Low Nanogram Per Liter Determination of Halogenated Nonylphenols, Nonylphenol Carboxylates, and Their Non-Halogenated Precursors in Water and Sludge by Liquid Chromatography Electrospray Tandem Mass Spectrometry

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A new LC-MS-MS method for quantitative analysis of nonylphenol (NP), nonylphenol carboxylates (NPECs), and their halogenated derivatives: brominated and chlorinated nonylphenols (BrNP, ClNP), brominated and chlorinated nonylphenol carboxylates ($BrNPE_1C$ and $ClNPE_1C$) and ethoxycarboxylates ($BrNPE_2C$ and $ClNPE_2C$) in water and sludge has been developed. Electrospray negative ionization MS-MS was applied for the identification of above mentioned compounds. Upon collision-induced dissociation, their deprotonated molecules gave different fragments formed by the cleavage of the alkyl moiety and/or (ethoxy)carboxylic moiety. For halogenated compounds a highly diagnostic characteristic pattern of isotopic doublet signals was obtained and fragmentation yielded, in addition to above mentioned ions, $[Br]^-$ and $[Cl]^-$, respectively. Quantitative analysis was done in the multiple reaction monitoring (MRM) mode, using two specific combinations of a precursor-product ion transitions for each compound. Additionally, for halogenated compounds two specific channels for each transition reaction, corresponding to two isotopes, were monitored and the ratio of their abundances used as an identification criterion. The method has been validated in terms of sensitivity, selectivity, accuracy, and precision and was applied to the analysis of water and sludge samples from drinking water treatment plant (DWTP) of Barcelona (Catalonia, NE Spain). Halogenated NP and NPECs were detected in prechlorinated water in concentrations up to 315 ng/L, $BrNPE_2C$ being the most abundant compound. In the DWTP effluent non-halogenated compounds were detected at trace levels (85, 12 and 10 ng/L for NP, NPE_1C, and NPE_2C, respectively), whereas concentration of halogenated derivatives never exceeded 10 ng/L. Nonylphenol, brominated and chlorinated NPs were found in flocculation sludge in concentrations of 150, 105, and 145 μg/kg, respectively. Acidic polar metabolites were found in lower concentrations up to 20 μg/kg. (J Am Soc Mass Spectrom 2003, 14, 516–527) © 2003 American Society for Mass Spectrometry

Non-ionic surfactants, nonylphenol polyethoxylates (NPEOs), have been widely used in the last 40 years as detergents, emulsifiers, dispersants, antifoamers and pesticide adjuvants. The biodegradation of NPEOs under aerobic conditions yields mainly short ethoxy chain oligomers ($NPEO_1$ and $NPEO_2$), whereas under anaerobic conditions fully deethoxylated nonylphenol (NP) is also formed. Further transformation leads to acidic metabolites formed by oxidation of the ethoxy chain (nonylphenol carboxylates; NPECs) as well as oxidation of the branched alkyl chain [1–4]. During the chlorination process at drinking water treatment plants (DWTP) [5–8] and wastewater treatment plants (WWTP) [9, 10] the formation of halogenated derivatives, such as ring-brominated and chlorinated NPEOs, NPECs, and NPs, have been reported.

Toxicity of NP and short ethoxy chain NPEOs to aquatic organisms [11], lipophilic properties that lead to bioaccumulation in aquatic food chain [12], and ability

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to mimic endogenous hormone 17β-estradiol [13, 14] are well documented. However, little is known about environmental significance and toxicology of brominated and chlorinated alkylphenolic compounds. Maki et al. [15] determined that, both BrNPEOs and BrNPECs, show higher acute toxicity to *Daphnia magna* than their non-brominated precursors NPEOs and NPECs. A recent study, employing recombinant yeast assay (RYA) and enzyme linked receptor assay (ELRA) for the determination of estrogenic and anti-estrogenic activity, showed that halogenated compounds retained a significant affinity for the estrogen receptors suggesting that they may be still able to disturb the hormone imbalance of exposed organisms [16]. This was especially clear for halogenated NPECs, which acted as true anti-estrogens in the RYA.

The presence of alkylphenolic compounds in the environment has become of increasing concern globally and efforts have been made to determine their concentration levels in WWTP and in aquatic environments. However, studies to date have largely focused on short chain NPEOs, NPECs, and NPs, while fewer reports have included halogenated metabolites. One of the reasons for this is the low relative abundance of these compounds (generally less than 10% of the total pool of alkylphenolic compounds) and unavailability of appropriate analytical methods for their identification and quantification.

The first attempts to analyse halogenated alkylphenolic compounds were carried out using Fast Atom Bombardment-Mass Spectrometry (FAB-MS) [5, 7, 8, 17], which proved to be a reliable tool for the identification of halogenated metabolites in raw and drinking water, but not for their quantification. Recently, some efforts have been made to quantify these compounds in complex environmental and wastewater samples. Methods applied included gas chromatography-mass spectrometry (GC-MS) [10, 18, 19] (after appropriate derivatization) and reversed-phase liquid chromatography with electrospray mass spectrometry (LC-ESI-MS) [6, 20, 21]. ESI permitted the direct determination of the full range of halogenated NPEOs metabolites (i.e., XNPEOs, XNPECs and XNPs), as well as their precursors in aqueous and solid samples, thus obviating the necessity to methylate them. However, using "soft ionization" LC-MS, under conditions giving solely molecular ions, the identification of halogenated compounds is difficult since the chlorinated derivatives ($ClNPEO_n$ and $ClNPE_nC$) have the same molecular mass as brominated compounds with one ethoxy group less ($BrNPEO_{n-1}$ and $BrNPE_{n-1}C$) [6]. Moreover, in the analysis of real-world samples $ClNPE_1C$ was obstructed by a severe isobaric interference of linear alkylbenzene sulfonate ($C_{11}LAS$), which is often found in environmental and wastewater samples in concentrations several orders of magnitude higher than those of halogenated alkylphenolic compounds.

Thus, to obviate the matrix interference and interference of known and unknown compounds that may cause deviations when only a single stage of mass selectivity is used, more selective methods, such as tandem mass spectrometry are needed. However, although considered as one of the most powerful techniques for structure interpretation and quantification, LC-MS-MS has been seldom used in the analysis of acidic and neutral metabolites of NPEOs [2, 22], and has not been applied thus far to study their halogenated derivatives.

In the present work, a tandem mass spectrometric investigation of halogenated NPECs, NPs and their precursors (non-halogenated analogs) was carried out. Electrospray negative ionization MS-MS was applied for the identification of acidic and neutral NPEOs metabolites. From the observed ion fragmentation pathways a reliable and sensitive quantification method, that overcomes the main drawbacks on existing methods, is developed. The method was applied to study occurrence of halogenated alkylphenolic compounds derived from chlorination treatment in DWTP of Barcelona (Spain). To our knowledge this is the first LC-MS-MS method that permits analysis of halogenated NPs and NPECs at low nanogram per liter level.

