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

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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 substitutes for BPA. In 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 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, consuming small volumes of the sample and organic solvents.

Method of chromatographic separation was optimized by performing planned series of trial and errors tests (including e.g. gradient, chromatographic column selection, software optimization of ESI and MS/MS working parameters). 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 87,6-138,2%. Matrix effects were mitigated with the help of matrix-matched calibration curves prepared for every batch of samples. Results obtained after analysis of 245 real serum samples indicates that human beings are exposed to different BPA analogues, that are present in the environment and in common, daily use products.

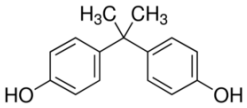
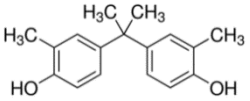
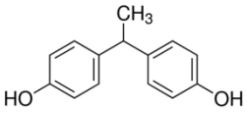
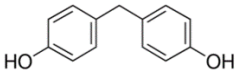
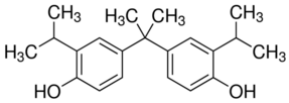
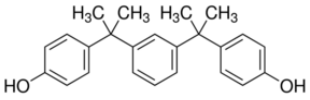
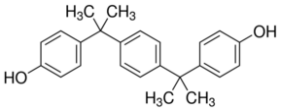
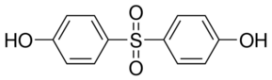
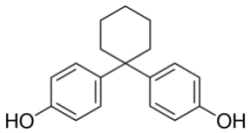
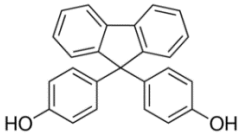
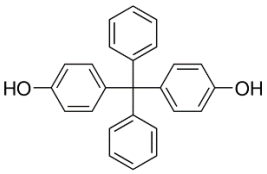
keywords: bisphenol A, BPA analogues, blood serum analysis, liquid chromatography, tandem mass spectrometry, endocrine disrupters

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 (Rissma and Adli, 2014). Bisphenols (BPs) are the chemicals that have recently been the subject of growing interest due to their endocrine disrupting properties. 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 (tin linings and other food contact materials, water pipes, powder paints, toys etc.). Nowadays, BPA annual production reaches over 6 million tons and the demand for this compound is predicted to increase over next years (Kadasala et al., 2016).

The effects exerted by BPA on human health have been extensively studied and its estrogenic activity is one of the best known upshots. 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 (Huanga 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). Detailed information including chemical structure and IUPAC names of bisphenol A analogues that are subjected to present study are given in Table 1.

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	13595-25-0		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

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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 have been documented to be commercially applied (Chen et al., 2016). Bisphenol S and bisphenol F 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., 2010, Hsieh and Hsu, 2015, Ochiai and Masuda, 2009, Teichert et al., 2014, Wagner et al., 2015, Zouta et al., 2014). Currently, only the bisphenol A applications are regulated by legislative standards in European Union, United States and Canada (Yang et al., 2014a). 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, 2013, Yang et al., 2014). Unfortunately, the understanding of the environmental, biological and health impact of BPA analogues is still very scarce. The environmental abundance of BPs undoubtedly indicates that humans are 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. Because of lack of scientific data concerning analytical methods for determination of wide spectrum of BPA analogues, the aim of this research was to develop easy, fast, highly sensitive and robust method for human biomonitoring of these chemicals. To encompass the range of bisphenols that are commonly present in environment constituents and may pose health risk, the total number of 11 bisphenols were determined in human serum by 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 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 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 (Bhandari et al. 2015, Lee et al., 2015, Song et al., 2014, Yamazaki et al., 2015), introducing new methods for BPs biomonitoring seems to be justified due to constant human exposure to them. In addition, the data concerning 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 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). Blank bisphenol-free normal human serum reference material was 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

A total of 245 serum samples were collected by personnel of Medical University of Gdansk from adult female patients suffering from various disorders of endocrine nature. Informed consent has been obtained from all patients who participated in clinical investigations. Samples were collected in glass vials and stored in -80ºC.

