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Determination of nonylphenol isomers in landfill leachate and municipal wastewater using steam distillation extraction coupled with comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry

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ABSTRACT

4-Nonylphenols (4-NPs) are known endocrine disruptors and by-products of the microbial degradation of nonylphenol polyethoxylate surfactants. One of the challenges to understanding the toxic effects of nonylphenols is the large number of isomers that may exist in environmental samples. In order to attribute toxic effects to specific compounds, a method is needed for the separation and quantitation of individual nonylphenol isomers. The pre-concentration methods of solvent sublimation, solid-phase extraction or liquid–liquid extraction prior to chromatographic analysis can be problematic because of co-extraction of thousands of compounds typically found in complex matrices such as municipal wastewater or landfill leachate. In the present study, steam distillation extraction (SDE) was found to be an effective pre-concentration method for extraction of 4-NPs from leachate and wastewater, and comprehensive two-dimensional gas chromatography (GC × GC) coupled with fast mass spectral data acquisition by time-of-flight mass spectrometry (ToFMS) enhanced the resolution and identification of 4-NP isomers. Concentrations of eight 4-NP isomers were determined in leachate from landfill cells of different age and wastewater influent and effluent samples. 4-NP isomers were about 3 times more abundant in leachate from the younger cell than the older one, whereas concentrations in wastewater effluent were either below detection limits or <1% of influent concentrations. 4-NP isomer distribution patterns were found to have been altered following release to the environment. This is believed to reflect isomer-specific degradation and accumulation of 4-NPs in the aquatic environment.

1. Introduction

Technical nonylphenol (tNP), ~86–94% of which is composed of 4-nonylphenols (4-NPs), is a complex mixture [1]. 4-NPs are known endocrine disruptors and are by-products of the microbial degradation of nonylphenol polyethoxylates, widely used nonionic surfactants [2]. Including all stereoisomers, there are 550 possible 4-NPs due to branching of the nonyl group. However, tNP is believed to contain less than ~30 4-NP isomers [1].

Previous investigators have reported 4-NPs in complex environmental matrices such as wastewater effluent and landfill leachate [3,4]. The composition of 4-NP isomers in the environment can vary due to variations in the compositions of tNPs from different manufacturers [1,5,6] or because of differences in degradation rates [7]. The transformation of 4-NP isomers by certain NP-degrading microorganisms has been correlated with their α-substitution pattern [8]. Moreover, the estrogenic potency of individual nonylphenol isomers is dependent upon the structure of the alkyl group [8–11]. To understand the environmental fate and toxic effects of nonylphenols, an isomer-specific method for determination of 4-NPs is required [12].

The similar physico-chemical properties of nonylphenol isomers make gas chromatographic separation difficult. Co-elution of isomers can lead to bias during quantitation of individual 4-NPs by selected ion monitoring (SIM) GC/MS analysis [13]. The presence of some 4-NP isomers in tNP has been confirmed [5,14,15], and several isomers have been used for bioassay and environmental analyses [7,11]. Thousands of compounds can be co-extracted from landfill leachate and municipal wastewater by conventional methods such as solvent sublimation, solid-phase extraction or liquid–liquid extraction. This can make identification and accurate quantitation of nonylphenol isomers difficult. Moreover, we found the cartridges were often blocked or serious emulsification during SPE (solid phase extraction) or LLE (liquid–liquid extraction) processes due to the worst case matrices of influent and landfill leachate [16,17]. To solve them, filtration processes or diluted

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sample are often demanded, which could bring errors for NP isomers determination. Steam distillation extraction (SDE) can minimize the co-extraction of low volatility high molecular weight compounds (such as lipids) [18] and has been used successfully to pre-concentrate nonylphenols in environmental samples [19].

Comprehensive two-dimensional gas chromatography (GC × GC) is well suited for monitoring complex mixtures of hydrophobic organic contaminants in environmental matrices (e.g. polychlorinated biphenyls, polychlorinated dibenzodioxins and furans, and pesticides) [20]. Recently, GC × GC/ToFMS has been proposed as a promising technology for the isomer-specific determination of 4-NPs [12]. Ieda et al. [21] reported 102 peaks in tNP and measured two 4-NP isomers in river water samples by GC × GC/MS. Moeder et al. [22] applied GC × GC/ToFMS to improve the separation of 4-NP isomers and their biodegradation products. Recently, Eganhouse et al. [1] demonstrated a significant advantage of GC × GC/ToFMS over GC/MS for the quantitation of certain 4-NP isomers and described the potential of this method to reveal previously unrecognized 4-NPs.

