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Determination of nonylphenol ethoxylates in wastewater samples with SPME/GC-MS

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ABSTRACT

Nonylphenol ethoxylates (NPnEOs) are non-ionic surfactants that have been widely used in the cleaning industry over the past decades. At the same time, they pose a major environmental threat due to their toxicity and estrogenic activity. During the wastewater treatment process, they degrade and form more persistent and toxic metabolites that are detected mainly in the plant effluent. In the present work headspace solid-phase microextraction (SPME) with in-sample derivatization was used with dimethyl sulfate as methylating agent in order to quantify nonylphenol ethoxylates in wastewater samples. The selection of the appropriate fiber coating material and the optimization of the desorption conditions were necessary in order to enhance the SPME performance. The analytes were successfully isolated using 65 μm PDMS/DVB fiber and they were separated by means of gas chromatography with mass spectrometry detector (GC/MS) with the injector at 270°C. The method was linear over a wide range of concentrations (0.05–480 μg/l depending on the compound). The limits of detection for the compounds of interest were found to be in the microgram per liter range. For nonylphenol the lower limit of detection was obtained (0.02 μg/l), while for NP1EO, NP2EO and NP1EC limits of detection were 0.61, 3.2 and 0.29 μg/l respectively. Samples from the Municipal wastewater treatment plant of Chania (Crete, Greece) were collected from different stages of treatment and were analyzed for the presence of nonylphenol ethoxylates. Nonylphenol, which is considered to be the most abundant metabolite, was detected in all samples at low concentrations.

Keywords: SPME; Derivatization; Nonylphenol ethoxylates; Surfactants

1. Introduction

Alkylphenol ethoxylates (APEOs) belong to the category of non-ionic surfactants and are formatted during the reaction of an alkylphenol with ethylene oxide [1]. For the production of APEOs nonylphenol (NP) and octylphenol (OP) are used [2]. Nonylphenol ethoxylates have been widely used in domestic detergents and cleaning industry. They are also employed as emulsifiers, dispersants, antifoamers, dyeing assists, stabilizers, lubricants, spermicides and pesticide adjuvants. They are present in the aquatic environment through the effluents of the wastewater treatment plants [3].

Nonylphenol ethoxylates degrade during the wastewater treatment process, forming more persistent and toxic metabolites which are detected mainly in the plant effluent [4,5]. Biodegradation of NPnEO occurs with progressive shortening of the ethoxylate chain [6,7]. The ethoxylates with 0, 1 and 2 ethoxylate units (NP, NP1EO, NP2EO) are considered to be the metabolic products of NPnEO, together with carboxylated compounds (NP1EC, NP2EC) [4].

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Nonylphenol polyethoxylates are considered to be endocrine disrupting compounds [8]. Toxicity and estrogenic activity is increasing with the shortening of the ethoxylate chain [3]. They are often detected in the final effluents of sewage treatment plants, with nonylphenol being the most abundant, [3,9–14], due to their persistence and inefficiency of third-stage treatment. The effluent discharges pose a major environmental risk to the natural and human environment. In Greece, they have been detected in different stages of the wastewater treatment process [15–17], as well as in industrial wastewater [18].

Chromatographic separations are the only way to quantify the levels of NPnEOs in the environmental samples. Gas chromatography with mass spectrometric detector (GC/MS) is used for the determination of short ethoxy chain nonylphenol ethoxylates and carboxylated metabolites [19–21].

Regarding the sample pretreatment, conventional methods have been developed, which involve solid-phase extraction (SPE) and liquid-liquid extraction (LLE). The use of large volumes of organic solvents, the elevated cost and the laborious work in order to pre-concentrate the analytes are major negative aspects of these methods [22]. Nowadays, microextraction methods tend to be used very often for the pretreatment of environmental samples for the analysis of NPnEOs [17,23–26]. The pretreatment procedure following microextraction has become very fast with very good performance.