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 (-20ºC). All glassware was pre-washed with methanol. Seven-point (0,05, 0,1, 0,25, 0,5, 1, 2,5 and 5 ng/mL) matrix-matched calibration curves were prepared using bisphenol-free blood serum. Internal standard (IS) concentration was kept at 25 ng/mL in all calibration samples. Fresh calibration solutions were prepared for every batch of samples. Purchased blank

normal human serum, used for preparation of the calibration curves, was analyzed and confirmed to be free of bisphenols.

2.4 Sample preparation

Sample preparation step was conducted with precautions intended to minimize sample contamination. All used glassware was previously flushed with MeOH and all plastics were made of high quality polypropylene to avoid bisphenols passing into the samples. In order to extract the analytes from serum samples, the modified liquid-liquid extraction method described was used. The 500 µL of serum was mixed with 1,5 mL of ACN and 10 µL of IS solution (2,5 µg/mL) was added. Samples were shaken for 30 seconds and left for 10 minutes in room temperature for complete protein precipitation. After that, 250 mg of anhydrous $MgSO_4$ was added to each sample that were then vortexed to remove water, followed by centrifugation (6000 rpm, 2 minutes). Supernatants were transferred to clean glass tubes and evaporated under the gentle stream of nitrogen in water bath (42^{o}C), to the final volume of about 150 µL. The residue was diluted 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 were prepared in the same way as other samples for every batch in triplicate along with system blanks. Chromatograms of procedural blanks are given in Supplementary Figure 1. in Supplementary Materials.

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). Conditions of ion fragmentation were optimized for all analytes with the help of LabSolutions v.5.85 Software. Detailed information on ion transitions, MS/MS operational parameters and ion source parameters are given in Supplementary Material (Supp. Tab.1). **Analytes standard solutions including IS were first separately injected directly into MS/MS instrument to determine pseudomolecular ions. Then product ion scan was performed to find and identify specific molecular fragments. Finally, software automatic optimization of voltages for specific MRM transition was performed. Ion source parameters was adjusted manually to obtain the best signal intensity for all analytes.**

2.6 Separation conditions

Chromatographic separation was carried out using UPLC Nexera X2 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 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 minutes, before increasing to 75% over 10,5 minutes and further **gradient** increased to 100% over 4 minutes. Following this, mobile phase composition was set-back to starting conditions and maintained for 5 minutes for column re-equilibration.

For the determination of BPA and BPS the mobile phase consisted of H_2O (component A) and MeOH (component B) without additives. Isocratic elution of 50% B was used.

Ascentis® Express (C18 15cm x 2.1mm, 2.7µm) with guard column (0,5cm x 2,1mm, 2,7µm), mobile phase flow of 0,55 mL/min, 50ºC of thermostated column compartment and injection volume of 5µL 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 Figure 1. More detailed information on separation conditions will be discussed in the next section.

