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Application News

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Gas Chromatography Mass Spectrometry

High-Sensitivity Analysis of Nonylphenol in River Water Using GC-MS/MS

Nonylphenol (NP) is used as a raw material for the production of surfactants, and as an antioxidant used to protect rubber and plastics, etc. However, in recent years, it has been specified as a substance that can cause endocrine disruption in the environment (environmental hormone).
NP, a type of alkylphenol, can theoretically exist as 211 structural isomers. Among these, the main component that is generated by the reaction of nonene (trimer of propylene) with phenol is the branched 4-nonylphenyl (4-NP).
Analysis is conducted by solid phase extraction – gas chromatography – mass spectrometry, and quantitation is conducted by (1) establishing the composition ratio of the 13 isomers included in a 4-NP standard mixture, (2) calculating the concentration of each of the detected 13 isomers using a calibration curve generated for each isomer, and (3) multiplying each isomer by the corresponding composition ratio, and calculating the sum.
However, when conducting GC/MS measurement of each isomer separately, the type of the analytical column or the instrument sensitivity may adversely affect the peak of the low-composition-ratio twelfth isomer.
Therefore, we investigated the use of a high *m/z* selectivity triple quadrupole gas chromatograph-mass spectrometer (GC-MS/MS). By optimizing the MS/MS analytical conditions, selective detection of thirteen 4-NP isomers was achieved with high sensitivity. Further, in the analysis of NP in river water, which typically contains many contaminants, analysis was possible without adversely affecting identification accuracy, even when omitting the cleanup procedure that may reduce the recovery rate.

Preparation of Standard Solution

For the nonylphenol standard mixture, we used a 4-nonylphenol standard (Code No.: 28640-96, Kanto Chemical), a 4- (3,6-dimethyl-3-heptyl) phenol-$^{13}C_6$ standard solution (Code No.: 043-32861, Wako Pure Chemical Industries), and a p-n-nonylphenol-d4 standard (Code No.: 141-07081, Wako Pure Chemical Industries).
The standard solutions for generating a calibration curve were prepared at concentrations of 0.01, 0.05, 0.1, and 0.5 µg/mL, respectively, and for all of the calibration curve standard solution series, 4- (3,6-dimethyl-3-heptyl) phenol-$^{13}C_6$ (surrogate) was prepared to obtain a concentration of 0.1 µg/mL, and p-n-nonylphenol-d4 (internal standard) was prepared to obtain a concentration of 0.1 µg/mL.

Analytical Conditions

This instrument system and the instrument parameters used are shown in Table 1, and the ions and transitions used for measurement are shown in Table 2. The GCMS-TQ8040, even as a single GC-MS, is an instrument that can perform high-sensitivity analysis. Therefore, we acquired data by switching between the GC-MS/MS and GC-MS modes.

Table 1 Analytical Conditions of GC-MS and GC-MS/MS

Triple Quadrupole Gas Chromatograph Mass Spectrometer: GCMS-TQ8040

GC		MS	
Column	: Rxi-5ms (30 m × 0.25 mm I.D., 0.25 µm)*1	Ion Source Temperature	: 230 °C
Glass Insert	: Single gooseneck liner, with wool*2	Interface Temperature	: 280 °C
Injection Port Temperature	: 250 °C	GC-MS	
Injection Mode	: Splitless	Measurement Mode	: Q3 SIM
Sampling Time	: 1 min	Event Time	: 0.3 sec
Sample Injection Volume	: 2 µL	GC-MS/MS	
Control Mode	: Linear velocity – constant (40 cm/sec)	Measurement Mode	: MRM
Oven Temperature	: 50 °C (1 min) → (8 °C/min) → 300 °C (3min)	Loop Time	: 0.3 sec
High-Pressure Injection	: 200 kPa (1.5 min)		

