Journal of Chromatography B, 877 (2009) 4042–4046

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Sensitive and selective method for the determination of bisphenol-A and triclosan in serum and urine as pentafluorobenzoate-derivatives using GC–ECNI/MS

Tinne Geens[a], Hugo Neels[a], Adrian Covaci[a,b,*]

[a] Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Belgium
[b] Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Belgium

ARTICLE INFO

Article history:
Received 13 July 2009
Accepted 13 October 2009
Available online 22 October 2009

Keywords:
Bisphenol-A
Triclosan
Serum
Urine
Pentafluorobenzoylchloride-derivatives
GC–MS

ABSTRACT

The development and validation of an analytical method is presented for the determination of bisphenol-A (BPA) and triclosan (TCS), two ubiquitous contaminants, in serum and urine. The glucuronidated metabolites were first turned into their free forms to determine total BPA and TCS. The determination consisted of a solid-phase extraction on Oasis HLB cartridges followed by an extractive derivatization with pentafluorobenzoylchloride. The extract was then purified on 10% (w/w) acidified silica and analyzed by gas chromatography–mass spectrometry in electron-capture negative ionization mode. Monitored ions were *m/z* 616 and 406 for BPA and *m/z* 482 and 287 for TCS, respectively. Limits of quantification were 0.5 ng/mL in serum and 0.2 ng/mL in urine for BPA and 0.1 ng/mL in serum and 0.05 ng/mL in urine for TCS. Method recoveries were between 76 and 110%, while repeatability was below 20%. The method was applied on 20 serum and 20 urine samples. The detection frequency in serum was 10% and 55% for BPA and TCS, respectively. BPA and TCS could be detected in all urine samples with median concentrations of 1.25 ng BPA/mL (range 0.58–5.20 ng/mL) and 1.71 ng TCS/mL (0.18–672 ng/mL).

1. Introduction

Bisphenol-A (BPA, 4,4′-(propan-2-ylidene)diphenol) and triclosan (TCS, 2,4,4′-trichloro-2′-hydroxydiphenyl ether) are two high-volume chemicals (>1000 tons produced or imported in the EU) used in a wide range of applications. BPA is mainly used as a monomer in the production of polycarbonate and epoxy resins. Polycarbonate is a hard and transparent plastic used in reusable drinking bottles and baby bottles, electrical and electronic household and office equipment. Epoxy resins are mainly found in coil coatings and as coating in food and drink cans [1]. The concern about human exposure to BPA has risen since recent findings that BPA has estrogenic effects in *in vivo* animal experiments at low doses which are within the expected human exposure range [2]. Following oral administration, BPA appears to be rapidly absorbed from the gastro-intestinal tract and undergoes a practically complete first pass metabolism in the liver by conjugation with glucuronic acid. Subsequently, the formed glucuronide is rapidly cleared from the blood and eliminated through urine [3]. TCS is a broad spectrum, synthetic, antimicrobial agent, which is widely used in personal care products such as shampoos, soaps and toothpaste [4]. The major concern for the excessive use of TCS regards the potential risks of generating TCS-resistant pathogenic microorganisms or the selection of resistant strains [5]. For TCS, a similar metabolism as BPA was proposed [5].

Due to their ubiquitous presence in the environment and possible adverse effects, biomonitoring studies are necessary to make an assessment of the exposure. BPA and TCS have already been detected in serum [6,7], urine [8,9], cord blood [10] and human milk [7,11]. Recently, different studies proposed urine as the most appropriate matrix for the biomonitoring of these phenolic compounds [12]. Since BPA and TCS are rapidly metabolized and almost completely urinary excreted, a 24-h collection or a single spot urine sample can be used for the assessment of the daily exposure to these compounds [12,13].