Solid-phase microextraction (SPME) is one of the alternatives to the traditional methods of extraction. It is a fast, low-cost technique that does not require special laboratory equipment and organic solvents. A polymer or adsorbent-coated fused-silica fiber is exposed either directly to the sample (immersion SPME) or in the vapor phase (headspace SPME). The analytes are adsorbed to the fiber and when equilibrium is reached, the fiber is transferred to the GC injector or the SPME-HPLC interface for separation and quantitation [27]. The parameters that most affect SPME efficiency are the fiber coating material, the extraction time, the extraction temperature and the ionic strength of the solution. [27]

The extraction of polar compounds from environmental matrices as well as their separation on a chromatographic column is often a difficult task and is achieved using derivatization. SPME can be combined with derivatization, thus making the analysis even simpler. Derivatization reactions may take place in the sample vial simultaneously with extraction (in-sample derivatization), in the GC injector port after the extraction, or on fiber prior to extraction [28]. On-fiber derivatization was compared with in-sample derivatization [23] and the later was suggested as the most appropriate method for the determination of nonylphenol ethoxylate metabolites. The derivatizing agent was first added to the vial containing the sample; the derivatives were extracted by SPME and introduced into the analytical instrument. Dimethyl sulfate was used as a derivatization reagent, forming methoxy (NPEOs) and methyl ester (NP1EC) derivatives. HS-SPME was employed for the isolation of analytes. Addition of sodium hydroxide was necessary, due to the strong acidic character of DMS which can cause serious damage in the chromatographic column. Higher derivatization yields were obtained by using DMS and NaOH in a ratio 0.2 ml/1 ml (DMS/NaOH). This method was applied for the determination of the compounds in tap and river water.

The main objective of this work is to apply the SPME method in order to determine the nonylphenol ethoxylates in wastewater samples. Modifications were made to the analytical method adopted by literature [23], in order to be used as routine analysis method. The main problem of the method was that the fiber coating was not so resistant in alkaline pH (necessary condition for the derivatization reaction to take place). Therefore, it could only be used for the analysis of a small number of samples. This problem was solved in this work by using another coating material.

2. Experiments

2.1. Reagents

For the SPME/GC-MS analysis of the nonylphenol ethoxylate metabolites, nonylphenol (NP) and a mixture of nonylphenol monoethoxylate and nonylphenol diethoxylate (POE-1 to 2 nonylphenol) were purchased from Chem Service. Nonylphenoxy acetic acid (NP1EC) was purchased from Dr. Ehrenstorfer, GmbH. The stock solutions were prepared monthly by weighing in dichloromethane, at a concentration of 10,000 mg/l. The standard solutions were prepared by dilution weekly or daily, depending on their concentration, in methanol. Reference standard solution for NP1EC had a concentration of 10 mg/l in acetone and each time the appropriate volume was used without any dilution. The spiked water sample solution that was used for the optimization of the SPME procedure had a concentration of 6 μg/l of NP, 30 μg/l of NP1EO, 30 μg/l of NP2EO and 4 μg/l of NP1EC. All the solutions were kept at 4°C in the dark and brought to room temperature before use.

Dichloromethane, and methanol, were of GC grade, purchased from Merck. Acetone was of PESTANAL grade (FLUKA). The derivatization reagent Dimethyl Sulfate (DMS) was purchased from Sigma-Aldrich. 4*n*-nonylphenol and 4*n*-nonyloxybenzoic acid that were used as internal standards were purchased from Supelco and Sigma-Aldrich respectively. Sodium chloride and

sodium hydroxide were of analytical grade provided by Fluka. Sodium chloride was cleaned with sonication with dichloromethane for 30 min and then heated overnight in 180°C to remove impurities.

2.2. Sample collection

Grab samples were obtained from the Municipal wastewater treatment plant of Chania (Crete, Greece) at different stages of the treatment process (raw, primary effluent, secondary effluent and chlorinated effluent). The samples were collected in amber glass bottles and were analyzed immediately in order to determine their quality characteristics and the compounds of interest. In each sample pH, chemical oxygen demand (COD) and total suspended solids (TSS) were determined. Chemical oxygen demand and total suspended solids were measured according to standard methods 5220D and 2540D, respectively [29].

In cases where preservation of the samples was necessary, formaldehyde solution was added (1 ml/100 ml of sample). Formaldehyde has been suggested in order to prevent biological degradation of the compounds of interest, if their analysis was not performed on the day of sampling [2,30].

