DETERMINATION OF DICARBOXYLIC DEGRADATION PRODUCTS OF NONYLPHENOL POLYETHOXYLATES IN WATER SAMPLES BY SPE AND GC-MS AFTER METHYLATION

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Abstract

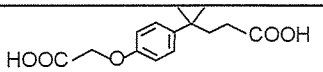
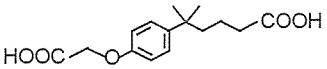
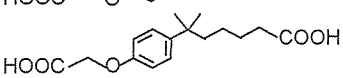
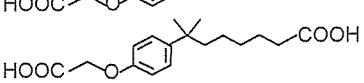
The synthesis of four selected dicarboxylic degradation products (CAPECs) of NPnEOs was successfully accomplished for the first time. The selected isomers have an α,α-dimethyl configuration (expressed as "dm" in their abbreviation), five to eight C atoms and a carboxyl group in the alkyl chain, and a carboxymethoxy acid group (dm-CA_{5-8}P1ECs). A reliable method combining SPE, derivatization and GC-CI-MS was then developed for the measurement, in river and sewage effluent water, of those selected model compounds and other dicarboxylic metabolites (CA_{5-8}P1ECs). The derivatization of terminal carboxyl groups was successful with (trimethylsilyl)diazomethane. The best extraction conditions were obtained using an Oasis HLB cartridge as a sorbent bed and 4 ml of *tert*-butyl methyl ether and methanol elution mixture (MTBE/methanol (9:1, v/v)). The method detection limits of 0.03-0.07 μg/l for dm-CA_{5-8}P1ECs were attained in 500 ml pure water. The high recoveries of typically > 89% for each isomer (in pure water, river water and sewage effluent water) indicated the high performance of the method. Although dm-CA_{5-8}P1ECs were not detected in the collected water samples, 21 isomers of CA_{5-8}P1ECs were identified by CI-MS and the tentative structures of six out of them were elucidated, mainly limited to the branch at α-C atom, by studying the EI-mass spectra. The relative concentrations of individual CA_{5-8}P1EC metabolites were calculated based on dm-CA_{5-8}P1ECs. The results showed that the main degradation on the nonyl chain occurred via the elimination of two carbon-units and the concentrations in Japan were much lower than those in Taiwan and Italy.

Keywords: Derivatization, Dicarboxylic degradation products, GC, nonylphenol polyethoxylates, SPE, water analysis.

1. Introduction

The dicarboxylic degradation products (CAPECs) of nonylphenol polyethoxylates (NPEOs) have been recently recognized to occur in different environmental compartments [1-10] at relatively high, microgram per liter, levels [1-6, 9]. Particularly, those CAPECs that contain 5-8 C atoms and a carboxyl group in the alkyl chain are believed to be the most commonly occurring metabolites in an aqueous environment, as they appeared to be extremely persistent species [3-5]. However, hindered by the lack of authentic standards and effective analytical methods, the literature on the occurrence and behavior of the CAPECs in the environment is still rather scarce compared with other degradation metabolites such as nonylphenols (NP), short chain NPEOs and NPECs.

Table 1. Structures and abbreviations of synthesized CA_{5-8}P1ECs in this study

No.	Chemical name	Abbreviation	Structure
1	4-(4'-carboxy-2'-methyl-2'-butyl)phenoxyacetic acid	dm-CA_5P1EC	
2	4-(5'-carboxy-2'-methyl-2'-pentyl)phenoxyacetic acid	dm-CA_6P1EC	
3	4-(6'-carboxy-2'-methyl-2'-hexyl)phenoxyacetic acid	dm-CA_7P1EC	
4	4-(7'-carboxy-2'-methyl-2'-heptyl)phenoxyacetic acid	dm-CA_8P1EC	

In this study, we reported the development of four model CAP1ECs that are characterized by an α,α-dimethyl configuration, 5-8 C atoms and a carboxyl group in the alkyl chain, and an ethoxy acetic acid group (Table 1). The mass spectra of the model compounds from the GC-MS by applying electron ionization (EI) and isobutane chemical ionization (isobutane CI) were extensively interpreted. In addition, the development of a reliable analytical method combining SPE, derivatization, and GC-isobutane CI-MS for the determination of dm-CA_{5-8}P1ECs in water was described. The method was validated for pure water, river water, and sewage effluent water and then was applied for the determination of the corresponding CA_{5-8}P1EC metabolites. Besides, the structural elucidation of the detected CA_{5-6}P1EC isomers other than dm-CA_{5-8}P1ECs by EI mass spectral interpretation was attempted.