This paper presents the development of a method for the determination of total (free + conjugated) BPA and TCS which can be applied for both serum and urine analysis. The method is based on solid-phase extraction (SPE) followed by derivatization with pentafluorobenzoylchloride (PFBCl) and analysis by gas chromatography coupled with electron-capture negative ionization mass spectrometry (GC–ECNI/MS). Furthermore, this study investigated the stability of BPA and TCS towards sulphuric acid as a clean-up step. To our knowledge, this is the first time that use of

* Corresponding author at: Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium. Tel.: +32 3 265 2498; fax: +32 3 265 2722.
E-mail address: adrian.covaci@ua.ac.be (A. Covaci).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.10.017

Table 1
Chromatographic detection parameters of the target compounds, the ^{13}C-labeled internal standards and the recovery standard PBB-80.

Compound	Retention time (min)	Molecular mass of PFB-derivative	Quantification ion (*m*/*z*)	Qualification ion (*m*/*z*)
PBB-80	12.24	–	79	81
TCS	12.21	482	482	287
^{13}C-TCS	12.21	494	494	299
BPA	16.72	616	616	406
^{13}C-BPA	16.72	628	628	418

acidified silica in the purification method for the analysis of BPA from biological matrices is reported. The proposed method was finally applied for the determination of BPA and TCS in serum and urine samples.

2. Materials and methods

2.1. Chemicals and materials

Standard solutions of BPA and $^{13}C_{12}$-labeled BPA were purchased from Cambridge Isotope Laboratories (Andover, MA, USA), TCS and $^{13}C_{12}$-TCS were from Wellington Laboratories (Guelph, ON, Canada). 3,3,5,5-Tetrabromobiphenyl (PBB-80) was purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). All solvents used (dichloromethane (DCM), methanol (MeOH), *n*-hexane and iso-octane) were of SupraSolv grade (Merck, Darmstadt, Germany). Oasis HLB (60 mg/3 mL; Waters, Milford, MA, USA) was used for solid-phase extraction (SPE) and polypropylene cartridges (3 mL, Supelco) filled with silica gel acidified with H_2SO_4 (10%, w/w) were used for clean-up. Deconjugation was performed with β-glucuronidase/aryl sulphatase (Merck), while derivatization was performed with pentafluorobenzoylchloride (PFBCl; 99% purity, Sigma–Aldrich).

2.2. Sample preparation

2.2.1. General procedures

One millilitre of serum or 3 mL urine was fortified with 2.50 ng ^{13}C-labeled internal standard. To determine total (free and conjugated) BPA and TCS, the sample was treated with 50 μL β-glucuronidase/sulphatase in the presence of 750 μL of 1 M Na-acetate (pH 4.5) and held at 40 °C for 30 min. Subsequently, 200 μL of formic acid was added followed by sonication for 15 min. The Oasis HLB columns were prewashed and conditioned successively with 3 mL DCM, 6 mL MeOH and 3 mL milliQ H_2O. The samples were transferred on the SPE columns after which the columns were washed with 3 mL milliQ H_2O and dried for 10 min using a vacuum manifold. The analytes were then eluted with 5 mL MeOH–DCM (1:1, v/v) and the eluate was evaporated until dryness using N_2. The extractive derivatization was carried out in a two layer system of 1 mL milliQ H_2O and 2 mL hexane. 50 μL of 2 M KOH was added for ionization of the target compounds and 50 μL PFBCl (5% in hexane, v/v) was used as derivatization reagent. The solution was vortexed for 2 min and, after phase-separation, the hexane layer was transferred. Then, another 2 mL hexane was added to the aqueous phase and the cycle was repeated to obtain a quantitative transfer. After evaporation until the combined hexane layers are dry, the analytes were reconstituted in 100 μL recovery standard PBB-80 (100 pg/μL in *i*-octane).

After elution from the SPE cartridge, the extract containing lipids and other impurities was cleaned up with sulphuric acid. A comparison was made between the passage over 10% (w/w) and 44% (w/w) acidified silica (AS). Moreover, a comparison was made between the use of the clean-up step immediately before and after derivatization.

2.2.2. Evaluation of clean-up before derivatization

For the evaluation of the clean-up step before derivatization, AS was added to a 3 mL empty cartridge and, after application of the extract, the compounds were eluted with 12 mL DCM. The recovery was calculated for the ^{13}C-labeled compounds based on the recovery standard PBB-80, which was added right before the instrumental analysis.

