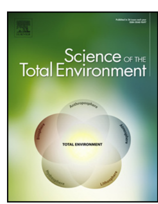
Science of the Total Environment 628–629 (2018) 1362–1368

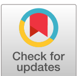
Contents lists available at ScienceDirect

Science of the Total Environment

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



Determination of trace levels of eleven bisphenol A analogues in human blood serum by high performance liquid chromatography–tandem mass spectrometry



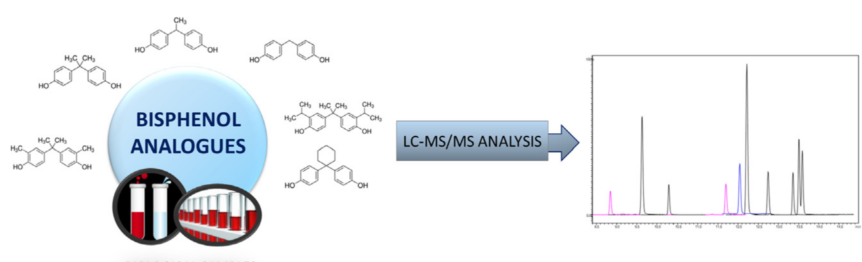
Katarzyna Owczarek [a], Paweł Kubica [a], Błażej Kudłak [a], Aleksandra Rutkowska [b], Aleksandra Konieczna [b], Dominik Rachoń [b], Jacek Namieśnik [a], Andrzej Wasik [a,*]

[a] Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, Narutowicza 11/12 str, 80-223 Gdańsk, Poland
[b] Department of Clinical and Experimental Endocrinology, Medical University of Gdańsk, Dębinki 7, 80-210 Gdańsk, Poland

HIGHLIGHTS

- Chromatographic separation of bisphenol A and 10 bisphenol A analogues
- Liquid-liquid extraction of bisphenol A analogues from 245 human serum samples
- Matrix-matched calibration to compensate for matrix effects

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 September 2017
Received in revised form 12 February 2018
Accepted 12 February 2018
Available online xxxx

Editor: Yolanda Picó

Keywords:
Bisphenol A
BPA analogues
Blood serum analysis
Liquid chromatography
Tandem mass spectrometry
Endocrine disrupting chemicals

ABSTRACT

Chemicals showing structural or functional similarity to bisphenol A (BPA), commonly called BPA analogues, have recently drawn scientific attention due to their common industrial and commercial application as a substitute for BPA. In the European Union, the use of BPA has been severely restricted by law due to its endocrine disrupting properties. Unfortunately, it seems that all BPA analogues show comparable biological activity, including hormonal disruption, toxicity and genotoxicity. Until now, the knowledge about human exposure to BPA analogues is scarce, mainly due to the lack of the data concerning their occurrence in human derived biological samples. This study presents the development of an analytical method for determination of trace levels of eleven BPA analogues in human blood serum samples. The method involves fast and simple liquid-liquid extraction, using low sample and solvent volumes. Chromatographic separation of analytes was optimized using one-factor-at-a-time approach (mobile phase composition, gradient shape, chromatographic column selection, separation temperature, etc.).

The method allows for effective separation of the analytes, even in the case of configurational isomers (bisphenol M and bisphenol P). The calibration curves for all analytes were linear in the range tested. The limits of detection and quantitation were in the range of 0.0079 ÷ 0.039 ng/mL and 0.024 ÷ 0.12 ng/mL respectively. Compound-dependent recovery values were in the rage of 88 ÷ 138%. Matrix effects were mitigated with the help of matrix-matched calibration curves prepared for every batch of samples. Results obtained after the analysis of 245 real human blood serum samples indicate that human beings are exposed to different BPA analogues, that are present in the environment and in common, daily use products.

© 2018 Elsevier B.V. All rights reserved.

* Corresponding author.
E-mail address: wasia@pg.edu.pl (A. Wasik).

https://doi.org/10.1016/j.scitotenv.2018.02.148
0048-9697/© 2018 Elsevier B.V. All rights reserved.

1. Introduction

In the last years endocrine disrupting compounds (EDCs) have become the chemical group of special concern due to their ability to interfere with hormonal system and ubiquitous presence (Rissman and Adli, 2014). Bisphenols (BPs) are the chemicals that have recently been the subject of growing interest due to their endocrine disrupting properties (Konieczna et al., 2015). BPs contain two *p*-hydroxyphenyl functionalities in their molecular structure and include several analogues, of which bisphenol A (BPA) is the most commonly used and known. Since early 1950s, BPA has been used in plastic industry for the production of epoxy and polycarbonate resins (Vogel, 2009) being commonly used as raw materials in the manufacturing of a broad spectrum of everyday use products (i.e. tin linings and other food contact materials, water pipes, powder paints, toys etc.). Nowadays, the annual production of BPA reaches over 7.7 million tons and the demand for this compound is predicted to increase over the next years (Industry Experts, 2016).

