Journal of Chromatography A, 1340 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

In-port derivatization coupled to different extraction techniques for the determination of alkylphenols in environmental water samples

J. Cavalheiro[a,b], M. Monperrus[b], D. Amouroux[b], H. Preud'Homme[b],
A. Prieto[a], O. Zuloaga[a,*]

[a] Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country, P. K. 644, 48080, Bilbao, Spain
[b] Laboratoire de Chimie Analytique Bio-inorganique et Environnement, Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux, CNRS UMR 5254, Université de Pau et des Pays de l'Adour, Hélioparc Pau Pyrénées, 2, av. P. Angot, 64053, Pau cedex 9, France

ARTICLE INFO

Article history:
Received 24 December 2013
Received in revised form 2 March 2014
Accepted 4 March 2014
Available online 11 March 2014

Keywords:
In-port derivatization
Large volume injection
Alkylphenols
Water samples
Different extraction techniques

ABSTRACT

Large volume injection (LVI)-in port silylation coupled to gas chromatography–mass spectrometry (GC–MS) for the determination of alkylphenols (APs) in water samples applying four different extraction approaches was evaluated. Among the variables studied for in-port derivatization, vent time, cryo-focusing temperature and the ratio solvent volume/*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) volume were optimized using an experimental design approach. Regarding the extraction techniques, different approaches previously optimized in the research group were tested. On the one hand different polymeric materials were tested: silicon rod (SR), polyethersulfone (PES) and polydimethylsiloxane (PDMS), the latter in the stir-bar sorptive extraction format (SBSE–PDMS). PES was chosen among the polymeric materials due to the higher recoveries (compared with SR) and lower price (compared to PDMS in the stir-bar sorptive extraction, SBSE–PDMS). Both MASE and PES protocols were selected at this point for further method validation and application to real samples. Finally, the developed methods were validated and applied to the determination of target analytes in various aqueous environmental matrices, including estuarine water and wastewater. Acceptable repeatability in the case of MASE (5–17%) and PES (7–21%) procedures and method detection limits (MDLs, 5–123 and 28–328 ng L^{-1} for PES and MASE, respectively) were obtained for most analytes. In terms of apparent recoveries in the presence of matrix, estuarine and effluent samples showed no significant matrix effect (apparent recoveries in the 73–121% for PES and 74–128% for MASE), while a stronger matrix effect was observed for influent wastewater samples (98–132% for PES and 65–156% for MASE). Both MASE and PES extractions combined with LVI-in-port derivatization–GC–MS were applied to the determination of APs in the estuary of Bilbao (Gulf of Biscay, Spain).

1. Introduction

The fate and presence of alkylphenols (APs) in freshwater ecosystems, wastewater and atmosphere were recently reported in different reviews [1–3]. They also depicted the behavior, toxicity and endocrine disrupting effects of APs and noted that waste discharges from sewage treatment plants (STW) and industries are the main sources of APs and alkylphenol ethoxylates (APEOs) in aquatic environments. APEOs belong to the group of nonionic surfactants and are used as detergents, emulsifiers, solubilizers, wetting agents, and dispersants. Nonylphenol polyethoxylates (NPEOs) account for about 80% of total APEOs, and octylphenol polyethoxylates (OPEOs) about 20% [4–8]. Since 1950, they have been used in a wide variety of industrial, agricultural and household applications, and in 1997 the worldwide production of APEOs was estimated at 500,000 t [2]. According to Mihaich et al. [9], a large share of APEO consumption is for industrial uses (about 55% of total APEOs), then about 30% of APEOs are used in industrial and institutional cleaners and detergents and about 15% in household and personal care products. APEOs are then deposited into sewage waters or directly released into the environment. They are biodegraded during sewage treatment processes in sewage treatment plants and partially in the environment by loss of the ethoxy groups, resulting in nonylphenols (NPs), octylphenols (OPs) and other

---

[*] Corresponding author. Tel.: +34 946013269; fax: +34 946013500.
E-mail address: olatz.zuloaga@ehu.es (O. Zuloaga).

http://dx.doi.org/10.1016/j.chroma.2014.03.013
0021-9673/

Table 1
Abbreviations, retention time (t_r, min) and m/z fragments of the target analytes.

Family	Analyte (abbreviation)	t_r (min)	m/z
Alkylphenols	4-*tert*-Octylphenol *(4tOP)*	14.2	207, 208
	Nonylphenols (technical mixture) (NPs)	15.0–15.6	207, 208, 221
	4*n*-Octylphenol (4nOP)	15.8	179, 278
	Nonylphenol monoethoxylated (NPEO1)	17.4–18.0	265, 251
	Nonylphenol diethoxylated (NPEO2)	19.1–19.7	295, 309
Surrogates	[$^2H_4$]-nonylphenol ([$^2H_4$]-NP)	16.5	183, 296

The first ion was used as quantifier and the second as qualifier.

mono-, di- and tri-ethoxylates (NPEO1, NPEO2 and NPEO3, respectively). For these reasons, a clear picture of the levels of these compounds in environmental samples is compulsory.

