Combined Cation Exchange - Liquid-Liquid Extraction Method for GC-MS Determination of 4-Nonylphenol Content in Water

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An efficient and highly selective combined cation exchange – liquid-liquid extraction method coupled with GC-MS determination is proposed for the separation and concentration of 4-nonylphenol from various water matrices. The method has good precision expressed as repeatability: mean value 97.28% with a RSD value of 4.20%. Recovery of alkylphenols from spiked samples varies from 92 to 101% and the values obtained are higher than with other methods currently in use. The method was successfully applied for the determination of 4-nonylphenol from various water matrices.

Keywords: 4-nonylphenol, cation exchange – liquid-liquid extraction procedure, GC-MS

Nonylphenols and their ethoxylates are non-ionic surfactants generally used as detergents, emulsifier, stabilizers, wetting agents [1]. Alkylated *p*-nonylphenol is a starting material for the synthesis of collector reagents for ion flotation [2]. There are several papers and reports relating to the toxicity and estrogenic activities of nonylphenols [3]. Due to its endocrine disrupting potential, nonylphenol (NP) has been included in the list of priority substances in the field of water policy which is part of the Water Framework Directive of the European Commission (EC) and its application have been limited in some developed countries [4]. The matrices where these compounds have been detected are extremely various. One can find such non-ionic surfactants in river water, seawater, aquatic sediments and sediment samples [5–8]. One of the main degradation products of alkyl phenol polyethoxylates is 4-nonylphenol (further noted by 4NP), which is more persistent in the environment than their ethoxylates parent: with a half-life of about 2 months in water and even years in sediments [9].

Usually nonylphenols are determined by HPLC [10-12], LC-MS [13,14] or by GC-MS [15-19]. By means of GC-MS method nonyphenols could be analysed without derivatization that constitutes a huge advantage, but, at lower concentration, peak tailing might occur and give low sensitivity and reproducibility [20]. For this reason many works have focused on the separation and concentration of nonylphenols in order to be determined by GC-MS.

The literature reports analytical methods for the isolation and preconcentration of 4-nonylphenol. The methods include: liquid–liquid extraction, LLE [21], solid-phase extraction, SPE [22], solid phase microextraction, SPME [23], batch, magnetic solid phase extraction, MSPE [24]. Recently, different membrane extraction techniques have been reported as an alternative to the SPE and LLE extraction [12,25,26]. In the standardised procedure [21] 1000 mL of water sample is acidified at *p*H 2 and then extracted with 40 mL of toluene for at least 4 h using a shake device. However there are no reports on the applications of ion exchange separation of nonylphenols.

This paper reports an original combined procedure of cation exchange – liquid-liquid extraction for the separation and concentration of nonylphenols, specially 4-nonylphenol, from various water matrices followed by GC-MS determination.

Experimental part

Apparatus

The analytical instrument was a Thermo Scientific gas chromatograph (Focus CG) coupled with an ion trap mass spectrometer (PolarisQ) and Autosampler TriPlus AS.

Chromatographic conditions

The capillary column was a DB-5 (5%-phenyl)-methylpolysiloxane, non-polar, bonded and cross-linked having 30 m length, with inner diameter 0.25 mm and 0.25 µm film thickness. The carrier gas was helium, at a constant flow rate of 1.0 mL/min. A splitless injection was used to inject the sample. Injection port temperature was 250°C. The transfer line temperature was 280°C and the temperature program as follows: at 100°C for 1 min; then to 200°C at 10 °C/min; then to 250°C at 7°C/min then at 250°C for 10 min [21]. The MS detector was used in both scan and selected ion mode (SIM). The ion selected for identification and quantification of 4NP has m/z = 107.

Reagents

All the reagents were of analytical or better quality and have been purchased from Sigma – Aldrich company. Helium for GC-MS system (99.9995%) was purchased from Linde Gaz-Romania. Water for chromatography (18 MW) was obtained using both reverse-osmosis and ultra clear UV systems (SG Wasseraufbereitung und Regenerierstation GmbH). The 4-nonilfenol (98,5%) were purchased from Supelco. Dowex 50-X4 strongly acidic cation exchange resin (Dow Chemical Co. – USA) was used. Inorganic anions salt were purchased from Merck. Polycyclic aromatic hydrocarbons calibration mix was purchased from Supelco. Standards solutions for AAS of Na (I), K(I), Ca(II), Mg(II), Al(III), Cd(II), Co(II), Cr(VI), Fe(II), Fe(III), Mn(II), Pb(II), Ni(II), Zn(II), Hg(II) ions in azotic acid were purchased from Merck.

