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Analytica Chimica Acta 530 (2005) 245–252
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Determination of alkylphenol and bisphenol A in beverages using liquid chromatography/electrospray ionization tandem mass spectrometry

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Received 6 July 2004; received in revised form 21 September 2004; accepted 21 September 2004
Available online 8 December 2004

Abstract

A comprehensive analytical method based on liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) with negative ionization mode has been developed for measuring of alkylphenols and bisphenol A in beverage samples. Concentration and clean up of samples were performed on 200 mg OASIS HLB solid extraction cartridges. The effects of mobile phases and additives on ionization were assessed. The recoveries for each compound ranged from 76.7 to 96.9% and reproducibilities were represented as having relative standard deviation (R.S.D.) below 10%. The limits of quantification (LOQ) of the method under multiple-reaction monitoring (MRM) acquisition mode were 0.04, 0.03 and 0.2 ng L^{-1} for 2 L of mineral drinking water and 2.0, 1.8 and 8.0 ng L^{-1} for 50 mL of soda beverages.

Keywords: Alkylphenol; Bisphenol A; LC–ESI–MS/MS

1. Introduction

During the last decade, the presence of alkylphenol (AP) and bisphenol A (BPA) has become a public concern due to their massive use, ubiquitous occurrence and persistency in the environment. In addition, in vitro and in vivo bioassay studies have identified that alkylphenol, especially nonylphenol (NP) and 4-*tert*-octylphenol (OP), as well as bisphenol A elicited estrogenic potency and chronic toxicity [1–5]. There is increasing evidence that the above intermediates are capable of inducing synthesis of the yolk protein vitellogenin in male rainbow trout [6,7].

OP and NP are widely used as intermediates to produce surfactant (anionic and non-ion surfactant) and stabilizer of ethylcellulose resin, oil-soluble phenol resin and esters. These compounds are also discharged as metabolites of alkylphenol ethoxylates into the environment mainly by biodegradation from sewage treatment plants [8]. BPA is mainly used as a monomer in the preparation of epoxide resins, polycarbonate plastics and as an antioxidant or stabilizer in polyvinylchloride. Releases into the environment mainly occur during manufacturing. The occurrence of OP, NP and bisphenol A has widely been studied in aquatic environment, sediment, agricultural soil, fish, alga, and birds and these compounds even have been found in the atmosphere [9–16]. The results of these studies also indicated that not only the semivolatile NP and OP, but also the nonvolatile BPA could transform between different phases and different matrices. As additives of plastics and resin, alkylphenols and BPA are not chemically bound to the polymer, these compounds may be transferred into the surrounding medium, such as foods and beverages. Klaus et al. made the first comprehen-

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doi:10.1016/j.aca.2004.09.086

sive survey on the occurrence of NP in 60 kinds of foodstuff in Germany, and found that NP is ubiquitous in food [17]. Previous studies indicated that alkylphenol and BPA could migrate from packaging material such as rubber products and polyvinylchloride into food simulants and foods [18–20]. Except for the occupational exposure, the food, drinking water and beverages are the possible route of human exposure.

However, there have been few studies about the alkylphenol and BPA in beverages [21], which have mainly been limited to mineral water based on liquid–liquid extraction following liquid chromatography with coulometric detection with higher sensitivity (0.25 pmol for (BPA) and 1.0 pmol for (*p*-NP)) and low selectivity and specificity. Current analytical methods for detection of NP and OP and BPA are generally based on gas-chromatography mass spectrometry, which is tedious and time-consuming because of its long derivation process [22,23]. In addition, the accuracy of quantitative data often cannot be ensured for the effect of complex matrix on the derivation. Recently, the use of electrospray tandem mass spectrometry was promising for its little sample preparation and it has high selectivity and specificity. Reversed phase LC coupled with MS detector using selective-ion monitoring mode was recently used to determinate NP, OP and BPA in fish [24], which is lack of specificity because of the interference of matrix.