The effects exerted by BPA on human health have been extensively studied and its estrogenic activity is one of the best known upshots. Due to its phenolic structure BPA is able to interact with estrogen receptors and therefore may exert estrogenic actions (Konieczna et al., 2015) leading to ovarian dysfunction (Rutkowska and Rachoń, 2014) or even estrogen dependent cancers (Rachoń, 2015). Besides that, the vast number of other adverse effects have been proven, including neural and developmental disorders (Arbuckle et al., 2016; Kundakovic et al., 2013), alternation of thyroid function (Ahmed, 2016), metabolic disorders (Stojanoska et al., 2017) and suspicion of increasing the risk of Parkinson disease (Huang et al., 2014). Hazardous implications of BPA presence are not only limited to humans. Especially, the homeostasis of aquatic ecosystems can be disrupted in various ways such as feminization of many wildlife species or developmental and behavioral alternations (Bhandari et al., 2015). Therefore, several endocrine societies have published their position statements on its adverse health risks and stressed the need of governmental authorities in establishing the laws of minimizing the exposure to these endocrine disrupting chemicals (Grob et al., 2015; Hunt et al., 2016; Rutkowska et al., 2015).

Detailed information including chemical structures and IUPAC names of BPA analogues under the study are given in Table 1.

Due to the growing doubts concerning ecological and long-term health implications, new BPA-related chemicals were considered to be safer alternatives for industrial applications. The total number of 16 bisphenols has been documented to be commercially applied (Chen et al., 2016). Bisphenol S (BPS) and bisphenol F (BPF) are nowadays the most commonly used BPA substitutes, predominantly in the manufacturing of epoxy resins, polyesters and polycarbonate plastics. Other analogues are also used in plastic industry to produce dental sealants, pesticides, thermal papers, food container's inner coatings, toys, lacquers, powder paints, flame retardants, personal care products, thermosensitive materials and others (Hada et al., 2013; Hsieh and Hsu, 2015; Ochiai and Masuda, 2013; Teichert et al., 2014; Wagner et al., 2015; Zouta et al., 2014). Currently, only the BPA applications are regulated by legislative standards in European Union, United States and Canada (Yang et al., 2014). BPA, BPE, BPF, BPS, BPP, BPZ, TBBPA, TCBPA, and BPAF were detected in sludge, surface water and indoor dust (Bhandari et al., 2015; Lee et al., 2015; Song et al., 2014; Yamazaki et al., 2015). BPA, BPB, BPE, BPF, BPP, BPS, BPZ BPAF, and BPAP were detected in foodstuffs (Liao and Kannan, 2012; Yang et al., 2014). Unfortunately, the understanding of the environmental, biological and the health impact of BPA analogues is still very scarce. The environmental abundance of BPs undoubtedly indicates that humans are

Table 1
Basic information on bisphenol A analogues.

Compound/molecular weight [g/mol]	CAS number	Structure	IUPAC name
BPA 228.29	80-05-7		2,2-Bis(4-hydroxyphenyl)propane
BPC 256.34	79-97-0		2,2-Bis(4-hydroxy-3-methylphenyl)propane
BPE 214.26	2081-08-5		1,1-Bis(4-hydroxyphenyl)ethane
BPF 200.23	620-92-8		4,4′-Methylenediphenol
BPG 312.45	127-54-8		2,2-Bis(4-hydroxy-3-isopropylphenyl)propane
BPM 346.46	13,595-25-0		4,4′-(1,3-Phenylenediisopropylidene)bisphenol
BPP 346.46	2167-51-3		4,4′-(1,4-Phenylenediisopropylidene)bisphenol
BPS 250.27	80-09-1		4,4′-Sulfonyldiphenol
BPZ 268.35	843-55-0		4,4′-Cyclohexylidenebisphenol
BPFL 350.41	3236-71-3		4,4′-(9-Fluorenylidene)diphenol
BPBP 352.43	1844-01-5		1,1-Bis(4-hydroxyphenyl)-1,1-diphenylmethane

constantly exposed to the wide spectrum of these chemicals, but the data concerning the presence of BPA analogues in human-derived samples is still very limited.

Human exposure to mentioned chemicals include dietary (as a most probable) and non-dietary (inhalation, dermal) routes. Human exposure to bisphenol analogues other than BPA is not well characterized. Available data concerning bisphenols levels in body fluids and tissues is very limited so far. Majority of studies has been conducted on the urine where BPA, BPAF, BPB, BPF, BPS, BPP and BPZ have been measured.

Among sampling material of human origin, blood (and its derivatives - plasma and serum) is a matrix that carries most valuable information about short-term exposure due to its contact with all body cells and tissues. Due to the of lack of scientific data concerning analytical methods for the determination of a wide spectrum of BPA analogues, the aim of this research was to develop an easy, fast, highly sensitive and robust method for human biomonitoring of these chemicals. To encompass the range of bisphenols that are commonly present in the environment constituents and may pose health risk, the total number of 11 bisphenols was determined in human serum samples using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Analytes have been selected on the basis of the probability of their occurrence in both the environment and common goods and previous research concerning their genotoxicity, estrogenicity or toxicity (Chen et al., 2002; Rivas et al., 2002). Taking into consideration that most of bisphenols other than BPA may also exhibit similar biological activity (Chen et al., 2002; Rivas et al., 2002; Rosenmai et al., 2014; Sui et al., 2012) and number of their industrial and commercial applications is increasing, introducing new methods for BPs biomonitoring seems to be justified due to the constant human exposure to them. In addition, the data concerning the occurrence and concentration levels of BPA analogues in human derived samples is very limited or absent. To the best of authors' knowledge, this is the first report on the determination of eleven BPA analogues in human blood serum samples.