Stock solution of 4NP in toluene

Solution of 2.5 $mg.mL^{-1}$ of 4NP was prepared by dissolving the appropriate amount of them in a known volume of toluene 95%.

Working solution

Dilutions of stock solution in 5 mL or 10 mL volumetric flasks have been done in toluene in order to obtain working

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solutions having the concentrations of 4NP ranging between 0.09 – 2.5 μg.mL^{-1}.

Solutions of inorganic cations

Stock aqueous solution of 1 mg.mL^{-1} of each metallic cation: Na (I), K(I), Ca(II), Mg(II), Al(III), Cd(II), Co(II), Cr(VI), Fe(II), Fe(III), Mn(II), Pb(II), Ni(II), Zn(II), Hg(II) were prepared by dilution in purified water using corresponding AAS standards.

Working solutions

Working solutions of inorganic cations 0.05 mg.mL^{-1} were prepared by dilution in water just before use.

Stock solution of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) calibration mix has the concentration of 10 μg.mL^{-1} of each PAH in acetonitrile.

Working solution

Dilutions with acetonitrile of 1 mL of stock PAH solution in a 10 mL volumetric flask were made. The final concentration of each PAH was 1μg.mL^{-1}.

Preparation of cation exchange resin

The ion exchange separation took place in a glass column 15 cm long, 1 cm in diameter, provided with stopcocks at bottom and funnel with ground joint at top. A quantity of 8 g of strongly acidic cation exchange resin Dowex 50-X4 swollen in distillated water were transferred in the column and then purified, activated in H_3O^+ form (with 4 mol·L^{-1} HCl solution) and finally washed with distilled water up to negative Cl$^-$ test.

Procedure

Studies on the synthetic samples

In a volumetric flask of 1000 mL volumes of 1 mL of each working solutions: 0.05 mg.mL^{-1} of metallic cation, 1 μg.mL^{-1} of 4NP and 1 μg.mL^{-1} of PAH were added. The flask was filled to the mark with water for chromatography. A volume of 900 mL from this sample solution was passed, with a flow rate of 0.4 mL.min^{-1}, through the cation exchange column. All the free-of-cations effluent was collected in a 1000 mL volumetric flask. After the complete passage of the solution, the column was washed with water for chromatography and the effluent was collected in the same 1000 mL volumetric flask up to mark. The *p*H of this free-of-cations solution was adjusted to 11-12 with a 4 mol.L^{-1} NaOH solution. Volumes of 500 mL of this water sample free of cations were extracted twice with 20 mL of hexane. After the separation, the aqueous phases that contain only the sample's anions, including phenolates, were collected together in a 1000 mL Berzelius flask. The *p*H of this solution was adjusted to ~ 2.5 with 4 mol.L^{-1}HCl solution and the sample was extracted for four times with 20 mL fraction of toluene. The organic layers were put together, dried with anhydrous sodium sulphate, filtered and then concentrated using an evaporation device. The concentrate was transferred quantitatively with toluene in a 10 mL volumetric flask. The solution obtained was analyzed by GC-MS.

Regeneration of the column

100 mL of 4 mol.L^{-1} HCl were passed through the column with a flow rate of 0.4 mL.min^{-1} in view to exchange all the cations retained in resin with H_3O^+ ions. Finally the column was washed with water for chromatography up to negative Cl$^-$ test. Such a column could be considered regenerated and ready to be used for another separation.

Verification of performance parameters of the method

Linearity was studied according to the international rules [27-28] in concentration range 0.09 – 2.5 μg.mL^{-1} of 4NP.

Selectivity was studied on synthetic samples containing 0.05 mg.L^{-1} of metallic cation, 1 μg.L^{-1} of 4NP and 1 μg.L^{-1} of PAH. The synthetic samples were analysed according to the procedure described.

Precision expressed as repeatability was estimated on 6 synthetic samples prepared as described when the selectivity of the method was investigated [27-28].

