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Dispersive liquid–liquid microextraction applied to isolation and concentration of alkylphenols and their short-chained ethoxylates in water samples

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ABSTRACT

Dispersive liquid–liquid microextraction (DLLME) coupled with high-performance liquid chromatography with fluorescence detector was applied for the determination of alkylphenols and their short-chained ethoxylates in water samples. Development of DLLME procedure included optimisation of some important parameters such as kind and volume of extracting and dispersing solvents. Under optimised conditions 50 μL of trichloroethylene in 1.5 mL of acetone were rapidly injected into 5 mL of a water sample. After centrifuging the organic phase containing the analytes was taken for evaporation with a gentle nitrogen purge and reconstituted to 50 μL of acetonitrile. The aliquot of this solution was analysed with the use of HPLC. For octylphenol (OP) and octylphenol ethoxylates (OPEOs) linearity was satisfactory in the range 8–1000 $\mu g\,L^{-1}$ and for nonylphenol (NP) and nonylphenol ethoxylates (NPEOs) linearity was in the range from 50 to about 3000 $\mu g\,L^{-1}$. Limit of quantitation was 0.1 $\mu g\,L^{-1}$ for OP and OPEOs and 0.3 $\mu g\,L^{-1}$ for NP and NPEOs. Satisfactory recoveries between 66 and 79% were obtained for environmental samples. The results showed that DLLME is a simple, rapid and sensitive analytical method for the preconcentration of trace amounts of alkylphenols and their ethoxylates in environmental water samples.

1. Introduction

Alkylphenol ethoxylates (APEOs) are one of the most widely used class of non-ionic surfactants. However, their biodegradation is difficult and leads to accumulation of the short-chained homologues of APEOs, their carboxylic derivatives as well as alkylphenols (APs) [1,2]. These biodegradation products are known to demonstrate estrogenic activity [3,4] which is of great concern to authorities. Several reports on APs and APEOs were published recently [5–9]. Also, the European directive 2003/53/EC was issued reducing the possibility of use of nonylphenol (NP) and nonylphenol ethoxylates (NPEOs) [10]. No such regulations were, however, made for octylphenol (OP) and octylphenol ethoxylates (OPEOs). Considerably lower use of OP and OPEOs than NP and NPEOs could be probable reason for this lack of regulations. Also, environmental monitoring showed lower concentrations of OP and OPEOs than NP and NPEOs [11–13].

Since 2000, the use of solid phase extraction for isolation of APs and APEOs from the aqueous solution has been reported by most of the papers [12–19]. Some papers reported the use of liquid–liquid extraction [20,21]. The use of other techniques for isolation of these analytes from the aqueous solution was limited. Here, the use of polytetrafluoroethylene capillary trap [22], solid phase microextraction [23] and steam distillation–solvent extraction [24] can only be mentioned.

An interesting alternative to the above mentioned sample isolation methods emerges from the latest developments of dispersive liquid–liquid microextraction (DLLME)—a new technique of sample isolation from the aqueous solution [25,26]. This technique is based on a ternary component solvent system in which a mixture of two organic solvents is added to a water sample. The first of these solvents (a dispersing solvent) is freely soluble in water (e.g. methanol, acetonitrile) and the second one (an extracting solvent) is a high density low water soluble liquid (e.g. chlorobenzene, carbon disulphide). A stable dispersion is formed after the injection of organic solvents to water. This facilitates extraction of analytes from the water sample to the dispersed phase. Then the dispersion is broken by centrifugation. As a result the analytes of interest can be found dissolved in the extracting solvent on the bottom of the centrifuge tube [25,26]. The dispersive liquid–liquid microextraction was successfully used for isolation of water contaminants from environmental samples. Rezaee et al. [25] used DLLME for the analysis of polycyclic aromatic hydrocarbons in surface water. Berijani et al. [26] presented a DLLME procedure for isolation of organophosphorus pesticides from river, well and farm water. Other examples of DLLME usage in environmental analysis include determination of pesticides [27–32], halogenated organic contaminants [33–36], phtalate esters [37,38], antimicrobial agents [39], bisphenol A [40]

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as well as organophosphorus flame retardants and plasticizers [41].