2.2.3. Evaluation of clean-up after derivatization

For the clean-up examination after derivatization, the hexane layer was immediately transferred on a cartridge containing AS. Subsequently, the compounds were eluted with 6 mL DCM. Analytes were reconstituted in a solution containing the recovery standard PBB-80 prior to analysis.

2.3. Instrumental analysis

The analysis of the PFB-derivatives was performed with a Hewlett-Packard 6890 GC coupled to a HP 5973 MS operated in electron-capture negative ionization mode. The extract (2 μL) was injected in cold pulsed splitless mode (injector temperature 90 °C for 1.25 min, then increased at 700 °C min^{-1} to 290 °C, pulse pressure 15.0 psi, time pulse 1.25 min, and splitless time 1.25 min). The GC column was a 30 m × 0.25 mm × 0.25 μm DB-5 column (J&W Scientific). The oven temperature program was 90 °C for 1.25 min, then 25 °C min^{-1} to 300 °C, kept for 8 min. Helium was used as carrier gas at constant flow (1.0 mL min^{-1}) with methane as moderating gas. Dwell times were 30 ms. The ion source, quadrupole, and interface temperatures were 170, 150, and 300 °C, respectively, and the electron multiplier voltage was 2100 V. Compounds with their respective retention times and the ions used for quantification and confirmation are summarized in Table 1.

3. Results and discussion

3.1. Derivatization

The derivatization procedure with PFBCl is based on previously described methods [7,14]. Kuch and Ballschmiter [14] also proved that the two extractions with 2 mL hexane were sufficient to completely transfer the formed derivatives from the aqueous phase to the organic phase. The introduction of five (for TCS) and ten (for BPA) fluorine atoms turns the analytes into highly electrophilic derivatives. This results in very high sensitivity for ECNI-MS detection. Moreover the PFB-derivatives are rapidly formed, are stable over time and are stable on 10% AS (see below). During the extractive derivatization step, polar substances stayed in the aqueous phase and therefore the derivatization also provided an additional purification. A full-scan chromatogram of a standard mixture of $^{13}C_{12}$- and $^{12}C_{12}$-derivatives is presented in Fig. 1 with their corresponding mass spectrum. The dominant ion formed was $[M]^-$ for both BPA–PFB- and TCS–PFB-derivatives. For TCS–PFB, the confirmation ion was $[M-C_6F_5CO]^-$, while, for BPA, the confirmation ion was $[M-C_6F_5COCH3]^-$.

