DOI: 10.17707/AgricultForest.65.1.24

Biljana PETANOVSKA-ILIEVSKA, Mirjana S. JANKULOVSKA*, Lenche VELKOSKA-MARKOVSKA, Jasmina SPASOVSKA [1]

DETERMINATION OF BISPHENOL A IN BEVERAGES BY RP-HPLC

SUMMARY

Bisphenol A (BPA) is a monomer widely used in the production of polycarbonate, *epoxy resins, diacrylates* and phenolic resins. A small quantity of BPA can migrate into the food and thus it can be potential hazard for human health and environment. Therefore, quantitative determination of BPA is of a great importance. A fast, simple, precise and economic RP-HPLC method with UV-DAD detection for quantitative determination of BPA in beverages was developed. Three different analytical columns were tested: Hypersil ODS (250 mm x 4.6 mm; 5 μm), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm). Analyzed beverages were packed in plastic bottles and small glass bottles closed with a cops coated with epoxy resin on inside. For quantitative determination of BPA following experimental conditions were established: mobile phase consisted of acetonitrile/water 50/50 (*v*/*v*), flow rate of 1 mL/min, column temperature of 25 ^{o}C, injection volume of 5 μL and UV detection at 200 nm. The method was developed in an isocratic manner and with a reversed phase column. Prior the analyses the samples were filtrated through syringe filters Spartan – T with pore size 0.45 μm. The following parameters were determined: retention time, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, selectivity and sensitivity. The RP-HPLC method with UV-DAD detection can be successfully used for quantitative determination of BPA in non-alcoholic beverages without pre-treatment. The BPA was not detected in the analyzed beverages.

Keywords: Bisphenol A, RP-HPLC, UV-DAD detection, non-alcoholic beverages.

INTRODUCTION

Food packaging allows a lot of benefits mainly related to food quality and safety such as: extension of shelf life, protection of deterioration caused by microorganisms, light, oxygen, pests that can cause disease and so on. On the other hand, packaging can be source of chemical contaminants into food and beverages which have negative influence of food safety (Barens *et al*., 2007).

[1]Biljana Petanovska - Ilievska, Mirjana S. Jankulovska *(corresponding author: jankulovska_m@yahoo.com), Lenche Velkoska-Markovska, Jasmina Spasovska, Ss. Cyril and Methodius University, Faculty of Agricultural Sciences and Food, Skopje, NORTH MACEDONIA
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Chemical migration is the transition of certain chemical compounds from food packaging materials into food under certain conditions such as: the composition of the packaging material, the nature and degree of the contact, the food nature, the contact temperature, and duration of the contact. Among other plastic materials, polycarbonates (PC) are widely used in the manufacture of containers for packaging of milk, water, bottles closed with cops and so on, while epoxy resins are used as an interior protective layer for food and beverage cans. Bisphenol A (BPA) is a chemical monomer used as a main component in the production of polycarbonate and epoxy-phenolic resins and because of that it is a potential migrant from packaging into food (Almeida *et al*., 2018). The migration of BPA traces from both polycarbonate-based packaging and epoxy resins into food depends mainly on liquid composition and pH (Brede *et al*., 2010; Nerin *et al*., 2016). Hence, BPA is consumed each day with food *i.e.* humans are exposed to BPA. Literature data showed that BPA possess estrogenic activity *i.e*. disturbs the function of endocrine system which causes cancer, heart diseases, diabetes and hypomethylation of genes promoters (Almeida *et al*., 2018).

Migration of chemical compounds European Union is regulated with EU Directives. European Commission with Regulation (EU) 2018/213 strengthens the restriction of Bisphenol A (BPA) in certain food contact materials and articles (Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011). This regulation included a new restriction of BPA in food contact varnished and coated materials and articles. In addition, Annex I of Regulation (EU) No 10/2011 is amended by lowering migration limit of BPA in plastic food contact materials and articles. It also included a new prohibition of BPA in polycarbonate drinking cups or bottles intended for infant and young children. According to this annex specific migration limit for BPA in plastic food contact materials and articles is lowered from 0.6 mg/kg to 0.05 mg/kg (Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018).