Experimental

Standards and Reagents

NPE_1C and NPE_2C were synthesized according to the method described elsewhere [19]. Technical grade 4-NP and 4-nonyloxy benzoic acid, used as an internal standard was obtained from Aldrich (Milwaukee, WI).

BrNP was synthesized using elemental bromine according to the method described by Reinhard et al. [9]. ClNP was prepared by chlorination of nonylphenol using sulfuryl chloride according to the method of Stokker et al. [23]. $BrNPE_1C$ and $ClNPE_1C$ were synthesized by reacting brominated and chlorinated NP, respectively, with chloroacetic acid in the presence of sodium hydride and dimethylformamide as a solvent. These two synthesized compounds rendered $BrNPEO_1$ and $ClNPEO_1$ by reduction with lithium aluminum hydride in ether solution. $BrNPEO_2$ and $ClNPEO_2$ were synthesized by reacting BrNP and ClNP, respectively, with 2-(2-chloroethoxy)ethanol in the presence of NaOH in water. Finally, $BrNPE_2C$ and $ClNPE_2C$ were obtained from $BrNPEO_2$ and $ClNPEO_2$, respectively, by oxidation with Jones reagent [9].

Water Samples

Raw water entering the DWTP Sant Joan Despí (Barcelona, Spain) and water samples after each treatment step (i.e., prechlorination, rapid sand filtration, groundwater dilution, ozonation, granulated active carbon filtration, and final chlorination) were collected as grab samples in Pyrex borosilicate amber glass containers, previously rinsed with high-purity water.

NP, NPECs, and their halogenated derivatives were

Table 1. List of MRM channels, cone and collision energies applied to ESI-MS-MS for the analysis of nonylphenol, nonylphenol carboxylates, and their halogenated derivatives

	MRM 1 (Quantification)			MRM 2 (Confirmation)			
Compound	Precursor (*m/z*) → Product (*m/z*)	Cone (V)	Collision (eV)	Precursor (*m/z*) → Product (*m/z*)	Cone (V)	Collision (eV)	Ratio MRM1/ MRM2
NP	219 → 133	30	30	219 → 147	30	30	9.2
BrNP	297 → 79	30	30	297 → 211	30	30	12.3
	299 → 81			299 → 213			
ClNP	253 → 167	30	30	253 → 181	30	30	10.5
	255 → 169			255 → 183			
NPE_1C	277 → 219	10	30	219 → 133	30	30	1.8
$BrNPE_1C$	355 → 297	10	30	355 → 79	30	30	1.0
	357 → 299			357 → 81			
$ClNPE_1C$	311 → 253	10	30	253 → 167	30	30	3.5
	313 → 255			255 → 169			
NPE_2C	219 → 133	30	30	321 → 219	10	30	1.2
$BrNPE_2C$	297 → 79	30	30	399 → 297	5	20	2.0
	299 → 81			401 → 299			
$ClNPE_2C$	253 → 167	30	30	355 → 253	5	20	5.5
	255 → 169			357 → 255			

isolated from water samples using solid-phase extraction (SPE). A more detailed description of the SPE method is given elsewhere [6]. Briefly, 500 mL of water samples were loaded onto preconditioned Accubond C18 cartridges (J and W Scientific, Folsom, CA). Cartridges were air-dried under vacuum, and were eluted with 2 × 4 mL of methanol. The eluates were taken gently to dryness under a nitrogen steam and reconstituted in 500 μL of methanol.

Sludge Samples

Sludge from DWTP of Barcelona, obtained from prechlorinated raw water after flocculation with aluminium sulfate and mixed in a minor proportion with sludge coming from the washing of sand filters, was collected in precleaned amber glass bottles. The suspension (concentration of dry matter 3.5 to 5 g/L) was centrifuged at 4500 rpm, and the solid matter was separated and frozen at −20 °C before being freeze-dried.

Pressurized liquid extractions (PLE) were carried out using a Dionex ASE 200 (Dionex, Idstein, Germany) as described elsewhere [24]. Briefly, 1 g sub-sample of freeze-dried sludge was mixed with Na_2SO_4 and filled into 11 mL extraction cells. Extraction was carried out with acetone/methanol (1:1, vol/vol) under following conditions: temperature of 75 °C, pressure 1500 psi, heating time 5 min, two cycles of static extraction (5 min). As a final step, the cell was purged with gaseous nitrogen. The total volume of extract was ~20 ml. Extracts obtained by PLE, were concentrated to an approximate volume of 1 ml using a rotary vacuum, redissolved in 100 ml of HPLC water and subsequently purified by SPE using LiChrolute C18 cartridges (Merck, Darmstadt, Germany), as described elsewhere [6].

Chromatographic Conditions

Analyses were performed on a Waters 2690 series Alliance HPLC (Waters, Milford, MA) with a quaternary pump equipped with a 120 vial capacity sample management system. The analytes were separated on a narrow-bore 3 μm, 55 × 2 mm i.d. C_{18} reversed phase column Purospher STAR RP-18 endcapped (Merck, Darmstadt, Germany). The sample injection volume was set at 10 μL. A binary mobile phase gradient with methanol (A) and water (B) was used for analyte separation at a flow rate of 200 μL/min. The elution gradient was linearly increased from 30% A to 85% A in 10 min, then increased to 95% A in 10 min and kept isocratic for 5 min.

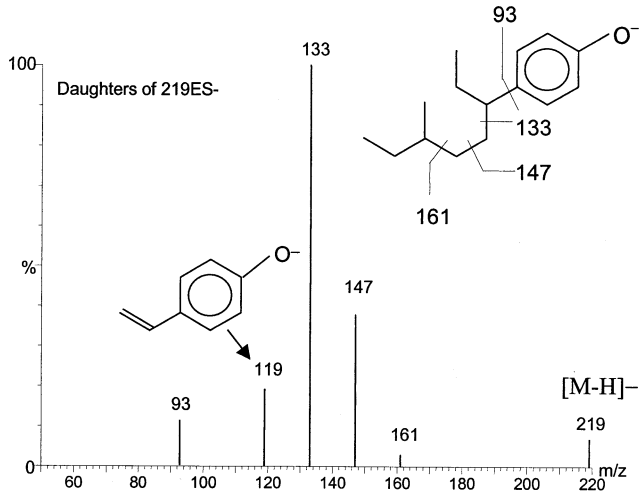

Figure 1. Product ion scan of NP with *m/z* 219 and the proposed fragmentation scheme under CID conditions obtained using argon as collision gas at collision energy of 40 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.