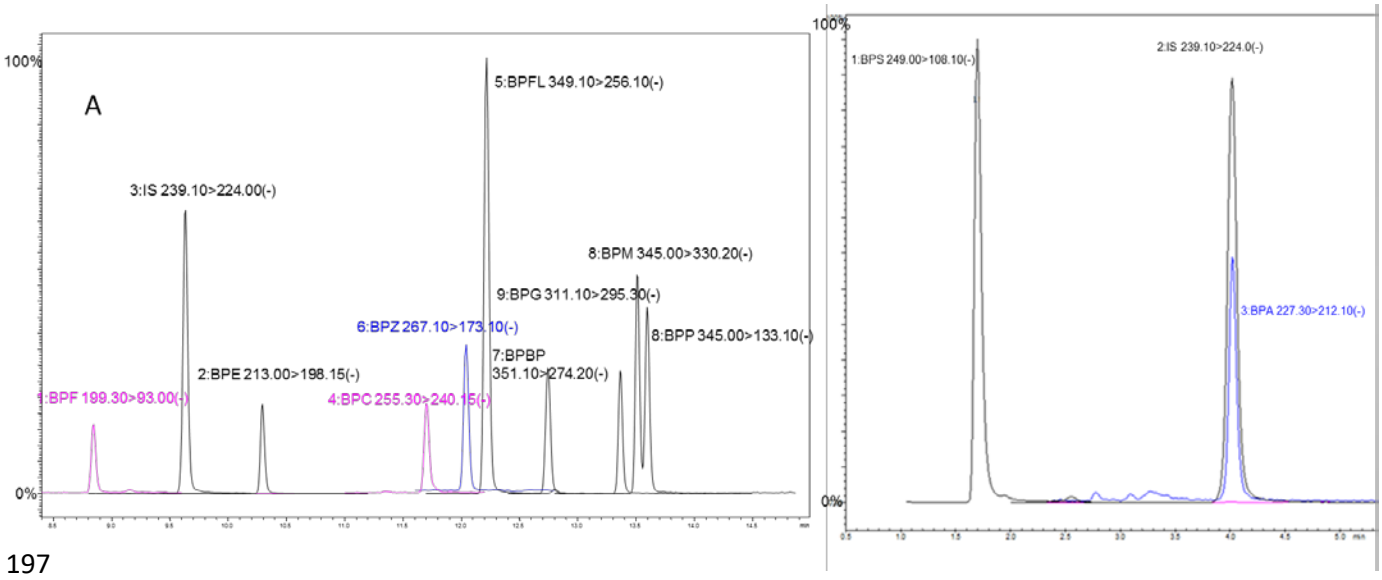

Fig. 1 Chromatograms obtained after analysis of calibration solutions; (A) mixture of 9 bisphenols, (B) mixture of BPA and BPS.

3. Results and discussion

3.1 Extraction conditions

General problem in bisphenols analysis is ion suppression, 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. Modification of liquid-liquid extraction with anhydrous $MgSO_4$ enhanced peaks intensity, due to precipitation of water soluble interferences, which caused signal suppression. Smaller amount of water in the liquid phase could also improve transport of analytes into the extractant.

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 (15cm x 2,1mm, 2,7µm) column with guard column (0,5cm x 2,1mm, 2,7µm), 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 main organic components of the mobile phase, however, in the case of ACN, peak broadening, peak shape deterioration along with the strong signal suppression were noted. The probable cause of response decrease in the presence of ACN is lower surface tension of the MeOH, that is conductive to desolvation of electrospray droplets (Caballero-Casero et al., 2002, Regueiro et al., 2015), hence methanol was selected as the mobile phase component. 5, 10, 20 and 25 mM amonium acetate, 0,01, 0,05 and 0.1% v/v

formic acid, acetic acid and ammonia were tested separately as the mobile phase additives to A and B components. The most promising results (in terms of response and peak shapes) were obtained when applying the latter. Buffer and acid solutions caused signal suppression.

All analytes are similar in molecular structure and two of them – namely BPM and BPP, are configurational isomers (Tab.1). In order to gain enhanced chromatographic separation of mentioned analytes, the initial content of the organic mobile phase component was kept at 5% for 1,5 minutes and was slowly increased up to 75%. Optimum column oven temperature was set to 50ºC (range from 30 – 50ºC), providing best peak shapes and separation.

Addition of ammonia to the mobile phase resulted in sensitivity decrease and shifting the bisphenol S signal towards system void time. Moreover, low initial content of methanol and long analysis and conditioning time caused the enrichment of bisphenol A, derived from system elements, on the front of separation column (Wilczewska et al., 2016). 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 calibration curves.

3.3 Method validation

The performance of both analytical methods was evaluated in terms of linearity, limits of detection (LODs) and quantification (LOQs) and recoveries. 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 accuracy of lowest concentration points, 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 equation $LOD=(3,3S_b)/a$, where: S_b – standard deviation of intercept of the calibration curve, a – slope of the calibration curve.