2.3. Instrumentation-SPME equipment

The SPME procedure was performed with a manual SPME holder (for GC use). For the SPME/GC analysis, 65 μm PDMS/DVB stable-flex fiber was used and Carbowax/Divinylbenzene (CW/DVB), Polyacrylate (PA) and Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) were also checked for their performance. All the necessary SPME equipment was purchased from Supelco. Each fiber was conditioned before use according to the manufacturer's recommendations.

For the separation and quantitation of the analytes during SPME/GC-MS analysis a Shimadzu QP5050 gas chromatograph system, equipped with mass spectrometer (MS) detector was used. The detector was operated in electron ionization positive mode (70 eV). The chromatographic column was DB-5MS, 30 m × 0.32 mm × 0.32 μm (Supelco). The sample was injected in splitless mode and helium was used as a carrier gas with a flow of 2.8 ml/min. Interface temperature was 290°C. The initial column temperature was 70°C and was maintained for 3 min. It was increased at 160°C with a rate of 20°C/min and finally reached 280°C with a rate of 10°C/min, where it was kept for 10 min. Injector temperature was 270°C. The chromatographic method was adopted from reference [23].

2.4. SPME procedure

The volume of sample that was used for HS-SPME was 20 ml. Attempts have been made to perform the extraction with higher sample volumes in order to increase the analyte peak responses, but the results were less reproducible. This was attributed to the transfer of drops from the solution to the fiber due to the elevated temperature that was used for the extraction. Moreover, the lifetime of the fiber coating material was reduced. The sample was transferred to a 40-ml vial containing 4.33 g of NaCl and a Teflon-coated magnetic stir bar. Sodium hydroxide was added to the sample (700 μl of 5 M solution) and the vial was sealed with screw cap and Teflon lined septum. Through the septum 2 μl of the internal standard mixture (5 ppm 4*n*-nonylphenol and 1000 ppm 4*n*-nonyloxybenzoic acid) and 140 μl of the methylation reagent (dimethyl sulfateDMS) were added. The vial was placed in a magnetic stirrer inside a thermostated water bath at 60°C. The 65 μm PDMS/DVB fiber was exposed to the headspace of the sample for 1 h, with continuous magnetic stirring at 700 rpm. When the equilibrium was reached, the fiber was retracted from the sample and was immediately transferred into the gas chromatograph injector. Thermal desorption at 270°C and separation of the compounds followed.

2.5. Quantitative analysis

For the calibration of the instrument 5-point calibration curves were plotted. The fraction of the area response for a given concentration versus the area of the internal standard (A/Ais) was plotted against the concentration for each compound separately. The A/A is fraction was calculated by the average of triplicate injections of extracted spiked standards into ultrapure water with known concentrations of the metabolite. Internal standards (*n*-NP and *n*-nonyloxybenzoic acid) were used for the quantitation.

3. Results and discussion

3.1. Fiber choice

Four different fiber coatings were tested for the extraction of the nonylphenol ethoxylate metabolites from aqueous samples: Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) 65 μm, Carbowax/ Divinylbenzene (CW/DVB) 70 μm, Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm and Polyacrylate (PA) 85 μm. A volume of 20 ml of a standard mixture of the compounds in Milli-Q water (6 μg/l of NP, 30 μg/l of NP1EO, 30 μg/l of NP2EO and 4 μg/l NP1EC) was methylated with dimethyl sulfate (DMS) and extracted for 60 min in 60°C, with continuous

stirring. The injector temperature was 250°C and the fiber was removed from the injector after 20 mins, to avoid carryover. Sodium hydroxide was added to the sample (700 μl of 5 M solution). Sodium chloride was also added to the sample.

As shown in Fig. 1 the compounds can be successfully isolated from the water sample using CW/DVB fiber. The same fiber is suggested to be the most suitable for the extraction by Diaz and Ventura [23]. The manufacturer suggests that the pH range for the usage of CW/DVB fiber is 2–9. The pH of the solution during extraction is around 11 due to the sodium hydroxide that is added to the sample. Under these conditions, the CW/DVB fiber was found to be very fragile and could last only for a few extractions even when the headspace mode was applied. Low pH adjustment did not give the desirable results. The methylation products are being hydrolyzed at lower pH values thus resulting in decreasing the extraction efficiency.

