Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Chromatography A, 1121 (2006) 154–162

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Sensitive gas chromatographic–mass spectrometric method for the determination of phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives in wastewater samples

Oscar Ballesteros, Alberto Zafra*, Alberto Navalón, José Luis Vílchez

Research Group of Analytical Chemistry and Life Sciences, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avda. Fuentenueva s/n, E-18071 Granada, Spain

Received 22 February 2006; received in revised form 8 April 2006; accepted 10 April 2006
Available online 11 May 2006

Abstract

Phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives are the suspected endocrine disrupters or mutagens. These compounds, commonly called endocrine disrupter chemicals (EDCs), are widely used as plastic additives, lacquers, resins, or surfactants and can be usually found in environmental samples, namely wastewaters. An accurate and reproducible gas chromatographic–mass spectrometric (GC–MS) method is proposed to measure these compounds in wastewater samples of Granada city (Spain). A solid-phase extraction with LiChrolut RP-18 cartridges was carried out and the elution was performed with a diethyl ether/methanol mixture. After isolation, solvent was removed and a silylation step was carried out using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Phthalate esters and silylated compounds were identified and quantified by GC–MS using a ZB-5 MS column. Bisphenol F was used as a surrogate. Quantification limits found were between 20 ng L^{-1} for 4-nonylphenol and 400 ng L^{-1} for benzylbutyl phthalate while inter and intra-day variability was under 5% in all cases. Recoveries for spiked samples were over 95% and under 105%. The method was validated using standard addition calibration and recovery assays.

Keywords: Phthalate esters; Alkylphenols; Bisphenol A; Bisphenol A chlorinated derivates; Gas chromatography–mass spectrometry (GC–MS); Environmental analysis

1. Introduction

Since the mid-1990s, a wide range of adverse effects associated with a long list of man-made chemicals, called endocrine disrupting chemicals (EDCs) have been observed in humans and wildlife [1,2]. Such effects are cumulative and it is possible that they only will appear in subsequent generations. Therefore, the resulting effects may be irreversible, threatening sustainable human development. Most EDCs are synthetic organic chemicals introduced into the environment by way of anthropogenic inputs and are therefore ubiquitous in aquatic environments receiving wastewater effluents. Aware of the problem, both European Union and the US Environmental Protection Agency (EPA) have authored a “priority” list of substances for further evaluation of their role in endocrine disruption [3,4] and indicated the need to assess the levels and effects of EDCs. Among others, phthalate esters, alkylphenols and bisphenol A (BPA) are the potential active compounds [5,6].

As food and feed may contain some of these widely used products as a result of diffuse environmental pollution and direct uptake by animals via food or air, a potential bioaccumulation and transfer through the food chain is possible. Phthalate esters are plasticizers used in food handling and storage, and some of them are considered to be ubiquitous pollutants but present slight endocrine-disrupting properties [7]. BPA is used in the synthesis of polycarbonated plastics, epoxy adhesives and can-coating materials, and is known to migrate from can coatings into food [8]. Further, bisphenol A chlorinated derivatives are by-products yielded in the reaction between BPA and free chlorine used in disinfecting procedures, mainly in wastewater treatments [9]. Alkylphenols, represented by 4-(*tert*-octyl)phenol, 4-*n*-octylphenol and 4-*n*-nonylphenol are the reported by-products of alkylphenol polyethoxylates (APEOs). They are non-ionic surfactants often added to soaps, paints, herbicides and pesti-

---

* Corresponding author. Tel.: +34 958 243326; fax: +34 958 243328.
E-mail address: azafra@ugr.es (A. Zafra).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.04.014

cide formulations. Each of these four representative groups of phenolic compounds has been reported to be non-biodegradable, effective EDCs and has been detected in wastewater, sewage and groundwater [10–13].

Therefore, it is of crucial importance to devise an analytical methodology for detecting and quantifying these compounds in environmental samples. Different analytical methods have been developed for analysing EDCs from wastewater samples. The most common include liquid chromatography [14–16], capillary electrophoresis [17] or gas chromatography coupled with mass spectrometry (GC–MS) [18–23]. The analytical methods proposed in the literature apply derivatization procedures before GC–MS analysis. Different reagents have been used to derivatize EDCs, including pentafluorobenzyl (PFBr), *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) or *N*-(*tert*-butyldimethylsilyl)-*N*-methyl-trifluoro acetamide (MTBSTFA) that lead to the formation of trimethylsilyl (TMS) and tributylsilyl (TBS) derivatives. These are often chosen because they are stable and allow the sensitivity [24–27] to be improved.