*1 Code No: 13423 *2 Code No: 567366

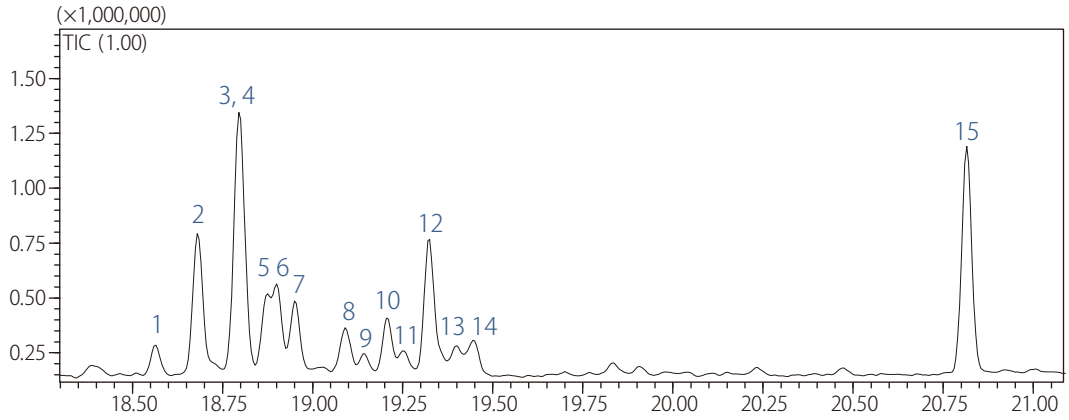
Table 2 Monitoring Ions of GC-MS and GC-MS/MS

ID#	Compound Name	GC-MS		GC-MS/MS	
		Target Ion	Ref. Ion	Target Ion	Ref. Ion
NP1	4-(2,4-dimethylheptane-4-yl) phenol	121	163	163.00 >107.10	163.00 >121.10
NP2	4-(2,4-dimethylheptane-2-yl) phenol	135	220	135.00 >107.10	135.00 >95.10
NP3	4-(3,6-dimethylheptane-3-yl) phenol	135	107	135.00 >107.10	135.00 >95.10
NP4	4-(3,5-dimethylheptane-3-yl) phenol	149	191	149.00 >107.10	149.00 >121.10
NP5	4-(3,5-dimethylheptane-2-yl) phenol	135	163	135.00 >107.10	135.00 >95.10
NP6	4-(3,5-dimethylheptane-3-yl) phenol	149	191	149.00 >107.10	149.00 >121.10
NP7	4-(3-ethyl-2-methylhexane-2-yl) phenol	135	220	135.00 >107.10	135.00 >95.10
NP8	4-(3,4-dimethylheptane-4-yl) phenol	163	121	163.00 >107.10	163.00 >121.00
NP9	4-(3,4-dimethylheptane-3-yl) phenol	149	107	149.00 >107.10	149.00 >121.10
NP10	4-(3,4-dimethylheptane-4-yl) phenol	163	121	163.00 >107.10	163.00 >121.10
NP11	4-(2,3-dimethylheptan-2-yl) phenol	135	220	135.00 >107.10	135.00 >95.10
NP12	4-(3-methyloctane-3-yl) phenol	191	163	191.00 >107.00	191.00 >121.20
NP13	4-(3,4-dimethylheptane-3-yl) phenol	149	107	149.00 >107.10	149.00 >121.10
Surr.	4-(3,6-dimethyl-3-heptyl) phenol-$^{13}C_6$	155	113	155.00 >113.10	155.00 >127.10
I.S.	p-n-nonylphenol-d4	111	224	224.00 >111.10	224.00 >98.10

Separation of 13 Nonylphenol Isomers

In the analysis of 4-nonylphenol, the composition ratio of each of the thirteen isomers must be calculated in advance using a GC-FID. We therefore conducted several analyses of a 4-NP standard mixture to investigate and determine the GC conditions which could be used to separate all of the thirteen isomers.
Factors such as the type of analytical column, linear velocity of the carrier gas, and column oven temperature program, etc. can affect the separation, and should therefore be considered.

As a result of this study, all of the thirteen isomers were separated using an Rxi-5ms analytical column (30 m × 0.25 mm I.D., 0.25 µm) and a carrier gas linear velocity of 40 cm/sec. (The analytical conditions are listed in Table 1.)
Fig. 1 shows the total ion current chromatogram obtained from measurement of a 0.5 µg/mL 4-nonylphenol standard solution using the GC-MS Q3 scan mode.