2. Experimental

2.1. Materials and standards

Analytical standards of BPA, BPC, BPE, BPF, BPG, BPM, BPP, BPS, BPZ, BPFL and BPBP were purchased from Sigma-Aldrich (St. Louis, USA, 99% purity). Acetic acid, formic acid and ammonia were purchased from Sigma-Aldrich (St. Louis, USA). $MgSO_4$ was obtained from Eurochem BGD (Tarnów, Poland). Internal standard ^{13}C-labeled BPA (ring-13C12) was supplied by Cambridge Isotope Laboratories Inc. (UK). Normal human serum (cat. no. S1-M EMD Millipore) was also obtained from Sigma-Aldrich (St. Louis, USA). Acetonitrile (ACN) and methanol (MeOH), used during the sample preparation procedure and as a mobile phase components, were LC-MS grade, obtained from Merck KGaA (Darmstadt, Germany). Ultrapure water was produced by the Milli-Q Gradient A10 system equipped with an EDS-Pak cartridge for removing endocrine disrupting compounds (Merck-Millipore).

2.2. Samples

The study has been conducted according to the Declaration of Helsinki for Medical Research involving human subjects and was approved by the Ethics Committee of the Medical University of Gdańsk (permission number NKBBN 198/2012). In total 245 women aged 18–40 years took part in the study. They were all informed about the purpose of it and gave a written consent to participate. Venous blood samples were drawn after an overnight fast (>8 h) between 7 am and 9 am at the medical diagnostic laboratory based in the city centre of Gdańsk (Bruss, ALAB group, Poland), which possesses current ISO certificates and has an accreditation in the field of medical laboratory diagnostics. All the procedures were conducted with precautions intended to minimize the risk of sample contamination with BPA analogues. Blood was drawn from the antecubital vein using a vacuum blood collection system directly into a 7 mL glass tubes (no additives) and within 30–90 min was centrifuged at the speed of 2500 rpm for 15 min. After the centrifugation process, sera were transferred into 2 mL glass vials using a plastic disposable (Pasteur) transfer pipette made of BPA-free low-density polyethylene (LDPE) and were closed with a polypropylene (PP) screw cup with a polytetrafluoroethylene (PTFE) septum. The samples were then stored at −70 °C for further analyses.

2.3. Preparation of standards and calibration

Individual stock solutions (0.5 mg/mL) of all analytes were prepared by dissolving accurately weighted amounts of analytical standards in ACN. Working solution was obtained by mixing the stock solutions and diluting the mixture with ACN. All solutions were stored in a freezer (−20 °C). All glassware was pre-washed with methanol. Seven-point (0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ng/mL) matrix-matched calibration curves were prepared using normal human blood serum. Before use, the serum used for preparation of the calibration curves, was analyzed for the presence of analytes under the study. It was found free from all studied bisphenols except for small amounts of BPBP (see Supplementary Fig. 1), therefore the calibration data for this compound were corrected to account for this fact, i.e. average blank peak area of BPBP was subtracted from average peak areas of other calibration points. Internal standard (IS) concentration was kept at 25 ng/mL in all calibration samples. Fresh calibration solutions were prepared for every batch of samples.

2.4. Sample preparation

Sample preparation step was conducted with precautions intended to minimize sample contamination. All glassware was washed with MeOH and all plastics were made of high quality polypropylene to avoid contamination of the samples by bisphenols. In order to extract the analytes from serum samples, the following liquid-liquid extraction method was used. 500 μL of serum was placed in a clean glass tube and mixed with 1.5 mL of ACN and 10 μL of IS solution (2.5 μg/mL). Samples were shaken for 30 s and left for 10 min in room temperature to complete protein precipitation. After that, 250 mg of anhydrous $MgSO_4$ was added and each sample was vortexed to remove water. After centrifugation for 2 min (6000 rpm, 3864*g*) supernatants were transferred to clean glass tubes and evaporated under the gentle stream of nitrogen in a water bath (42 °C), to the final volume of about 150 μL. The residue was mixed with 250 μL of mobile phase (MeOH:H_2O, 50:50, 0.01% v/v NH_3), vortexed again and transferred to chromatographic vials for analysis. Procedural blanks spiked with IS, along with system blanks were prepared for every batch in triplicate in the same way as other samples. Example of a procedural blank chromatogram is given in Supplementary Fig. 1.

2.5. MS/MS and separation conditions

All analyses were performed with Shimadzu triple quadrupole LC-MS/MS system (LCMS-8060, Shimadzu, Japan) equipped with an electrospray ionisation source (ESI) working in the negative multiple reaction mode (MRM). LabSolutions v.5.85 software suite was used for data acquisition and processing. Detailed information on ion transitions, MS/MS operational parameters and ion source parameters are given in Supplementary material (Supp. Table 1). Optimal conditions for MRM transitions were established using an automated procedure built into the LabSolutions software suite. In short, it consisted of injecting standard solutions of analytes (one at a time) into the spectrometer, finding a precursor ion ($[M-H]^-$), fragmentation of precursor, selecting most intense product ions and finally automatic optimisation of detector voltages. Ion source parameters (gas flows, temperatures) were adjusted

manually using one-factor-at-a-time approach to obtain the best signal intensity for all analytes.