In the literature there are known a lot of methods for determination of BPA in food, beverages and samples of biological materials (Ballesteros-Gómez *et al*., 2009). Due to the complexity of the matrices and the low concentrations of BPA there is constant need for development and improvement of analytical techniques for precise and accurate determination of BPA. One of the most used methods is chromatographic method, *i.e*. liquid and gas chromatography [Xu-Liang, 2012; Aristiawan *et al*., 2015). Using the chromatographic methods BPA can be determined in food for infants and different food samples such as fruits, vegetables, fishes, vines, beverages, as well as drinking water (Tanigawa *et al*., 2011; Yoshida *et al*., 2010; Cao *et al*., 2010; Taskeen and Naeem, 2010; Braunrath *et al*., 2005). Each method for determination of BPA in food and beverages includes sample clean-up by liquid-liquid extraction or solid-phase extraction followed by chromatographic determination with different detectors (Struckhofova and Marki, 2006; Sadeghi *et al*., 2016; Rykowska *et al*., 2004). To

assess potential human health risks caused by BPA exposure, it is therefore essential to start from accurate data on BPA levels in foodstuffs, at very low concentrations (Ballesteros *et al*., 2009).

Therefore, the aim of our investigation was developed and validation of reverse phase - high performance liquid chromatography (RP-HPLC) method for quantitative determination of BPA in beverages packed in plastic bottles and glass bottles closed with metallic caps coated from the inside with epoxy resin. The investigation was made in order to confirm if there is a migration from the packaging material into beverages, and if the migration quantities exceed the quantities prescribed by the EU Directive (Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011; Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018).

MATERIAL AND METHODS

Reagents and instrumentation

The analytical standard of bisphenol A (BPA) with purity 99 % was produced by Sigma Aldrich (Germany). The acetonitrile for mobile phase was produced by Sigma Aldrich (Germany) was with HPLC grade. The ultra-pure water purified by Water purification System TKA Smart 2 Pure 12 UV/UF was used. Liquid chromatography analysis were performed on an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), auto sampler (G1329B), a thermostatted column compartment (G1316A), UV/Vis diode array detector (G1316B) and ChemStation software. Following analytical columns were tested: Hypersil ODS (250 mm x 4.6 mm; 5 μm) – Sigma Aldrich(Germany), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm), Merck (Germany) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm), Merck (Germany). The mass was measured using analytical balance with 0.1 mg accuracy by Mettler (Zürich, Switzerland). Ultrasonic bath "Elma" (D-7700 SINGEN/Htw., Germany) was applied for a better dissolution of a stock solution.

Standard and working solutions of BPA

A mass of 0.037 g of the analytical standard of BPA was measured and transferred into a volumetric flask of 10 mL which was filled up to the mark with acetonitrile. The solution was degassed by ultrasonic bath for 15 minutes and then stored at 4 ^{o}C. In order to prepare working solution 1 mL of a standard solution was transferred into volumetric flask of 10 mL and filled up with acetonitrile/water 50/50 (*v*/*v*). Linearity of the method was tested with five working solutions prepared in beverage (clear apple juice) with addition of known amount of BPA as follows: 300 ng/mL (solution 1), 420 ng/mL, (solution 2), 600 ng/mL (solution 3), 720 ng/mL (solution 4), and 900 ng/mL (solution 5). Each of these solutions was injected into HPLC system in triplicate with volume of 5 μL. The method of standard addition was used in order to evaluate the

accuracy of the method for quantitative determination of BPA. For that purpose, a known amount of BPA: 600, 720 and 900 ng/mL was added in clear apple juice. An apple juice sample in which BPA was not added was used as a blank. A 5 μL of all samples were injected into the HPLC system in triplicate.

Sample preparation

Six clear apple juices produced by different manufactures анд frequently consumed in Macedonia were analyzed. Three of them mainly consumed by infants were packed in glass bottles closed with metallic caps coated from the inside with epoxy resin. The apple juice beverages consumed by adults were packed in glass bottle (one sample beverage) and in plastic polycarbonate packaging material (two sample beverages). The samples for analyses were marked as A, B, C, D, E and F. Prior to HPLC analyses all samples were filtrated through syringe filters Spartan – T with pore size 0.45 μm. A volume of 5 μL of each sample was injected into the HPLC system in triplicate.