The Polyacrylate fiber (PA) gave satisfactory results and it was used for the optimization of the parameters that affect SPME efficiency. However, significant variation in fiber durability was observed during the experiments. Based on these experiments, for the calibration of the instrument and the analysis of samples the 65 μm PDMS/DVB fiber was used, which was found to be the most stable and durable under the above experimental conditions.

When the extraction was completed, thermal desorption of the compounds took place in the GC chromatograph injector port. The parameters that affect the desorption of the compounds are referred to as "desorption conditions" [27]. This term includes desorption temperature and desorption time. Both parameters needed to be adjusted for the complete desorption of the analytes, thus avoiding cross contamination after the reuse of the fiber (carryover effects). An increase of the injector temperature can also increase the analytes peak responses. For PDMS/DVB fiber that was used in the current research a comparison of the performance of the HS-SPME method with three different injector temperatures was done and the results are shown in Fig. 2.

The best results for all compounds were obtained when the PDMS/DVB fiber was used at 270°C, which was the maximum allowable usage temperature of the material according to the manufacturer. Desorption time was 10 min. No carryover effects were observed. Blank samples were run from time to time in order to eliminate any possible contamination between the analyses.

Typical chromatograms (MICMulti Ion Chromatogram) are presented in Fig. 3. The analytes were branched compounds (mixtures of isomers), so they appear in the chromatograph as a group of peaks. Their identification was based on the retention time taking into account the whole time range e.g., for NP1EO is from 13 min to 14.5 min. These time values are shown in the MIC chromatographs.

The ions chosen for the quantitation of the analytes were m/z 121, 135 and 149 for NP, m/z 179 and 193 for NP1EO, m/z 223 and 237 for NP2EO and m/z 207, 235 and 292 for NP1EC. The internal standards were quantified using m/z 121 and 234 for *n*-NP and m/z 278 for *n*-nonyloxylbenzoic methyl ester.

3.2. Method validation

The fraction of the area response for a given concentration versus the area of the internal standard (A/Ais) was plotted against the concentration separately for each compound. The sum of areas for the selected ions of each methylated compound was calculated for quantitation. The analytes showed good linearity and correlation (R^2 = 0.99 for 5-point calibration curves), while the limits of detection for all the compounds were in the low microgram per liter range. In Table 1 all the qualitative characteristics of the method are presented. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated for the analytes. Limit

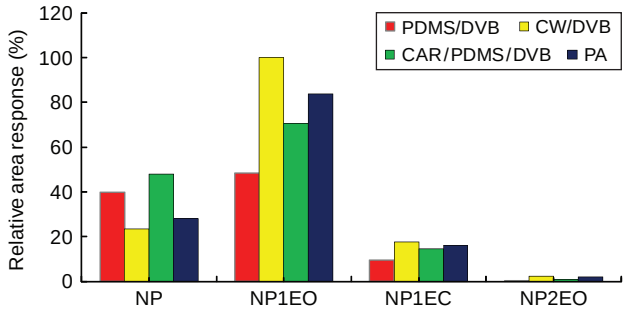

Fig. 1. Comparison of four commercial SPME fibers for their extraction efficiency. Sample: Spiked Milli-Q water sample.

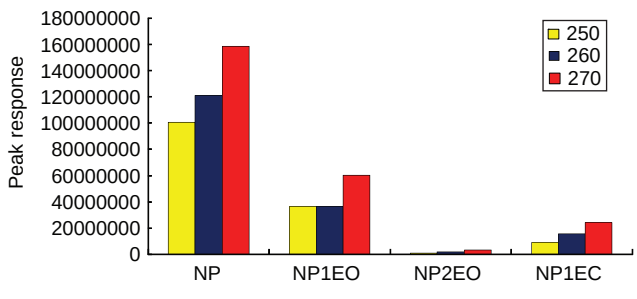

Fig. 2. Comparison of the performance of 65 μm PDMS/DVB stable flex fiber in different injector temperatures (°C). Sample: Spiked Milli-Q water sample.