In our previous work, optimal conditions for characterization of tNP using GC × GC/ToFMS were established [1]. The objective of this study was to investigate the feasibility of pre-concentrating 4-NPs and 4-*tert*-octylphenol (4-*t*-OP), a degradation product of octylphenol polyethoxylate, from landfill leachate and municipal wastewater by SDE and determine concentrations of eight 4-NP isomers (commonly found in tNP) and 4-*t*-OP using GC × GC/ToFMS. The 4-NP isomers targeted for analysis comprise 36–46% of tNP [1] and are known to differ in estrogenic potency [8]. Our aim was to determine if SDE-GC × GC/ToFMS could provide information that would be useful in studies of isomer-specific degradation and accumulation of 4-NPs in landfill leachate and municipal wastewater.

2. Materials and methods

2.1. Materials

2.1.1. Reagents and chemicals

In this study we follow the systematic numbering system developed by Guenther et al. [12] to identify individual 4-NP isomers. All solvents (isooctane, methanol, dichloromethane) were pesticide grade (Burdick & Jackson, USA) and were used without further purification. Technical nonylphenol was obtained from Fluka. 4-*t*-OP and individual synthetic 4-NP isomers (such as 4-NP_9, 4-NP_{65}, 4-NP_{112} and 4-NP_{152}) were ≥99% pure, whereas 4-NP_{194} and 4-NP_{36} were obtained as an 86/14 mixture and 4-NP_{111} consisted of two chromatographically resolvable diastereomers, 4-NP_{111a} and 4-NP_{111b}, present as a 44.9/55.1 mixture. 4-*n*-Heptylphenol (4-*n*-HP) and 4-*n*-octylphenol (4-*n*-OP), used as recovery surrogates (RS1, RS2, respectively), were ≥ 98% pure, 2,4,6-trimethylphenol, 2,4,6-tribromophenol and 4-*n*-nonylphenol (4-*n*-NP), used as internal standards (IS1, IS2, IS3, respectively), had purities >99%.

2.1.2. Preparation of standard solutions

Multipoint calibration standard solutions (seven levels) were prepared in isooctane. The solutions contained 4-NP_9, 4-NP_{65}, 4-$NP_{111(a,b)}$, 4-NP_{112}, 4-NP_{152}, 4-$NP_{194/36}$, 4-*t*-OP, 4-*n*-HP and 4-*n*-OP at concentrations ranging from 0.2 to 104 ng/μL and 2,4,6-trimethylphenol (IS1), 2,4,6-tribromophenol (IS2) and 4-*n*-NP (IS3) at 20 ng/μL. The recovery surrogate solution contained 8 ng/μL of 4-*n*-HP (RS1) and 4-*n*-OP (RS2) in isooctane.

2.1.3. Environmental sample collection

Unfiltered 2-liter grab samples of influent and effluent were collected at the North Canadian Waste Water Treatment Plant (NCWWTP; OK, USA) in December 2008. This plant uses aerobic activated sludge and disinfection treatment. Two leachate samples were obtained in December 2008 from an active landfill in Oklahoma City, OK, USA. This landfill is one of four major landfills operating in the Oklahoma City metropolitan area, which has a population of approximately 560,000 residents [23]. The municipal solid waste (MSW) deposited in this landfill is non-hazardous and is from residential and commercial sources, and the landfill is equipped with a liner and leachate-collection system. Two landfill cells were sampled. One had received MSW from 1993 to 2006 that had been capped and closed for 3 years ('moderate age' leachate). The other cell was still open and being filled with MSW ('new age' leachate) at the time of sampling. Leachate was collected into a 20-l bucket from a valve tapped into the previously purged leachate collection/discharge trunk line. A peristaltic pump was then used to transfer leachate samples from the bucket to 2-l amber glass bottles (previously combusted at 425 °C for 4 h). The bottles were covered with aluminum foil and placed into ice-filled coolers, which were transported to the laboratory of the United States Geological Survey (USGS, Reston, VA) within 2 days. All samples were stored in a refrigerator at 4 °C and extracted within 7 days.