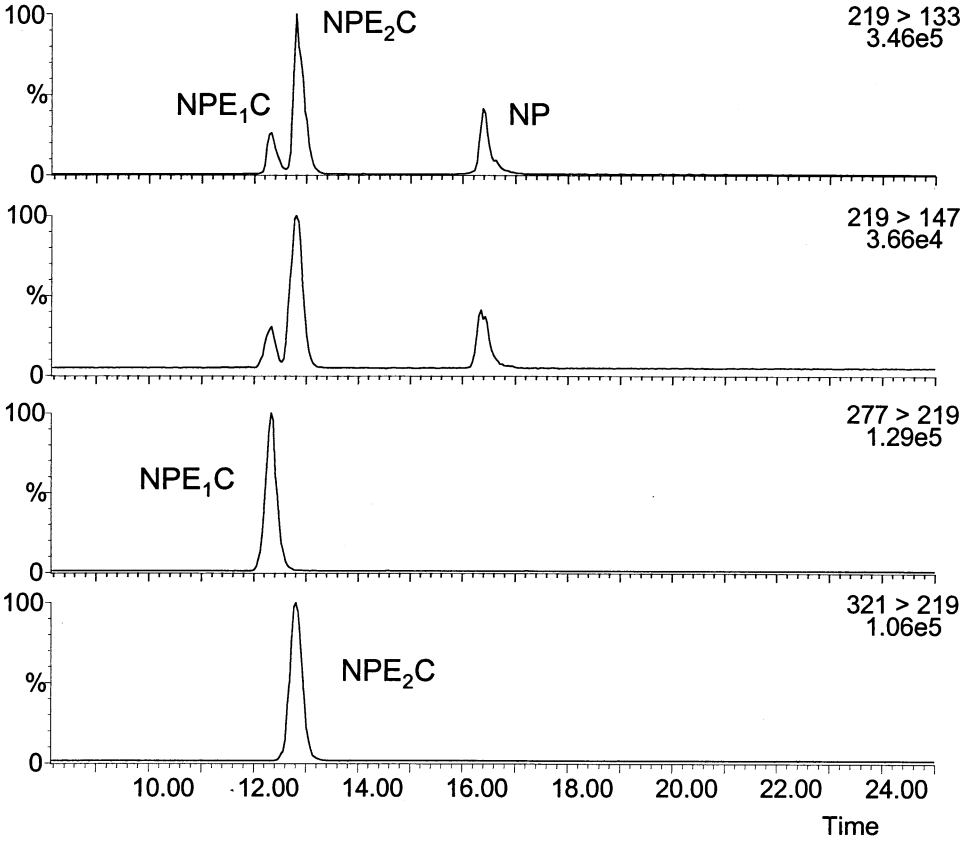

Figure 2. MS/MS chromatograms of raw effluent (river water) treated in Barcelona drinking water treatment plant corresponding to nonylphenolic compounds (MRM mode: 219 → 133 and 219 → 147 for detection of NP, NPE_1C, and NPE_2C; 277 → 219 for NP_1EC and 321 → 219 for NP_2EC).

Mass Spectrometry

A bench-top triple quadrupole mass spectrometer Quattro LC from Micromass (Manchester, UK) equipped with a pneumatically assisted electrospray probe and a Z-spray interface was used for this study. Capillary voltage was set at −2.8 kV, extractor lens 7 V and RF lens 0.6 V. The source and desolvation temperatures were 150 and 350 °C, respectively. The nitrogen (99.999% purity) flows were optimized at 50 L/h for the cone gas and 540 L/h for desolvation gas. For each analyte the values of the voltages applied to the cone, focusing lenses, collision cell, and quadrupoles were optimized by continuous infusion of a standard solution (1 μg/mL) via a syringe infusion pump Kd Scientific 100 (Boston, MA) at a constant flow-rate of 20 μL/min. All ESI mass spectral data were acquired with Masslynx NI software (version 3.5).

MS scans. For one stage MS scans the cone voltage was varied from −10 to −50 V according to the type of experiment performed and analyte studied. Full-scan mass spectra were recorded between *m/z* 30 and 500, with scan duration of 1 s/scan and an interscan time of 0.1 s.

MS/MS scans. The cone voltage was set to a value, which resulted in maximum abundance of the pseudo molecular ion (see Table 1). The argon collision gas was maintained at a pressure of 5.8×10^{-3} mbar. The optimum collision energy was chosen after performing MS/MS product ion scans on $[M - H]^-$ over a range of energies between 10 and 50 eV. The electron multiplier was set at 600 V. For experiments performed in MRM mode scan time was 1 s/scan, and the dwell time ranged from 50 to 200 ms, depending on the number of transition channels monitored (from 10 to 20).

Quantification

Quantitative analyses were done in MRM mode. The extent of ion suppression of MS signal was determined using 4-nonyloxy benzoic acid as an internal standard. The results (see Discussion) showed very limited signal reduction (less than 15% for sludge and negligible for water samples), thus the quantification was performed using external calibration.

Initially, a series of injections of target compounds in the concentration range from 1 ng/mL to 10 μg/mL was used to determine the linear concentration range. Calibration curves were generated using linear regres-