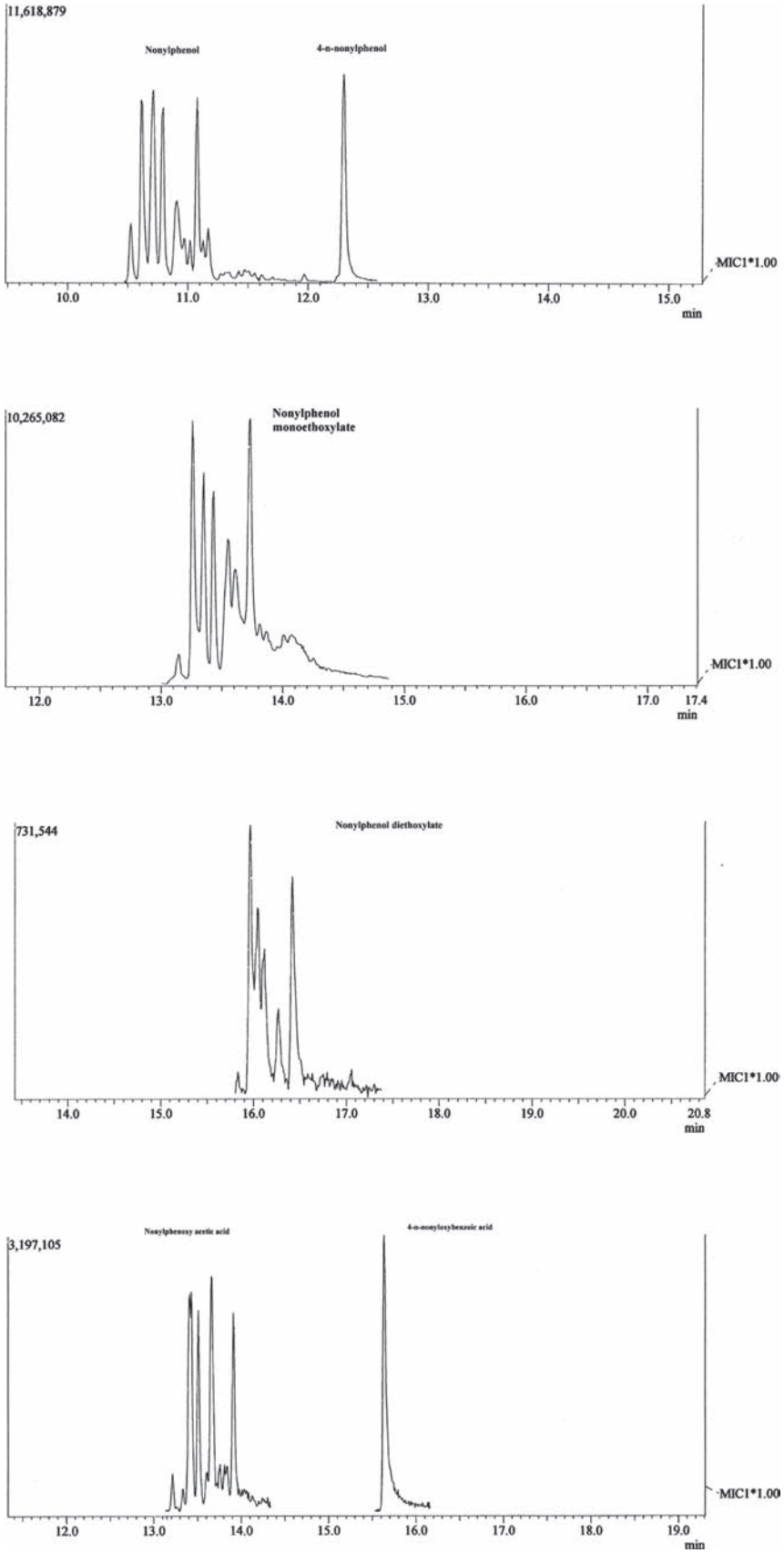

Fig. 3. Multi ion chromatograms (MIC) of the methylated products of nonylphenol ethoxylate metabolites after extraction from spiked aqueous standards with HS-SPME method.

