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

ARTICLE *in* JOURNAL OF CHROMATOGRAPHY A · AUGUST 2006

Impact Factor: 4.17 · DOI: 10.1016/j.chroma.2006.04.014 · Source: PubMed

4 AUTHORS:

Oscar Ballesteros
University of Granada
71 PUBLICATIONS **1,187** CITATIONS
SEE PROFILE

A. Zafra Gomez
University of Granada
98 PUBLICATIONS **1,196** CITATIONS
SEE PROFILE

Alberto Navalón
University of Granada
153 PUBLICATIONS **2,411** CITATIONS
SEE PROFILE

José Luis Vílchez
University of Granada
172 PUBLICATIONS **2,717** CITATIONS
SEE PROFILE

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

Table 1
SIM mode characterization and structural assignments of the fragments

Compound	t_r (min)	Fragment (*m/z*)
DMP	4.23	194 $[M]^+$, 163 $[M - OMet]^+$, 133 $[M - OMet - OMet + H]^+$
DEP	5.30	177 $[M - OEt]^+$, 149 $[M - OEt - Et + H]^+$
4-OP	5.68	278 $[M]^+$, 263 $[M - Met]^+$, 207 $[M - OTMS]^+$
4-*t*-OP	7.00	278 $[M]^+$, 263 $[M - Met]^+$, 179 $[M - C_7H_{15}]^+$
4-NP	7.70	292 $[M]^+$, 277 $[M - Met]^+$, 179 $[M - C_8H_{17}]^+$
DBP	7.93	223 $[M - But + 2H]^+$, 205 $[M - OBut]^+$, 149 $[M - OBut - But + H]^+$
BPF	9.32	344 $[M]^+$, 329 $[M - Met]^+$, 179 $[M - Ph - OTMS]^+$
BPA	9.86	372 $[M]^+$, 357 $[M - Met]^+$
BBP	11.32	205 $[M - OBut]^+$, 149 $[M - OBenzyl - But + H]^+$
Cl-BPA	11.47	406 $[M]^+$, 391/393[a] $[M - Met]^+$
Cl_2-BPA	12.70	440/442[a] $[M]^+$, 425/427[a] $[M - Met^a]^+$
BEHP	12.94	279 $[M - OEtHex]^+$, 149 $[M - OEtHex - EtHex + H]^+$
Cl_3-BPA	13.82	474/476[a] $[M]^+$, 459/461[a] $[M - Met^a]^+$
DOP	14.88	279 $[M - OOct]^+$, 149 $[M - OOct - Oct + H]^+$
Cl_4-BPA	15.21	508/510[a] $[M]^+$, 493/495[a] $[M - Met^a]^+$

[a] Cl-35/Cl-37 isotopes.

network mass selective detector (MSD). A capillary column ZB-5 MS Zebron (30 m × 0.25 mm I.D.; 0.25 μm film thickness) with phenyl character from Phenomenex was used. For qualitative determinations, the MSD was operated in full-scan mode from *m/z* 50–550 and for quantitative determinations, the MSD it was operated in selected ion-monitoring (SIM) mode. The mass spectrometer was calibrated every day before use with perfluorotributylamine (PFTBA) as a calibration standard. Data acquisition and integration were carried out with the HPCHEM chromatography software.

The injector port of the GC was set at 250 °C. The silylated samples were automatically injected using the splitless-injection mode. The transfer line of the GC to the MS was set at 270 °C, and the electron impact (EI) ion source of the MS was set at 250 °C. The ionization energy was 70 eV. The GC oven temperature program applied was as follows: the initial oven temperature was set at 120 °C, held for 1 min, then the temperature was increased to 230 °C and from 230 °C up to 260 °C via ramp of 15 and 30 °C min^{-1}, respectively, and maintained at 260 °C for 8 min. The carrier gas was high-purity helium (99.999%) with a constant flow of 1 mL min^{-1}. A solvent delay time of 3.5 min was used to protect the ion multiplier of the MS instrument from saturation. SIM mode was used to carry out measurements. The characteristic ions listed in Table 1 were used for the quantification of the EDCs.

SPE was carried out on a Supelco vacuum manifold for 12 columns connected to a Supelco vacuum tank and to a vacuum pump.

Statgraphics software package [28] was used for the statistical analysis of data.

3. Results and discussion

3.1. Preconcentration procedure

Determinations at the trace level require a preconcentration step of the samples to reach these levels of concentration. A solid-phase extraction procedure was selected as appropriate to extract analytes from the samples. The extraction was optimized by adjusting parameters that influence analyte adsorption and desorption, e.g., nature of stationary phase and eluent.