2. Materials and methods

Unless otherwise noted, all reagent grade chemicals and solvents for the synthesis were purchased from Wako (Osaka, Japan), TCI (Tokyo, Japan) and Kanto Chemical (Tokyo, Japan), and used without further purification. *N,O*-Bis(trimethysilyl)acetamide (BSA) and (trimethylsilyl)diazomethane (2 M in hexane) were from Wako and Aldrich (Milwaukee, WI, USA), respectively. The methyl alcohol-d_4 (CD_3OD) and pyrene-d_{10} were from Isotec (USA) and Kanto Chemical, respectively. The silica gel [BW-127ZH (100-270 mesh)] was from Fuji Silysia (Aichi, Japan). All glassware was rinsed with purified water and pesticide grade solvents before used.

River water samples were collected from the Neya River (Oct. 2004) and Kanzaki River (Nov. 2004) in Osaka, Japan, where high concentrations of the short ethoxy chain NPnEOs degradation products have been measured. The effluent water was collected from the Minami-Suita Sewage Treatment Plant (Minami-Suita STP) (Nov. 2004) in Osaka, Japan, one of the water treatment plants located upstream from the sampling point at the Kanzaki River. Six-liter water samples were collected and shipped within a period of 1 h to the laboratory. Upon arrival, the samples were immediately filtered by 0.45 μm HA membrane filters (Millipore, USA), adjusted to pH ~ 3 using 5 M HCl (Wako), and stored at 4 °C until analysis.

3. Results and discussion

Synthesis of dm-CA_{5-8}P1ECs

NP in the environment is a mixture of isomers of the nonyl group. Based on the high-resolution GC analysis of NP, Wheeler *et al.* [11] recognized that isomers having an α,α-dimethyl structure in the alkyl chain were predominant (accounting for 48.6% of the total para-isomers). Furthermore, the α,α-dimethyl structure was reported to have a relatively high estrogenic activity among the isomers of NP [12]. Similar to NP, CAP1ECs have been recognized as the metabolites of NPnEOs in the environment via oxidation of the ethoxy and alkyl side chains [7, 10]. Therefore, CAP1ECs are expected to encompass isomeric mixtures of branched carboxyalkyl structures that are impossible to presently prepare in the laboratory. The information on the predominance and estrogenicity of the α,α-dimethyl structure of NP prompted us to synthesize this structural type of CAP1ECs to aid in the more accurate determination of these metabolites in the environment. Because of their similar structures (only the number of carbon atoms in the alkyl chain varied), the four model CAP1ECs were effectively synthesized by the same reaction sequence as shown in Figure 1. The products were purified by column chromatography on silica gel, and their structures and purities were confirmed by GC-MS and ^1H- and ^{13}C-NMR.

EI-Mass spectra of dm-CA5-8P1ECs

For all the trimethylsilyl and methyl derivatives, the most significant ions were at m/z = 265 and 207, respectively, corresponding to the α,α-dimethyl structures via the benzylic cleavage of the carboxyalkyl chain (Figure 2). The other fragment ions showed very low intensities (less than 5% abundance). The fragmentation pathway of the dm-CA_{5-8}P1ECs was similar to those reported for the metabolites such as NP, short ethoxy chain NPnEOs and NPnECs as reported by Wheeler *et al.* [11]. The mass spectra interpretation of the dm-CA_{5-8}P1ECs agreed well with those of the CAPEC metabolites mainly extracted from different environmental samples in previous reports [1, 2, 4, 5, 13].

Isobutane CI-mass spectra of dm-CA5-8P1ECs

Table 2 shows the major fragment ions and their abundances for the methyl and trimethylsilyl dm-CA_{5-8}P1ECs upon analyzing 500 μg/l standard solutions by isobutane CI-MS. Interestingly, the abundances of the ions resulting from the same derivatization and fragmentation were not united except for **D** (100% abundance) in the trimethylsilylation and **B** (100% abundance) in the methylation although the only structural difference was the length of the alkyl chain. From the resulting fragment ions, the fragmentation pathway of the dm-CA_{5-8}P1ECs was proposed as shown in Figure 2. The fragmentation included a proton transfer chemical ionization and simultaneously occurring electron ionization. These results confirmed the fragmentation pathway interpreted by Ding and Tzing [2] except for the formation of ions **E**.