DLLME is rarely used in combination with HPLC although the first attempt to combine DLLME with HPLC [42] was successfully presented already one year after introduction of DLLME. This rare use of DLLME–HPLC can be attributed to problematic injection of chlorinated solvents to HPLC column in both normal and reversed phase analyses in comparison to ease of their use in gas chromatography. Chlorinated solvents can be in some instances replaced with low density extracting solvents. Recently, this procedure was successfully used for development of DLLME–HPLC method applied for analysis of selected alkylphenols in sea water samples [43].

In the present paper a method for quantitative determination of both alkylphenols and their ethoxylates in water samples is developed. The analytes are isolated from the water matrix using DLLME with chlorinated extracting solvents and subsequently analysed using high-performance liquid chromatography with fluorescence detection.

2. Materials and methods

2.1. Reagents and chemicals

Standards of 4-tert-octylphenol and nonylphenol were both from Aldrich (USA). Alkylphenol Target Analyte Mix containing all the analytes of interest used for peak identification was from Fluka (Switzerland). Two mixtures of alkylphenol ethoxylates were used together with alkylphenol standards for recovery studies. The first mixture containing octylphenol ethoxylates with an average ethoxylation degree 1.5 was obtained from Serva Feinbiochemica GmbH & Co (Germany) as Triton X-15. The second mixture containing nonylphenol ethoxylates with an average ethoxylation degree 1.5 was purchased from Aldrich as Igepal CO-210. HPLC-grade acetonitrile and methanol were from J.T. Baker (The Netherlands). The HPLC-grade water was prepared by reverse osmosis in a Demiwa system from Watek (Czech Republic), followed by double distillation from a quartz apparatus. Only freshly distilled water was used.

All of the reagents used as the extracting solvents in the experiments were of analytical grade. Chloroform ($CHCl_3$), carbon tetrachloride (CCl_4) and chlorobenzene (C_6H_5Cl) were from Fluka. Trichloroethylene ($Cl_2C{=}CHCl$) and tetrachloroethylene ($Cl_2C{=}CCl_2$) were from Merck (Germany). Analytical grade acetone and ethanol used as dispersing solvents were obtained from J.T. Baker. Sodium chloride was purchased from POCh (Poland).

2.2. Chromatography

A chromatographic system from Dionex (USA) consisting of a P580 A LPG gradient pump, an ASI-100 autosampler, an STH 585 oven and an RF 2000 fluorescence detector was used. 30 μL samples were injected into a 150 mm × 4.6 mm I.D. analytical column packed with 4 μm Inertsil ODS3 from GL Sciences (Japan) with a guard column (10 mm × 4.0 mm I.D.) packed with 4 μm C18. The mobile phase used for the analysis consisted of methanol, acetonitrile and water (50:15:35). The time of separation was 24 min in isocratic elution mode, at a flow-rate of 1.8 mL min^{-1} at 35 °C. Signal response was measured by fluorescence detector at wavelengths set at 225 nm for excitation and 300 nm for emission.

2.3. Dispersive liquid–liquid microextraction procedure

5 mL of water sample was placed in a 10 mL glass test tube with a conical bottom. 1.5 mL of acetone (dispersing solvent) containing 50 μL of trichloroethylene (extracting solvent) was injected rapidly into the sample solution using a 2 mL syringe. In this step, the extraction solvent was dispersed into the aqueous sample as very fine droplets and a cloudy solution was formed in the test tube. Then, the mixture was centrifuged for 10 min at 5000 rpm. The dispersed fine particles of extraction phase were sedimented in the bottom of the test tube. The sedimented phase was withdrawn with a 50-μL micro-syringe. The extract was evaporated with a gentle nitrogen purge, reconstituted to 50 μL of acetonitrile and injected into HPLC for analysis.

2.4. Method performance

Linearity of the method was tested in a wide range for all the analytes. For octylphenol and octylphenol ethoxylates it was tested in the range 8–1000 μg L^{-1} and for nonylphenol and nonylphenol ethoxylates in the range from 30 to about 3000 μg L^{-1}. At least nine calibration levels were included in each calibration line.

