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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-