Derivatization conditions

Many factors such as reaction time, medium, derivatizing reagent, and water content were reported to affect the complete derivatization of the NPnEOs degradation products; therefore, the initial work involved optimizing the derivatization reaction and verifying complete derivatization.

The derivatization of dm-CA_{5-8}P1ECs (at 500 μg/l concentrations) was initially attempted with 50 μl of BSA or (trimethylsilyl)diazomethane, which was reported to be effective for the derivatization of the carboxylic degradation products [14-16]. The media were methyl acetate (in the case of BSA) and a mixture of MTBE/methanol (9:1, v/v) (in the case of (trimethylsilyl)diazomethane), similar to the eluants used for eluting target compounds, including carboxylic acids, from a sorbent bed (as described by Hu *et al.* [17]. Figure 3 shows the time profile of the derivatization of dm-CA_{5-8}P1ECs with BSA (a) and (trimethylsilyl)diazomethane (b). The methyl derivatives formed by the use of (trimethylsilyl)diazomethane obviously showed higher responses than the trimethylsilyl derivatives formed by the use of BSA; though the completion of the derivatization using (trimethylsilyl)diazomethane took longer time (2 h) than that using BSA (about 1 h). The yields of the derivatized samples were observed to be unchanged at 4 °C for as long as 20 days.

The amount of reagent and amount of water in the medium were not critical to the complete derivatization with (trimethylsilyl)diazomethane (Figure 4). Similar amount of methyl derivatives were recognized when various amounts of (trimethylsilyl)diazomethane (20, 50, and 100 μl) were used. In addition, the same results were obtained when a series of derivatization reaction was conducted with (trimethylsilyl)diazomethane in the presence of 0.1 to 10% water and water free. These revealed a significant advantage of the derivatization of dm-CA_{5-8}P1ECs with (trimethylsilyl)diazomethane over BSA (the reaction of the carboxylic degradation products (such as NPnECs) with BSA could be easily quenched in the presence of water).

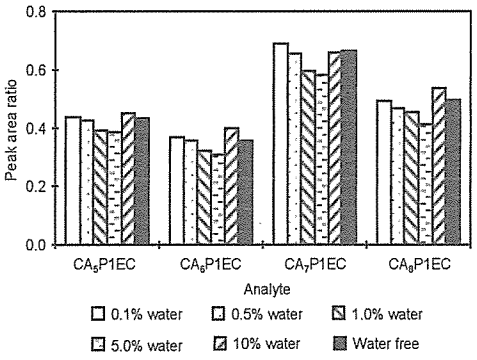


Figure 4. Effect of water content on the methylation of analytes.

The obtained results made us choose (trimethylsilyl)diazomethane as the derivatization reagent, and the amount of 50 μl was selected for the derivatization over a period of 2 h.

Quantitation

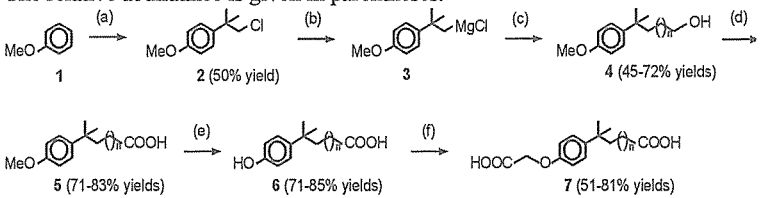
The quantitative analysis was performed by the internal standard method using the sum of peak areas of two ions of the target compound (in bold, Table 2) and the peak area of base peak ion m/z 213 of pyrene-d_{10}. Seven-point calibration curves of each of the dm-CA_{5-8}P1ECs over the established concentration range (0.01-1.0 μg/ml for dm-CA_{5-6}P1ECs and 0.02-2.0 μg/ml for dm-CA_{7-8}P1ECs) gave good fits ($R^2 > 0.995$) (Table 3).