The instrumental limit of detection (LOD) and the instrumental limit of quantitation (LOQ) were calculated on the basis of signal to noise (S/N) ratio. The S/N = 3 was used for calculation of LOD and the S/N = 10 for calculation of LOQ. Similar procedure was used for calculation of method LOD and LOQ. However, here LOD and LOQ were calculated from the sample at concentration level close to limit of quantitation subjected to DLLME procedure.

A blank recovery test was performed to verify the possibility of contaminations from laboratory glassware and solvents. Recoveries of the analytes were tested for real water samples spiked with alkylphenols and their ethoxylates which were subjected to DLLME procedure and injected into HPLC. Precision was calculated from the recovery test results.

3. Results and discussion

3.1. Selection of the extracting solvent and the dispersing solvent

High recovery of analytes in DLLME depends mainly on choice of the extracting solvent and the dispersing solvent. A proper extracting solvent has to meet several requirements. It should demonstrate (a) low solubility in water, (b) potential for extracting analytes and (c) possibility of direct injection into chromatographic system or ease of evaporation. Here, mostly chlorinated solvents can be found in the literature as the extraction solvents [25–29,31–45,37–41] although the use of carbon disulphide [25,34–36], bromobenzene [29] and ionic liquid [30] has also to be mentioned.

Similarly the dispersing solvent has to fulfil several requirements. Basically, it has to (a) be miscible with both the water sample and the extracting solvent and (b) enable separation of the extracting solvent from a dispersion formed in the water sample. Good examples of the dispersing solvents are acetone, acetonitrile and methanol. Usually, at least two of these solvents are used for DLLME optimisation [25–41,44,45]. Other examples of the dispersing solvents include ethanol [28,29,33,38] and tetrahydrofuran [28,32,34,39].

A series of extracting solvents and three dispersing solvents were taken for selection of the best extracting system. The analytes were extracted from 5 mL water sample by addition of 50 μL of the extracting solvent in 1 mL of the dispersing solvent. The dispersion formed in a glass centrifuge tube was centrifuged, the extract was taken from the bottom of the tube, evaporated with a gentle nitrogen purge and reconstituted to 50 μL of acetonitrile.

The average recovery for extraction performed in triplicate and standard deviation (SD) are presented in Table 1. This table contains results obtained for several chlorinated extracting solvents and three dispersing solvents. Use of chloroform led to the lowest recoveries in all tested extracting systems. Moreover, for chloro-

Table 1
Percentage extraction recovery (R [%]) and standard deviation (±SD [%]) of different dispersing and extracting solvents. Each sample was prepared three times.

Dispersing solvent	Extracting solvent	OP	OPEO1	OPEO2	NP	NPEO1	NPEO2
Ethanol	Chloroform	–	–	–	–	–	–
	Chlorobenzene	61 ± 6	68 ± 6	64 ± 6	65 ± 4	62 ± 5	58 ± 6
	Tetrachloroethylene	52 ± 5	61 ± 5	60 ± 6	67 ± 7	70 ± 8	66 ± 7
	Trichloroethylene	63 ± 3	66 ± 1	63 ± 2	63 ± 2	63 ± 1	61 ± 2
	Carbon tetrachloride	58 ± 3	63 ± 3	58 ± 4	59 ± 4	59 ± 4	60 ± 5
Acetone	Chloroform	38 ± 4	43 ± 5	40 ± 7	47 ± 5	48 ± 6	47 ± 6
	Chlorobenzene	65 ± 8	71 ± 8	69 ± 9	69 ± 10	68 ± 9	64 ± 9
	Tetrachloroethylene	59 ± 5	63 ± 6	60 ± 5	65 ± 4	64 ± 5	58 ± 5
	Trichloroethylene	75 ± 3	75 ± 4	71 ± 6	75 ± 5	73 ± 2	73 ± 4
	Carbon tetrachloride	70 ± 3	73 ± 4	73 ± 5	74 ± 5	74 ± 1	74 ± 7
Acetonitrile	Chloroform	45 ± 5	49 ± 6	46 ± 5	49 ± 6	51 ± 5	48 ± 5
	Chlorobenzene	59 ± 1	64 ± 1	61 ± 1	64 ± 2	61 ± 2	57 ± 1
	Tetrachloroethylene	64 ± 2	72 ± 6	63 ± 4	72 ± 9	66 ± 6	66 ± 6
	Trichloroethylene	67 ± 9	69 ± 7	62 ± 6	67 ± 6	64 ± 7	63 ± 8
	Carbon tetrachloride	65 ± 3	69 ± 4	64 ± 2	68 ± 4	69 ± 3	70 ± 11