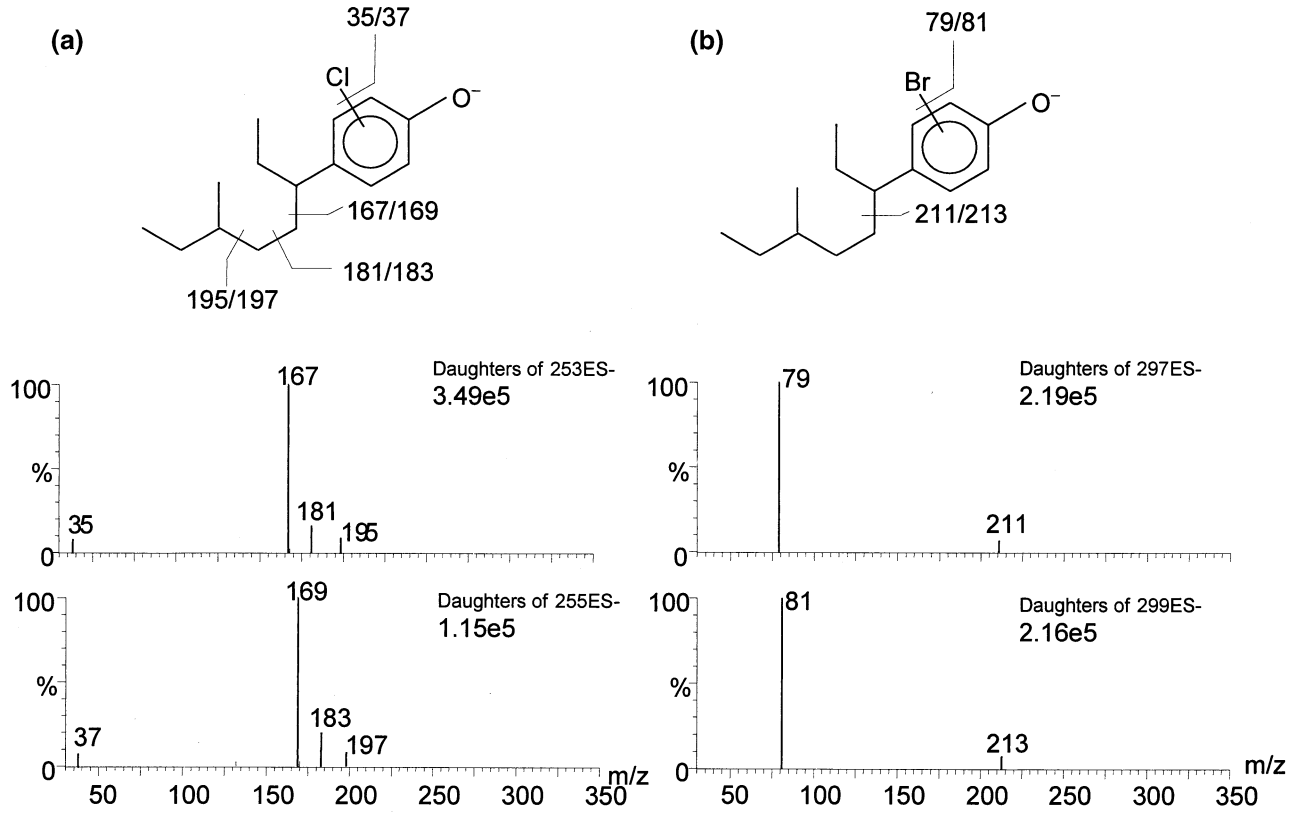

Figure 3. CID spectra and the proposed fragmentation pattern of (**a**) ClNP (precursor ions *m/z* 253 and 255) and (**b**) BrNP (precursor ions *m/z* 297 and 299) obtained at collision energy of 30 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.

sion analysis and over the established concentration range (0.01–1 μg/mL) gave good fits ($r^2 > 0.990$). Five-point calibration was performed daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8–10 injections.

Results and Discussion

Mass Spectrometry—Optimization of Experimental Conditions

A preliminary study was carried out using the single quadrupole mode under full-scan conditions and the negative ionization. The cone voltage was adjusted to give the maximum abundance of deprotonated molecule $[M - H]^-$, which were chosen as precursor ions in further MS-MS experiments, performed with the purpose of finding the best instrumental conditions for the identification of target compounds.

NP and NPECs. The product ion scan of $[M - H]^-$ for NP evidenced fragmentation of the side chain of deprotonated molecule (Figure 1), resulting in sequential loss of CH_2 groups (*m/z* 14), down to specie with *m/z* 93. The most abundant fragments with *m/z* 133 and 147 resulted from the loss of C_6H_{14} and C_5H_{12}, respectively.

The product ion spectra of deprotonated molecule at *m/z* 277 (for NPE_1C) and *m/z* 321 (for NPE_2C), showed the intense signal at *m/z* 219, corresponding to $[M–CH_2COO–H]^-$ and $[M–CH_2CH_2OCH_2COO–H]^-$, respectively, as reported previously by other authors [2, 22]. Additional fragments at *m/z* 133 and 147 were formed by the fragmentation on the side chain, as described above for NP. Thus, specific transitions at *m/z* 277 → 219 and *m/z* 321 → 219 could be used to monitor NPE_1C and NPE_2C, respectively, while MRM channels at *m/z* 219 → 133 and *m/z* 219 → 147 are characteristic for both NP and NPECs, and could be used to monitor all these compounds: However, in the latter case good chromatographic separation is essential, as depicted in Figure 2.

Halogenated NP and NPECs. Owing to the presence of chlorine and bromine atoms in the molecules, halogenated derivatives yielded a characteristic pattern of isotopic doublet signals, which was a highly diagnostic fingerprint for this group of compounds.

The main fragmentation process of ClNP was similar to those observed for NP. The predominant reaction was the side chain fragmentation that resulted in sequential loss of 14 Da, with the most abundant fragments at *m/z* 167 for ^{35}Cl and *m/z* 169 for ^{37}Cl with the