Table 2. Major fragment ions and their relative abundances of dm-CA_{5-8}P1ECs in CI-MS

CAP1EC	Trimethylsilyl derivative								Methyl derivative							
	MW	A	B	C	D	E	F	G	MW	A	B	C	D	E	F	G
dm-CA_5P1EC	410		187	97	321		410	265	294		**129**	97	**263**		294	207
			(45)	(5)	(100)		(5)	(25)			(100)	(5)	(68)		(7)	(20)
dm-CA_6P1EC	424	425	201	111	335			265	308		**143**	111	**277**		308	207
		(4)	(78)	(5)	(100)			(31)			(100)	(8)	(8)		(4)	(19)
dm-CA_7P1EC	438	439	215	125	349	291		265	322		**157**	125	**291**	233		207
		(3)	(65)	(14)	(100)	(43)		(29)			(100)	(14)	(11)	(7)		(22)
dm-CA_8P1EC	452	453	229	139	363	305		265	336		**171**	139	**305**	247		207
		(3)	(9)	(6)	(100)	(22)		(28)			(100)	(17)	(89)	(23)		(30)

All entries are the m/z values.

The relative abundance is given in parentheses.



Key: (a) H_2SO_4 (50 mol%), $CH_2=C(CH_3)CH_2Cl$ (0.5 equiv.), 20 °C, 12 h; (b) CH_3I (300 µl), Mg turnings (1.3 equiv.), THF, reflux, 6 h; (c) CuI (0.65 mol%), $Br(CH_2)_{n+1}OH$ (0.4 equiv.), THF, reflux, 4 h; (d) Jones reagent (CrO_3 (2 equiv.) and H_2SO_4/H_2O), acetone, room temperature, 10 h; (e) HBr/AcOH (4 equiv.), 60 °C, 60 h; (f) NaOH (10 equiv.), $ClCH_2COOH$ (5 equiv.), ethanol, reflux, 8 h; n = 1 – 4.

Figure 1. Reaction sequence for the synthesis of dm-CA_{5-8}P1ECs.

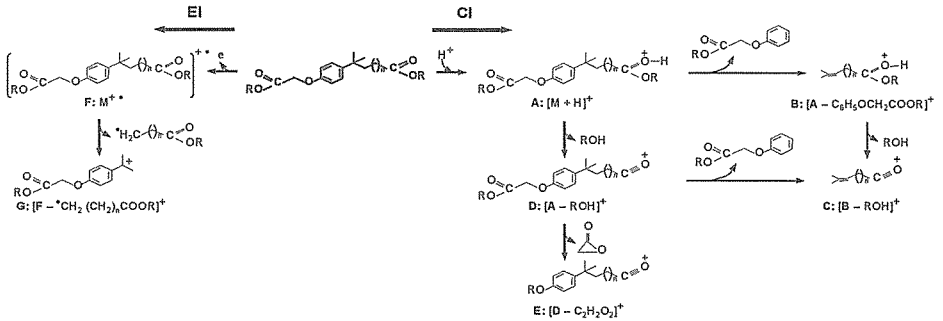


Figure 2. General scheme for the fragmentation of dm-CA_{5-8}P1ECs in CI-MS (n = 1-4; R = methyl or trimethylsilyl).

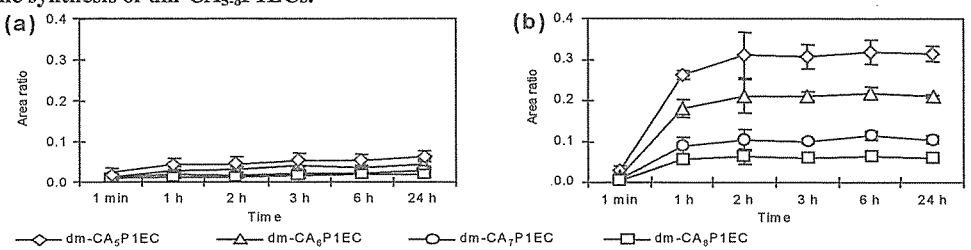


Figure 3. Time profile of the derivatization with BSA (a) and (trimethylsilyl)diazomethane (b) (error bars indicate standard deviations determined from triplicate samples; area ratio was calculated by the sum of peak areas of characteristic ions (in bold, Table 2) of individual CA_{5-8}P1ECs divided by the peak area of base peak at m/z 213 of pyrene-d_{10}).