The method development in the analysis of organic pollutants such as of APs should not only focus on the development of highly sensitive and selective methods but also in environmentally friendly procedures (green chemistry) that minimize solvent consumption. Sample preparation/pretreatment is one of the most time consuming and laborious steps in analytical procedures. Many sample preparation protocols still rely on simple classical techniques such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) for liquid samples. In the case of LLE large volumes of sample and organic solvent are typically needed, as well as repeated extractions for sufficient enrichment. In the case of SPE, repeated extractions are not performed but elution volumes are usually higher than those used in “greener” extraction techniques. The common need of further clean-up and concentration steps make them laborious, time-consuming and prone to analyte losses. Furthermore, the high-purity organic solvents used are expensive, usually toxic and harmful to the environment (e.g. ozone-layer-destroying chlorinated solvents), and substantial quantities of solvent waste have to be handled.

With the objective of avoiding the disadvantages present in the traditional extraction techniques mentioned before, a wide variety of microextraction techniques using many different approaches have been presented over the last years. The first applications used capillaries with polymer coatings (such as polydimethylsiloxane, PDMS) as the sorptive phase for analyte extraction and were developed in the mid-1980s [10]. Similar approaches were presented later as solid-phase microextraction (SPME). Later on, several techniques using bars or rods were developed, stir bar sorptive extraction (SBSE) [11–13] or silicone rod (SR) extraction [14], among others. Thereafter, SR extraction was applied mainly to the extraction of chlorobenzenes [15], polybrominated diphenyl ethers (PBDEs) [16] and some pharmaceuticals [17]. Nevertheless, due to the poor extraction efficiency of PDMS based polymeric materials for polar compounds, some authors have already proposed new devices or new materials for sorptive purposes including monolithic materials [18–23], molecularly imprinted polymers [24–28], restricted access materials [29–33] or polyethersulfone (PES) [34–36].

Another recent alternative for miniaturized preconcentration of analytes is based on the use of membranes that protect the acceptor phase (extraction solvent) where the analytes from the donor phase (sample) are concentrated. Jönsson and Mathiasson introduced the use of porous membranes with this aim [37–39] and such membranes have been successfully applied to the extraction of both polar and non-polar compounds using different approaches. Another alternative is the use of non-porous membranes which were first described by Hauser and Popp as membrane-assisted solvent extraction (MASE) for the determination of organochlorine compounds in water samples [40]. MASE uses low volumes of organic solvent (400–1000 μL) and, combined with large volume injection (LVI), provides good limits of detection (LODs). It has already been successfully applied to the extraction of a variety of organic pollutants [41–47].

In order to improve the resolution and detection of certain analytes containing –OH, $–NH_2$ or –COOH groups, among others, by gas chromatography (GC) a derivatization step is recommended. Derivatization process either increases or decreases the volatility of the compound of interest. It also reduces analyte adsorption in the GC system and improves detector response, resolution and peak symmetry. Alkylation, acylation and silylation are common derivatization reactions for GC analysis [48]. Most derivatization reactions are performed “off-line” in a reaction vessel that is separated from the GC analysis hardware [49]. Off-line silylation reaction is performed after the extraction of the target analyte from the water sample and requires, thereby, an additional sample processing step and additional time for sample analysis. Off-line silylation procedures suffer from experimental errors such as the loss of analyte through evaporation and re-suspension steps, the contamination of samples during work-up, and the interference of water in the reaction system, since silylating reagents and the resulting derivatives are extremely sensitive to the presence of water [50–52]. As an alternative to off-line derivatization, “on-line” derivatizations are found. On-line derivatization can eliminate time consuming sample-processing steps, decrease the amounts of valuable and/or toxic reagents and solvents that would otherwise be needed, and increase the speed and the efficiency of the analysis [53,54]. On-line derivatizations where the derivatization reaction is simultaneously carried out with the analysis step by injecting the sample/reagent mixture directly into the hot GC inlet are known as inlet-based derivatizations or “in-port” derivatizations and the derivatization occurs in the gas-phase [48,55].

In this sense, the purpose of the present work is to test different extraction techniques (SR, PDMS in the stir-bar sorptive extraction format: SBSE–PDMS, PES and MASE) previously developed in the research group for the determination of APs in environmental water samples and to optimize the in-port silylation as an alternative to off-line derivatization.

2. Experimental

2.1. Reagents and materials

The mixture of nonylphenols (NPmix, Pestanal) was obtained from Riedel-de Haën (Seelze, Germany) and Igepal CO-210 (nonylphenol monoethoxylate, NPEO1) and Igepal CO-520 (nonylphenol diethoxylate, NPEO2) and [$^2H_4$]-nonylphenol ([$^2H_4$]-NP) from Sigma-Aldrich (Milwaukee, WI, USA). 4-*tert*-octylphenol (4*t*OP) and 4-*n*-nonylphenol (4*n*OP) were supplied by Alfa-Aesar (Karlsruhe, Germany). Abbreviations and suppliers are included in Table 1.

Ethyl acetate (HPLC grade, 99.8%), methanol (HPLC grade, 99.9%), dichloromethane (HPLC grade, 99.8%) toluene (HPLC grade, 99.8%) and acetone (HPLC grade, 99.8%) were obtained from Labscan (Dublin, Ireland) and acetonitrile (HPLC, 99.9%) from Sigma-Aldrich (Steinheim, Germany).