1 NP1 4- (2,4-dimethylheptane-4-yl) phenol
2 NP2 4- (2,4-dimethylheptane-2-yl) phenol
3 Surr. 4- (3,6-dimethyl-3-heptyl) phenol-$^{13}C_6$
4 NP3 4- (3,6-dimethylheptane-3-yl) phenol
5 NP4 4- (3,5-dimethylheptane-3-yl) phenol
6 NP5 4- (3,5-dimethylheptane-2-yl) phenol
7 NP6 4- (3,5-dimethylheptane-3-yl) phenol
8 NP7 4- (3-ethyl-2-methylhexane-2-yl) phenol
9 NP8 4- (3,4-dimethylheptane-4-yl) phenol
10 NP9 4- (3,4-dimethylheptane-3-yl) phenol
11 NP10 4- (3,4-dimethylheptane-4-yl) phenol
12 NP11 4- (2,3-dimethylheptan-2-yl) phenol
13 NP12 4- (3-methyloctane-3-yl) phenol
14 NP13 4- (3,4-dimethylheptane-3-yl) phenol
15 I.S. p-n-nonylphenol-d4

Fig. 1 Total Ion Current Chromatogram of 4-Nonylphenol Standard Solution (0.5 µg/mL)

Analysis of 4-Nonylphenol Standard Solution

The results of measurement of a 0.01 µg/mL 4-nonylphenol standard solution (calibration curve lowest concentration) using the GC-MS Q3 SIM mode and the GC-MS/MS MRM mode, respectively, are shown in Fig. 2. The 12th isomer (NP12), having a low composition ratio and low sensitivity, was difficult to detect using the Q3 SIM mode. Without any adverse background effect associated with the analytical column, a fifty-fold improvement in sensitivity was achieved using an optimized MRM mode.

To confirm the quantitative performance in MRM mode, repeat analyses were conducted to evaluate the analytical precision and calibration curve linearity (correlation coefficient: R) in the Q3 SIM mode and MRM mode. The results are shown in Table 3. The calibration curve linearity was excellent, with R=0.9999 or higher for all the components. In addition, good repeatability results of 6.01 % (NP12) or less were obtained.