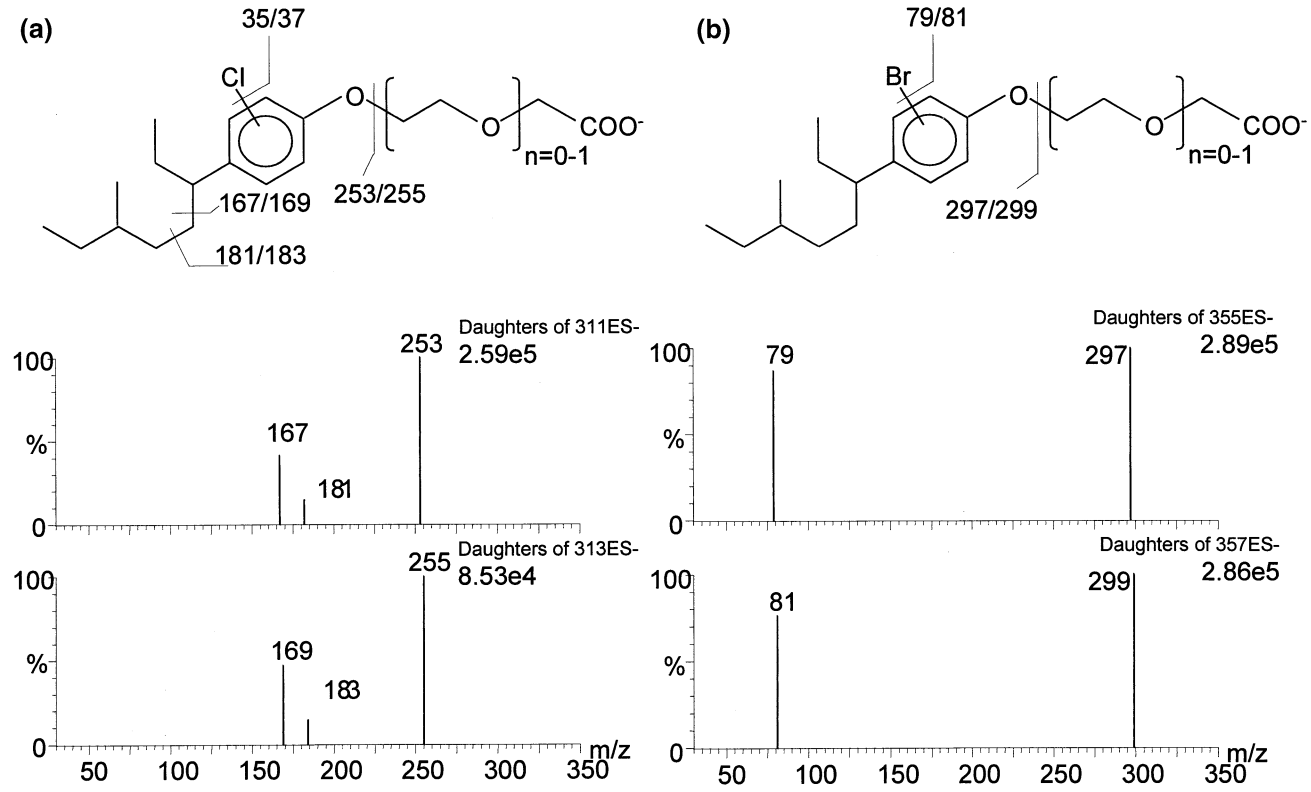

Figure 4. CID spectra and the proposed fragmentation pattern of (**a**) $ClNPE_1C$ (precursor ions *m/z* 311 and 313) and (**b**) $BrNPE_1C$ (precursor ions *m/z* 355 and 357) obtained at collision energy of 30 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.

relative ratio of intensities of 3.03 (Figure 3a). Fragment corresponding to $[Cl]^-$ was produced only when sufficient collision energy was applied. The intensity of this ion was not very pronounced, but nevertheless remained useful for the identification of chlorinated NP.

The brominated NP showed a markedly different fragmentation pathway (Figure 3b). At collision energy of 40 eV the product ion spectra of BrNP (*m/z* 297/299) yielded intense signals at *m/z* 79 and 81 corresponding to the $[Br]^-$ (ratio of isotopes 1.02), while the fragmentation of the side chain was suppressed and resulted just in a low-intensity fragment at *m/z* 211/213 produced after the loss of C_6H_{14}.

For halogenated NPE_1Cs and NPE_2Cs (Figure 4) the predominant reaction was loss of CH_2COO and $CH_2CH_2OCH_2COO$, respectively, that resulted in intense signals at *m/z* 253/255 for ClNPECs and *m/z* 297/299 for BrNPECs. Further fragmentation yielded the same ions as described above for halogenated NPs, i.e., $[^{79}Br]^-$ and $[^{81}Br]^-$ for brominated NPECs and *m/z* 167/169 and *m/z* 181/183 for ClNPECs. Such difference in the mechanism of fragmentation of chlorinated and brominated compounds is presumably the consequence of the lower energy of a $Br–C_6H_5$ (benzene) bond compared to a $Cl–C_6H_5$ bond.

BrNPECs yielded $[Br]^-$ at low collision energies (10 eV), as shown in Figure 5a for $BrNPE_2C$, while ClNPECs produced $[Cl]^-$ only at higher collision energy (50 eV) (Figure 5f). At collision energy of 50 eV the *m/z* 79/81 ion was the sole fragment ion observed for $BrNPE_2C$, while chlorinated analog showed, in addition to *m/z* 167/169, fragments at *m/z* 131 and *m/z* 145. These two fragments, observed as product ions of both $^{35}ClNPE_2C$ and $^{37}ClNPE_2C$, respectively, did not show characteristic isotopic pattern and were tentatively assigned as fragments formed by the cleavage of ethoxycarboxylated moiety, chlorine and by the cleavages in the alkyl moiety.

From the observed fragmentation pathways the dominant dissociation reaction channels were chosen (Table 1). The cone voltage of each transition was optimized to a value that gave the maximum abundance of the precursor ion, whereas the collision energy was set to a value that resulted in the maximal intensity of the product ion. Generally, deprotonated molecules where chosen as precursor ions, with the exception of NPE_2C and halogenated NPE_2Cs. Even at low cone voltages (5–10 V), in-source CID was obtained for these compounds and their spectra displayed low abundance of deprotonated molecules. Thus, for these compounds the most intense MRM channels were those monitoring the fragmentation of "the first generation" products (e.g., 219 → 133 for NPE_2C, 297/299 → 79/81 for $BrNPE_2C$, and 253/255 → 167/169 for $ClNPE_2C$).