RESULTS AND DISCUSSION

UV spectrum of BPA

Bisphenol A (BPA) is frequently used name for chemical compound with IUPAC name 4,4'-(propane-2,2-diyl)diphenol. It is an organic compound which belongs to the phenolic group of compounds. At aqueous solution at pH below 7, BPA exist in undissociated form. A molecule of BPA is stable in solid phase, but in the air, water and soil BPA undergoes aerobic degradation. UV spectrum of BPA recorded in mobile phase of acetonitrile/water 50/50 (*v*/*v*) is presented in the Fig. 1.

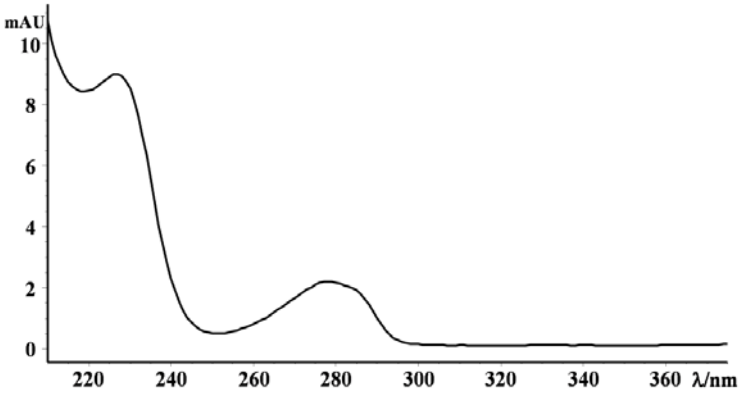

Figure 1: UV spectrum of BPA, acetonitrile/water 50/50 (*v*/*v*)

In the obtained UV spectrum of BPA were noticed two absorption bands with different intensity. First one is more intensive and it is placed at 227 nm, while the absorption maximum of the second less intensive band is located at 275 nm. However, at a wavelength of 200 nm the intensity is even greater and for that reason the chromatographic process of quantitative determination of BPA was

followed at 200 nm. The UV spectrum of a pure analytical standard of BPA was used for identification of BPA in the samples.

Matrix effect

Chromatographic analyses were performed in order to investigate matrix effect which can influence the accuracy of the applied method for quantitative determination of BPA (Niessen *et al*., 2006; Matuszewski, 2006; Silvestro *et al.*, 2013). Hence, it is very important to evaluate the influence of the co-eluting peaks onto the chromatographic response of BPA. For that purpose chromatographic analyses were performed on a standard solution of BPA and beverage in which 600 ng/mL of BPA was added. The calculation of the matrix factor (MF) can be performed as ratio between the response of the analyte present in matrix and response of pure analytical standard. If MF = 1 the matrix has no influence on the response of the investigated compound, if MF < 1 the response of the investigated compound is decreasing, if MF > 1 the response of the investigated compound is increasing.