2.6. Separation conditions

Chromatographic separation was carried out using the UPLC Nexera ×2 system (Shimadzu, Japan) consisting of degasser DGU-20A5R, controller CBM-20A, binary pump LC-30AD, autosampler SIL-30AC and column oven CTO-20AC. Two chromatographic methods were applied to determine the analytes. BPC, BPE, BPF, BPG, BPM, BPP, BPZ, BPFL, BPBP were separated using gradient of H_2O (mobile phase A) and MeOH (mobile phase B), both modified with 0.01% v/v of ammonia. Initial conditions of 5% B were kept for 1.5 min, then the content of B component was increased to 75% over 10.5 min and further increased to 100% over 4 min. Following this, mobile phase composition was set-back to starting conditions and maintained for 5 min for column re-equilibration. For graphical illustration of mobile phase gradient see inset of Fig. 1.

During the determination of BPA and BPS the mobile phase consisted of H_2O (component A) and MeOH (component B) without additives. Isocratic elution using 1:1 mixture of components A and B was used.

Ascentis® Express (C18 15 cm × 2.1 mm, 2.7 μm) with guard column (0.5 cm × 2.1 mm, 2.7 μm), mobile phase flow of 0.55 mL/min, 50 °C of thermostated column compartment and injection volume of 5 μL were applied for separation of analytes in case of both methods.

Examples of chromatograms obtained after analysis of calibration solutions using both methods are given in the Fig. 1. More detailed information on separation conditions will be discussed in the next section.

3. Results and discussion

3.1. Extraction conditions

General problem in bisphenol analysis is ion suppression or enhancement, resulting from the presence of matrix components. In case of such complex biological matrix as blood serum, procedure was focused on removal of its elements. ACN and MeOH were tested as the extraction solvents that are also needed for precipitation of endogenous proteins. Eventually, ACN was chosen due to significantly higher recovery of analytes. Drying of the extract with anhydrous $MgSO_4$ enhanced peaks intensities due to precipitation of water soluble interferences which could caused signal suppression. Salting-out effect could also help to improve the transport of analytes from aqueous phase into the organic solvent.

3.2. Separation and detection of the analytes

The goal of the conducted research was to develop analytical method for separation and determination of 11 bisphenols in blood serum samples. Ascentis® Express C18 (15 cm × 2.1 mm, 2.7 μm) column with guard column (0.5 cm × 2.1 mm, 2.7 μm), packed with core-shell technology particles, was chosen due to its high separation efficiency and relatively short analysis time.

MeOH and ACN were tested as the main organic components of the mobile phase, however, in the case of ACN, peak broadening, peak shape deterioration along with the much smaller response were noted. This phenomenon has been observed for several other compounds determined in ESI negative ion formation mode (Gioumouxouzis et al., 2015). 5, 10, 20 and 25 mM ammonium acetate, 0.01, 0.05 and 0.1% v/v formic acid, acetic acid and ammonia were tested separately as the mobile phase additives. The most promising results (in terms of response and peak shapes) were obtained when applying the latter. Buffer and acid solutions have caused signal suppression.

In case of BPS the addition of ammonia to the mobile phase resulted in decrease of sensitivity and shifting the BPS signal towards system void time. Moreover, low initial content of methanol as well as long analysis and conditioning time caused the enrichment of BPA, derived from system elements, on the front of separation column (Wilczewska et al., 2016).

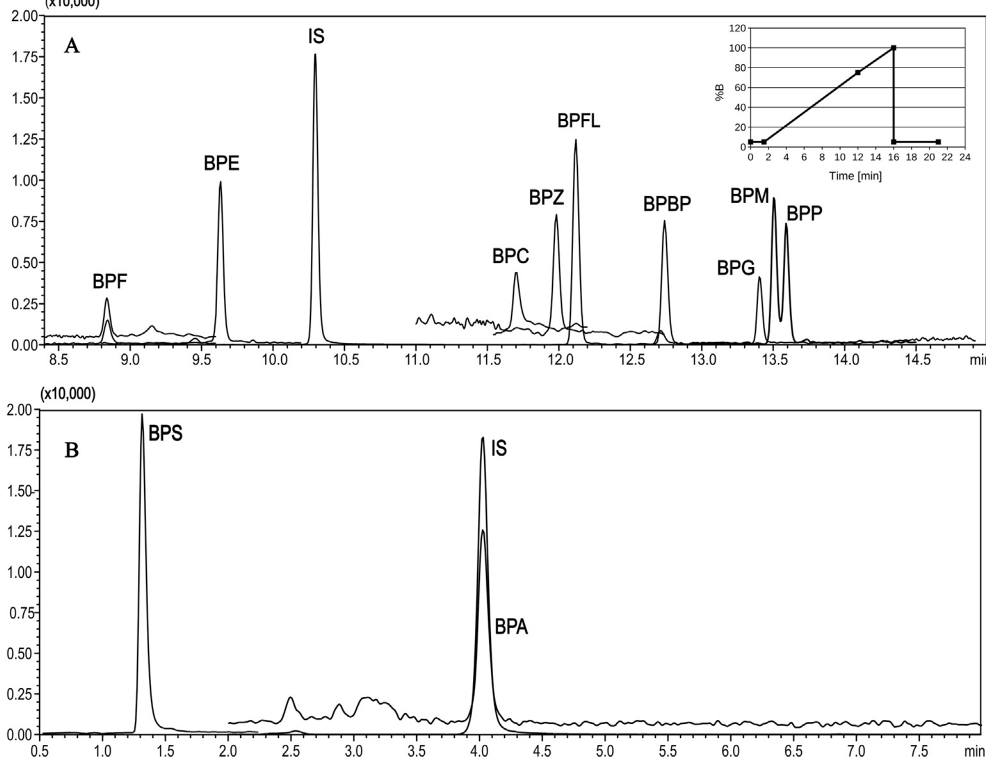


Fig. 1. Chromatograms obtained after analysis of 1 ng/mL calibration solution; (A) separation of 9 bisphenols (mobile phase gradient in the inset), (B) separation of BPA and BPS. For clarity IS trace has been scaled down by the factor of 0.05.