In further calculations of LOQ values it was assumed that $LOQ = 3xLOD$. 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 serum free from bisphenols. Six independent chromatographic runs were carried out for each of three concentration levels. Obtained recoveries vary from 87,6% for BPC up to 138,2% for BPZ. All relative standard deviations are 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 matrix effects.

Matrix effects for each analyte were also evaluated and are given in Suppl. Table 2 (Supplementary Material). The enhancement of signal in the range of 5% for BPA and BPZ up to 29% for BPP were observed, which is another significant premise for use of matrix-matched calibration curves.

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

Analyte	**Recovery (RSD) [%] of analyte (n=6)**			**Detection and quantification 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)	0,008	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	103,2 (12)	106,0 (2,9)	101,0 (2,6)	0,009	0,028
BPS	96,0 (15)	96,5 (3,7)	101,6 (3,0)	0,022	0,067

4. Analysis of real samples

Proposed methods were successfully applied 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 sample chromatograms are given in the Figure 2. Analytes were found in over 50% of serum samples except for BPC, BPZ, BPFL and BPBP. Bisphenol A, bisphenol G and bisphenol S 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 health issues. 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).

Tab. 3 Information on the results obtained for the real 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	0,18	0,071-3,8
BPE	55,1	59,7	0,15	0,053-0,828
BPF	49,8	65,0	0,12	0,052-0,845
BPG	60,5	70,4	0,19	0,050-1,190
BPM	58,8	65,8	0,21	0,057-1,104
BPP	52,7	66,3	0,14	0,057-0,917
BPZ	37,5	45,3	0,24	0,053-1,415
BPFL	7,8	23,5	0,070	0,050-1,597
BPBP	32,9	39,5	0,40	0,13-2,846
BPA	86,4	91,4	0,12	0,050-4,1
BPS	68,7	72,0	1,1	0,073-4,8

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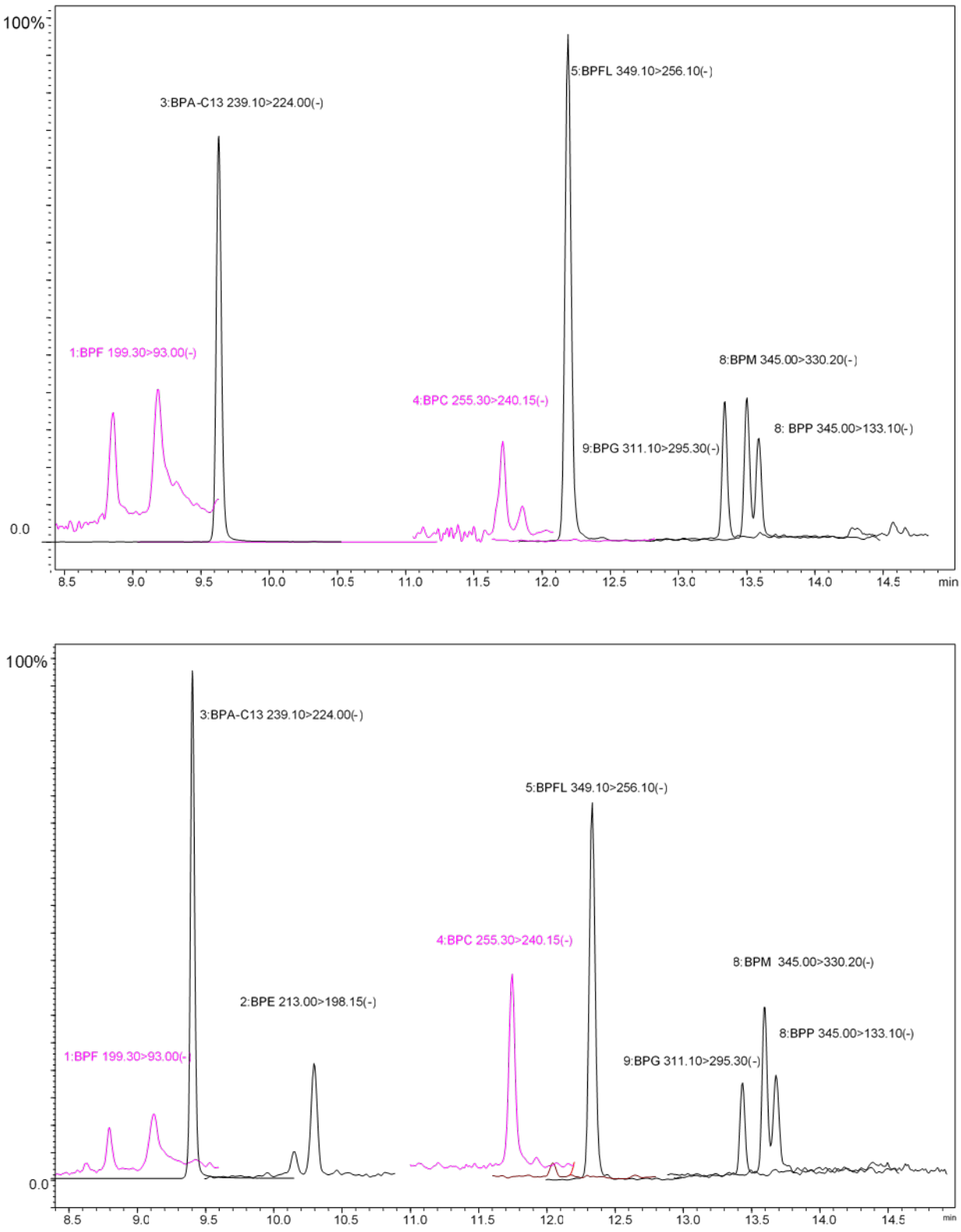