form:ethanol mixture no separation from the water phase was obtained and thus no results were obtained for this extracting system. Also the use of ethanol as the dispersing solvent led to lower recoveries than the use of acetone or acetonitrile. The use of acetonitrile as the dispersing solvent with chloroform or tetrachloroethylene enabled to gain higher recoveries than the use of acetone with both these extracting solvents. On the contrary, the use of acetonitrile as the dispersing solvent with chlorobenzene, trichloroethylene or carbon tetrachloride led to lower recoveries than the use of acetone with all these three extracting solvents. Here, it is worth noting that typical solvent selection in DLLME procedure is usually performed using step by step procedure—first the extracting solvent is selected and then the dispersing solvent [25–29,32–38,40,41]. The results obtained here could suggest that all the pairs of extracting—dispersing solvents should be used in the optimisation for proper selection of the solvents. Nevertheless, this procedure is time consuming and is not used in many studies [31].

The highest recoveries during solvent selection were obtained for extracting system (a) trichloroethylene with acetone and (b) carbon tetrachloride with acetone. The mixture of trichloroethylene with acetone was taken for further experiments due to higher toxicity of carbon tetrachloride than trichloroethylene.

3.2. Effect of volumes of the extracting solvent and the dispersing solvent

Optimisation of volumes of the extracting solvent and the dispersing solvent is a further step in development of a DLLME procedure. Both these volumes can influence formation of dispersion and thus have to be optimised. Effect of the dispersing solvent volume was studied by performing experiment in which four different volumes of acetone: 0.5, 1.0, 1.5 and 2.0 mL were used. The extracting solvent volume was kept constant at 50 μL. The results presented in Fig. 1 show almost constant recovery of the analytes for volumes from 1.0 to 2.0 mL. Much lower recovery was found for 0.5 mL of acetone. Thus, 1.5 mL of acetone was taken for further experiments as this volume enabled to obtain high recovery and stable conditions of the DLLME process.

Effect of the extracting solvent volume was tested for five volumes of trichloroethylene. The range from 30 to 70 μL of the extracting solvent volume was tested. The results obtained in this experiment (Fig. 2) proved high influence of the extracting solvent volume on the recovery of the analytes. The highest recovery of all the analytes was obtained for the extracting solvent volume in the middle of the tested range i.e. 50 μL. This volume was used in further experiments.

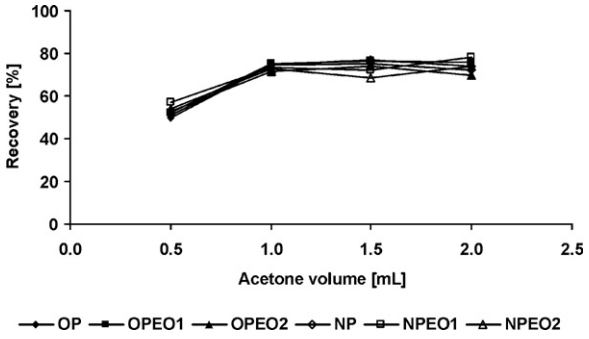

Fig. 1. Effect of the volume of the dispersing solvent on the recovery of the analytes for octylphenol, octylphenol mono- and diethoxylates, nonylphenol, nonylphenol mono- and diethoxylates. Extraction conditions: water sample volume—5 mL, dispersing solvent—acetone, extracting solvent—trichloroethylene and extracting solvent volume—50 μL.