Table 3. Linear regression parameters for calibration curves and method detection limits

Compound	Concentration range (μg/ml)	Slope	Intercept	R^2	MDL (μg/l)
dm-CA_5P1EC	0.01-1.0	4.72	- 0.008	0.999	0.03
dm-CA_6P1EC	0.01-1.0	5.46	0.001	0.995	0.03
dm-CA_7P1EC	0.02-2.0	4.47	- 0.049	0.998	0.06
dm-CA_8P1EC	0.02-2.0	2.09	- 0.024	0.997	0.07

Solid-phase extraction

Initial work involved a test with two types of SPE cartridge in accordance with two corresponding well-developed methods for the determination of NPnEOs degradation products (an Oasis HLB cartridge using MTBE/methanol (9:1, v/v) elution mixture [17] and a Bond Elut C18-HF cartridge (Varian, 500 mg, 3ml) using methyl acetate eluant. The Oasis HLB cartridge showed high recoveries (> 80%) for various volumes (100, 200, and 500 ml) of spiked pure water and Neya River water samples. Similar range of sample volumes was used for evaluating the performance of Bond Elut C18-HF cartridge; however, low recoveries of dm-CA_{5-8}P1ECs (from 36 to 72%, in average) were recognized for the 500 ml Neya River sample (Table 4). The wide range retention of organic compounds, including the amount of clogging, on the Bond Elut C18-HF could be the reason for increasing the chance of target compound breakthrough and limiting the sample volume.

Table 4. Recoveries of analytes from different volumes of spiked Neya River sample using C18-HF

Sample volume (ml)	Recovery (%)			
	dm-CA_5P1EC	dm-CA_6P1EC	dm-CA_7P1EC	dm-CA_8P1EC
100 ml	97 (12)	116 (11)	113 (12)	92 (15)
200 ml	84 (11)	94 (7.1)	91 (9.3)	94 (7.2)
500 ml	70 (14)	72 (23)	66 (5.6)	36 (20)

The relative standard deviation (R.S.D.) is given in parentheses (n = 3).

Spiked level = 200 ng/l (except for CA_7P1EC + CA_8P1EC = 400 ng/l).

In addition, the color of the extract and the background level of the mass chromatogram were considered as indicators of the success of the extraction. A colorless extract and a lower background in the mass chromatogram of a sample extracted by an Oasis HLB cartridge compared with the brown-yellow extract and higher background in the mass chromatogram by the Bond Elut C18-HF (data not shown) indicated the advantage of the extraction conditions using the Oasis HLB cartridge and MTBE/methanol mixture. For these reasons, the Oasis HLB cartridge and the MTBE/methanol elution mixture were selected, and the remaining results discussed hereafter were obtained using these extraction conditions.

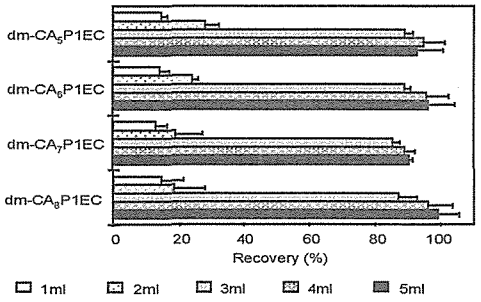


Figure 5. Recoveries of dm-CA_{5-8}P1ECs at different elution volumes (error bars indicate standard deviations determined from triplicate samples).

Two-ml samples of pure water spiked with 250 μg/l target compounds (except for dm-CA_{7-8}P1ECs = 500 μg/l) were used to determine the optimum elution volume. The average recoveries of target compounds at different elution volumes are shown in Figure 5. A volume of 4 ml was sufficient to elute most of the dm-CA_{5-8}P1ECs from the cartridge (89-96% recoveries).

Method validation

The performance of the analytical method was tested by examining the recoveries of dm-CA_{5-8}P1ECs at two concentration levels, 0.20 μg/l (except for dm-CA_{7-8}P1ECs = 0.40 μg/l) and 4.0 μg/l (except for dm-CA_{7-8}P1ECs = 8.0 μg/l), from pure water, river water and sewage effluent water. The two spiked levels

were chosen as they were considered representative of the level that could be found in river and sewage effluent water in Japan (μg/l level) and were above the expected method detection limit. The entire analytical procedure of extraction, derivatization, and GC-MS analysis as described in the experimental section was performed in five replicates for each water matrix.