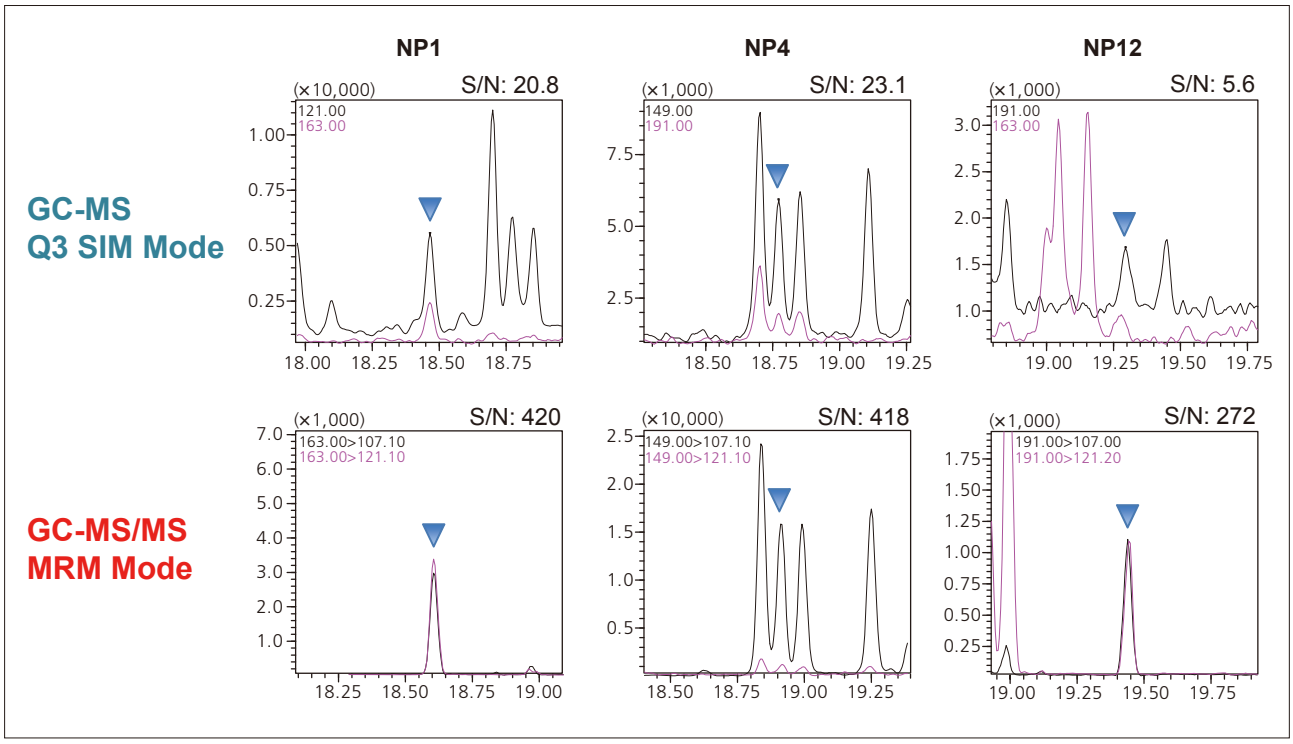


Fig. 2 SIM Chromatograms (Top) and MRM Chromatograms (Bottom) of NP1 and NP4, NP12 (0.01 µg/mL Standard Solution)

Table 3 Repeatability and Linearity of Calibration (0.01 µg/mL, n=5)

Isomer No.	GC-MS				GC-MS/MS			
	Average (µg/mL)	Standard Deviation	%RSD	Correlation Coefficient: R	Average (µg/mL)	Standard Deviation	%RSD	Correlation Coefficient: R
NP1	0.01005	0.00015	1.47	0.999999	0.01037	0.00038	3.64	0.999997
NP2	0.00980	0.00036	3.69	0.999993	0.00992	0.00014	1.42	0.999994
NP3	0.01015	0.00016	1.58	0.999999	0.01015	0.00025	2.47	0.999997
NP4	0.01037	0.00016	1.51	0.999998	0.01025	0.00024	2.34	0.999998
NP5	0.00980	0.00024	2.44	0.999999	0.00994	0.00027	2.74	0.999999
NP6	0.00986	0.00036	3.62	0.999999	0.00992	0.00032	3.23	0.999993
NP7	0.01029	0.00034	3.31	0.999992	0.00983	0.00029	2.95	0.999995
NP8	0.01033	0.00034	3.27	0.999997	0.00984	0.00021	2.15	0.999997
NP9	0.00941	0.00013	1.41	0.999992	0.01014	0.00030	2.97	0.999995
NP10	0.01034	0.00028	2.75	0.999995	0.00989	0.00010	1.05	0.999998
NP11	0.01026	0.00027	2.60	0.999996	0.01005	0.00013	1.31	0.999992
NP12	**0.01019**	**0.00077**	**7.52**	**0.999986**	**0.00985**	**0.00059**	**6.01**	**0.999954**
NP13	0.01012	0.00025	2.47	0.999999	0.01007	0.00035	3.52	0.999992

Analysis of 4-Nonylphenol in River Water

Fig. 3 shows the pretreatment process that was used for the analysis of river water. For the solid phase column, the Oasis HLB Plus Short Cartridge (Code No.: 186000132, Waters) was used, and the AQUALoader Twin SPL698T (Code No.: 6030-69804, GL Sciences) was used for high-pressure solid phase extraction. In this method, we omitted the post-elution cleanup procedure which uses silica gel.

The recovery rates for the surrogate standard (Surr.) added to purified water and actual river water samples are shown in Table 4. The recoveries were lower in the two river water samples than in the distilled water, but as they are in the 50 – 120 % range, the loss due to adsorption was assumed to be minimized at the pretreatment stage.

Next, we spiked a pretreated river water sample with the 4-nonylphenol standard solution to obtain a final concentration of 0.05 µg/mL, and then verified the effect due to the contaminant components. As shown in Fig. 4 (upper tier), when measurement was conducted using the Q3 SIM mode, identification was difficult due to the effects of co-eluting contaminants. Fig. 4 (lower tier) shows the results of measurement of these components using the MRM mode. In this case, peak identification was easy because selective separation according to *m/z* eliminated the interference due to contaminants.