2.2. Analytical methods

2.2.1. Steam distillation extraction

4-NPs were extracted in a closed-loop apparatus (Modified Nielson–Kryger unit, Ace Glass, USA) for continuous steam-distillation and solvent-extraction of the distillate. One-and-a-half liter water samples were refluxed for 6 h after adding 120 μL of the recovery surrogate solution. Ten mL of isooctane was used as the extraction solvent. The extracts were passed through anhydrous sodium sulfate and concentrated to ~0.5 mL by rotary evaporation. The extracts were then concentrated under a stream of N_2 gas until dry and immediately taken up in 250 μL of the internal standard solution prior to analysis by GC/MS and GC × GC/ToFMS. The wastewater influent sample extract, which contained high concentrations of organic substances, required dilution using isooctane prior to analysis.

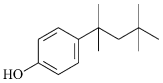
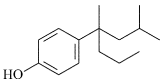
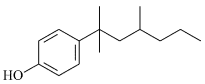
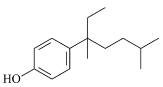
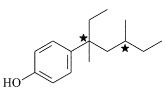
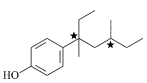
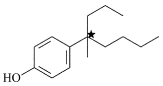
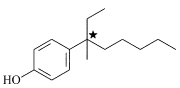
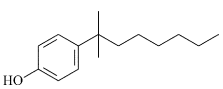
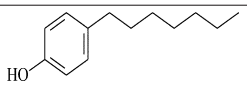
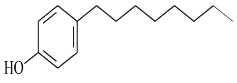
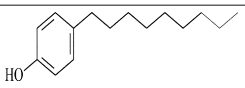
2.2.2. Gas chromatography/mass spectrometry

GC/MS analyses were carried out on an Agilent 6890 gas chromatograph (Agilent, USA) interfaced to an Agilent 5973 quadrupole mass spectrometer. The fused silica capillary column was coated with DB-5ms (30-m length, 0.25-mm i.d., and 0.25-μm film thickness, J&W Scientific). The injection port was maintained at 275 °C, and one-μL sample was injected in splitless mode followed by a 1-min purge. The column oven temperature was held at 70 °C for 1 min, then programmed at 25 °C/min to 130 °C, followed by 2 °C/min to 290 °C, and held for 7 min. Samples were analyzed in full scan mode over the mass interval 50–500 u. Helium was used as carrier gas at 100 kPa and the ionization potential was 70 eV. Temperatures of the ion source and the transfer line were 250 °C and 275 °C, respectively. The ions used for quantitation of 4-NPs, recovery surrogates and internal standards are shown in Table 1 along with calibration curve statistics. The total concentration of 4-NPs (Σ4-NPs) was determined by comparing the summed areas for m/z 107, 121, 135 and 149 (within the elution range of these compounds), to the summed area of m/z = 107 and 220 for the internal standard (4-*n*-NP). 4-*t*-OP concentrations were based on the summed areas for m/z = 107 and 135. Data processing was carried out using Agilent ChemStation software version D.01.00, Build 75 (Agilent Technologies).

2.2.3. GC × GC/ToFMS

An Agilent model 6890N gas chromatograph interfaced to a Pegasus III time-of-flight mass spectrometer (LECO Corporation, USA) was used for GC × GC/ToFMS analysis. The GC × GC analyses

Table 1
Quantitation ions for Σ4-NPs by GC/MS and for 4-NP isomers by GC × GC/ToFMS.

Compounds	GC/MS	Compounds		GC × GC/ToFMS	
Targeted analyte	Quantitation ions	Targeted analyte	Structures	Unique mass	Quantitation ions
4-*t*-OP	107 + 135	4-*t*-OP		135	107 + 135
Σ4-NPs	107 + 121 + 135 + 149	4-NP_{194}		121	121 + 107
		4-NP_{36}		135	135 + 107
		4-NP_{112}		107	107 + 121 + 149
		$4NP_{111a}$		121	107 + 121 + 149
		4-NP_{111b}		107	107 + 121 + 149
		4-NP_{152}		121	107 + 121
		4-NP_{65}		107	107 + 121 + 149
		4-NP_9		135	135 + 107
Compounds	GC/MS	Compounds		GC × GC/ToFMS	
Recovery surrogate	Quantitation ions	Recovery surrogate	Structures	Unique mass	Quantitation ions
4-*n*-HP	107 + 192	4-*n*-HP		107	107
4-*n*-OP	107 + 206	4-*n*-OP		107	107
Compounds	GC/MS	Compounds		GC × GC/ToFMS	
Internal standard	Quantitation ions	Internal standard	Structures	Unique mass	Quantitation ions
4-*n*-NP	107 + 220	4-*n*-NP		107	107