Several commercially available SPE sorbents (LiChrolut RP-18, Oasis HLB, Strata-X and Isolute ENV+) were tested for extraction of analytes from aqueous samples.

The role of eluent nature was also investigated. Due to the different polarities of the analytes, mixtures of dichloromethane, hexane, diethyl ether, methanol and acetonitrile were assayed. The higher extraction yields of these chemicals for 500 ng L^{-1} spiking samples were obtained for LiChrolut RP-18 and a diethyl ether/methanol mixture (9:1, v/v).

Finally, the sample pH influence before charging in SPE cartridge was also studied in the range 2.0–5.0. The higher recoveries were obtained at pH 3.0. Therefore, samples were acidified before extraction with *o*-phosphoric acid concentrate and adjusted at pH 3.0 by addition of 2 M NaOH solution.

A mixture of diethyl ether/methanol (9:1, v/v), a drying time of 20 min at room-temperature and a pH sample of 3.0 were selected in order to obtain an adequate analyte elution and a higher extraction yield.

3.2. Derivatization method

Trimethylsilyl derivatives of the target EDCs were obtained using a BSTFA/TMCS mixture as silylation reagent. This reagent was selected because of its fast reactivity with compounds containing hydroxyl groups, its high volatility resulting in non-coelution of early eluting peaks, low thermal degradation and good solubility in common organic solvents of the derivatized compounds. Derivatized samples presented an improved separation of the analytes under GC–MS analysis because of their higher volatility and lower interaction with the stationary phase.

The percentage of silylation agent (BSTFA/TMCS 99:1, v/v) in ethyl acetate, the temperature of process and the reaction time are related to each other; consequently these variables were optimised by using the response surface methodology. A three-factor central composite face-centred experimental design was applied [29]. The maximum response while simultaneously varying these three parameters was obtained. All calculations were carried out with the Statgraphics software package.

The BSTFA/TMCS concentration was studied from 0 to 100%, at a temperature from 25 to 95 °C and the reaction time was varied between 0 and 60 min. A 50% of silylation agent in ethyl acetate (v/v), 30 min and 60 °C were the procedural conditions selected, near to the optimum values obtained for all compounds.

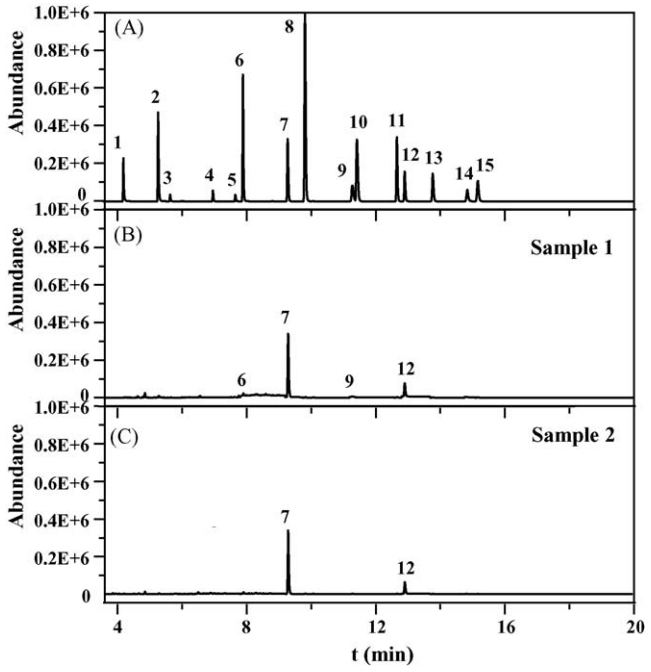

Fig. 1. Chromatograms of (A) standard mixture of phthalate esters, bisphenol A and bisphenol A chlorinated derivatives and alkylphenols; (B) urban wastewater sample 1; (C) urban wastewater sample 2. 1, DMP; 2, DEP; 3, 4-OP-TMS; 4, 4-*t*-OP-TMS; 5, 4-NP-TMS; 6, DBP; 7, BPF-TMS (IS); 8, BPA-TMS; 9, BBP; 10, Cl-BPA-TMS; 11, Cl_2-BPA-TMS; 12, BEHP; 13, Cl_3-BPA-TMS; 14, DOP; 15, Cl_4-BPA-TMS.