Analysis of water samples

Water samples were collected according to the general rules [29]. Different water matrices were analysed: sub-surface water sampled in the south of Bucharest and river water taken from the section of the *Sabar* River, west and south Bucharest. The samples were filtered through a Millipore membrane of 0.45 μm pore size and stored at 4°C. The samples were analysed within 48 hours after the sampling.

Results and discussion

The standardised procedure for the separation of 4NP from various aqueous matrices require in the first step of the analysis the adjustment of the *p*H at 2.00 [21]. At this *p*H 4NP is protonated and could be extracted in organic solvents. However, at this *p*H not only 4NP is soluble in organic solvents but all the organic molecular species and even some organic acids are so. Unfortunately, this step in separation is not at all selective.

As the main objective of this work was to elaborate a selective separation and concentration procedure of 4NP from various water matrices, the proposed method is based on the acidic property of 4NP, which is a weak acid having the pK_a around 10.70 [30]. If the *p*H of water sample is greater than 11.50 the 4NP is in its fenolate form. Solvent extractions of this sample ensure the removal of almost all organic compounds that are not in an anionic form at this *p*H. But, at *p*H greater than 8 the cations present in water precipitate as hydroxides and the water analyse becomes more complicated.

Cation-exchange separation

The first step in this proposed procedure is the removal of cationic species using a strongly acidic cation exchange resin in H_3O^+ form. The water sample at its usual *p*H (5-6) is first purified through a cation exchange resin. This step in separation is critical: not only the inorganic cations were removed from the samples but also some cationic organic species; the effluent contains all the neutral molecules, including alkyl phenols, and anions.

The cation exchange resin is regenerated using both 4 mol.L^{-1} HCL solution and water for chromatography. In the acidic effluent obtained one can determine by an appropriate technique (i.e. FAAS) the content of all cations found in the sample of water analysed.

Liquid-liquid extraction

The obtained free-of-cations effluent is then treated with 4 mol.L^{-1} NaOH solution in order to reach a *p*H value around 12. At this *p*H one can find in the sample molecules and anionic forms of 4NP, others alkylated phenols and organic acids depending on their pK_a values. A solvent extraction using 4 portions of 20 mL of hexane ensure the removal of

Table 1

SIGNIFICANT LINEAR REGRESSION PARAMETERS OF 4 NP GC-MS DETERMINATION

Parameter	***Value***
Slope, *b*	-4619.962
Intercept, *a*	57327.714
Standard deviation of the slope, s_b	1748.856
Standard deviation of the intercept, s_a	2413.010
Correlation coefficient, *R*	0,9963
Standard deviation	4370.704
Degree of freedom	10

Table 2

DETERMINATION OF 4NP IN SYNTHETIC SAMPLES

Sample	**Cation exchange –L-L extraction***			**SPE extraction [32]**			**L-L extraction*[21]**		
	Amount added µg.mL^{-1}	Amount detn. µg.mL^{-1}	Recovery %	Amount added µg.mL^{-1}	Amount detn. µg.mL^{-1}	Recovery %	Amount added µg.mL^{-1}	Amount detn. µg.mL^{-1}	Recovery %
1.	0.0025	0.0023	92.00	0.0025	0.0021	84.00	0.0025	0.0018	80.00
2.	0.0532	0.0529	99.43	0.0532	0.0524	98.49	0.0532	0.0443	83.27
3.	0.0151	0.0152	100.7	0.0151	0.0142	94.03	0.0151	0.0122	80.79
4.	0.1125	0.1111	98.75	0.1125	0.1005	89.33	0.1125	0.0097	86.22
5.	0.1515	0.1534	101.3	0.1515	0.1423	93.93	0.1515	0.1262	83.33
6.	0.1726	0.1717	99.48	0.1726	0.1582	91.66	0.1726	0.1403	81.29
7.	0.2108	0.2092	99.24	0.2108	0.2092	99.24	0.2108	0.1944	92.22

Note:*toluene as solvent; all the results are based on a single analysis.