were carried out using a two-stage quad jet thermal modulator with a modulation period of 9 s. A DB-5ms capillary column (0.25-mm id × 1.0-μm film thickness × 30-m length; Agilent, USA) was used as the first-dimension column. The second-dimension column was coated with Supelcowax-10 (0.1-mm id × 0.1-μm film thickness × 2.0-m length; Supelco, USA). Two-μL samples of steam distillation extract were injected in split mode (split ratio = 1:1) at an injection temperature of 275 °C. Split, rather than splitless, injection was employed because optimal first-dimension separation of the nonylphenol isomers was obtained under isothermal (as opposed to oven temperature programmed) conditions. The low overall concentrations of the targeted analytes in some samples necessitated a lower-than-typical split ratio and a larger-than-typical injection volume. Nevertheless, analytical precision was found to be acceptable (Table 2, this study and the reference [1]). The main oven was held at 170 °C for 110 min, then programmed to 250 °C at 10 °C/min, and held isothermally for 120 min. The secondary oven was maintained at 230 °C for 110 min, then programmed to 250 °C at 10 °C/min, and held isothermally for 120 min. Helium was used as carrier gas at a constant flow of 1.42 mL/min. The Pegasus III ToFMS was operated under electron impact conditions at 70 eV. Mass spectra were recorded in the mass interval of 35–350 u with an acquisition rate of 50 Hz. The identification of 4-NP isomers in tNP by GC × GC/ToFMS was described by Eganhouse et al. [1]. Target compounds in the landfill leachate and municipal wastewater samples were identified by matching deconvoluted mass spectra and retention times against the synthetic NP isomers. Eight synthetic 4-NP isomers, 4-*t*-OP, and the two recovery

Table 2
Effectiveness of the SDE extraction method and MDL determined by GC/MS and by GC × GC/ToFMS.

GC/MS								GC × GC/ToFMS			
Compounds	15–1000 ng/μL tNP	Milli-Q water				Field samples		Compounds	MDL_{est} (ng/L)	0.2–100 ng/μL individual isomers	Field samples
	Linear correlation coefficient (R^2)	200 ng/L (n = 10)[a]			2000 ng/L (n = 3)[b]	4000 ng/L (n = 28)[c]				Linear correlation coefficient (R^2)	4000 ng/L (n = 28)[c]
		MDL (ng/L)	R (%)	RSD (%)	R (%) RSD (%)	R (%)	RSD (%)				R (%) RSD (%)
4-*n*-HP (RS1)	0.998	121	66.1	15.2	78.4 1.95	83.4	0.14	4-*n*-HP (RS1)	0.37	>0.999	95 8.6
4-*n*-OP (RS2)	0.998	119.4	66.5	15	76.8 2.21	80.6	0.17	4-*n*-OP (RS2)	0.16	0.997	101 18.3
4-*t*-OP	0.998	88.7	76.5	14.1	85.4 3.09	—	—	4-*t*-OP	12.02	0.996	— —
Σ4-NPs[d]	0.998	795.4	87.8	12.5	88.2 1.61	—	—	4-NP_{194}	14.03	0.998	— —
								4-NP_{36}	1.96	0.998	— —
								4-NP_{112}	15.06	0.998	— —
								4NP_{111a}	0.67	0.996	— —
								4-NP_{111b}	0.97	0.992	— —
								4-NP_{152}	9.11	0.996	— —
								4-NP_{65}	10.29	0.995	— —
								4-NP_{9}	3.83	0.996	— —

Note: R = average recovery; RSD = relative standard deviation, MDL = method detection limits, MDL_{est} = estimated method detection limits.
[a] The concentration of recovery surrogates (RS1 and RS2) and 4-*t*-OP is about 200 ng/L and tNP about 2255 ng/L.
[b] The concentration of recovery surrogates (RS1 and RS2) and 4-*t*-OP is about 2000 ng/L and tNP about 16,040 ng/L.
[c] The concentration of recovery surrogates (RS1 and RS2) is about 4000 ng/L, 4-*t*-OP and tNP not spiked.
[d] Σ4-NPs = summed concentration of 4-NPs in tNP. See Section 2.2.2 below for explanation.

surrogates were determined using the internal standard method (using 4-*n*-NP as internal standard) and a 7-level linear calibration curve with forcing through the origin (correlation coefficients provided in Table 1) using LECO ChromaTOF software (v. 3.32; cf. Eganhouse et al. [1]).