Therefore BPA and BPS were determined with separate method using a mobile phase consisting of MeOH:H_2O without additives. Isocratic flow of relatively high elution strength (50% v/v MeOH) mobile phase provided accurate results and good linearity of the calibration curves.

3.3. Method validation

The performance of both analytical methods was evaluated in terms of linearity, limits of detection (LODs) and quantitation (LOQs) and recoveries. The obtained results are presented in Table 2 and in Suppl. Table 2 of Supplementary material. For both methods the linear calibration equations were obtained from 7-point calibration curves, that were made by plotting the ratios of analyte peak area to IS peak area versus corresponding concentrations. Calibration curves were linear in the tested concentration range from 0.05 to 5 ng/mL. To increase the accuracy at the lowest concentration range, the weighting factor 1/x was applied to every calibration curve.

The LOD values were evaluated on the basis of matrix-matched calibration curves analyzed in triplicates, using the $LOD = (3.3S_b)/a$ equation, where: S_b – standard deviation of intercept of the calibration curve, a – slope of the calibration curve (ICH Harmonized Guidelines, 2005).

In further calculations of LOQ values it was assumed that $LOQ = 3 \times LOD$. LOD values were in the range from 0.0079 ng/mL for BPG to 0.039 ng/mL for BPBP, which indicate that proposed analytical method is highly sensitive towards BPA analogues. More detailed information on validation parameters are given in Suppl. Table 2 of Supplementary material.

To evaluate the recoveries, spiked samples were prepared according to the described procedure using normal human blood serum. Six independent chromatographic runs were carried out for each of three concentration levels. Obtained recoveries varied from 88% for BPC up to 138% for BPZ. All relative standard deviations were below 10% and are within the range of 1.2% to 7.8%. Recovery discrepancies observed for some bisphenol analogues confirm, that preparing matrix-matched calibration curves during analysis of real samples is highly justified in order to minimize its impact on the end results.

Total matrix effects (i.e. comprising of recovery and ion suppression/enhancement) for each analyte were also evaluated and are given in Suppl. Table 2 (Supplementary material). In case of some compounds noticeable enhancement of the signal was observed (27% for BPC and 29% for BPP). The reason for this effect could be a coelution with some unknown compound enhancing ionisation efficiency for these analytes.

Despite the fact that recoveries and magnitude of matrix effects differ among analytes the use of matrix-matched calibration curves allows us to compensate for these phenomena and obtain reliable results.

Table 2
Recovery values obtained for three independent concentrations of spiked quality control (QC) samples.

Analyte	Recovery (RSD) [%] of analyte (n = 6)			Detection and quantitation limits (n = 3)	
	0.05 ng/mL	0.5 ng/mL	1 ng/mL	LOD [ng/mL]	LOQ [ng/mL]
BPC	89.8 (3.4)	87.6 (1.9)	88.7 (2.3)	0.021	0.061
BPE	96.0 (3.7)	95.5 (3.0)	106.1 (2.9)	0.011	0.032
BPF	123.4 (4.2)	120.7 (3.6)	118.7 (2.8)	0.012	0.037
BPG	103.7 (1.4)	104.4 (1.7)	103.9 (1.2)	0.0080	0.024
BPM	90.3 (2.3)	90.7 (3.2)	93.4 (4.8)	0.018	0.054
BPP	105.1 (6.5)	103.5 (7.8)	105.8 (2.6)	0.019	0.056
BPZ	132.4 (1.5)	138.2 (1.9)	134.6 (1.9)	0.017	0.051
BPFL	98.3 (3.3)	99.1 (2.7)	99.6 (2.8)	0.014	0.041
BPBP	99.2 (2.7)	98.9 (1.9)	99.0 (1.7)	0.039	0.12
BPA	103 (12)	106.0 (2.9)	101.0 (2.6)	0.0090	0.028
BPS	96 (15)	96.5 (3.7)	101.6 (3.0)	0.022	0.067

4. Analysis of real world samples

Proposed methods were successfully used to analyse 245 real human blood serum samples in order to determine the analytes' content and to assess the human exposure to 11 BPA analogues for the first time. The results are summarized in Table 3 and examples of real world sample chromatograms are given in the Fig. 2. Analytes were found in over 50% of serum samples except for BPC, BPZ, BPFL and BPBP. BPA, BPG and BPS were the most often occurring analogues. Beyond the problem of constant human exposure, the presence of bisphenols in blood is important in the terms of possible adverse health issues (Konieczna et al., 2015; Rachoń, 2015). There are scientific proofs that these compounds have the ability to induce eryptosis (suicidal death of erythrocytes) (Maćczak et al., 2016) or biochemical and morphological alternations in mononuclear cells of peripheral blood (Michałowicz et al., 2015).