3.3. Effect of the ionic strength

The addition of salt to the sample may influence the efficiency of extraction in the DLLME process. The recovery of the analytes from the sample solutions was tested for several different concentrations of sodium chloride ranging from 0 to 9%. The other DLLME parameters were used according to the results of former optimisation steps i.e. 1.5 mL of acetone as the dispersing solvent and 50 μL of trichloroethylene as the extracting solvent. The results show constant recovery from samples containing 1–3% of salt. Further increase of salt concentration caused small decrease of the recov-

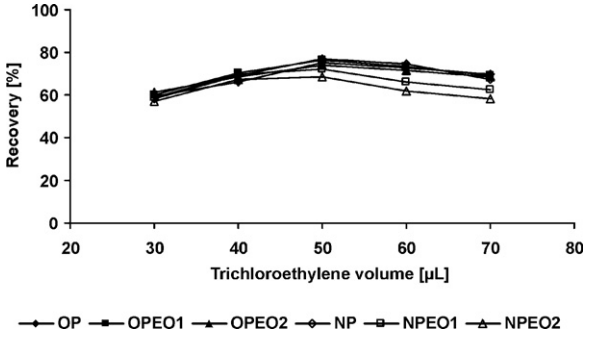

Fig. 2. Effect of the volume of the extracting solvent on the recovery of the analytes for octylphenol, octylphenol mono- and diethoxylates, nonylphenol, nonylphenol mono- and diethoxylates. Extraction conditions: water sample volume—5 mL, dispersing solvent—acetone, dispersing solvent volume—1.5 mL and extracting solvent—trichloroethylene.

Table 2
Linearity, instrumental and method limit of detection, instrumental and method limit of quantitation, recovery and precision (calculated as standard deviation—SD) obtained during validation of the method of analysis of octylphenol (OP), octylphenol monoethoxylate (OPEO1), octylphenol diethoxylate (OPEO2), nonylphenol (NP), nonylphenol monoethoxylate (NPEO1) and nonylphenol diethoxylate (NPEO2). Linearity was tested for standard solutions. Instrumental limit of detection and quantitation calculated from injections of standard solutions were expressed in concentration of solution injected into chromatographic system. Method limit of detection and quantitation calculated from injections of sample solutions from DLLME procedure (at concentration level close to limit of quantitation) were expressed in concentration of sample solution taken for DLLME. Recoveries and precision were calculated for water samples extracted with 50 μL of trichloroethylene in 1.5 mL of acetone.

Parameters	OP	OPEO1	OPEO2	NP	NPEO1	NPEO2
Calibration curve range ($\mu g\,L^{-1}$)	8–1000	8–942	7–834	30–3000	26–3216	23–2298
Correlation coefficient (r^2)	0.9999	0.9999	1.0000	0.9999	0.9993	0.9992
Instrumental limit of quantitation ($\mu g\,L^{-1}$)	7	7	7	26	26	25
Instrumental limit of detection ($\mu g\,L^{-1}$)	2	2	2	8	8	8
Method limit of quantitation ($\mu g\,L^{-1}$)	0.10	0.08	0.09	0.33	0.28	0.32
Method limit of detection ($\mu g\,L^{-1}$)	0.03	0.02	0.03	0.10	0.08	0.10
Recovery [%] ($n = 3$)	75	76	74	77	72	68
Precision (SD) [%] ($n = 3$)	5	4	4	4	4	4

Table 3
Concentrations of alkylphenols and alkylphenol ethoxylates in environmental samples and recovery of the analytes.

Analyte	Tap water		River water		Well water	
	Found ($\mu g\,L^{-1}$) ±SD ($\mu g\,L^{-1}$)	Recovery (%) ±SD (%)	Found ($\mu g\,L^{-1}$) ±SD ($\mu g\,L^{-1}$)	Recovery (%) ±SD (%)	Found ($\mu g\,L^{-1}$) ±SD ($\mu g\,L^{-1}$)	Recovery (%) ±SD (%)
OP	ND	72 ± 3	0.1 ± 0.01	67 ± 2	ND	66 ± 5
OPEO1	ND	74 ± 3	0.3 ± 0.07	70 ± 1	ND	71 ± 5
OPEO2	ND	79 ± 9	0.1 ± 0.03	73 ± 1	ND	72 ± 7
NP	ND	72 ± 1	<LOQ	69 ± 1	ND	67 ± 7
NPEO1	ND	73 ± 3	0.5 ± 0.17	71 ± 1	ND	71 ± 5
NPEO2	ND	75 ± 3	0.3 ± 0.10	69 ± 2	ND	69 ± 8

Each sample was prepared three times. ND—not detected and <LOQ—found below limit of quantitation.