This paper describes a comprehensive method for the quantitation of NP, OP and BPA in mineral water, pure drinking water and soda beverages stored in plastic bottles or cans using solid phase extraction clean-up followed by reversed phase LC coupled with tandem mass spectrometry. Considering the effect factors on the sensitivity, the sample preparation, the mobile phase compositions and additives were optimized. The aim of this study was to determine the NP, OP and BPA in mineral water, pure drinking water and soda beverages commercially available in Beijing, China.

2. Experimental

2.1. Standards and reagents

Organic solvents such as dichloromethane, and methanol of pesticide residue analytical grade were purchased from Merck (Darmstadt, Germany). Standard BPA (>99%) and technical purity NP were both purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). OP and internal standard 4-*n*-NP of 99% purity were both from Dr. Ehrenstorfer Gmbh (Augsburg, Germany, 99%). Ammonium hydroxide solution (PA, 25%) was obtained from Beijing Chemical Co. Ultra pure water was made by the Milli-Q ultrapure system (Millipore, Bedford, MA, USA). All standards were stored at −20 °C. Oasis HLB solid phase extraction cartridges with 200 mg *N*-vinylprolidone-divinylbenzene copolymer and Sep-Pak octadecylsilica cartridges with 500 mg materials were purchased from Waters (Milford, MA, USA). Supleclean ENVI-Carb solid phase extraction cartridges (GCB) and a 6 cm × 1.4 cm i.d. polypropylene tube (500 mg) were purchased from Supelco (Bellefonte, PA). To avoid the contamination of NP, OP and BPA, no APE detergents and plastics were allowed to be used, all the glassware was baked for 4 h at 400 °C prior to use. In addition, procedural blanks were conducted for each batch of samples to ensure minimal contamination.

Stock solutions were prepared for all standard substances at 1000 mg L^{-1} in methanol. Spiking and calibration mixtures at various concentration levels were obtained by combining aliquots of stock solutions and by subsequent dilution with methanol and stored at 4 °C.

2.2. Sample collection and preparation

Thirteen kinds of beverages including mineral water, pure drinking water and soda beverages were purchased from supermarkets in Beijing. They were stored unopened until analysis at 4 °C. They were all stored in plastic bottles or cans.

Before extraction, 0.1 mL of 4-*n*-NP with 200 μg L^{-1} concentration level was spiked into samples as internal standard. Analytes were extracted from 2 L of mineral water and pure drinking water and from 50 mL of other soda beverages. Prior to loading of the samples, the Sep-Pak C-18 and Oasis HLB cartridges were conditioned sequentially with 10 mL methanol, and 10 mL water with pH 3. A GCB cartridge was conditioned sequentially with 10 mL of CH_2Cl_2/CH_3OH (80:20 (v/v)), 10 mL CH_3OH, and 10 mL water with pH 3. The acidified sample (pH 3) was forced through cartridges with a flow rate of 10 mL min^{-1}. After the sample had passed through the cartridges, 5 mL methanol–water (40:60 (v/v)) was used to wash the interference, the vacuum was reduced and then the cartridges were dried by a gentle nitrogen stream. It should be noted that the residual water must be removed especially for GCB cartridge because trace water may affect the recovery significantly. The analytes were eluted with the corresponding eluant. Finally, the residues were dried under a gentle nitrogen stream, and reconstituted with 1 mL mobile phase.

2.3. LC–MS/MS analysis

Identification and quantification of analytes were carried out using an alliance 2695 (Waters, USA) liquid chromatography equipped with a Quattro Ultima Pt (Micromass, UK) tandem mass spectrometer. A Symmetry C-18 column (150 mm × 2.1 mm i.d., 3.5 μm) was used for LC separation. The column oven was at 40 °C, the flow rate was 0.2 mL min^{-1}, and the injection volume was 10 μL. Methanol and water with 0.1% ammonia were used as mobile phases. The methanol was linearly increased from 10 to 55% in 10 min, then increased to 85% in 10 min and held for 7.5 min, finally brought back to 10% and held for 15 min to the next injection. The mass spectrometer was operated in negative mode electrospray ionization in multiple-reaction monitoring (MRM) mode. The capillary voltage was main-