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287 Fig.2 Examples of chromatograms obtained after the analysis of a real blood serum samples

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. Although bisphenols other than BPA do not seem to be safer alternative, 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 2015 Asimakopoulos et al. determineded 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., 2015). 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 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 Deceuninck et al. Only BPA and BPS were found in concentrations 0,002-1,16 ng/g and 0,23 ng/g respectively. 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 analytes have been found in concentrations corresponding to the results presented.

In this paper the development of a novel analytical LC-MS/MS method for determination of a broad spectrum of bisphenol A analogues was described. Sample preparation procedure consisting of liquid-liquid extraction is a routine and inexpensive approach that consumes low volumes of the sample (500µL) 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.

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ELECTRONIC SUPPLEMENTARY MATERIALS

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

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Suppl. Tab. 1 Optimized MS/MS conditions for negative mode MRM analysis for target analytes

Compound	Precursor ion M-H [m/z]	Quantitation ion [m/z]	Confirmation [m/z]	Q1 PreRod bias* [V]	Collision energy [V]*	Q3 PreRod bias* [V]
BPA	227.3	212.1	133.1	11	20	13
BPS	249.0	108.1	92.0	12	27	10
BPC	255.3	240.15	147.1	13	20	10
BPE	213.0	198.15	119.1	10	18	18
BPF	199.3	93.0	105.1	13	23	13
BPG	311.1	295.3	175.2	22	35	29
BPM	345.0	330.2	133.1	17	29	14
BPP	345.0	330.2	133.1	17	29	14
BPZ	267.1	173.1	145.1	12	27	10
BPFL	349.1	256.1	215.1	12	27	11
BPBP	351.1	274.2	258.2	12	25	25
13C-BPA	239.1	224.0	138.0	11	20	13

Nebulizing gas flow [L/min]	**Heating gas flow [L/min]**	**Interface temperature [ºC]**	**DL temperature [ºC]**	**Heat block temperature [ºC]**	**Drying gas flow [L/min]**
3	10	300	250	400	10