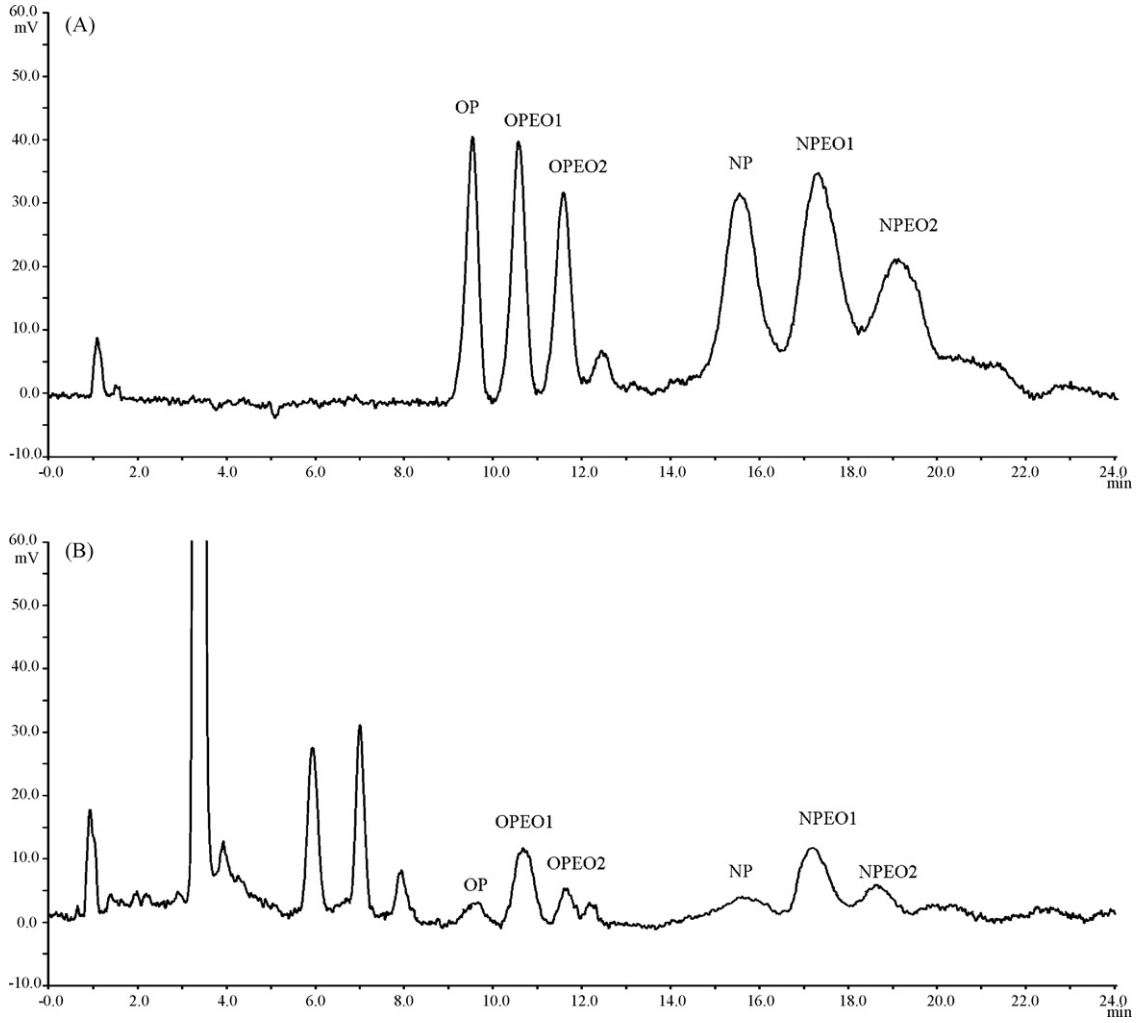

Fig. 3. Typical chromatogram of (A) a standard solution and (B) a river water sample solution.

ery. This effect was reported by Montes et al. [44]. The obtained results can be explained by higher viscosity of the sample solution which slows down mass transfer kinetics for non-polar compounds. This trend matches also with that reported earlier for SPME studies [45]. Therefore no salt was added in further experiments.