The purpose of the study was to develop an accurate and reproducible multiresidue method to detect trace amounts of BPA and its chlorinated derivatives, alkylphenols (4-NP, 4-*t*-OP and 4-OP), and the most important phthalates esters (dimethyl, diethyl, di-*n*-butyl, dioctyl, butylbenzyl, and bis(2-ethylhexyl) phthalate) in wastewater samples. In order to isolate analytes and clean up samples, a solid-phase extraction (SPE) procedure with different sorbents was assayed. Samples taken from different points of Granada city were analysed as a preliminary step and the method validation was proved by recovery assays in spiked wastewater samples.

2. Experimental

2.1. Reagents and standards

All reagents were of analytical grade unless specified otherwise. Water was purified with a Milli-Q plus system (Millipore, Bedford, USA).

Methanol, hexane, ethanol, ethyl acetate, diethyl ether, dichloromethane, sodium sulphate anhydrous, *o*-phosphoric acid and sodium hydroxide were supplied from Panreac (Barcelona, Spain). All solvents and reagents were checked to ensure they were free of contamination from compounds (phthalate esters).

4-Nonylphenol (4-NP), 4-octylphenol (4-OP), 4-*tert*-octylphenol (4-*t*-OP), bisphenol F (BPF), bisphenol A (BPA) and tetrachlorobisphenol A (Cl_4-BPA) were supplied by Sigma-Aldrich (Madrid, Spain). Monochloro, dichloro and trichloro bisphenol A (Cl-BPA, Cl_2-BPA, Cl_3-BPA) were synthesized in our laboratory.

Phthalate esters dimethyl (DMP), diethyl (DEP), di-*n*-butyl (DBP), butylbenzyl (BBP), bis(2-ethylhexyl) (BEHP) and di-*n*-octylphthalate (DOP) were supplied by Fluka (Buchs, Switzerland).

SPE sorbents assayed for our purpose were silica-based bonded C_{18} cartridges LiChrolut RP-18 (500 mg, 3 mL) from Merck (Darmstadt, Germany), polymeric cartridges Oasis HLB (60 mg, 3 mL) from Waters (Dublin, Ireland), and Strata-X (200 mg, 6 mL) from Phenomenex (Torrance, CA, USA) and hydroxylated copolymers of polystyrene–divinylbenzene cartridges Isolute ENV + (200 mg, 3 mL) from Isolute (Symta, Madrid, Spain).

A mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchloro silane (TMCS) (99:1, v/v), supplied by Supelco (Bellefonte, PA, USA), was used as the silylation reagent.

Stock standard solutions (100 mg L^{-1}) of each EDC were prepared in absolute ethanol and stored in dark bottles at 4 °C until use, remaining stable for at least three months. These solutions were used to spike the water samples.

2.2. Sample preparation

Urban wastewater samples were collected from different points in the city of Granada. They were placed in glass bottles previously cleaned with nitric acid (1:1, v/v) and the usual precautions were taken to avoid contamination. Samples were centrifuged at 3000 rpm for 10 min and filtered through a 0.22 μm cellulose acetate disk filter. Samples were stored in the dark at 4 °C until treatment was performed, which occurred within 48 h of sample collection in all cases. The analysis was performed with the minimum possible delay and as described in Section 2.3.

2.3. Extraction and derivatization

Prior to extraction, the wastewater samples were spiked with bisphenol F as a surrogate at a concentration of 2.5 μg L^{-1}. The SPE cartridges were conditioned with 5 mL of diethyl ether, 5 mL of methanol and 5 mL of deionized water on an SPE manifold at a rate of 1–2 mL min^{-1}. Five hundred millilitres of wastewater was passed through the SPE cartridges at a flow rate of 2–3 mL min^{-1}. Then, the cartridges were cleaned with 3 mL of 10% methanol in water and dried under vacuum for 20 min. The EDCs were eluted from the sorbents with 6 mL of a mixture of diethyl ether/methanol (9:1, v/v) at a flow rate of 1 mL min^{-1}. Finally, eluents were evaporated to dryness at 50 °C under a stream of nitrogen.

Thirty microlitres of a mixture of ethyl acetate and BSTFA/TMCS (1:1, v/v) were added into the reaction vial in order to resuspend the residue and to carry out the derivatization. Only alkylphenols, bisphenol A and its chlorinated derivatives are suitable for derivatization due to their chemical structure. Next, the vials were closed and slightly heated at 60 °C for 30 min. Once the derivatization process was complete, 1 μL of the reaction mixture was injected into the GC–MS system.

2.4. Apparatus and software

The GC–MS analysis was performed using a 6890 Agilent (Agilent Technologies, Wilmington, USA) gas chromatograph with a 7683 series injector and a quadruple mass filter 5976