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Short communication

Determination of phenolic xenoestrogens in water by liquid chromatography with coulometric-array detection

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Abstract

A sensitive method for the simultaneous determination of phenolic xenoestrogens such as bisphenol A, 2,4-dichlorophenol, 4-*tert*.-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*n*-heptylphenol, 4-octylphenol, 4-nonylphenol was developed using reversed-phase LC and coulometric-array detection. Stepwise gradient elution with phosphoric acid in water–acetonitrile was used. The calibration curves were linear in the range of 5.0 (or 10.0)–1000 ng ml^{-1} with correlation coefficients of 0.9978–0.9999, the limits of detection were 0.01–0.02 ng ml^{-1}. Sample clean-up was performed by solid-phase extraction (SPE) using 3M Empore™ extraction disks. Three commercial sorbents, C_{18}, SDB-XD (styrene–divinylbenzene polymer) and SDB-RPS (sulfonated styrene–divinylbenzene polymer) were compared. The highest recoveries were obtained with SDB-RPS. They were above 70% with a relative standard deviation of less than 15%. The proposed method was applied to the determination of phenolic xenoestrogens in various water samples.

Keywords: Xenoestrogens; Phenolic compounds

1. Introduction

A number of environmental chemicals have been implicated in the abnormal sexual development of reptiles [1], in feminized responses of male fish [2], and in an increased incidence of human breast cancer [3], as well as the attenuation of sperm count and fertility [4]. Many of these xenoestrogens are phenolic compounds that were reported to have estrogenic activity from in vitro and in vivo assay [4–6]. These phenolic compounds are commonly used in many industrial processes [7,8]. Accordingly, it is highly possible that xenoestrogenic compounds leach into the environment. In the present study, the emphasis is on the combined determination of xenoestrogenic compounds such as alkylphenols, bisphenol A (BPA) and chlorophenols. GC–MS was initially used as an analytical technique for determination of xenoestrogens, even though derivatization was required [9,10]. However, there are only a few methods that do not require derivatization for simultaneous determination of phenolic xenoestrogens in water. Therefore, our purpose was application of the procedure to a number of water samples by using liquid

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chromatography with multi-electrode electrochemical detection (LC–ED) and solid-phase extraction (SPE).

2. Experimental

2.1. Materials and standard solution

BPA, 2,4-dichlorophenol (2,4-DCP), 4-*tert*.-butylphenol (BP), 4-*n*-pentylphenol (PP), 4-*n*-hexylphenol (HexP), 4-*n*-heptylphenol (HepP), octylphenol (OP) and nonylphenols (NP) standards and pesticide analytical grade of methanol, acetonitrile and acetone were purchased from Kanto Chemical Industries (Tokyo, Japan). Laboratory water was purified with a Milli-Q gradient A10 Elix with EDS polisher system water purification (Millipore, Bedford, MA, USA). These compounds were pretreated using three SPE disks having a size of 47 mm/0.5 mm: C_{18}, SDB-XD (styrene–divinylbenzene polymer), and SDB-RPS (sulfonated styrene–divinylbenzene polymer) from Empore™ (3M, USA).

Stock standard solutions of 1.0 mg ml^{-1} each were prepared in methanol. A stock solution of reference standards was prepared by methanol–water (50:50, v/v).

2.2. Instrumentation and condition

LC–ED consisted of an L-6300 pump (Hitachi, Tokyo, Japan), an autosampler model 460, a column oven, a multi-electrode electrochemical detector containing cells from Coul Array model 6210 and the database from Coul Array System Win 32 vol. 1.0 (ESA, Chelmsford, MA, USA). The cell potentials of ED consisted of an increasing array (Ch_1–Ch_8; 150, 250, 350, 450, 550, 650, 750, and 850 mV). The confirmation was achieved by comparing the matching retention time and the peak ratio (R_a) between each standard and sample [11]. Separation was achieved on a Capcell Pak UG 120 C_{18} (4.6× 150 mm) column (Shiseido, Tokyo, Japan). The column oven was controlled at 40 °C. The 30-μl samples were injected by an auto-sampler. The flow-rate was 1.0 ml min^{-1}. Stepwise elution provided complete resolution of the compounds of interest. Mobile phase A was 0.5% phosphoric acid–acetonitrile (60:40, v/v) and mobile phase B, 0.7% phosphoric acid–acetonitrile (40:60, v/v). Mobile phase A was used for the first 13 min after injection and next, mobile phase B between 13 and 35 min.

2.3. Sample preparation (SPE)

The analytes were pretreated using three kinds of SPE disks: C_{18}, SDB-XD and SDB-RPS. In order to prevent the analytes from taking their ionic form, the water samples were acidified to pH 3.0 with 1.0 *M* HCl. The disks were conditioned with 50 ml of methanol followed by 30 ml of pure water adjusted to pH 3.0 using 1.0 *M* HCl. One liter of each sample was passed through the disks. Then, 100 ml of methanol at a low flow-rate was used to elute the retained compounds. The methanol extracted was evaporated to 1 ml with a rotary evaporator. Then, it was adjusted in 2.0 ml of methanol. The obtained samples were measured by LC–ED. All water samples were analyzed immediately after collection or opening the bottles.