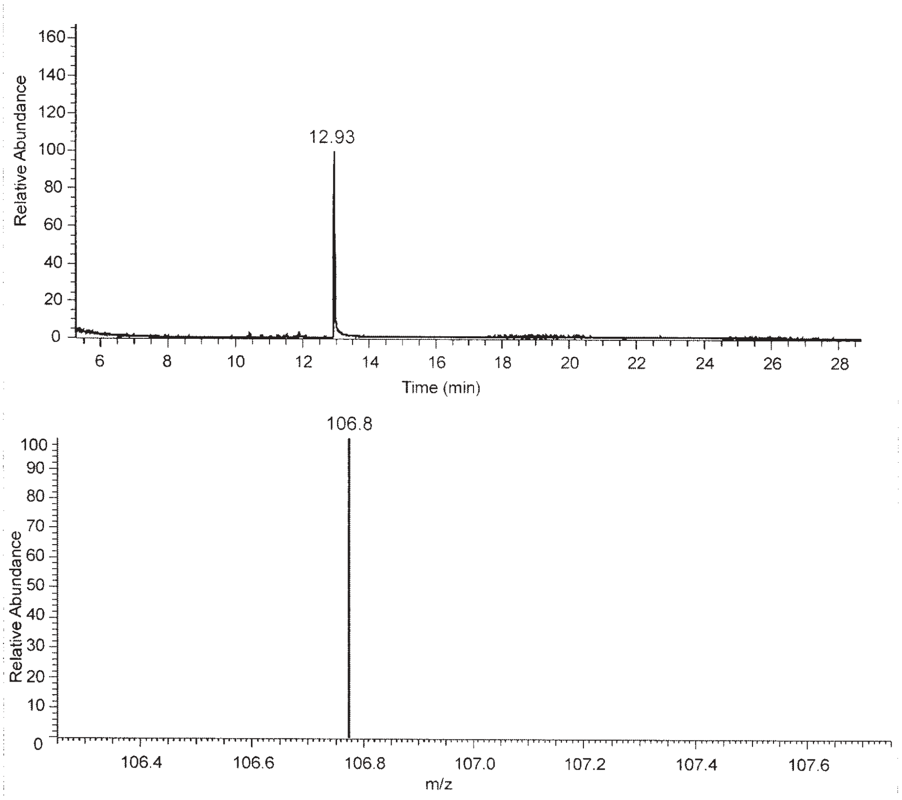

Fig. 1. GC-MS analysis of 4-nonylphenol: a. Chromatogram; b. MS recoded in SIM mode

non ionic species from this free-of-cations sample. The aqueous phase obtained is then treated with 4 mol.L^{-1} HCl solution until the *p*H reaches 2.5. At this *p*H the 4NP is completely protonated and can be extracted in organic solvents. Volumes of 20 mL of toluene were utilised for the extraction. An efficient extraction were obtained when four fraction of 20 mL of toluene were utilised.

Salts effect on the extraction

The influence of sodium and chlorine ions on the efficiency of extraction was investigated as it is known that the extraction efficiency increases in presence of salts. The addition of small volumes of both 4 mol.L^{-1} HCl and NaOH solutions is equivalent with small amounts of salt added and does not influence the extraction yield.

GC-MS determination

In this study the GC-MS method used has been largely studied in literature [21] and was applied without any modification. For this reason only the linearity of GC-MS method was checked [27-28]. The mass spectrum was recorded in SIM mode, with m/z=107 for the selected ion. Figure 1 presents a GC-MS analysis of 4-nonylphenol. The results obtained when the linearity of GC-MS method was

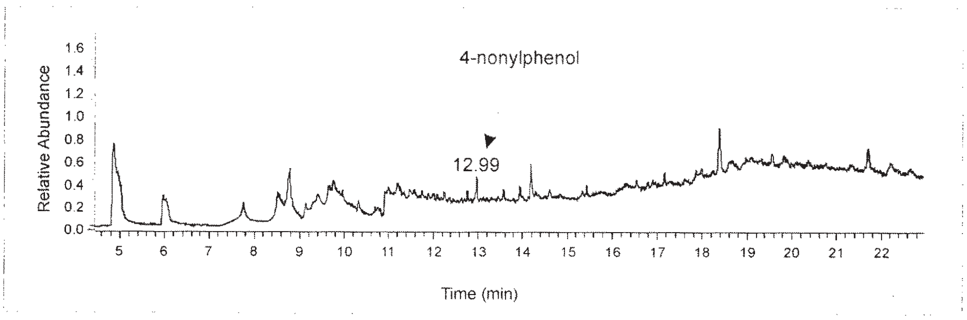

Fig. 2. Representative GC recorded in scan mode for a sample of *Sabar* River (west Bucharest) treated by proposed method