3.3. Gas chromatographic–mass spectrometric analysis

An increment in the signal-to-noise ratio for alkylphenols, bisphenol A and its chlorinated derivatives was clearly observed in the derivatized sample. Fig. 1 shows a characteristic chromatogram obtained in SIM mode for silylated and non-silylated compounds. Fifteen peaks together with corresponding internal standard appear in the chromatogram, at retention times between 4.20 and 15.20 min. Corresponding, in order of elution, to DMP, DEP, silylated 4-OP, silylated 4-*t*-OP, silylated 4-NP, DBP, internal standard (silylated BPF), silylated BPA, BBP, silylated Cl-BPA, silylated Cl_2-BPA, BEHP, silylated Cl_3-BPA, DOP and silylated Cl_4-BPA, respectively.

Another feature of the application of derivatization reactions is that trimethylsilyl derivatives produce ions with higher *m*/*z* in the GC–MS system in contrast to those obtained from underivatized compounds. The selection of high mass fragments as quantification ions is of great interest, particularly when complex matrices are to be analysed, due to the decreased likelihood of interferences. The mass spectra obtained in scan mode are shown in Figs. 2 and 3.

Silylated BPA shows the base peak at 372 *m*/*z* corresponding to the molecular ion and it was used as target ion and the peak at 357 [M − 15] was the qualifier ion. The molecular ion peak of silylated chlorinated compounds appears at 406, 440, 476 and 508 *m*/*z*, respectively, whereas the base peak corresponding to loss of a methyl group is at 391, 425, 459 and 493 *m*/*z*, respectively. For alkylphenols, qualifier ion was 179 *m*/*z* for *t*-OP and 4-NP and 207 *m*/*z* for 4-OP. The base peak corresponding to molecular ion was 278, 292 and 278 *m*/*z*, respectively. Finally, phthalate esters show a characteristic peak at 149 *m*/*z* except for DMP that shows a 163 *m*/*z*, the most abundant fragment. For the surrogate, bisphenol F, the ions used were those corresponding to 344 and 329 and 179 *m*/*z*, respectively. Table 1 shows the main fragments selected for SIM mode analysis.

3.4. Analytical performance

Calibration graphs for samples treated according to the analytical procedure described above were made using SIM mode. Bisphenol F was used as surrogate. Linearity of the calibration graphs was tested according to the Analytical Methods Committee [30]; the *lack-of-fit* test was applied to two replicates and two injections of each standard. The results for the intercept (*a*), slope (*b*), correlation coefficient (R^2) and probability level of the *lack-of-fit* test, P_{lof} (%), are summarised in Table 2.

The analytical performance parameters assessed for the overall assay were linearity, precision, accuracy, sensitivity and selectivity.

3.4.1. Linearity

The response of compounds was checked in the range of application of the analytical method by linear regression analysis by the least-squares method of peak area ratio of analyte/surrogate against different analyte concentrations. The responses were linear in the range of concentrations evaluated for all compounds.

3.4.2. Accuracy

The accuracy was determined for the overall assay by measuring the percentage of recovery after the addition of known amounts of standard to the wastewater samples. The recovery studies were carried out (five replicates) by spiking 500 mL volumes of wastewater samples with the analytes, at three concentration levels. The recoveries of the tested compounds were between 95 and 105% in all cases as shown in Table 3. These recoveries were very good considering the amount of sample and the low concentration of analytes. The values indicate that compounds are quantitatively extracted using this method.

3.4.3. Precision

The precision expressed as relative standard deviation (RSD), at three concentration levels, was obtained from a repeated analysis, five times, of a spiked extract during the same day (repeatability) and in different days (reproducibility). Repeatability, as relative standard deviation, is lower than 5% in all cases as is shown in Table 3. Data indicate that the analytical method is repeatable and reproducible.

3.4.4. Sensitivity

Limits of quantitation were calculated in order to determine if analytes are present in real samples. In this paper, criteria for method performance have been proposed that include the decision limit, CC_α, the detection capability, CC_β and limit of