Table 1
Parameters for validating the HS-SPME with in-sample derivatization method for the analysis of nonylphenol ethoxylate metabolites

Compound	Linear range (μg l^{-1})	R^2	RSD%	LOD (μg l^{-1})	LOQ (μg l^{-1})
Nonylphenol (NP)	0.05–1	0.9996	6.60	0.02	0.06
Nonylphenol monoethoxylate (NP1EO)	2–30	0.9970	13.55	0.61	1.95
Nonylphenol diethoxylate (NP2EO)	10–480	0.9953	18.80	3.01	9.6
Nonylphenoxy acetic acid (NP1EC)	1–32	0.9992	9.57	0.29	0.92

Table 2
Qualitative characteristics of SPME methods for the analysis of NPnEOs

Method	Sample matrix and analytes	Linear range (μg/l)	LOD	Reference
HS-SPME with in-sample derivatization	NP, NP1EO, NP2EO, NP1EC in treated water and river water	NP: 0.06–35 NP1EO: 0.50–0.45 NP2EO: 1.2–6.0 NP1EC: 0.9–6.0	NP: 0.02 NP1EO: 0.2 NP2EO: 0.4 NP1EC: 0.3	[23]
Immersion SPME withHS derivatization (on-fiber silylation with BSTFA)	NP in river water and fish blood serum	1–100	0.003	[24]
Immersion SPME coupled with GC/MS	NP in pure water, model wastewater and effluent of a pilot scale wetland plant	0.27–153	0.2 (for pure water)0.4 (for model wastewater in accordance to DIN 38412T24) 0.8 (for effluent of a pilot scale wetland plant)	[13]
HS-SPME with on-fiber derivatization (Derivatization reagent: MTBSTFA with 1% TBDMCS)	NP in spiked chlorinated tap water, detergent water and lake water	0.005–1.04	0.00158-0.00385	[25]
Immersion SPME with derivatization in the injection port with BSTFA	NP in seawater	0.5–50	0.0141	[26]
HS-SPME with in-sample derivatization with dimethyl sulfate	NP, NP1EO, NP2EO and NP1EC in wastewater	NP: 0.05–1 NP1EO: 2–30 NP2EO: 10–480 NP1EC: 1–32	NP: 0.02 NP1EO, NP2EO: NP1EC:	Current study

of detection is defined as the concentration that gives signal three times higher than the noise and was calculated by using the triplication of the average signal-to-noise (S/N) at low concentrations. The quantitation limit (LOQ) was calculated at 10 × S/N. LODs were at the low μg l^{-1} level for all ethoxamers. Repeatability of the method was checked with the calculation of relative standard deviation (%RSD). The %RSD was calculated for the average of three injections of extracted aqueous spiked standards of the same concentration in different days. Matrix effects were not taken into consideration, since the recovery of the analytes with this method was between 95 and 102%.

A comparison of the method presented in the current study with similar methods already published was done and it's briefly summarized in Table 2.

Most of the methods that were found in prevailing literature use SPME to determine nonylphenol, usually with other endocrine disrupting compounds. The method that was optimized and used in the current study for the analysis of the wastewater samples can be directly compared with the method developed

by Diaz et al. [23], which also provided simultaneous determination of all the metabolic products of nonylphenol polyethoxylate surfactants. In the HS-SPME method with in-sample derivatization that was developed from Diaz et al. [23] detection limits of NP and NP1EC have the same value as the ones obtained in the current work (0.02 μg l^{-1} for NP and 0.3 μg l^{-1} for NP1EC). Detection limits for NP1EO and NP2EO are higher (Table 2) in the present study than the ones obtained from Diaz et al. [23] (0.2 μg l^{-1} for NP1EO and 0.4 μg l^{-1} for NP2EO). The present work provides a wider linear range for the analysis of all the metabolites besides NP. Moreover, the 65 μm PDMS/DVB fiber proved to be robust enough for the extraction of approximately 40 samples without any deterioration, in contrast to CW/DVB that was used from Diaz et al., which was not resistant in the pH that methylation reaction took place.

3.3. Sample analysis

The HS-SPME method with in-sample derivatization was applied for the analysis of wastewater samples. The samples were collected from the Chania (Crete) Municipal wastewater treatment plant at different stages of the treatment process (raw, primary effluent, secondary effluent and chlorinated effluent). In each sample physicochemical parameters such as pH, total suspended solids (TSS) and chemical oxygen demand (COD) were determined and the results are presented in Table 3. The values of the parameters were within the typical value range for a municipal wastewater treatment plant. Subsequently, the samples were filtered and analyzed for the presence of nonylphenol ethoxylate metabolites, immediately after their collection (Table 4).