The initial test was performed with pure water. The high recovery (94-98 and 89-100% for the 0.20 and 4.0 μg/l spiked samples, respectively) and the low relative standard deviation values (less than 10%) indicated the high performance of the method (Table 5). The method detection limits were relatively low, ranging from 0.03 to 0.07 μg/l (Table 3).

The method performance was then tested by examining the recoveries of dm-$CA_{5\text{-}8}$P1ECs from the river and sewage effluent water matrix (Neya River and Minami-Suita STP). The high recovery and low relative standard deviation obtained for both spiked concentration levels (Table 5) indicated that this method is possible application to real environmental samples.

Table 5 Recoveries (%) of dm-$CA_{5\text{-}8}$P1ECs spiked into different water samples and their average concentrations (μg/l)

Sample	dm-CA_5P1EC	dm-CA_6P1EC	dm-CA_7P1EC	dm-CA_8P1EC
Pure water				
Recovery from 0.20 μg/l-spiked sample	94 (7.4)	98 (4.2)	98 (5.9)	95 (7.9)
Recovery from 4.0 μg/l-spiked sample	89 (8.2)	93 (4.9)	100 (6.3)	97 (9.3)
Neya River water				
Average concentration	n.d.	n.d.	n.d.	n.d.
Recovery from 0.20 μg/l-spiked sample	94 (7.3)	106 (5.5)	99 (9.8)	110 (5.9)
Recovery from 4.0 μg/l-spiked sample	98 (7.9)	96 (11)	102 (7.8)	99 (8.3)
Minami-Suita STP effluent water				
Average concentration	n.d.	n.d.	n.d.	n.d.
Recovery from 0.20 μg/l-spiked sample	84 (11)	94 (7.1)	91 (9.3)	94 (7.2)
Recovery from 4.0 μg/l-spiked sample	89 (7.3)	93 (7.7)	90 (10)	101 (9.1)

The relative standard deviation (R.S.D.) is given in parentheses (n = 5).

n.d. = Not detected.

Analysis of $CA_{5\text{-}8}$P1ECs based on the corresponding dm-$CA_{5\text{-}8}$P1EC isomers

Because the protonated molecular ions of the dm-$CA_{5\text{-}8}$P1ECs were absent under CI-MS conditions, the characteristic ions indicated in bold in Table 2 were used for the identification of unknown $CA_{5\text{-}8}$P1EC isomers in the collected samples. The characteristic ions could provide useful information on the carbon numbers in the carboxyalkyl chain and the identification of unknown isomers would be easily performed if they give similar mass spectra to the corresponding dm-$CA_{5\text{-}8}$P1ECs. For example, the peaks having ions m/z 129 and 263 were assigned as originated from CA_5P1EC isomers.

Because authentic standards of the isomeric mixtures of $CA_{5\text{-}8}$P1EC metabolites are not available, the GC-CI-MS analytical method developed for dm-$CA_{5\text{-}8}$P1ECs would give more advantage than the GC-EI-MS when used for the measurement of $CA_{5\text{-}8}$P1EC metabolites. The individual isomeric mixtures of the $CA_{5\text{-}8}$P1EC metabolites could not be distinguished using GC-EI-MS unless they were clearly separated by the GC system. The concentrations of the $CA_{5\text{-}8}$P1ECs in the collected samples could be calculated based on the dm-$CA_{5\text{-}8}$P1ECs, and the detection limits of dm-$CA_{5\text{-}8}$P1ECs could be assumed to be the same for the $CA_{5\text{-}8}$P1ECs. In addition, in the routine analysis of $CA_{5\text{-}8}$P1ECs, dm-$CA_{5\text{-}8}$P1ECs could be used as surrogates because they appeared to be absent in the environment.