2.2.4. Quality assurance/quality control

To assess the efficiency of the SDE method for extraction of NP and OP compounds in water samples, preliminary experiments were performed using GC/MS for quantitation (Table 2). In one experiment, tNP, 4-*t*-OP and the recovery surrogates (4-*n*-HP and 4-*n*-OP) were spiked into Milli-Q water at individual isomer concentrations of approximately 200 or 2000 ng/L and analyzed by GC/MS [*Note*: The experiment with the 200 ng/L spike concentrations was also used to determine GC/MS method detection limits as described below]. Higher concentrations of the recovery surrogates alone (4000 ng/L) were also spiked into 28 field samples to assess recoveries in the presence of the landfill leachate and municipal wastewater sample matrices (with GC/MS and GC × GC/ToFMS analysis).

As shown in Table 2, higher recoveries and better precision were obtained by GC/MS at the higher spiked concentrations. Recoveries of 4-NP isomers were greater than 66% and relative standard deviations (RSDs) were less than 15% even in the presence of co-extracted materials in environmental samples. Higher recovery rates were obtained in surrogate-spiked wastewater and landfill leachate samples analyzed by GC × GC/ToFMS (95% for 4-*n*-HP and 101% for 4-*n*-OP) than by GC/MS (65% for 4-*n*-HP and 48% for 4-*n*-OP). This may reflect the improved separation of analytes from interferences by GC × GC/ToFMS. Based on these data, we conclude that SDE is an efficient method for extraction of 4-*t*-OP and 4-NPs in the environmental sample matrices tested.

Method detection limits (MDLs) were determined using USEPA procedure 40 CFR 136, Appendix B, revision 1.11 (USEPA, 1986). An initial 'estimated MDL' for tNP by GC/MS was assumed to be between 10 and 20 times the minimum detection limit reported by Barber et al. [24]. Based on this estimated MDL, a laboratory standard solution containing the recovery surrogates and tNP was prepared in Milli-Q water at concentrations conforming to specifications in the USEPA procedure (nominally 200 ng/L for surrogates and 4-*t*-OP and 2000 ng/L for tNP) for processing through the entire procedure. Ten replicate analyses were used to determine the MDLs based on analysis by GC/MS, which are listed in Table 2. The MDLs of 4-*n*-HP, 4-*n*-OP, 4-*t*-OP and Σ4-NPs by GC/MS were 121 ng/L, 119 ng/L, 88.7 ng/L and 795 ng/L, respectively.

Three replicate runs based on spiking of Milli-Q water with higher concentrations of the analytes (nominally ~2000 ng/L for surrogates and 4-*t*-OP and ~16,000 ng/L for tNP; Table 2) were analyzed by GC/MS to assess method precision. Relative standard deviations were in all cases below 3.1%.

MDLs of the recovery surrogates (4-*n*-HP, 4-*n*-OP), 4-t-OP and the eight 4-NP isomers by the GC × GC/ToFMS method were estimated at a signal-to-noise ratio of 3 using the lowest level multipoint calibration standard solution. Concentrations of the targeted analytes in this solution ranged from 0.2 to 1.6 ng/μL. Estimated MDLs ranged from 0.16 to 15.06 ng/L and are listed for each analyte in Table 2. This was done using the ChromaTOF software. In practice, it is possible to estimate the portion of the peak that has been captured by each of the visible sub-peaks even for unknown compounds when the modulation ratio (relates modulation period) is known for a given region of the chromatogram [25]. However, it should be aware that estimated MDLs are preliminary and tentative. Errors generated during quantitation, particularly in samples with analyte concentrations near the detection limit, are affected by the phase shifting and period of modulation when sub-peaks are integrated and their areas are summed to yield total analyte peak