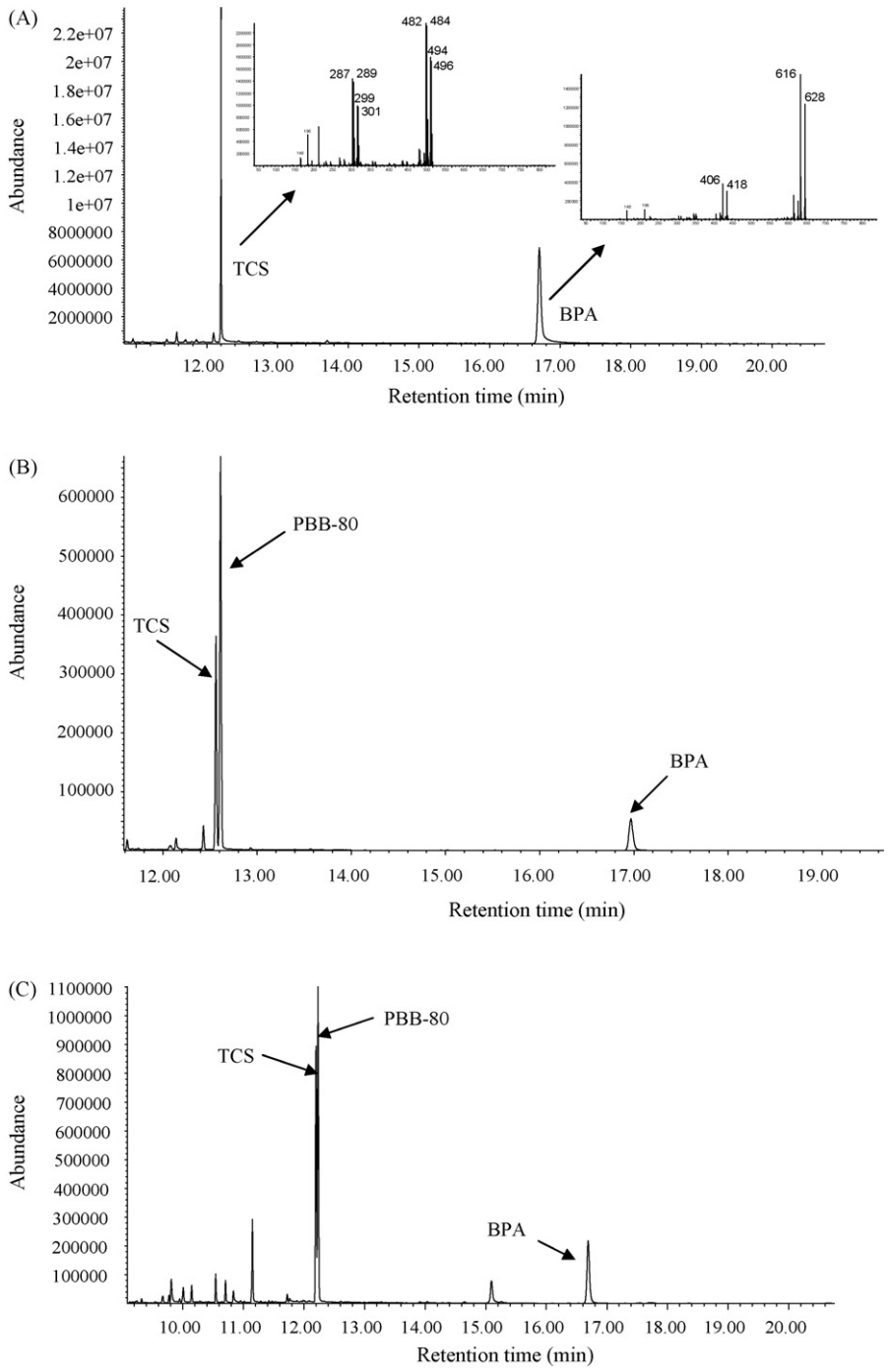

Fig. 1. Full-scan chromatogram (A) of a mixture of ^{13}C-labeled and native TCS and BPA standards (1 ng injected) as their pentafluorobenzoates with their corresponding mass spectrum and selected ion monitoring chromatograms of serum (B) and urine (C) samples.

3.2. Clean-up

Results of the clean-up methods based on purification with sulphuric acid are summarized in Table 2. Both investigated clean-up methods prior to derivatization gave high recoveries for TCS, however recoveries for BPA were below 20%. The stability of TCS in the presence of sulphuric acid had already been described by Allmyr et al. [7]. BPA showed low recoveries and it can be assumed that

Table 2
Recovery of clean-up step of respectively underivatized and derivatized PFB-compounds on different amounts of sulphuric acid (AS: acidified silica).

	Recovery underivatized compounds (%)		Recovery PFB-derivatives (%)	
	BPA	TCS	BPA	TCS
10% AS	17	100	94	96
44% AS	3	85	30	93

BPA is broken down. The PFB-derivative of BPA on the other hand showed a decreasing recovery with increasing concentrations of sulphuric acid (Table 2). In contrast with the underivatized BPA, the BPA–PFB-derivative was more stable in the presence of sulphuric acid and provided very good recoveries (94%) on 10% AS. On 44% AS, the BPA–PFB-derivative was mostly degraded and had only a recovery of 30%. Purification on AS results in cleaner extracts and improves the analytical sensitivity, therefore a clean-up step on 10% AS after the derivatization with PFBCl was implemented in the method. To our knowledge, a clean-up step on AS has not yet been reported for the determination of BPA.