Table 3
DETERMINATION OF 4NP IN REAL WATER SAMPLES

Sample	**Cation exchange –L-L extraction***			**L-L extraction*[21]**		
	Amount added µg.mL^{-1}	Amount detn. µg.mL^{-1}	Recovery %±RSD	Amount added µg.mL^{-1}	Amount detn. µg.mL^{-1}	Recovery %
SUB-SURFACE	0 0.0115	n.d. 0.0112 ± 0.011	97.39 ± 1.36	0 0.0115	n.d 0.9634	83.77
SURFACE	0 0.0840	0.0124 0.0912 ± 0.0002	94.05 ± 2.29	0 0.0840	n.d. 0.0853±0.0004	101.2 ± 4.70
WASTE	0 0.1157	0.162 0.2701±0.0003	94.78 ± 0.93	0 0.0269	0.1433 0.3714± 0.0007	84.75± 1.89

Note:*toluene as solvent; all the results are based on three determinations

evaluated are presented in table 1. The fact that the correlation coefficient is near 1 and the intercept around 0 constitutes a solid argument for a good linearity of this method.

Precision expressed as *repeatability* was estimated on 6 synthetic samples containing 0.05 mg.L^{-1} of each metallic cation, 1.00 µg.L^{-1} of 4NP and 1.00 µg.L^{-1} of PAH. The mean of the recovery was 97.28% with a RSD value of 4.20% that is an acceptable one based on the modified Horwitz [31] equation.

Analysis of synthetic samples

Synthetic samples that contain 4NP in various concentrations, ranging from very small to high ones were prepared and analysed. The samples also contain a constant amount of inorganic cations and PAH of 1.00 µg.L^{-1}, respectively. The samples were analysed by means of the proposed method and the results compared with those obtained using two consecrated procedures: SPE extraction [32] and liquid-liquid extraction, in one step and one portion of organic solvent [21]. The results obtained are presented in table 2.

One can observe that the recoveries for 4NP using the proposed combined cation exchange – liquid-liquid extraction method are better than those obtained when the two other methods were used. One can also noticed the very good recoveries obtained when water samples with small amounts of 4NP were analysed using this new method. In a concentration range of 0.0025 – 1 µg.L^{-1} for 4NP the mean recovery obtained by the combined cation exchange – liquid-liquid extraction method is around 97% while by SPE method and LLE are 91% and 82% respectively.

Analysis of real samples

Four samples of water were analysed by proposed procedure and the values obtained for 4NP were compared with those found by reference method [21]. In figure 2 the GC chromatogram recoded in full scan for the *Sabar* River sampled in the south of Bucharest, is presented. Results obtained are presented in Table 3. Values calculated for the recoveries of natural spiked samples are in good agreement with those obtained when synthetic samples were analysed. The obtained RSD values obtained are in accordance with the theoretical ones, as they can be calculated with Horwitz equation [31].

Conclusions

A new method based on a combined cation exchange – liquid-liquid extraction coupled with GC-MS is proposed for separation, concentration and GC-MS determination of 4-nonyl phenol (4NP) from various aqueous matrices. This study was developed starting from the acidic property of 4NP. Theoretical studies on aqueous system containing inorganic cations, neutral organic species, organic acids, phenols and alkyl phenols lead to the following steps of separation: (1) separation of the inorganic cations using a strongly acidic cation exchange resin, (2) adjustment of the *p*H value around 12 and solvent extraction using 4 portions of 20 mL of hexane to ensure the removal of non ionic species from the sample free of cations; (3) adjustment of *p*H of aqueous phase around 2,5 and solvent extraction of 4NP, that is completely protonated at this *p*H.

The method proposed was tested on synthetic samples. The method has good repeatability as mean of the recovery was 97.28% with a RSD value of 4.20%. Applied on real water samples the method leads to good results obtained for the recoveries, betters that those obtained with references methods, especially for low concentration of 4NP.

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