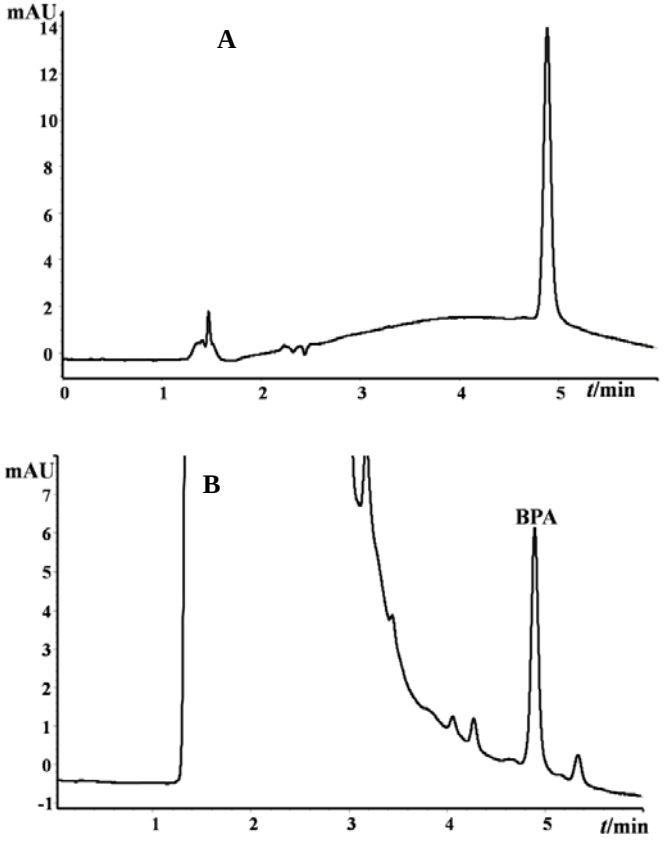

Figure 2: Chromatogram of BPA, Hypersil ODS (250 mm x 4.6 mm, 5 µm) column, mobile phase acetonitrile/water 55/45 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 ^{o}C analytical standard of BPA (A) and beverage (600 ng/mL BPA)) (B)

The matrix effect was tested with three different analytical columns: Hypersil ODS (250 mm x 4.6 mm; 5 μm), LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) and Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm). The obtained chromatograms recorded at 200 nm with the mobile phase acetonitrile/water (55/45, *v*/*v*) are presented in the Figs. 2-4.

From Fig. 2 it can be seen that the retention time of BPA was 4.88 min. The MF factor value for peak height was 0.438, while for peak area was 0.486, which suggested that the response of BPA obtained on a Hypersil ODS (250 mm x 4.6 mm; 5 μm) column with a mobile phase consisted of acetonitrile/water (55/45, *v*/*v*) was decreasing as a result of the influence of the matrix (Silvestro *et al*., 2013; Meyer, 2010).

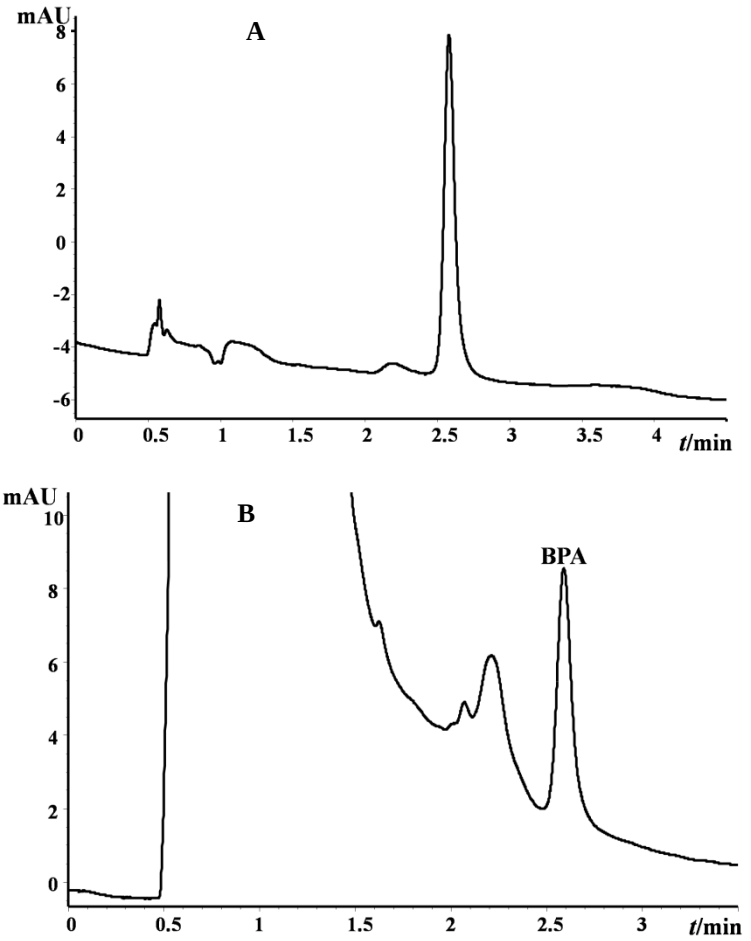

Figure 3: Chromatogram of BPA, LiChrospher 60 RP-Select B (125 mm x 4 mm, 5 μm) column, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 ^{o}C, analytical standard of BPA (A), beverage (600 ng/mL BPA) (B)