5. Conclusions

Presence of BPA and its analogues in different environmental matrices has been well studied by now, but there is still an insufficient scientific data on the occurrence of bisphenol analogues in the human and animal tissues/fluids. Bisphenols other than BPA doesn't seem to be its safer alternatives, therefore the interest in this research area has increased in the last decade. In the field of biomonitoring of BPs in human fluids and tissues the scientific data is still scarce although already noticeable. In 2016 Asimakopoulos et al. determined 8 bisphenols in human urine samples along with 49 other xenobiotics. Mean analytes concentrations varied from 0.05 ng/mL (for BPB and BPAF) up to 13.3 ng/mL (for BPS) (Asimakopoulos et al., 2016). Also in 2016 BPA and six other analogues (BPF, BPS, BPAP, BPAF, BPP, BPZ) were determined in human urine by applying novel DLLME technique coupled to LC-MS/MS. Mean BPA concentration was 2.8 ng/mL, while other analogues were found at much lower rates (2–10% of samples) (Rocha et al., 2016). In case of serum, plasma and blood, scientific data is even more limited. In 2009 BPA and BPB were determined in human serum at 0.79–7.12 ng/mL (BPA) and 0.88–11.94 ng/mL (BPB) concentration ranges (Cobellis et al., 2009). A broader range of analogues (BPA, BPB, BPC, BPE, BPF, BPPH, BPS, BPFL, BPP, BPM and BPZ) was determined in human breast milk in 2015. However, only BPA and BPS were found in concentrations 0.002–1.16 ng/g and 0.23 ng/g respectively (Deceuninck et al., 2015). More information on the comparison of the results obtained in this study to other studies are given in Supplementary Table 3. Because of very limited data on analogues selected for this research, this comparison has been extended to bisphenol derivatives and other biological matrices. Most of the analytes have been found in concentrations similar to the results presented.

In this paper the development of a novel analytical LC-MS/MS method for determination of a broad spectrum of BPA analogues was

Table 3
Information on the results obtained for the real world samples analysis.

Analyte	Quantification rate [% of all samples]	Detection rate > LOD [% of all samples]	Median [ng/mL]	Concentration range [ng/mL]
BPC	27.2	40.7	0.177	0.071 ÷ 3.800
BPE	55.1	59.7	0.154	0.053 ÷ 0.828
BPF	49.8	65.0	0.115	0.052 ÷ 0.845
BPG	60.5	70.4	0.188	0.050 ÷ 1.190
BPM	58.8	65.8	0.212	0.057 ÷ 1.104
BPP	52.7	66.3	0.142	0.057 ÷ 0.917
BPZ	37.5	45.3	0.235	0.053 ÷ 1.415
BPFL	7.8	23.5	0.070	0.050 ÷ 1.597
BPBP	47.8	53.9	0.37	0.12 ÷ 2.98
BPA	86.4	91.4	0.124	0.050 ÷ 4.017
BPS	68.7	72.0	1.135	0.073 ÷ 4.844

