP7	25	26

*ng/L

Acknowledgements

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