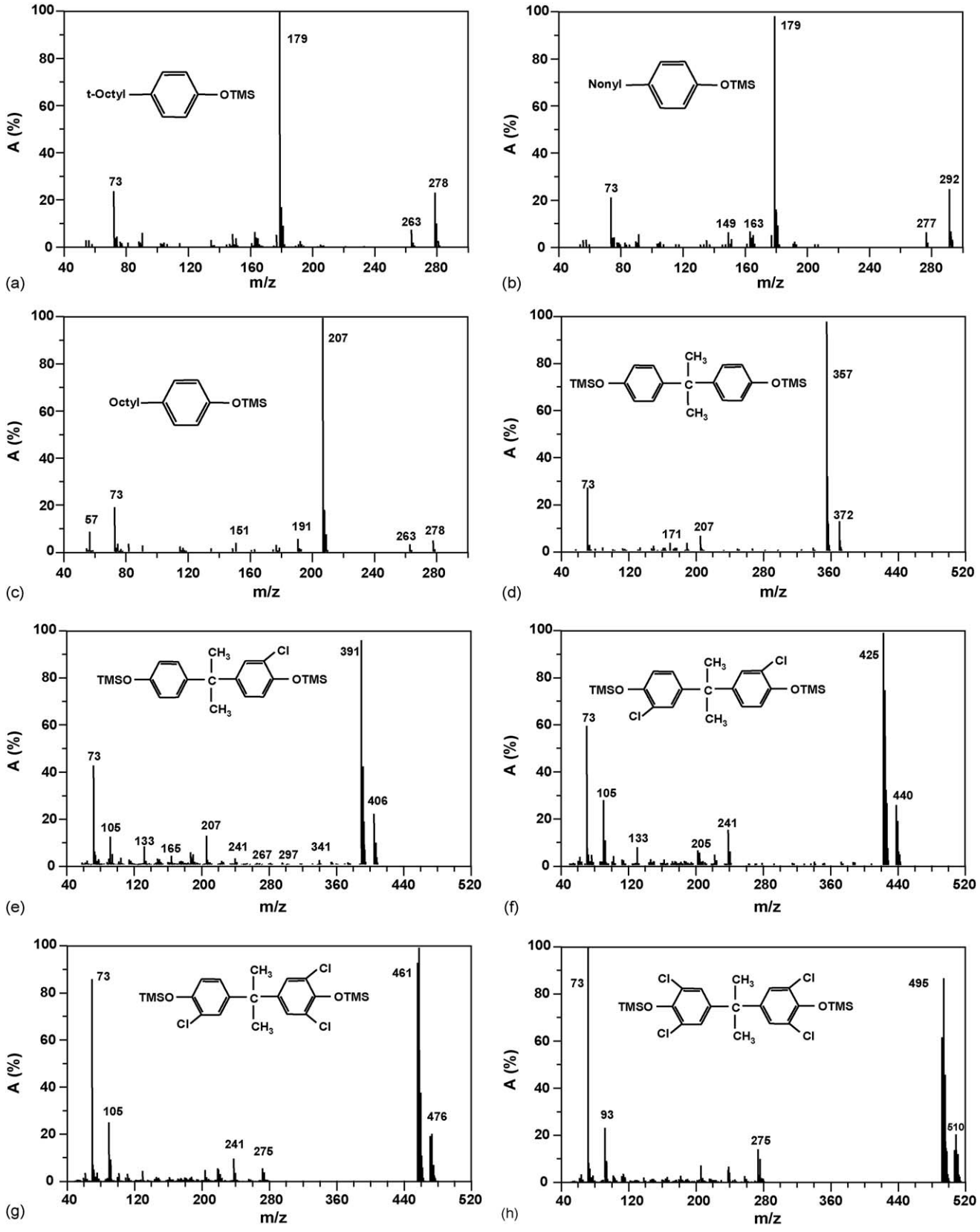

Fig. 2. Mass spectra of alkylphenols silylated (a) 4-*t*-OP-TMS; (b) 4-NP-TMS; (c) 4-OP-TMS), bisphenol A and bisphenol A chlorinated derivatives silylated (d, BPA-TMS; e, Cl-BPA-TMS; f, Cl_2-BPA-TMS; g, Cl_3-BPA-TMS; h, Cl_4-BPA-TMS).