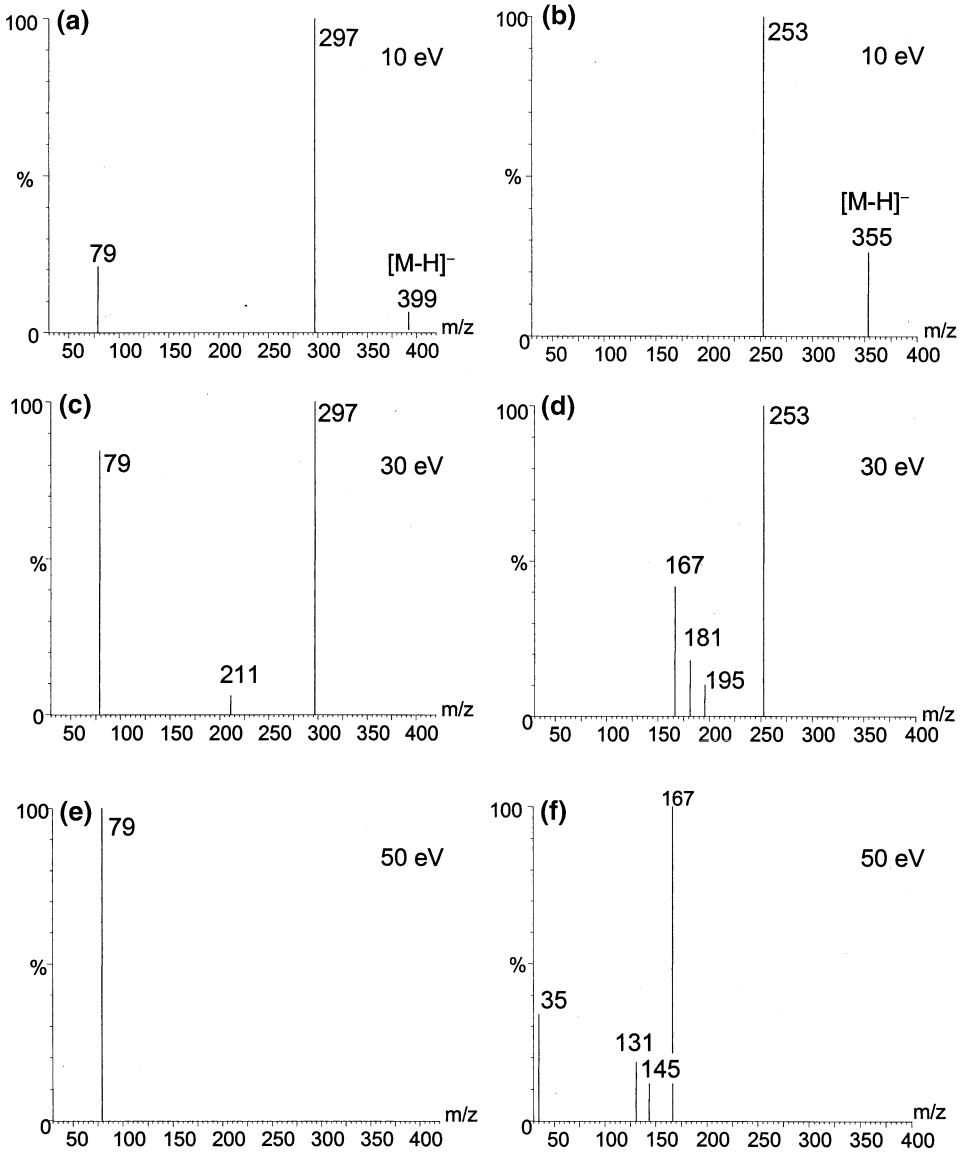

Figure 5. Product ion scans of $XNPE_2C$ obtained at 10, 30, and 50 eV: (**a**), (**c**), and (**e**) $^{79}BrNPE_2C$ (precursor ion *m/z* 399); (**b**), (**d**), and (**f**) $^{35}ClNPE_2C$ (precursor ion *m/z* 355).

Positive identification criterion of the target compounds was based on: (1) LC retention time of the analyte compared to that of a standard (±2%), (2) the ratio of abundances of two specific precursor ion → product ion transitions (within 10% of the ratios obtained for the standard). Additionally, for halogenated compounds a highly diagnostic criteria was the abundance ratio of characteristic isotopes (^{79}Br:^{81}Br = 1.02; ^{35}Cl:^{37}Cl = 3.03) and for each transition reaction two specific channels, corresponding to two isotopes, were monitored and the ratio of their abundances calculated.

High selectivity of the MS-MS detection of halogenated nonylphenolic compounds is shown in Figures 6 and 7, which display extracted MRM chromatograms for chlorinated and brominated compounds, respectively, detected in water and sludge samples from DWTP of Barcelona. A specificity of MRM mode permitted identification and quantification of halogenated NPECs, thus obviating isobaric interference of $ClNPE_1C$ and $C_{11}LAS$ (both having a base ion at *m/z* 311) observed using a LC-MS in selected ion monitoring (SIM) mode [6]. Note that broad and, in some cases multiple peaks, observed, e.g., for brominated compounds (Figure 7), reflect the multitude of isomers arising from the different branching of the nonyl entity and probably co-existence of different isomers of the benzene ring, i.e., 2-NP, which makes up to 10% of NP in industrial blends, and consequently different position of bromine and chlorine, respectively.

Other MS-MS modes, such as the precursor ion scan and neutral loss scan, were found to be less useful MS/MS scanning modes as they were 5 to 10 times less

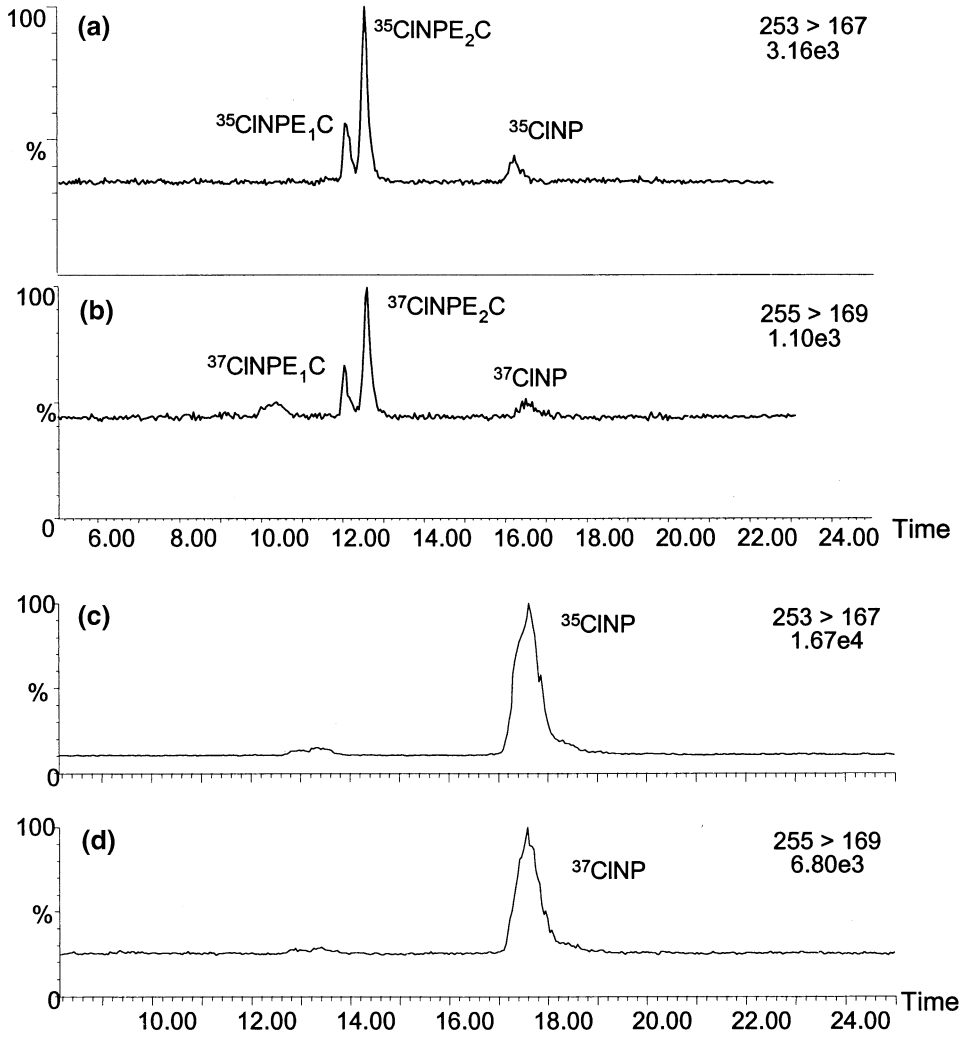

Figure 6. Detection of chlorinated nonylphenolic compounds in prechlorinated water [traces (**a**) and (**b**)] and flocculation sludge [traces (**c**) and (**d**)] from Barcelona drinking water treatment plant. (MRM mode: 253 → 167 and 255 → 169 for detection of ^{35}Cl and ^{37}Cl-nonylphenolic compounds, respectively).

sensitive than product ion MS-MS. The precursor ion scan of the *m/z* 133 yielded the $[M - H]^-$ ions of the respective NP, NPE_1C, and NPE_2C, together with small "isotopic" peaks one to several mass units higher. However, an acceptable mass chromatographic response was only obtained for nonylphenolic compounds, when present at concentrations higher than 0.5 $\mu g/L$. For less abundant halogenated compounds precursor ion scan of *m/z* 79/81 for brominated compounds and precursor ion scans of *m/z* 167/169 and 35/37 for chlorinated compounds were not applicable to real-world samples due to low sensitivity.