*** presented values refer to quantitation ion only**

Suppl. Tab. 2 Regression equations, LOD and LOQ for each analyte

Analyte	Calibration curve equation (7 points, n=3)	R^2	LOD [ng/mL]	LOQ [ng/mL]	Matrix effect[%]
BPC	y=0.0052x+0.00035	0.9975	0.021	0.061	27
BPE	y= 0.015x+0.00077	0.9992	0.011	0.032	7
BPF	y=0.0035x+0.00051	0.9996	0.012	0.037	17
BPG	y= 0.0046x+0.00034	0.9991	0.008	0.024	15
BPM	y=0.010x+0.00023	0.9986	0.018	0.054	15
BPP	y=0.0088x+0.000070	0.9997	0.019	0.056	29
BPZ	y=0.0082x+0.00097	0.9993	0.017	0.051	6
BPFL	y=0.024x+0.00079	0.9988	0.014	0.041	13
BPBP	y=0.0068x+0.0035	0.9968	0.039	0.120	19
BPA	y=0.092x+0.0015	0.9997	0.0093	0.028	6
BPS	y=0.065x+0.017	0.9999	0.022	0.067	12

$$ME = \left(\frac{a_m}{a_s} - 1\right) \times 100\%$$

where:

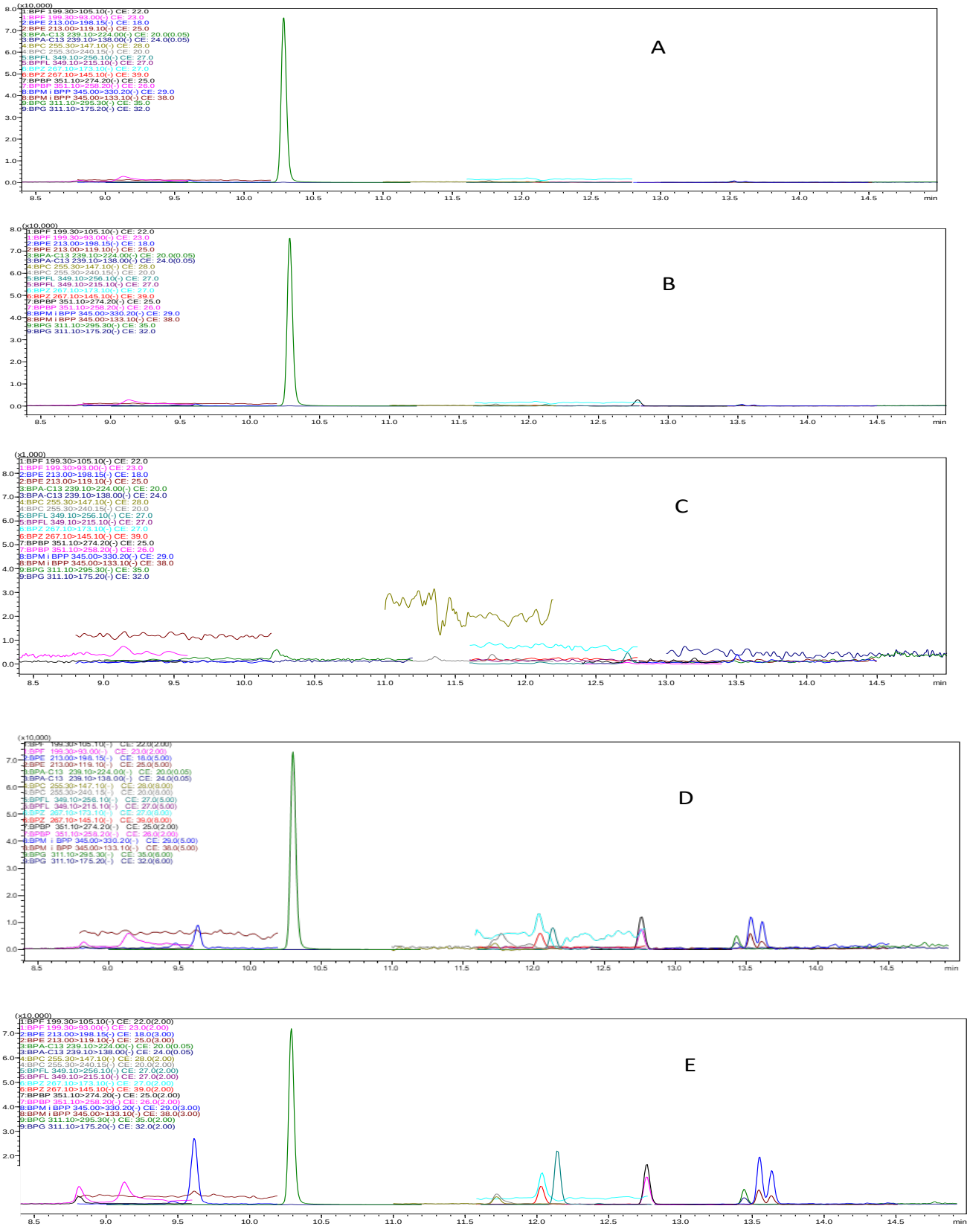
a_m is the slope of the extracts (matrix) spiked with analytes,