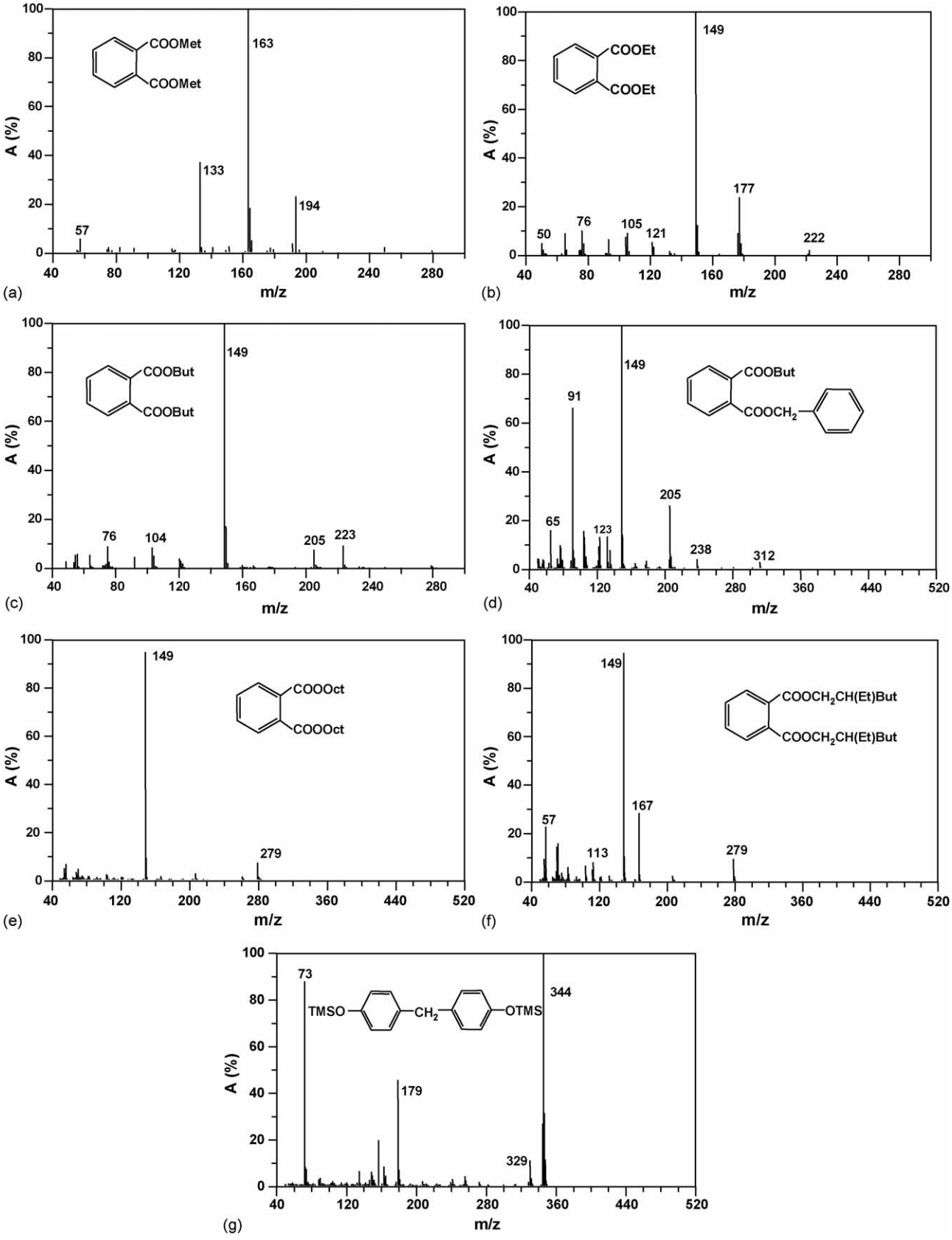

Fig. 3. Mass spectra of phthalate esters (a, DOP; b, DEP; c, DBP; d, BBP; e, DOP; f, BEHP) and internal standard, bisphenol F, silylated (g, BPF-TMS).