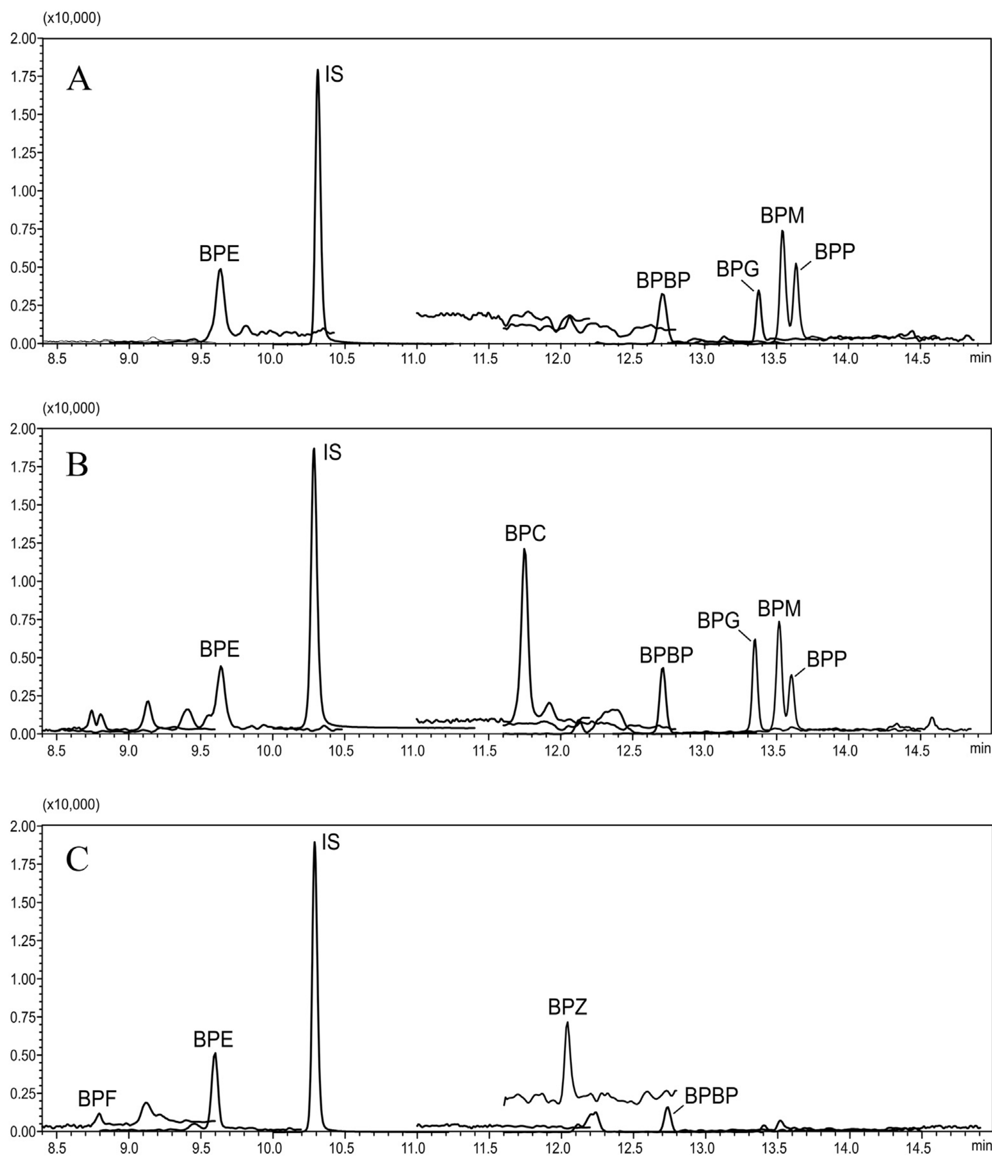


Fig. 2. Examples of chromatograms obtained after the analysis of a real world blood serum samples. For clarity IS trace has been scaled down by the factor of 0.05.

described. Sample preparation procedure consisting of liquid-liquid extraction is a routine and inexpensive approach that consumes low volumes of the sample (500 μL) and relatively low volumes of organic solvent. An addition of anhydrous $MgSO_4$ provided better peak shapes and response, due to removing water soluble interfering matrix compounds. The method is suitable to identify and effectively separate compounds of interest, even constitutional isomers (BPM and BPP), as well as to obtain very low detection and quantification limits. The developed method was successfully applied for the analysis of real human blood serum samples. To the best of authors' knowledge, this study is the first attempt to determine selected 11 bisphenol A analogues in human derived serum samples. Results indicate that the problem of the bisphenols occurrence in body fluids is still underestimated, and may lead to some adverse health issues. For this reason, the development and application of novel analytical procedures focused on bisphenols' human biomonitoring are of high scientific importance.

Acknowledgements

This study was financed from the funds of the Polish National Centre for Science (grant number 2014/15/B/NZ7/00999).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.02.148.

References

Ahmed, R., 2016. Maternal bisphenol A alters fetal endocrine system: thyroid adipokine dysfunction. Food Chem. Toxicol. 95, 168–174.

Arbuckle, T.E., Davis, K., Boylan, K., Fisher, M., Fu, J., 2016. Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6–11 years of age: CHMS 2007–2009. Neurotoxicology 54, 89–98.

Asimakopoulos, A.G., Xue, J., De Carvalho, B.P., Iyer, A., Abualnaja, K.O., Yaghmoor, S.S., Kumosani, T.A., Kannan, K., 2016. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. Environ. Res. 150, 573–581.

Bhandari, R.K., Deem, S.L., Holliday, D.K., Jandegian, C.M., Kassotis, C.D., Nagel, S.C., Tillitt, D.E., vom Saal, F.S., Rosenfeld, C.S., 2015. Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. Gen. Comp. Endocrinol. 214, 195–219.

Chen, M.-Y., Ike, M., Fujita, M., 2002. Acute toxicity, mutagenicity, and estrogenicity of bisphenol-a and other bisphenols. Environ. Toxicol. 17, 80–86.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity: a review. Environ. Sci. Technol. 50, 5438–5453.