3.3. Quality control

3.3.1. Calibration curve: linearity

For the calibration curves, ratios of peak area native compound/^{13}C internal standard were plotted against ratio of masses native compound/^{13}C internal standard. R^2 values for both compounds were >0.999. Seven calibration levels for both BPA and TCS with a range of 0.46–10.5 ng/mL for serum and 0.15–14 ng/mL for urine were plotted.

3.3.2. Limit of quantification

Small amounts of BPA and TCS could be detected in procedural blanks. Therefore, the method LOQs were calculated as 3× standard deviation of procedural blanks, taking into account the amount of sample used for the analysis (1 mL serum and 3 mL urine). Resulting LOQs for TCS and BPA are 0.1 and 0.5 ng/mL for serum and 0.05 and 0.20 ng/mL for urine, respectively. These method LOQs are similar or lower than those reported in the literature using online SPE LC–MS/MS or other GC–MS methods [6,15]. Instrumental LOQs, expressed as ten times the signal to noise ratio, were 0.04 pg injected for BPA and 0.02 pg injected for TCS. These low values are obtained due to the introduction of fluorine atoms during derivatization, to the very sensitive detection by ECNI-MS and to the clean extracts obtained after purification on AS.

3.3.3. Method recovery and precision

Method recovery and precision were determined by spiking 1 mL serum and 3 mL urine at two different concentration levels. Each fortification level for urine and serum was analyzed in triplicate on three successive days. The same pooled sample of serum and urine was used for the three days. Background levels in these samples were determined by analyzing them six times, and the average concentrations were subtracted from the obtained levels. Recoveries and within- (repeatability) and between-day (intermediate precision) precision were calculated and are summarized in Table 3. For BPA, the lowest spiking level was close to the LOQ and therefore a higher deviation (~20%) was seen. For the highest spiking level, which is closer to the levels found in real samples, a deviation of less than 10% is seen in most cases.

Table 4
Comparison of total BPA and total TCS in urine with other reported results in the literature (n = number of samples).

	Range (ng/mL)	n	Country	References
BPA	0.2–19.1	48	Japan	[16]
	<0.1–5.18[a]	394	US	[17]
	<0.3–19.8	30	US	[18]
	4.0–49	8	Spain	[19]
	<0.1–15.9[a]	2517	US	[20]
	0.58–5.20	20	Belgium	Current study
TCS	1.13–14.0	6	Japan	[21]
	<2.4–3790	2517	US	[9]
	0.18–672	20	Belgium	Current study

[a] 95th percentile.

3.4. Method application

The method was applied to 20 serum and 20 urine samples from Belgian adolescents. An example of a chromatogram of a serum and urine sample is presented in Fig. 1B and C. In the serum samples, BPA was detected in only 10% of the samples within a range of <LOQ–0.59 ng/mL. TCS could be detected in 55% of the samples within a range of <LOQ–9.2 ng/mL. In the urine samples, the detection rate was 100% for both compounds within a range of 0.58–5.20 ng/mL (median 1.25 ng/mL) and 0.18–672 ng/mL (median 1.71 ng/mL) for BPA and TCS respectively. In Table 4, our results are compared with results of total urinary concentrations reported in the literature. Similar to our results, TCS showed a wider concentration range than BPA. Both analytes had a concentration range comparable to data reported in other countries.

4. Conclusions

A reliable method is presented for the simultaneous determination of total BPA and TCS which can be applied both for serum and urine analysis. This method is an improvement of our previous method described by Dirtu et al. [22] for the determination of BPA and TCS in serum where, after SPE with Oasis HLB, Florisil was used as clean-up sorbent and pentafluoropropionic acid anhydride as derivatization reagent. The current presented method showed lower and more constant levels of the method blanks and provided cleaner extracts due to the clean-up with AS. Moreover, the combination of derivatization with PFBCl, analysis of the fluorinated derivatives by GC–ECNI/MS and a clean-up step on AS after derivatization makes this a more sensitive method. Since the BPA

Table 3
Quality control parameters for two spiking concentrations for serum and urine. The recovery and standard deviations (SD) are expressed as percentages.