Quantitative Analysis

Matrix effect. One of the problems to be solved when analyzing trace organics in complex matrices by LC-MS-(MS) is suppression of the analyte signal caused by high concentration of matrix components. The use of an internal standard can compensate, over a limited retention time window, for signal irreproducibility and correct quantitative data. However, it cannot compensate for the overall sensitivity reduction. Another approach to cope with matrix effect and to avoid an erroneous quantitation is aimed at the reduction of matrix components prior to the LC-MS-(MS) analysis applying a selective extraction and improved sample clean-up. Although the later strategy is laborious, it is still the most direct mean to avoid the loss of sensitivity.

Analytical procedure (SPE for water samples and PLE with SPE clean-up for sludge samples), applied in this work, yielded rather clean extracts that produced low background MS noise, as described in details elsewhere [24]. The extent of ion suppression was checked by adding an internal standard (4-nonyloxy benzoic acid) to all extracts and monitoring the transition reaction from *m/z* 263 $[M - H]^-$ to *m/z* 92 (retention time t_R = 14.7 min). In comparison to the signal

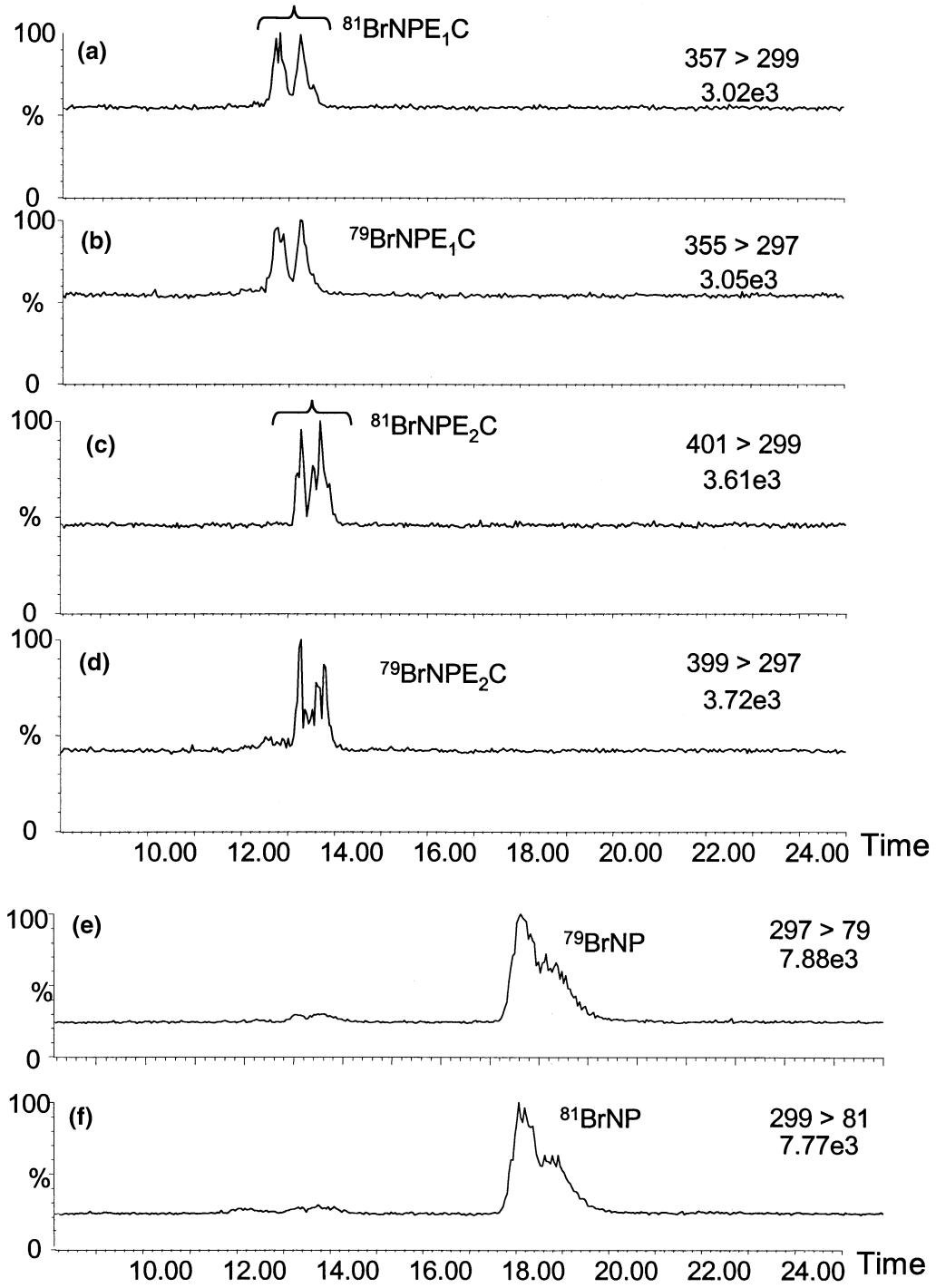

Figure 7. Detection of brominated nonylphenolic compounds in prechlorinated water [traces (**a**)–(**d**)] and flocculation sludge [traces (**e**) and (**f**)] from Barcelona drinking water treatment plant. MRM mode: (**a**) 357 → 299 and (**b**) 355 → 297 for $BrNPE_1C$; (**c**) 401 → 299 and (**d**) 399 → 297 for $BrNPE_2C$; (**e**) 297 → 79 and (**f**) 299 → 81 for BrNP.

intensity obtained in a standard solution (methanol), the flocculation sludge was the only sample that showed a reduced value (limited to 10 to 15% of ion suppression for all analyzed samples), while less complicated matrices (river water and drinking water) showed negligible loss of sensitivity.