Cobellis, L., Colacurci, N., Trabucco, E., Carpentiero, C., Grumetto, L., 2009. Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women. Biomed. Chromatogr. 23, 1186–1190.
Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C.-Y., Legrand, A., Boscher, C., Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 2485–2497.
Gioumouxouzis, C.I., Kouskoura, M.G., Markopoulou, C.K., 2015. Negative electrospray ionization mode in mass spectrometry: a new perspective via modeling. J. Chromatogr. B 998, 97–105.
Grob, K., Gürtler, R., Husøy, T., Mennes, W., Milana, M.R., Penninks, A., Roland, F., Silano, V., Smith, A., Poças, M. de F.T., et al., 2015. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: executive summary. EFSA J. 13, 3978–4599.
Hada, T., Shimizu, M., Aoki, Y., Endo, N., Ohhashi, H., Ishiguro, T., 2013. Heat-sensitive Recording Material and Method for Producing Same (Google Patents).
Hsieh, B.-Y., Hsu, J.-P., 2015. Photosensitive Resin Composition, Color Filter and Method for Manufacturing the Same, and Liquid Crystal Display Apparatus (Google Patents).
Huang, B., Jiang, C., Luo, J., Cui, Y., Qin, L., Liu, J., 2014. Maternal exposure to bisphenol A may increase the risks of Parkinson's disease through down-regulation of fetal IGF-1 expression. Med. Hypotheses 82, 245–249.
Hunt, P.A., Sathyanarayana, S., Fowler, P.A., Trasande, L., 2016. Female reproductive disorders, diseases, and costs of exposure to endocrine disrupting chemicals in the European Union. J. Clin. Endocrinol. Metab. 101, 1562–1570.
ICH Harmonized Guidelines, 2005. Validation of Analytical Procedures: Text and Methodology. Q2 R1 p. 1.
Industry Experts, 2016. Bisphenol-A — A Global Market Overview.
Konieczna, A., Rutkowska, A., Rachon, D., 2015. Health risk of exposure to bisphenol A (BPA). Rocz. Państw. Zakl. Hig. 66.
Kundakovic, M., Gudsnuk, K., Franks, B., Madrid, J., Miller, R.L., Perera, F.P., Champagne, F.A., 2013. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc. Natl. Acad. Sci. 110, 9956–9961.
Lee, S., Liao, C., Song, G.-J., Ra, K., Kannan, K., Moon, H.-B., 2015. Emission of bisphenol analogues including bisphenol A and bisphenol F from wastewater treatment plants in Korea. Chemosphere 119, 1000–1006.
Liao, C., Kannan, K., 2012. Determination of free and conjugated forms of bisphenol A in human urine and serum by liquid chromatography–tandem mass spectrometry. Environ. Sci. Technol. 46, 5003–5009.
Maćczak, A., Cyrkler, M., Bukowska, B., Michalowicz, J., 2016. Eryptosis-inducing activity of bisphenol A and its analogs in human red blood cells (in vitro study). J. Hazard. Mater. 307, 328–335.
Michałowicz, J., Mokra, K., Bąk, A., 2015. Bisphenol A and its analogs induce morphological and biochemical alterations in human peripheral blood mononuclear cells (in vitro study). Toxicol. in Vitro 29, 1464–1472.
Ochiai, T., Masuda, T., 2013. Thermal Recording Material and Method for Producing the Same (Google Patents).
Rachoń, D., 2015. Endocrine disrupting chemicals (EDCs) and female cancer: informing the patients. Rev. Endocr. Metab. Disord. 16, 359–364.
Rissman, E.F., Adli, M., 2014. Minireview: transgenerational epigenetic inheritance: focus on endocrine disrupting compounds. Endocrinology 155, 2770–2780.
Rivas, A., Lacroix, M., Olea-Serrano, F., Laïos, I., Leclercq, G., Olea, N., 2002. Estrogenic effect of a series of bisphenol analogues on gene and protein expression in MCF-7 breast cancer cells. J. Steroid Biochem. Mol. Biol. 82, 45–53.
Rocha, B.A., da Costa, B.R.B., de Albuquerque, N.C.P., de Oliveira, A.R.M., Souza, J.M.O., Al-Tameemi, M., Campiglia, A.D., Barbosa Jr, F., 2016. A fast method for bisphenol A and six analogues (S, F, Z, P, AF, AP) determination in urine samples based on dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. Talanta 154, 511–519.
Rosenmai, A.K., Dybdahl, M., Pedersen, M., Alice van Vugt-Lussenburg, B.M., Wedebye, E.B., Taxvig, C., Vinggaard, A.M., 2014. Are structural analogues to bisphenol A safe alternatives? Toxicol. Sci. 139, 35–47.
Rutkowska, A., Rachoń, D., 2014. Bisphenol A (BPA) and its potential role in the pathogenesis of the polycystic ovary syndrome (PCOS). Gynecol. Endocrinol. 30, 260–265.
Rutkowska, A., Rachoń, D., Milewicz, A., Ruchała, M., Bolanowski, M., Jędrzejuk, D., Bednarczuk, T., Górska, M., Hubalewska-Dydejczyk, A., Kos-Kudła, B., et al., 2015. Polish Society of Endocrinology Position statement on endocrine disrupting chemicals (EDCs). Endokrynol. Pol. 66, 276–285.
Song, S., Song, M., Zeng, L., Wang, T., Liu, R., Ruan, T., Jiang, G., 2014. Occurrence and profiles of bisphenol analogues in municipal sewage sludge in China. Environ. Pollut. 186, 14–19.
Stojanoska, M.M., Milosevic, N., Milic, N., Abenavoli, L., 2017. The influence of phthalates and bisphenol A on the obesity development and glucose metabolism disorders. Endocrine 1–16.
Sui, Y., Ai, N., Park, S.-H., Rios-Pilier, J., Perkins, J.T., Welsh, W.J., Zhou, C., 2012. Bisphenol A and its analogues activate human pregnane X receptor. Environ. Health Perspect. 120, 399.
Teichert, D., Conrad, L., Grötzinger, J., 2014. Epoxy Resin-Upgraded Cement-Bound Composition as Coating or Seal (Google Patents).
Vogel, S.A., 2009. The politics of plastics: the making and unmaking of bisphenol A "safety". Am. J. Public Health 99, S559–S566.
Wagner, S., Kramer, R.H., Konig, A., Roth, M., 2015. Flame-retardant Polyesters (Google Patents).
Wilczewska, K., Namieśnik, J., Wasik, A., 2016. Troubleshooting of the determination of bisphenol A at ultra-trace levels by liquid chromatography and tandem mass spectrometry. Anal. Bioanal. Chem. 408, 1009–1013.
Yamazaki, E., Yamashita, N., Taniyasu, S., Lam, J., Lam, P.K., Moon, H.-B., Jeong, Y., Kannan, P., Achyuthan, H., Munuswamy, N., et al., 2015. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol. Environ. Saf. 122, 565–572.
Yang, Y., Guan, J., Yin, J., Shao, B., Li, H., 2014. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 112, 481–486.
Zouta, K., Nakao, K., Takagi, Y., Ishii, H., 2014. Flame-Retardant Sheet or Film, Products Comprising the Same and Process for Production Thereof (Google Patents).