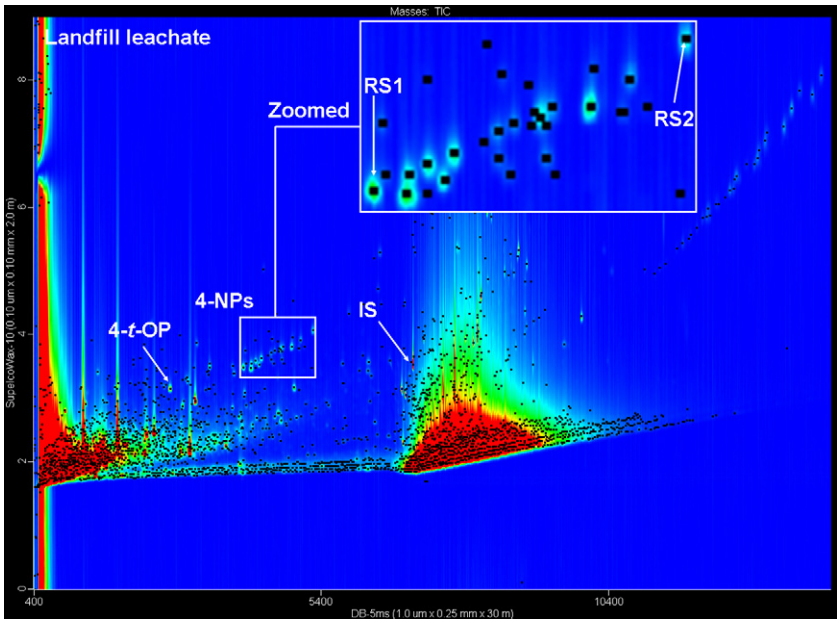


Fig. 1. 2D contour plot of landfill leachate analyzed by SDE-GC × GC/ToFMS.

area [25,26]. In the case of the present study (leachate, influent), concentrations were generally one to two orders of magnitude or greater than the estimated MDLs.

On a daily basis or for every batch of 8 samples, a procedural blank and spiked samples consisting of all reagents were run to check for interference and cross contamination. The concentrations of the targeted analytes in blank samples were always below the MDLs.

3. Results and discussion

3.1. 4-NP isomers in wastewater and landfill leachate samples

The organic matter in landfill leachate includes thousands of compounds such as phenols with low boiling point, steroids with high boiling points, hydrocarbons with high volatility (such as benzene) and carboxylic acids with strong polarity. In order to quantify targeted compounds, cleanup methods specific to the target compounds of interest are required. Fig. 1 is a 2D contour plot of landfill leachate analyzed by GC × GC/ToFMS. Peak markers, which appear as small black squares, indicate detectable sample components identified by ChromTOF, the data processing software. 4-NP isomers are presented in the zoomed part of the GC × GC chromatogram. Peak intensity, as defined by detector response, is represented by a color scheme from blue (zero, or baseline detector response) to red (most intense response). This chromatogram illustrates the fact that many compounds in complex environmental samples cannot be resolved by standard GC and that the 4-NPs co-elute with many sample co-extractants. However, GC × GC/ToFMS can yield a dramatically improved separation of the analytes of interest, and automated mass spectral deconvolution and identification of closely eluting peaks enables the identification of more than 30 alkylphenols in the 2D chromatogram.

The concentrations of 4-*t*-OP and eight 4-NP isomers in wastewater and landfill leachate samples determined using GC × GC/ToFMS are listed in Table 3. The summed concentration of the eight 4-NP isomers (ΣNP isomers) in leachate samples from the 'new age cell' is 3.91 μg/L, substantially lower than that in wastewater influent. The summed concentration of the eight synthetic 4-NP isomers in the 'new age' cell was about 3 times higher than that in the 'moderate age cell', presumably due to greater degradation of nonylphenols under the moderate age cell's conditions and/or attenuation due to a longer period of leaching.

Fig. 2A and B shows 2D contour plots of wastewater influent and effluent subjected to SDE-GC × GC/ToFMS analysis. It is obvious that many extractable substances have been removed during wastewater treatment. However, even if more than 90% removal rate for NPs were reported after wastewater treatment [27,28], NPs including unidentified NP isomers are often found in the environment [7] and are considered an indicator of domestic wastewater pollution [3]. Shown from Table 3, after waste treatment, removals above 99% of 4-*t*-OP and the 4-NP isomers have been observed. The summed concentration of eight 4-NP isomers (ΣNP isomers) in wastewater treatment plant (WWTP) influent was 38.8 μg/L, whereas the effluent contained 0.18 μg/L. Except for 4-NP_{152}, concentrations of the 4-NP isomers in the effluent were below the method detection limits, which indicates that aerobic activated sludge and disinfection treatment used in this plant is effective. Oxygen is believed to be a critical parameter for biodegradation of NPs and the bioaugmentation of sludge to improve their biodegradation [29]. It was reported that 56.2% of NP_{152} and >90% of NP_{36}, NP_{112}, NP_9, NP_{111} can be removed after 9 days of incubation by a specific aerobic organism [8]. Therefore, to remove NPs effectively, processes

Table 3
Concentrations of 4-*t*-OP and eight synthetic 4-NP isomers in wastewater and landfill leachate samples determined by GC × GC/ToFMS.