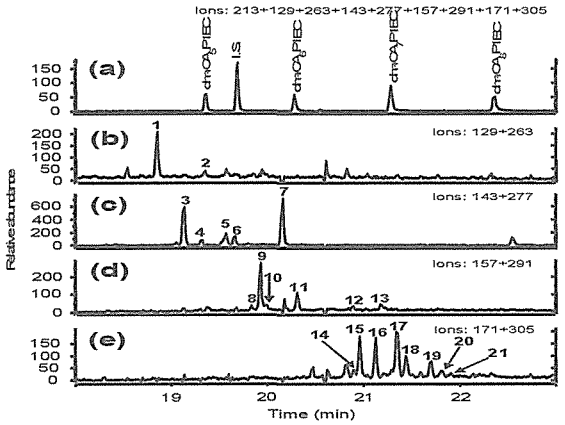


Figure 6. CI selected ion chromatograms of dm-$CA_{5\text{-}8}$P1ECs from standard solution (a) and isomers of CA_5P1EC (b), CA_6P1EC (c), CA_7P1EC (d), and CA_8P1EC (e) from Neya River.

Figures 6b-e show the CI selected ion chromatograms of the Neya River sample. Characterized by the same ions as the dm-CA_{5-8}P1ECs (except for the ion m/z 207 that was absent from some detected isomers), two isomers of the CA_5P1EC metabolite, five isomers of the CA_6P1EC metabolite, six isomers of the CA_7P1EC metabolite, and eight isomers of the CA_8P1EC metabolite were recognized (Figure 6). Surprisingly, 21 similar isomers were detected in the Kanzaki River and Minami-Suita STP effluent water.

In this study, the concentration of an isomer was calculated by assuming the same response factor between that isomer and the corresponding dm-CA_{5-8}P1EC isomer. The relative concentration of each CA_{5-8}P1EC metabolite was then obtained by summing the concentrations of individual isomers (except for isomers **8**, **10**, **12**, and **14** giving lower concentrations than the MDLs calculated for the corresponding dm-CA_7- and dm-CA_8P1EC). Table 6 shows the relative concentrations of CA_{5-8}P1EC metabolites in the collected samples. To our knowledge, this is the first report on the level of dicarboxylic degradation products in river water and STP effluent water in Japan.

The CA_6P1EC and CA_8P1EC metabolites were relatively dominant (at μg/l level), about 2 to 15 times higher than those of CA_5P1EC and CA_7P1EC, indicating that the main degradation on the nonyl chain occurs via the elimination of two carbon-units [2]. The concentrations of CA_{5-8}P1ECs from the Neya River were higher than those from the Kanzaki River, similar to other NPnEOs degradation products [18].

Investigating the behavior of NPnEOs in 40 full-scale STPs in Japan, Fujita *et al.* [16] reported the significant removal of NPnEOs and degradation products from a water phase along different treatment stages (approximately 60% after biological treatment and 70% after full treatment). The relatively high concentrations of CA_{5-8}P1EC metabolites in river water and STP effluent water recognized from this study allowed me to conclude that the dicarboxylic degradation products should play an important role in studying the behavior of NPnEOs in these STPs in addition to other factors such as mineralization and absorption into activated sludge flocs.

In order to evaluate the extent of contamination by CA_{5-8}P1ECs in Japan, the concentrations obtained in this study were compared with those from other countries where the data are available. The concentrations of CA_{5-8}P1ECs from the investigated rivers and STPs in Japan showed a much lower level, about one order of magnitude lower than those in Italy and one-to-two orders of magnitude lower than those in Taiwan.

Table 6 Relative concentrations (μg/l) of individual CA_{5-8}P1EC metabolites in collected water samples and their comparison with those from other countries

Sample	Location	CA_5P1EC	CA_6P1EC	CA_7P1EC	CA_8P1EC	Reference
Neya River	Osaka (Japan)	0.21 (5.4)	2.69 (7.2)	0.62 (6.7)	1.57 (4.1)	Present study
Kanzaki River	Osaka (Japan)	0.12 (6.3)	1.63 (8.1)	0.40 (8.1)	1.23 (10)	Present study
Minami-Suita STP effluent	Osaka (Japan)	0.14 (8.9)	2.05 (5.0)	0.44 (4.8)	1.82 (6.5)	Present study
Lao-Jie River	Chung-Li (Taiwan)		138		100	[2]
STP effluent	Taipei (Taiwan)		27		15	[2]
STP effluent (Cobis)	Rome (Italy)	2.4	7.7	2.3	12	[19]

The relative standard deviation (R.S.D.) is given in parentheses (n = 5).