a_s is the slope of the solvent.

Suppl. Tab. 3 Comparison of LOD and LOQ values for different studies concerning determination of bisphenol analogues and derivatives in biological samples of human origin.

Analytes	Matrix	Sample volume [µL]	Determination method	LOD and LOQ [ng/mL]	Measured concentrations [ng/mL]	Reference
BPA, BPC, BPE, BPF, BPG, BPM, BPP, BPS, BPZ, BPFL, BPBP	Serum	500	HPLC-ESI-MS/MS	LOD = 0,0079 - 0,039 LOQ = 0,024 - 0,12	0,05- 4,8*	This study
BPA, BPADS, BPAG, BPAMC, DCBPA, TCBPA,	Serum	500	LC-ESI-MS/MS	LOD = 0,003 – 0,02 LOQ = 0,01 – 0,05	BPA <LOQ- 0,588 BPAG<LOQ – 11,9 BPADS <LOQ- 1,77	Liao et al., 2012
BPA, BPB	Serum	300	LC-FD	LOD = 0,15 – 0,18 LOQ = 0,5 – 0,6	BPA 0,79-7,12 BPB 0,88-11,94	Cobellis et al., 2009
BPA, BPB, BPAP, BPAF, BPBP, BPC, BPE, BPPH, BPS, BPF, BPFL, BPZ, BPM, BPP	Breast milk	3 [g]	GC-ESI-MS	MDL = 0,001 – 0,03 MQL = 0,003 – 0,1	BPA 0,02-1,16 BPS 0,23	Deceuninck et al., 2015
BPA	Breast milk	1000	LC-MS/MS	LOD = 0,22	0,22-10,8	Zimmers et al., 2014
BPA, BPB, BPF, BPS, BPAF,TCBPA	Urine	-	LC-MS/MS	LOQ = 0,024-0,310	BPA <LOQ – 8,07 BPAF<LOQ – 0,217	Yang et al., 2014b
BPA, BPF, BPP, BPS, PZ, BPAF, BPAP	Urine	5000	LC-MS/MS	LOD = 0,005 – 0,2 LOQ = 0,02 – 0,5	BPA (mean) 2,8 Other analytes were found in very limited range of samples	Rocha et al., 2016

ore details on analytes concemtration ranges are given in Table 3.
MC – BPA monochloride, BPADC – BPA dichloride, BPATC – BPA trichloride, BPADG – BPA glucuronide, BPADS – BPA disulfate, PA – tetrachloro- BPA



pplementary Figure 1. Chromatogram examples for procedural blanks (A and B), system blank (C), samples of nk human serum spiked with low concentration of analytes 0,05 ng/mL (D) and 0,5 ng/mL (E)