Validation Parameters. Recoveries obtained using LC-MS-MS (MRM) were similar to those previously reported for LC-MS (SIM) [6, 24]. SPE of target analytes from water samples combined with LC-MS-MS detection yielded recoveries from 72 to 98% (see Table 2), whereas for solid samples PLE followed by SPE extract

Table 2. Instrumental detection limits (LOD_{inst}) of LC-MS and LC-MS-MS, method detection limits (LOD_{method}), and recoveries of combined SPE-LC-MS-MS for the analysis of target compounds in water samples and PLE-LC-MS-MS for solid samples

	LOD_{inst} (pg injected)		LOD_{method}		Recovery, % (RSD, $n = 3$)	
Compound	LC-MS (SIM)	LC-MS-MS (MRM)	Water samples (ng/L)[a]	Sludge samples (μg/kg)[b]	Water samples SPE-LC-MS-MS	Sludge samples PLE-LC-MS-MS
NP	60	10	1	0.5	85 (5)	81 (9)
XNP	50	15	2	1.0	72 (7) ClNP	60 (14) ClNP
					73 (7) BrNP	64 (11) BrNP
NPE_1C	100	30	2	1.5	98 (5)	78 (8)
$XNPE_1C$	150	50	2	1.5	90 (6) $ClNPE_1C$	71 (12) $ClNPE_1C$
					92 (7) $BrNPE_1C$	75 (9) $BrNPE_1C$
NPE_2C	100	30	2	1.5	88 (4)	68 (10)
$XNPE_2C$	200	50	2	1.5	84 (6) $ClNPE_2C$	
					79 (7) $BrNPE_2C$	n.d.

[a]SPE preconcentration factor 1000; 20 MRM channels (for other conditions see Experimental part).
[b]PLE, preconcentration factor 2; 20 MRM channel. %n.d. not determined.

clean-up and LC-MS-MS detection resulted in recoveries of 60 to 81% of target compounds. The relative standard deviation ($n = 3$) for water analysis was below 7% and for sludge below 14%. Intra-day variability of calibration curve slopes was minimal (<5%), while inter-day variability of daily calibration curve slopes over 1 week period ranged from 5 to 10% depending on the analyte.

The comparison of instrumental detection limits (LOD_{inst}) obtained using LC-MS and LC-MS-MS in SIM and MRM mode, respectively, and method detection limits (LOD_{method}) (MRM mode) are shown in Table 2. The LODs were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyses of a standard solution (LOD_{inst}) and real-samples (LOD_{method}), respectively, and on minimal value of signal-to-noise ratio of 3. The method LODs refer to SPE-LC-MS-MS procedure for water samples, based on a preconcentration factor of 1000, and PLE-LC-MS-MS for sludge samples (preconcentration factor 2). Values of LOD_{inst} obtained by LC-MS-MS were approximately 3–6 times lower than by LC-MS (SIM). The detection limits of the method proposed here for the analysis of target compounds in water samples fell down to 1–2 ng/L and in sludge samples ranged from 0.5 to 1.5 μg/kg, which is significant improvement in comparison to LODs reported previously for GC-MS [18] and LC-MS [6].

Analysis of Real Samples

The feasibility of LC-MS-MS method for the analysis of halogenated and non-halogenated NP and NPECs was evaluated by analyzing samples from DWTP Sant Joan Despí in Barcelona. The waterworks, with the daily production of 300,000 m^3 of drinking water, are situated at the Llobregat River downstream of a densely industrialized area. The whole river basin receives effluents from more than 30 WWTPs, which results in poor quality of the water entering to DWTP. Moreover, the specificity of the Llobregat River is a high concentration of bromide ions (average 0.7 mg/L, maximum 1.2 mg/L in 2000) [25] that arises from salt mine activities in the upper course of the river. As a consequence, it was already reported that treated (tap) water contains rather high concentration of brominated disinfection by-products [26].

NP and NPECs were found in the Llobregat River water entering the water treatment plant in concentrations of 1.5, 2.0, and 2.9 μg/L for NP, NPE_1C and the

Table 3. Concentrations found DWTP (Barcelona, Spain) samples

	Concentration in water (ng/L)						Flocculation sludge (μg/kg)
Compound	Raw water	Prechlorination	Sand filter	Ozonation	Carbon filter	Chlorination	
NP	1500	240	220	250	160	92	150
NPE_1C	2000	1640	1600	65	30	12	7.0
NPE_2C	2900	1960	2200	76	35	10	20
BrNP	n.d.	17	19	<5	n.d.	n.d.	105
ClNP	n.d.	9	12	<5	n.d.	n.d.	145
$BrNPE_1C$	n.d.	120	130	57	21	<5	n.d.
$ClNPE_1C$	n.d.	14	19	13	5	<5	n.d.
$BrNPE_2C$	n.d.	316	353	217	52	10	13
$ClNPE_2C$	n.d.	45	60	26	<5	<5	7.5

n.d. - not detected

most abundant NPE_2C, respectively, whereas their halogenated analogs were not detected as expected (Table 3). The following treatment process consists of prechlorination, flocculation with aluminum sulfate, sand filtration, groundwater dilution, ozonation, granular activated carbon filtration and a final chlorination. During the prechlorination steps, NP and NPECs were found to transform to halogenated derivatives. Both, brominated and chlorinated NP and NPECs were detected in prechlorinated water in concentrations up to 315 ng/L, being $BrNPE_2C$ the most abundant specie. The main part of these compounds, as well as of their precursors were removed by ozonation and subsequent activated carbon filtration. After final chlorination, in the treated water leaving the DWTP non-halogenated compounds were detected at trace levels (85, 12, and 10 ng/L for NP, NPE_1C, and NPE_2C, respectively) indicating the efficient removal of these compounds (95% of NP and more than 99% of acidic metabolites). Halogenated derivatives were identified but their concentrations never exceeded 10 ng/L.

The flocculation sludge (daily production 30,000 kg dry matter) was found to accumulate hydrophobic halogenated compounds and their precursors. NP, brominated and chlorinated NPs were found in concentrations of 150, 105, and 145 μg/kg, respectively. Acidic, polar metabolites were found in concentrations up to 20 μg/kg.

Conclusions

This work presents for the first time the application of liquid chromatography-tandem mass spectrometry to the analysis of halogenated nonylphenolic compounds and their precursors. MS-MS study of target compounds showed characteristic fragmentation pattern for each group of compounds that provided sufficient structural information and permitted the election of specific transition channels for their identification.

In addition to the advantages related to the quantification in MRM mode (high sensitivity and selectivity) the method allowed determination of isobaric target compounds (e.g., $BrNPE_1C$ and $ClNPE_2C$) and elimination of interference of co-eluting isobaric no-target compounds (e.g., $C_{11}LAS$). The method yielded very low detection limits: low ng/L level in water samples and low μg/kg level in sludge samples, thus providing a reliable and robust tool that can be used for routine analysis of estrogenic NP, NPECs, and their halogenated derivatives in aqueous and solid samples.

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