Table 2
Analytical and statistical parameters of the proposed method

Parameter	DMP	DEP	4-OP	4-*t*-OP	4-NP	DBP	BPA	BBP	Cl-BPA	Cl_2-BPA	BEHP	Cl_3-BPA	DOP	Cl_4-BPA
n	6	6	6	6	6	6	6	6	6	6	6	6	6	6
a	1.37×10^{-1}	2.05×10^{-1}	-2.81×10^{-2}	-4.74×10^{-3}	-1.10×10^{-2}	-1.78×10^{-2}	-1.50×10^{-1}	-5.32×10^{-2}	-9.42×10^{-2}	-6.01×10^{-2}	1.23×10^{-1}	-2.64×10^{-2}	-5.55×10^{-2}	-2.24×10^{-2}
S_a	4.67×10^{-3}	5.56×10^{-3}	5.25×10^{-4}	1.04×10^{-3}	6.68×10^{-4}	1.21×10^{-2}	1.28×10^{-2}	$5.32 \times 10{-3}$	5.72×10^{-3}	8.95×10^{-3}	4.22×10^{-3}	6.16×10^{-3}	4.80×10^{-3}	4.74×10^{-3}
b (L μg^{-1})	0.116	0.211	0.167	0.471	0.366	0.385	0.948	0.139	0.625	0.399	0.127	0.227	0.141	0.223
S_b (L μg^{-1})	1.65×10^{-3}	1.61×10^{-3}	1.97×10^{-3}	4.45×10^{-3}	3.72×10^{-3}	3.49×10^{-3}	4.12×10^{-3}	1.67×10^{-3}	2.67×10^{-3}	3.62×10^{-3}	1.39×10^{-3}	2.64×10^{-3}	1.82×10^{-3}	2.11×10^{-3}
LDR (μg L^{-1})	0.4–5.0	0.025–0.5	0.03–0.5	0.02–0.5	0.02–0.5	0.3–5.0	0.15–5.0	0.4–5.0	0.1–5.0	0.2–5.0	0.3–5.0	0.25–5.0	0.3–5.0	0.2–5.0
R^2 (%)	99.8	99.9	99.8	99.9	99.8	99.9	99.9	99.8	99.9	99.9	99.9	99.8	99.8	99.8
$S_{y/x}$	1.02×10^{-2}	1.20×10^{-2}	1.12×10^{-3}	2.25×10^{-3}	1.42×10^{-3}	2.62×10^{-2}	2.76×10^{-2}	1.09×10^{-2}	1.24×10^{-2}	1.93×10^{-2}	1.05×10^{-2}	1.34×10^{-2}	1.04×10^{-2}	1.03×10^{-2}
LIN (%)	98.6	99.9	98.8	99.1	99.0	99.1	99.6	98.8	99.8	99.1	98.9	98.8	98.7	99.1
CC_α (ng L^{-1})	90	6	7	5	4	70	30	90	20	50	80	60	80	50
CC_β (ng L^{-1})	150	10	12	8	7	120	50	150	40	90	130	100	130	80
LOQ (ng L^{-1})	400	25	30	20	20	300	150	400	100	200	300	250	300	200
P_{lof} (%)	20.2	20.8	75.7	8.9	5.7	18.9	62.4	20.0	44.1	18.4	20.3	40.0	29.0	81.5
Recovery (%)	44.9	89.8	76.8	67.0	79.9	84.5	102.0	88.1	99.4	97.1	86.3	65.4	90.2	91.0

n, calibration levels; *a*, intercept; S_a, intercept standard deviation; *b*, slope; S_b, slope standard deviation; R^2: determination coefficient; LDR, linear dynamic range; LIN, linearity; $CC_{\alpha,0.05}$, decision limit; $CC_{\beta,0.05}$, detection capability; $S_{y/x}$, regression standard deviation; LOQ, quantification limit; P_{lof}, *P*-value for *lack- of- fit* test.

Table 3
Recovery assay, precision (repeatability) and accuracy of target compounds in wastewater ($n = 5$)

Spiked (μg L^{-1})	Found ± SD (%, RSD)[a]	Recovery (%)
DMP		
0.50	0.48 ± 0.02 (4.2)	96.0
2.50	2.42 ± 0.15 (3.3)	96.8
5.00	5.25 ± 0.33 (4.4)	105.0
DEP		
0.100	0.098 ± 0.003 (3.1)	98.0
0.250	0.239 ± 0.012 (5.0)	95.6
0.500	0.490 ± 0.018 (3.7)	98.0
4-OP		
0.050	0.049 ± 0.002 (4.1)	98.0
0.250	0.241 ± 0.009 (3.7)	96.4
0.500	0.487 ± 0.021 (4.3)	97.4
4-t-OP		
0.050	0.052 ± 0.002 (3.8)	104.0
0.250	0.242 ± 0.012 (5.0)	96.8
0.500	0.517 ± 0.021 (4.1)	103.4
4-NP		
0.050	0.048 ± 0.003 (4.2)	96.0
0.250	0.262 ± 0.011 (4.2)	104.8
0.500	0.503 ± 0.019 (3.8)	100.6
DBP		
0.50	0.48 ± 0.01 (2.1)	96.0
2.50	2.42 ± 0.12 (3.3)	96.8
5.00	5.17 ± 0.12 (2.3)	103.4
BPA		
0.50	0.48 ± 0.02 (4.2)	96.0
2.50	2.48 ± 0.10 (4.0)	99.2
5.00	4.85 ± 0.29 (4.1)	97.0
DMP		
0.50	0.52 ± 0.02 (3.8)	104.0
2.50	2.42 ± 0.09 (3.7)	96.8
5.00	4.50 ± 0.16 (3.4)	95.0
DEP		
0.050	0.052 ± 0.002 (3.8)	104.0
0.250	0.260 ± 0.012 (4.6)	104.0
0.500	0.487 ± 0.024 (4.9)	97.4
4-OP		
0.100	0.105 ± 0.003 (2.9)	105.0
0.250	0.238 ± 0.012 (3.8)	95.2
0.500	0.480 ± 0.031 (3.5)	96.0
4-*t*-OP		
0.100	0.098 ± 0.003 (3.1)	98.0
0.250	0.243 ± 0.006 (2.5)	97.2
0.500	0.518 ± 0.019 (3.7)	103.6
4-NP		
0.100	0.105 ± 0.002 (2.9)	105.0
0.250	0.238 ± 0.009 3.8)	95.2
0.500	0.492 ± 0.023 (4.7)	98.4
DBP		
0.50	0.51 ± 0.02 (3.9)	102.0
2.50	2.60 ± 0.11 (4.2)	104.0
5.00	4.77 ± 0.19 (4.0)	95.4
BPA		
0.100	0.105 ± 0.004 (3.8)	105.0
0.250	0.238 ± 0.010 (4.2)	95.2
0.500	0.516 ± 0.018 (3.5)	103.2