	Concentration (ng/mL)	Day 1		Day 2		Day 3		Day 1-3	
		Recovery	SD	Recovery	SD	Recovery	SD	Recovery	SD
BPA									
Serum	0.46	97	17	89	14	94	21	93	16
	2.20	98	11	90	6	93	8	94	9
Urine	0.73	107	4	93	3	104	14	102	10
	2.20	103	5	98	1	97	1	100	4
TCS									
Serum	0.46	76	8	83	9	88	11	82	11
	2.20	91	4	93	3	95	4	93	4
Urine	0.73	87	17	110	17	86	13	95	19
	2.20	92	14	102	6	94	3	96	9

concentrations in serum are mostly below LOQ, urine seems to be a more appropriate matrix for biomonitoring studies and the subsequent exposure assessment.

Acknowledgements

Tinne Geens acknowledges the funds for Scientific Research Flanders (FWO) for a PhD fellowship and Adrian Covaci acknowledges FWO for a postdoctoral fellowship.

References

[1] European Commission, Updated European Risk Assessment Report: 4,4′-Isopropylidenediphenol (Bisphenol-A), 2008.
[2] W.V. Welshons, S.C. Nagel, F.S. vom Saal, Endocrinology 147 (2006) S56.
[3] W. Völkel, T. Colnot, G.A. Csánady, J.G. Filser, W. Dekant, Chem. Res. Toxicol. 15 (2002) 1281.
[4] European Commission, Report on Triclosan Antimicrobial Resistance, 2002.
[5] G. Sandborgh-Englund, M. Adolfsson-Erici, G. Odham, J. Ekstrand, J. Toxicol. Environ. Health A 69 (2006) 1861.
[6] X. Ye, L.J. Tao, L.L. Needham, A.M. Calafat, Talanta 76 (2008) 865.
[7] M. Allmyr, M.S. McLachlan, G. Sandborgh-Englund, M. Adolfsson-Erici, Anal. Chem. 78 (2006) 6542.
[8] W. Volkel, M. Kiranoglu, H. Fromme, Toxicol. Lett. 179 (2008) 155.
[9] A.M. Calafat, X. Ye, L.Y. Wong, J.A. Reidy, L.L. Needham, Environ. Health Persp. 116 (2008) 303.
[10] Y.J. Lee, H.Y. Ryu, H.Y. Kim, C.S. Min, J.H. Lee, E. Kim, et al., Reprod. Toxicol. 25 (2008) 413.
[11] X.Y. Ye, A.M. Bishop, L.L. Needham, A.M. Calafat, Anal. Chim. Acta 622 (2008) 150.
[12] W. Dekant, W. Voelkel, Toxicol. Appl. Pharmacol. 228 (2008) 114.
[13] J.S. Lakind, D.Q. Naiman, J. Exp. Sci. Environ. 18 (2008) 608.
[14] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[15] Z. Kuklenyik, J. Ekong, C.D. Cutchins, L.L. Needham, A.M. Calafat, Anal. Chem. 75 (2003) 6820.
[16] K. Ouchi, S. Wanatabe, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 780 (2002) 365.
[17] A.M. Calafat, Z. Kuklenyik, J.A. Reidy, S.P. Caudill, J. Ekong, L.L. Needham, Environ. Health Persp. 113 (2005) 391.
[18] X.Y. Ye, K. Zsuzsanna, L.L. Needham, A.M. Calafat, Anal. Bioanal. Chem. 383 (2005) 638.
[19] A. Garci-Prieto, M.L. Lunar, S. Rubio, D. Perez-Bendito, Anal. Chim. Acta 630 (2008) 19.
[20] A.M. Calafat, X.Y. Ye, L.Y. Wong, J.A. Reidy, L.L. Needham, Environ. Health Persp. 116 (2008) 39.
[21] M. Kawaguchi, R. Ito, H. Honda, N. Endo, N. Akanouchi, K. Saito, Y. Seto, H. Nakazawa, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 875 (2008) 577.
[22] A.C. Dirtu, L. Roosens, T. Geens, A. Gheorghe, H. Neels, A. Covaci, Anal. Bioanal. Chem. 391 (2008) 1175.