When LiChrospher 60 RP-Select B (125 mm x 4 mm; 5 μm) column with the mobile phase of acetonitrile/water (55/45, *v*/*v*) was used the retention time of BPA was 2.59 min, while the MF factor was 1.674 (peak height) and 1.645 (peak area). The obtained result suggested that in this case the response of BPA was increasing as a result of the influence of the co-eluting peaks (Silvestro *et al*., 2013; Meyer, 2010).

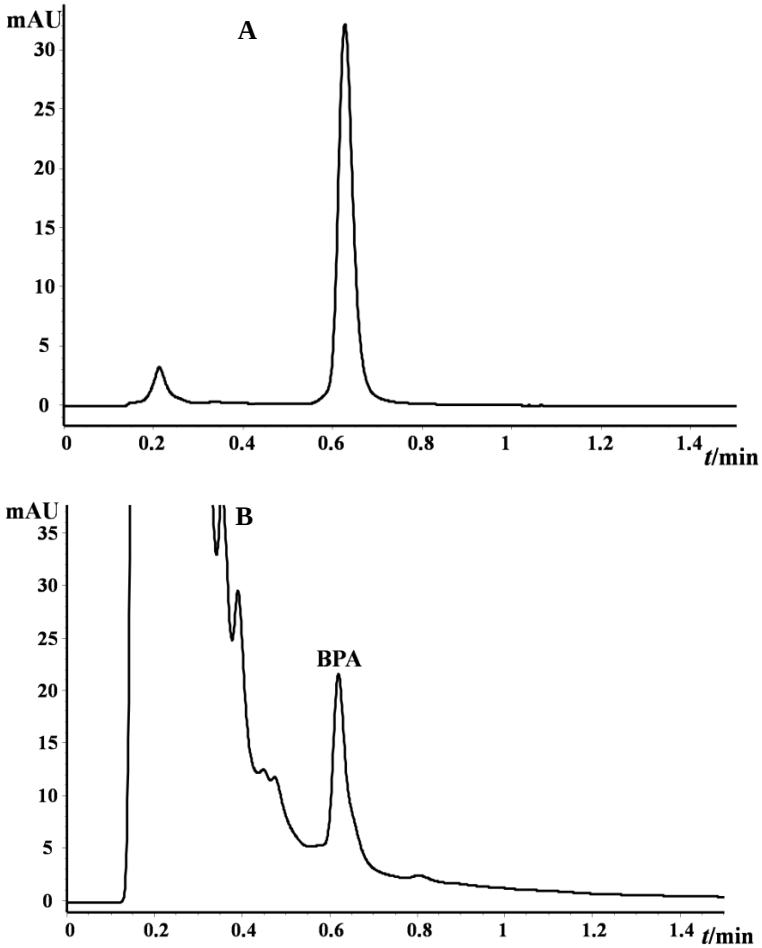

Figure 4: Chromatogram of BPA, Purospher® STAR RP-18 endcapped (30 mm x 4 mm, 3 μm) column, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature 25 °C, analytical standard of BPA (A), beverage (600 ng/mL BPA) (B)

The retention time of BPA was 0.63 min, while the MF factor value was 0.832 (peak height) and 0.936 (peak area) when Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) column and acetonitrile/water (55/45, *v*/*v*) as a mobile phase was used. This suggested that in this case the influence of the co-

eluting peaks on the response of BPA was insignificant (Matuszewski, 2006; Silvestro *et al*., 2013). Taking into consideration the obtained results with tested columns the best separation was achieved with Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) with retention time of 0.63 min, dead time of 0.215 and the retention factor (*k*') of 1.916.