SD, standard deviation; RSD (%), relative standard deviation.
[a] Mean of five determinations (μg L^{-1}).

Table 4
Determination of EDCs in urban wastewater samples[a]

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
DMP	nd	nd	nd	d	nd	nd
DEP	d	nd	nd	d	d	d
4-OP	nd	nd	d	nd	nd	nd
4-*t*-OP	d	d	d	nd	nd	d
4-NP	nd	nd	0.1	nd	nd	nd
DBP	0.3	nd	0.2	0.2	nd	nd
BPA	nd	nd	nd	nd	nd	nd
BBP	0.8	nd	d	nd	nd	nd
Cl-BPA	nd	nd	nd	nd	nd	nd
Cl_2-BPA	nd	nd	nd	nd	nd	nd
BEHP	1.1	0.5	0.4	0.9	0.7	d
Cl_3-BPA	nd	nd	nd	nd	nd	nd
DOP	nd	nd	nd	nd	nd	nd
Cl_4-BPA	nd	nd	nd	nd	nd	nd

nd, not detected (<$CC_{\beta,0.05}$); d, detected (<LOQ).
[a] Concentration found, mean of three determinations ($\mu g\,L^{-1}$).

quantification (LOQ) [31]. The decision limit is the limit from which it can be decided that a sample is contaminated with an error probability α. The detection capability is the smallest content of the analyte that may be detected, identified and/or quantified in a sample with an error probability β. Decision limit and detection capacity which are better adjusted to a statistical evaluation are implemented. Thus, LOQ, CC_α ($\alpha = 5\%$) and CC_β ($\beta = 5\%$) are also summarised in Table 2.

3.4.5. Selectivity

Compounds were quantified using selected ion recording mode (SIM). Analytes appear to be well resolved and free from interference peaks (Fig. 1). The identity of the chromatographic peak was confirmed not only by its retention time but also by its mass spectrum.

3.5. Validation and application of the method

Validation was performed according to the U.S. Food and Drugs Administration (FDA) guideline for bioanalytical assay validation [32]. Recovery assay was performed by comparing the analytical results for extracted urban wastewater samples, free of analytes, spiked at three concentration levels for the fourteen compounds studied. The concentration of each compound was determined by interpolation from the standard calibration curve within the linear dynamic range and compared with the added amount. A total absence of matrix interference was confirmed through analysis of two different lots of blank extract. The results are shown in Table 3.

The proposed method was applied to six urban wastewater samples picked up from different points in the city of Granada. The chromatograms obtained for the samples 1 and 2 are included in Fig. 1B and C. The obtained results, summarized in Table 4, show that the selected samples contain only low amounts of BEHP and DBP. Also, DEP, 4-*t*-OP, and BBP were detected in some samples. No BPA or chlorinated BPA derivatives seem to be present above the reported lower detection limits of this paper.

4. Conclusions

A highly sensitive, precise, reproducible and reliable method has been developed to study the levels of fourteen endocrine disrupter chemicals in wastewater. A solid phase extraction procedure is used for preconcentration and removal of interferences in conjunction with a silyl derivatization for the analysis of the compounds by GC–MS. The method has been successfully applied to the spiked and unspiked urban wastewater samples from different points of the Granada City, with different composition, consequently. Proper sample collection in conjunction with sound conservation practices prior to analysis allows for good recovery values in all cases as demonstrated by the validation procedure employed.