Structural elucidation of CA_{5-8}P1EC isomers detected in environmental samples

As mentioned, 21 isomers of the CA_{5-8}P1EC metabolites were detected in the collected samples using CI-MS. Given the same characteristic ions as dm-CA_{5-8}P1ECs (in bold, Table 2), their obtained CI-MS spectra did not give enough information for the structural elucidation of the carboxyalkyl chain. Similar circumstance was recognized when CI-MS-MS was used; the detected isomers having the same carboxyalkyl carbon number gave the same fragment ions as produced by the corresponding dm-CA_5-, -CA_6-, -CA_7-, or -CA_8P1EC isomer when either one of the characteristic ions (in bold, Table 2) was used as a precursor.

However, an attempt at EI-mass spectral interpretation aided in the elucidation of the carboxyalkyl chain structure of six isomers detected in the Neya River sample, though mainly limited to the structure of branch

at α-C atom (Figure 7). The peaks of the $CA_{5-8}P1EC$ isomers in the EI-total ion chromatogram were identified by comparing their retention time with those in the corresponding CI-selected ion chromatogram. Similar to the other NPnEOs degradation products, the benzyl cleavage of the carboxyalkyl chain in $CA_{5-8}P1EC$ isomers was favorable, and the formation of a substituted tropylium ion at m/z 179 was specific.

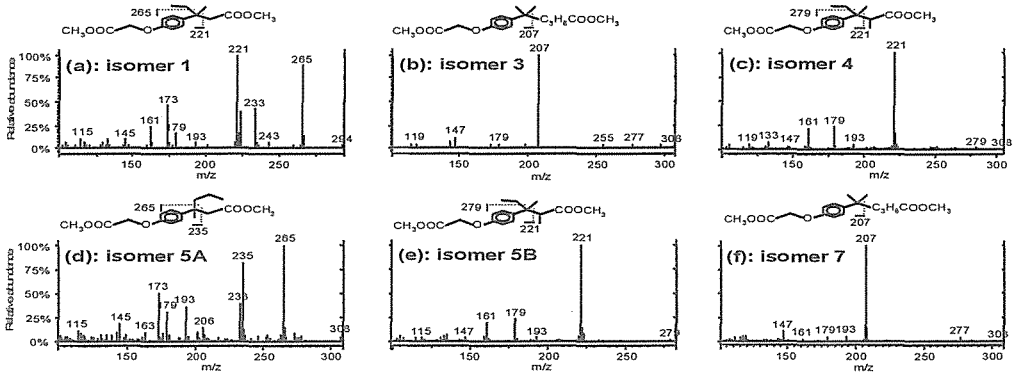


Figure 7. Mass spectra and tentative structures of $CA_{5-8}P1EC$ isomers detected in Neya River sample.

4. Conclusions

The Oasis HLB cartridge and MTBE/methanol (9:1, v/v) elution mixture were recognized to be applicable for the extraction of the CAPECs from the river and STP effluent water samples. (Trimethylsilyl)diazomethane shows advantage for the derivatization of the targets compared to the BSA. 21 isomers of the $CA_{5-8}P1EC$ metabolites were found in the Neya River, Kanzaki River, and effluent water of the Minami-Suita Sewage Treatment Plant when CI-MS was used; and the tentative structures of six out of them were elucidated, mainly limited to the branch at α-C atom, by interpreting the EI-mass spectra. The successful synthesis of four model dm-$CA_{5-8}P1ECs$ could play an important role in the studies of CAPECs, on which the report is relatively rare compared to those on other NPnEOs degradation products. Based on dm-$CA_{5-8}P1ECs$, the relative concentrations of $CA_{5-8}P1EC$ metabolites could be more correctly determined, offering advantages in the future investigations as well as analytical studies of these compounds and other $CA_{5-8}P1EC$ metabolites. Similar to the case of short ethoxy chain NPnEOs degradation products and their halogenated derivatives, further development of this method so that it can be used for other environmental matrix such as sediment is essential. The study on the sorption of $CA_{5-8}P1ECs$ onto activated sludge is also essential as this kind of NPnEOs degradation products are recognized predominant; it could be evaluated based on the results on the sorption of dm-$CA_{5-8}P1ECs$.

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