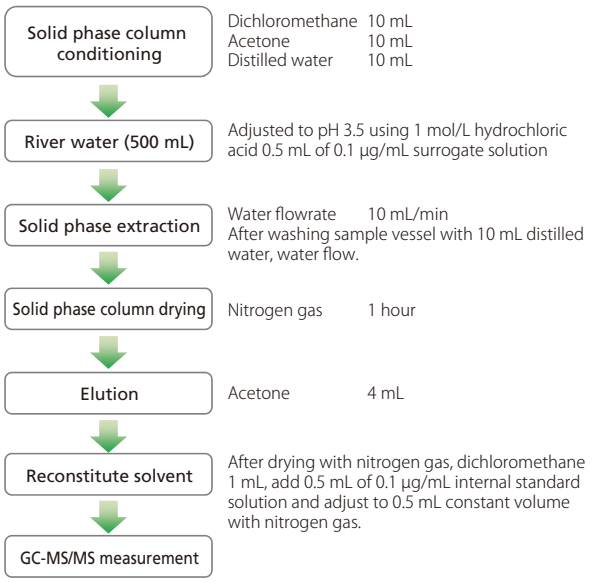


Fig. 3 River Water Sample Pretreatment Flow

Table 4 Surrogate Recovery

	Distilled Water	River Water 1	River Water 2
Recovery (%)	77.3	66.0	64.2

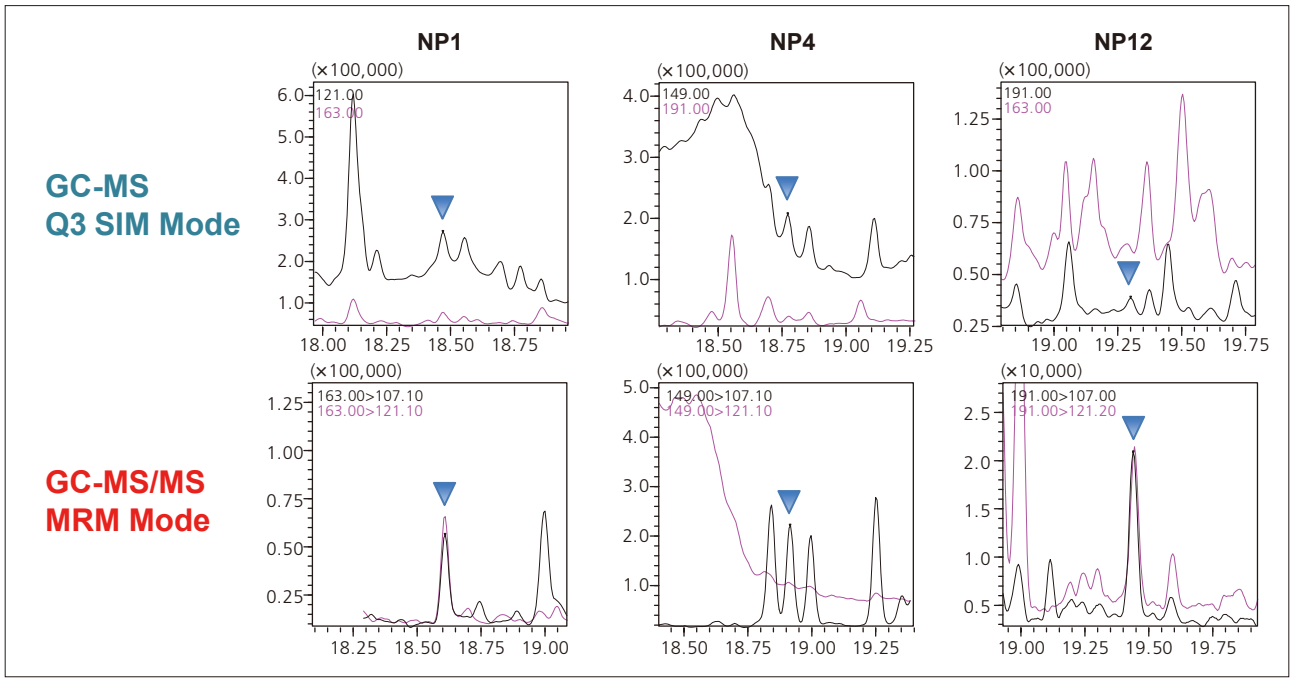


Fig. 4 SIM Chromatogram (Upper) and MRM Chromatogram (Lower) of Spiked River Water Sample

Conclusions

The sensitivity with respect to NP12 which has been problematic in isomer-specific quantitation using conventional nonylphenol analysis of water by GC-MS was improved by a factor of 50 through MRM mode optimization using GC-MS/MS.

Further, by using GC-MS/MS, peak identification, which is difficult by GC-MS due to the considerable interference from co-eluting contaminant components, can be significantly improved with real samples with the possibility of selective separation according to *m/z*. Further, even samples containing many contaminants, such as those found in river water, can be measured using a simplified pretreatment procedure without cumbersome cleanup.

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