Method validation

In order to validate the developed method the selectivity, linearity, precision, sensitivity and accuracy were tested. Selectivity of the method for determination of BPA was tested through comparison of the UV spectrum of pure analytical standard and the UV spectrum of BPA added in the analyzed beverage (Fig. 5). The obtained value for match factor was higher than 990 confirmed that the UV spectra originated from the same compound.

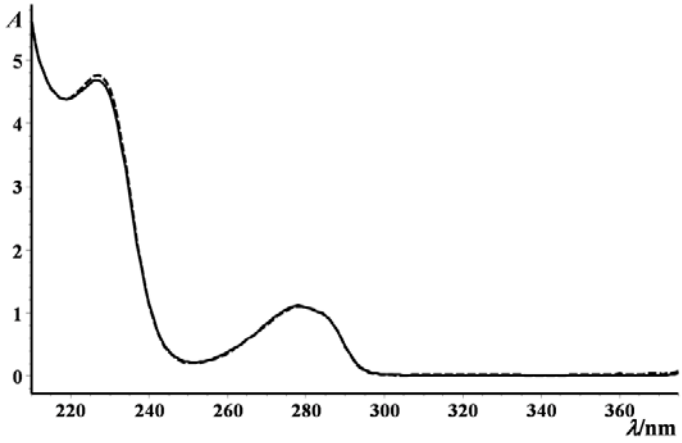

Figure 5: Overlapped UV spectra of analytical standard of BPA and beverage sample with addition of 600 ng/mL of BPA recorded at 200 nm

The linearity of the method was determined by construction of calibration curve which represented the dependence of the mass of BPA and the obtained response as peak height or peak area (Meyer, 2010). The linear dependence between the peak area and peak height on concentation of BPA was tested in the concentration range from 300 ng/mL to 900 ng/mL. The regression equations of BPA are given in Table 1. The coefficient of determination (R^2) value was 0.99 when peak area was used as dependent variable while when peak height was used as dependent variable its value was 0.98. According to literature data these values are satisfactory (Meyer, 2010).

Precision of the developed method was tested through intraday repeteability of the peak area and peak height of eight succesive injections (5 μL) of beverage in which a concentration of 600 ng/mL of BPA was added. The standard deviation (SD) and relative standard deviastion (RSD %) data were calculated using the obtained data for retention time, peak area and peak height (Table 2).

Table 1. Regression equations and R^2 values, 200 nm

Dependent variable	Regression equation	R^2
Peak area [mAU]	y = 15.545x – 3.7293	0.99
Peak height [mAU]	y = 7.2727x – 2.9171	0.98

Table 2. Chromatographic data (peak area, peak height and retention time), SD and RSD [%] of BPA (600 ng/mL), 200 nm

Parameter	1	2	3	4	5	6	7	8	$\bar{x}$	SD	RSD [%]
Retention time [min]	0.629	0.627	0.626	0.626	0.627	0.627	0.628	0.626	0.627	0.001	0.170
Peak area [mAU]	37.97	39.80	41.48	41.35	40.86	41.55	40.85	41.35	40.65	1.223	3.012
Peak height [mAU]	17.24	17.53	17.74	17.74	17.79	17.79	17.73	17.73	17.66	0.191	1.080

*mean value of three subsequent injection

According to manual for verification of the methods of AOAC (manual for the Peer Verified Methods) for compounds with mass of 0.01 pg/kg acceptable value of RSD is up to 30 % (AOAC Peer-verified methods program manual on policies and procedures, AOAC International, 1998). Hence, the obtained SD and RSD values for BPA were in the acceptable range.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated in order to determine the sensitivity of the RP-HPLC method. Their determination is significant for the samples containing very low concentration of compound of interest. As it is well known LOD can be determined as the lowest amount of analyte that can be detected above baseline noise (signal to noise ratio 3:1), while LOQ can be determined as the lowest amount of analyte which can be quantified above the baseline noise (signal to noise ratio 10:1). In order to determine the LOD, 100 μL from the stock solution of BPA were transferred into volumetric flask of 10 mL which than was filled up to the mark with acetonitrile/water in volume ratio 50:50 (*v*/*v*). From this solution a series of dilution were made and injected into chromatographic system. The smallest mass of BPA when it retained its spectral characteristics was 600 pg/mL. The chromatographic peak obtained at this manner was more than three times higher compared to the noise of the base line. With further dilution BPA lost its spectral characteristics. Thus, the determined value of LOQ was 2 μg/mL.