3.4. Real samples analysis

Several additional tests were necessary in order to analyse real water samples. Linearity of the method was calculated at nine or ten different concentration levels. Correlation coefficients obtained here were at least 0.999 (Table 2). There were also satisfactory both limit of detection and limit of quantitation calculated on the basis of signal to noise ratio (Table 2). The LODs obtained for OP and NP were lower than these reported by López-Darias et al. [43] who used a different DLLME procedure for isolation and preconcentration of alkylphenols. No other DLLME procedure was reported for isolation of APs and none for their ethoxylates. However, several different SPE procedures were reported. Careri et al. [14] reported SPE isolation procedure followed by HPLC analysis with fluorescence or MS–MS detection. Here, reported LODs for OP and NP were 0.2 and 0.3 $\mu g\,L^{-1}$, respectively for fluorescence detection or 0.08 and 0.02 $\mu g\,L^{-1}$, respectively for MS–MS detection. Cespedes et al. [15] reported LODs at similar level to these presented in Table 2. Slightly lower LODs (from 0.005 $\mu g\,L^{-1}$ for OP to 0.1 $\mu g\,L^{-1}$ for OPEO1 and NPEO1) were reported by Loss et al. [16]. Considerably lower LODs were obtained only by Loyo-Rosales et al. [17] who reported LODs from 0.02 $ng\,L^{-1}$ for OPEO2 and 0.03 $ng\,L^{-1}$ for NPEO2 to 0.2 $ng\,L^{-1}$ for NP and 0.3 $ng\,L^{-1}$ for OP. Surprisingly, LOQs reported in that study were much higher—from 2 $ng\,L^{-1}$ for NPEO2 to 9 $ng\,L^{-1}$ for OP and 10 $ng\,L^{-1}$ for NP. Nevertheless, these results were obtained by using a very long and complicated SPE procedure in which 4 L water sample was introduced to 6 mL SPE cartridge, eluted sequentially with three different organic solvents and evaporated from 36 mL to 0.5 mL.

A blank recovery test proved that the DLLME procedure introduced no interferences from the laboratory glassware and solvents. There were also tested recovery and precision of the method. The recoveries were in the range from 68 to 77% with satisfactory precision (Table 2).

The optimised method was applied in analysis of three water samples of different origin (tap water, river water and well water). Alkylphenols and their ethoxylates were found only in one of the tested samples—the river water sample (Table 3). Typical chromatograms of a standard solution and a sample solution containing APs and APEOs is presented in Fig. 3. Recovery of the analytes in the real water samples was tested to assess the matrix effects. The obtained recoveries were between 66 and 79% (Table 3) which indicates that the tested real water matrices had little effect on DLLME.

The concentrations of APs and APEOs obtained for the river water sample were similar to those reported in other papers. Nonylphenol and its ethoxylates were always reported at higher level than OP and OPEOs [15–17] which is obvious result of higher production of NPEOs than OPEOs [5–8]. Loss et al. [16] reported higher concentrations of NP and NPEOs than OPs and OPEOs in both Belgian and Italian rivers. Similarly, Cespedes et al. [15] reported higher concentration of NP than OP in Spanish rivers and Loyo-Rosales et al. [17] reported only NP and NPEOs in Back River (North America) while OP and OPEOs could not be quantified. These results correlate with the results reported in Table 3.

The APs and APEOs concentrations reported in Table 3 for Warta River are at higher level than those reported for Spanish and American rivers [15,17] and similar to those reported for Italian rivers [16]. Higher concentrations of NP and NPEOs were reported for selected Belgian rivers [16] and Italian rivers [18]. Also, high concentrations of NP were reported for most rivers studied in Taiwan [19]. Thus, Warta River results are between other reported results and are characteristic to medium industrialized area.

4. Conclusion

This paper outlined the successful development and application of the DLLME for analysis of endocrine disrupting alkylphenols and their ethoxylates in water samples. The application of DLLME to this group of analytes has not been reported before. The developed method offers several advantages such as simplicity, low cost, high enrichment and short time of sample preparation. Good performance of this method in analysis of real water samples demonstrates the possibility of its usage in routine analysis.

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