Acknowledgement

This work was supported by the Spanish Interministerial Commission of Science and Technology (CICYT) in its "Juan de la Cierva" program.

References

[1] F. Piva, L. Martini, Pure Appl. Chem. 70 (1998) 1647.
[2] K.L. Thorpe, T.H. Hutchinson, M.J. Hetheridge, M. Scholze, J.P. Sumpter, C.R. Tyler, Environ. Sci. Technol. 35 (2001) 2476.
[3] C.H. Groshart, P.C. Okkerman (BKH, Consulting Engineers), European Commission DG ENV, Informe M0355008/1786Q/10/11/00, 2000.
[4] Multi-Year Plan (FY2000-2012) for Endocrine Disruptor. Office of Research and Development, US Environmental Protection Agency, December 2003; http://www.epa.gov/osp/myp/edc.pdf.
[5] N. Casajuana, S. Lacorte, J. Agric. Food Chem. 52 (2004) 3702.
[6] G.G. Ying, B. Williams, R. Kookana, Environ. Int. 28 (2002) 215.
[7] R. Céspedes, M. Petrovic, D. Raldúa, U. Saura, B. Piña, S. Lacorte, P. Viana, D. Barceló, Anal. Bioanal. Chem. 378 (2004) 697.
[8] K. Mountfort, J. Tabata, S.M. Jickells, L. Castle, Food Addit. Contam. 14 (1997) 737.
[9] T. Yamamoto, A. Yasamura, Chemosphere 46 (2002) 1215.
[10] P. López-Roldán, M.J. López de Alda, D. Barceló, Anal. Bioanal. Chem. 378 (2004) 599.
[11] R.H. Rieck, LC–GC N. Am. 22 (2004) 914.
[12] M. Ahel, W. Giger, Anal. Chem. 57 (1985) 1577.
[13] A. Cohen, K. Klint, S. Bøwadt, P. Persson, J.A. Jönsson, J. Chromatogr. A 927 (2001) 103.
[14] M. Katayama, T. Sasaki, Y. Matsuda, S. Kaneko, T. Iwamoto, M. Tanaka, Biomed. Chromatogr. 15 (2001) 403.
[15] B. Shao, H. Han, J. Hu, J. Zhao, G. Wu, Y. Xue, Y. Ma, S. Zhang, Anal. Chim. Acta 530 (2005) 245.
[16] M. Petrovic, D. Barceló, J. AOAC Int. 84 (2001) 1074.
[17] K. Tsukagoshi, T. Kameda, M. Yamamoto, R. Nakjima, J. Chromatogr. A 978 (2002) 213.
[18] H.G.J. Mol, S. Sunarto, O.M. Steijger, J. Chromatogr. A 879 (2000) 97.
[19] I. Rodriguez, J.B. Quintana, J. Carpinteiro, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 985 (2003) 265.
[20] C. Kelly, J. Chromatogr. A 872 (2000) 309.
[21] M.I.H. Helaleh, Y. Takabayashi, S. Fujii, T. Korenanga, Anal. Chim. Acta 428 (2001) 227.
[22] W.H. Ding, C.C. Chiang, Rapid Commun. Mass. Spectrom. 17 (2003) 56.
[23] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.
[24] K. Schoene, H.-J. Bruckert, J. Steinhanses, A. Konig, Fresenius J. Anal. Chem. 348 (1994) 364.

[25] A. Shareef, C.J. Parnis, M.J. Angove, J.D. Wells, B.B. Johnson, J. Chromatogr. A 1026 (2004) 295.
[26] S. Nakamura, T. Hwee Sian, S. Daishima, J. Chromatogr. A 919 (2001) 275.
[27] D.D. Fine, G.P. Breidenbach, T.L. Price, S.R. Hutchins, J. Chromatogr. A 1017 (2003) 167.
[28] Statgraphics, Version Plus 5.0 Statistical Graphics Corporation and Manugistics, Rockville, MA, 2000.
[29] G.E.P. Box, W.G. Hunter, J.S. Hunter, Data Analysis and Model Building, Wiley, New York, 1978.
[30] Analytical Methods Committee, Analyst 119 (1994) 2363.
[31] Commission decision laying down performance criteria for the analytical methods to be used for certain substances and residues there of in live animals and animal products according to Council Directive 96/23/EC, Draft SANCO/1805/2000, 2000. Rev. 1.
[32] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Guidance for Industry, Bioanalytical Method Validation, May 2001, http://www.fda.gov/cder/guidance/index.htm.