Compounds	Leachate (μg/L)		WWTP[a] (μg/L)	
	New age cell	Moderate age cell	Influent	Effluent
4-*t*-OP	1.38	1.08	10.78	ND[b]
4-NP_{194}	0.22	0.14	2.76	ND
4-NP_{36}	0.94	0.12	12.95	ND
4-NP_{112}	0.92	0.29	7.75	ND
4NP_{111a}	0.48	0.12	6.08	ND
4-NP_{111b}	0.61	0.18	5.95	ND
4-NP_{152}	0.27	0.18	1.25	0.18
4-NP_{65}	0.23	0.21	1.27	ND
4-NP_9	0.25	ND	0.82	ND
ΣNP isomers	3.91	1.23	38.8	0.18

[a] WWTP = wastewater treatment plant.
[b] ND = below the method detection limit.

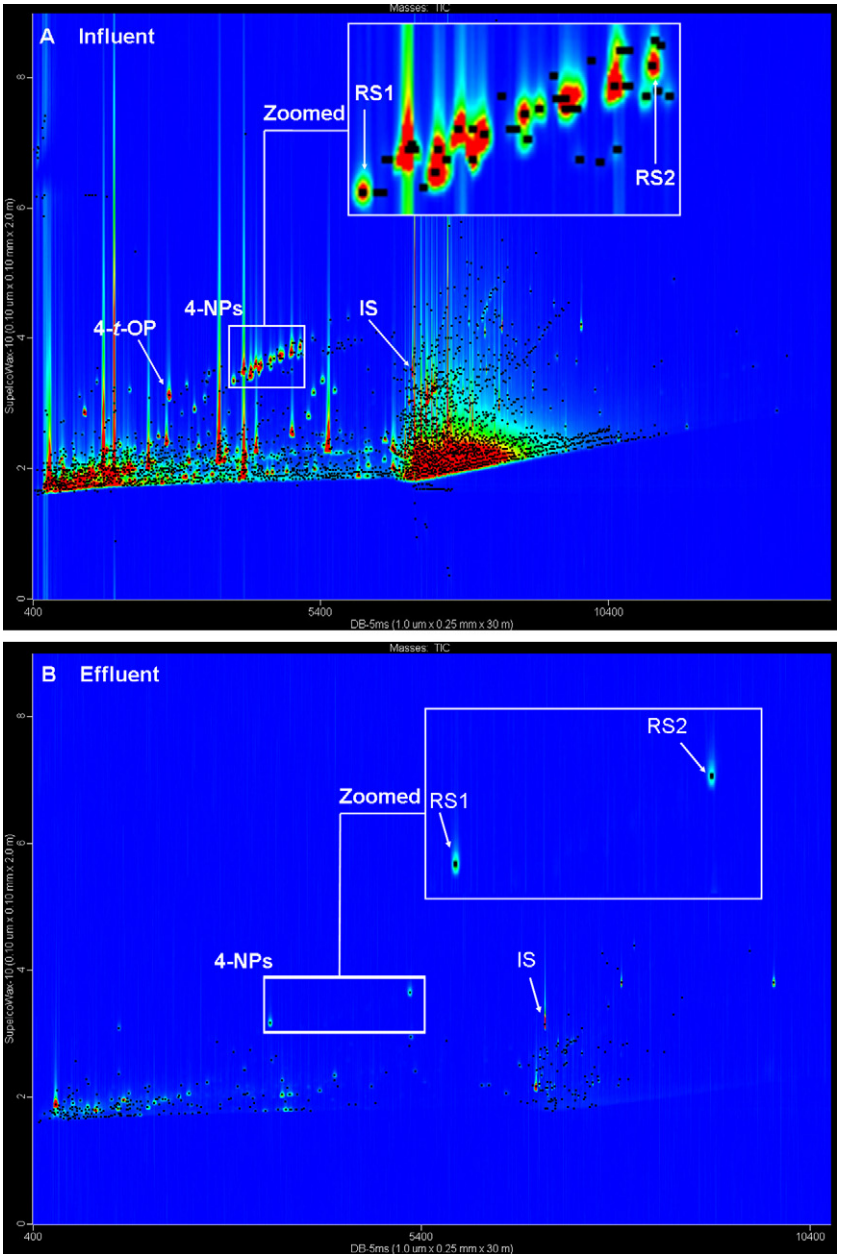


Fig. 2. Total ion chromatograms of (A) wastewater influent (2× diluted prior to analysis relative to effluent) and (B) wastewater effluent analyzed by SDE-GC × GC/ToFMS.