3. Results and discussion

3.1. LC–ED condition

We used phosphoric acid for mobile phase in order to adjust the pH [12]. Acetonitrile–phosphoric acid in water was used as the mobile phase. Hydrodynamic voltammograms (HVCs) of 2,4-DCP, BPA and BP under mobile phase A of 0.1–0.7% phosphoric acid were recorded. Optimisation conditions were obtained for mobile phase A with 0.5% phosphoric acid–acetonitrile (60:40, v/v). In the same way, HVCs of HepP, PP, HexP, NP, and OP were recorded. Based on the results, for mobile phase B, optimisation was 0.7% phosphoric acid–acetonitrile (40:60, v/v). Therefore, we decided to use stepwise mobile phase A and B, respectively (Fig. 1).

The detection limit (DL), within-run precision (relative standard deviation, RSD), and the linearity were determined using maximum signal electrode potential (Ch_5) for a given analysis. The linearity of these standards (r) was within the range of 5.0–1000 ng ml^{-1} (BPA, 2,4-DCP, BP, PP and HexP) and 10–1000 ng ml^{-1} (NP and OP). DL was $S/N = 10$

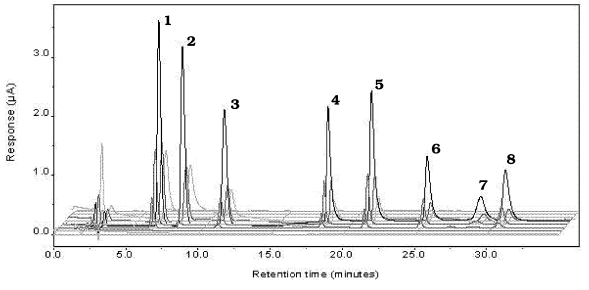

Fig. 1. Chromatogram showing the separation of the phenolic xenoestrogens from different classes using an oxidative array. The standard solution: concentration of 500 ng ml^{-1} (1=BPA, 2=2,4-DCP, 3=BP, 4=PP, 5=HexP, 6=HepP, 7=NP, 8=OP).

for detection analyses but 500 times concentrations was carried out by sample preparation. The results presented in the order DL/RSD/R_a/r were very good; BPA (0.01 ng ml^{-1}; 0.9%; 2.5; 0.999), 2,4-DCP (0.01 ng ml^{-1}; 0.5%; 53.1; 0.998), BP (0.02 ng ml^{-1}; 1.7%; 5.5; 0.999), PP (0.01 ng ml^{-1}; 0.6%; 2.3; 0.999), HexP (0.01 ng ml^{-1}; 1.0%; 2.1; 0.999), HepP (0.02 ng ml^{-1}; 0.4%; 2.2; 0.999), NP (0.02 ng ml^{-1}; 1.8%; 6.5; 0.997) and OP (0.02 ng ml^{-1}; 1.9%; 2.5; 0.999). The analytical performance was found to be reliable and suitable for routine analysis.

3.2. Sample pretreatment

We encountered difficulties in that phenolic xenoestrogens were detected when a blank preparation was used, suggesting risks arising from phenolic xenoestrogen contamination. The contamination is considered to be mostly attributable to the laboratory water stored into plastic containers and passed through plastic filters. Therefore, we investigated whether phenolic xenoestrogens would be detected in a sample of laboratory and pure water. Contamination of this water by BPA, BP, NP, and OP is examined. Recently, we reported that a trace amount of BPA was detected in Milli Q-purified water which is normally used in experiments [12]. In contrast, in Milli-Q water with EDS polisher system water purification they were not detected in blank test. Therefore, this water was found to be useful for analysis of trace levels of phenolic xenoestrogens.

Comparing the recoveries and RSDs gave a clear preference for each application. The recoveries were obtained by using water spiked with the standard. When an SDB-RPS phase was used, 2,4-DCP and the others showed higher recoveries than when an SDB-XD or C_{18} phase was used. Thus, an SDB-RPS phase is more suitable for aquatic samples than C_{18} or SDB-XD. The recoveries of 0.1 and 100 ng ml^{-1} of analyses from water using the SDB-RPS disk were obtained. The good recoveries and RSDs of water pre-treated are shown in Table 1. Therefore, we decided to use the SDB-RPS disk for pre-treatment of the phenolic xenoestrogens in water.

Table 1
Recoveries of analytes using SDB-RPS disk

Analyte	% Recovery±RSD (n=3)	
	0.1 ng ml^{-1}	100 ng ml^{-1}
BPA	93±2	104±1
2,4-DCP	75±15	101±1
BP	75±9	103±1
PP	90±6	101±1
HexP	81±3	100±1
HepP	97±6	100±0.4
NP	97±8	100±0.1
OP	100±5	100±1

3.3. Application

Trace concentrations of BPA, NP, and OP were not detected in two tap-water samples and in one of four drinking water samples (bought in Tokyo groceries). In addition, three samples of Japanese river water were analysed; here, xenoestrogens were detected in all cases (range: 0.01–0.17 ng ml^{-1}). These results are shown in Table 2.

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Table 2
Result of application in various kinds of water samples

Sample	Analyte concentration (ng ml^{-1})
Drink water	BPA (0.08), NP (0.04), OP (0.05)
River water 1	BP (0.01), NP (0.06)
River water 2	BPA (0.13), 2,4-DCP (0.01), BP (0.05), HepP (0.08), NP(0.13), OP(0.17)
River water 3	BPA (0.08), 2.4-DCP (0.01), BP (0.03), PP (0.03), PP(0.03), HepP (0.08), NP (0.04)

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