Nonylphenol which is the most stable and abundant metabolite was detected in all samples at low concentrations in contrast to the other metabolites which were not detected in any of the samples or they existed in concentrations below the method detection limit. The presence of nonylphenol in the wastewater could be explained by the fact that non-ionic surfactants are not the only source of nonylphenol in the environment. Concentrations of nonylphenol detected in wastewater effluents in Chania as compared to other Greek sewage treatment plants had values in the same order of magnitude [15,16].

The concentrations of nonylphenol that were detected in the effluent are lower than those detected in raw and treated wastewater. Elimination of the compounds during wastewater treatment process occurs either by biodegradation or by sorption to solids. Metabolic products of nonylphenol polyethoxylates have high $\log K_{ow}$ values (3.90–4.48) with nonylphenol having the highest one. Their solubility in water is limited [7]. Due to their lipophilic nature they tend to adsorb to particulate matter and to sewage sludge and this could be another possible reason for not detecting NP1EO, NP2EO and NP1EC in any of the samples. A recent research for the distribution of the compounds between particulate and dissolved phase in samples from Greek wastewater treatment plants showed that their fraction that was bounded to the solid phase was a lot higher in influent that in the effluent [15]. Almost 50% of the analytes was determined in the particulate phase in influent

Table 3
Physicochemical characteristics of the wastewater samples on the date of sampling (Municipal wastewater treatment plant of Chania)

Sampling date	Sample	pH	TSS (mg/l)	COD (mg/l)
10/10/2008	Raw wastewater	7.14	180	490
10/10/2008	Treated wastewater	7.53	142.5	502
10/10/2008	Secondary effluent	7.28	5.5	102
10/10/2008	Chlorinated secondary effluent	7.37	8	109

Table 4
Concentrations of nonylphenol ethoxylates in wastewater samples from Municipal wastewater treatment plant of Chania (Crete, Greece)

Compound	Raw wastewater (μg/l)	Treated wastewater (μg/l)	Secondary effluent (μg/l)	Chlorinated secondary effluent (μg/l)
NP	7.92	5.52	0.20	0.30
NP1EO	n.d.*	n.d.	n.d.	n.d.
NP2EO	n.d.	n.d.	n.d.	n.d.
NP1EC	n.d.	n.d.	n.d.	n.d.

*n.d: non-detected.

wastewater while this value was 18–35% in treated wastewater. In the same research [15] the analytes were determined in sewage sludge samples with mean values of 0.17, 12.3 and 6.14 μg/g dw for NP, NP1EO and NP2EO respectively. Concentrations of NP1EO and NP2EO were a lot higher than NP, but this was probably because 4-*n*-NP was used as a reference standard, so not all the isomers could be quantified. In another research that was conducted in Greece [31], the highest concentrations of NP in sludge were 93 μg/g dw (primary sludge sample) and the lowest was 3.6 μg/g dw (secondary sludge sample).

Based on the above information and the concentrations of NP that were detected in the influent and final effluent, it was estimated that the removal of nonylphenol during the wastewater treatment process was around 96%. The amount bound to solids in the final effluent according to these partitioning data would be lower than the effluent concentration. Since total suspended solids concentration in the final effluent was very low (8 mg/l), effluent discharges in the sea did not constitute at present a serious environmental threat.

4. Conclusions

Solid phase microextraction (SPME) was applied as a sample pretreatment step for the determination of nonylphenol ethoxylates and their metabolites in wastewater samples. SPME was applied in headspace with in-sample derivatization mode, coupled with GC-MS. The fiber coating material that was selected for the study was the 65 μm PDMS/DVB stable flex fiber which was found to be the most suitable under the experimental conditions, because of its effectiveness and durability. The SPME performance was enhanced with the thermal desorption of the compounds at 270°C. The method proved to be fast (70 min duration in total), simple and solventless. Detection limits were at the low μg/l range with the lower ones obtained for NP and NP1EC. Linearity of the method was ranging from 0.05–480 μg/l depending on the compound. Nonylphenol was detected in wastewater samples from all the stages of treatment. The highest concentration of NP that was detected was 7.92 g/l in raw wastewater. The secondary effluent concentration in NP was very low (0.2 μg/l). The amount of NP that was detected was very low as well in the chlorinated secondary effluent (0.3 μg/l).

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