including aerobic activated sludge treatment are often considered in WWTPs.

3.2. The degradation and accumulation behaviors of 4-NPs in the aquatic environment

The degradation and accumulation of 4-NP isomers in environmental samples can be examined by the alteration of isomer distribution patterns occurring after tNP is released to the environment. The abundance of individual 4-NP isomers relative to the summed concentration of the eight isomers in field samples and in commercial tNP (Fluka) (i.e. normalized distributions) is shown in Fig. 3. The normalized distributions of 4-NP isomers in the environmental samples are different from that of the commercial product (Fluka) and differences between 'moderate age cell' and 'new age cell' leachate samples are also found for most of the 4-NP isomers. For example, the concentration of 4-NP_9 in the leachate from the 'moderate age cell' is below the method detection limit, whereas it represents about 6.3% of the summed concentration of the eight 4-NP isomers in the 'new age cell'. By contrast, the normalized abundances of 4-NP_{194}, 4-NP_{152}, and 4-NP_{65} are greater in the leachate from the 'moderate age cell' than in the 'new age cell'. The results suggest that the distinct isomeric fingerprints depend on the extent of aging, which may be an expression of the dominant microbial metabolism to degrade specific 4-NPs or different molecular structures of the 4-NPs during leaching [8,30]. Changes of isomeric fingerprints in wastewater samples were also observed after treatment (i.e. effluent vs. influent). For example, 4-NP_9 appears to have been degraded effectively in the WWTP during activated sludge treatment. 4-NP_{194} and 4-NP_{65} were also removed during wastewater treatment even though their attenuation in landfill leachate was minimal. However, removal of 4-NP_{152} was incomplete under both landfill and WWTP conditions [*Note*: 4-NP_{152} was the only 4-NP detected in the WWTP effluent]. Together, these data suggest

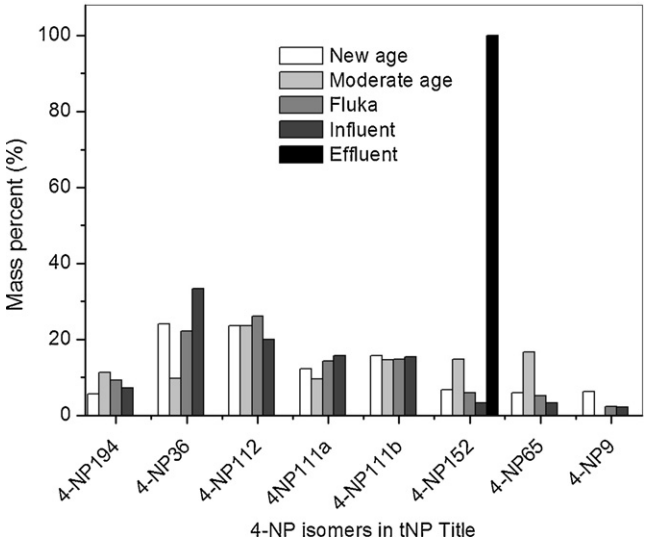


Fig. 3. Normalized abundances of 4-NP isomers in landfill leachate, commercial tNP (Fluka), and wastewater.

that degradation of 4-NP isomers depends on specific environmental conditions resulting in the accumulation of certain NP isomers in aquatic environments.

4. Conclusions

SDE is an effective method for extracting NPs from water samples. GC × GC/ToFMS presents advantages for the separation and quantification of NP isomers in complex environmental samples. Eight 4-NP isomers known to be present in tNP were found in landfill leachate and wastewater influent samples from Oklahoma City, OK, but the isomer abundances were variable and differed from that found in tNP. The results suggest the degradation rates of 4-NP isomers vary and depend on specific environmental conditions, which results in the accumulation of certain persistent 4-NPs in various aquatic environments.

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