In order to test the accuracy of the method for quantitative determination of BPA the method of standard addition was applied. The following concentrations: 600, 720 and 900 ng/mL of BPA was spiked into the beverage. Then 5 μL of each sample was injected into the chromatographic system in triplicate. Using the peak area data the analytical recovery was calculated (Table 3).

Table 3. Recovery data of the developed method, $n = 3$

Mass of BPA in the sample [ng]	Mass of BPA added in the sample [ng]	Total mass of BPA [ng]	Recovery [%]	RSD [%]
0	3.0	2.797	93.24	0.105
0	3.6	3.470	96.40	0.105
0	4.5	4.643	103.18	0.128

As it can be seen from the Table 3 the obtained recovery values ranged from 93.24 to 103.28 %. According to the analytical method criteria for the determination of bisphenol A in various matrixes the acceptable recovery is from 80 to 120 % with tolerance of 20 % (Barbalas and Garland, 1991).

Determination of BPA in samples

The developed method was used for determination of BPA in different samples of beverages frequently consumed and randomly selected from the Macedonian market. The samples were marked as A, B, C, D, E and F. Chromatograms of samples A and B are presented in Fig. 6.

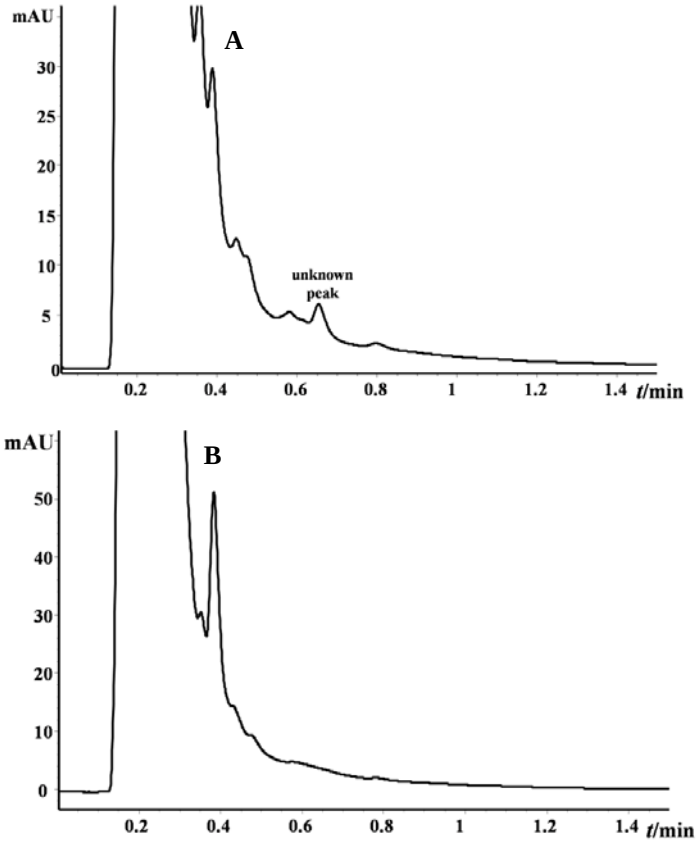

Figure 6: Chromatograms of beverages sample A (a) and sample B (b), Purospher® STAR RP-18 endcapped, 5 μL injected volume, mobile phase acetonitrile/water 50/50 (*v*/*v*), flow rate 1 mL/min, UV detection at 200 nm, temperature of 25 ^{o}C

In the chromatograms of the samples B, C, D and E there was not noticed peak with the retention time near the retention time of BPA, while in chromatograms of samples A and F a peak with retention time of 0.565 was noticed. In order to confirm if this chromatographic peak originates from BPA its UV spectrum was compared with the UV spectrum of pure analytical standard of BPA. From the compared spectra it is obvious that this peak do not have the same spectral characteristics as BPA *i.e*. the match factor was 899.561 (Fig. 7).

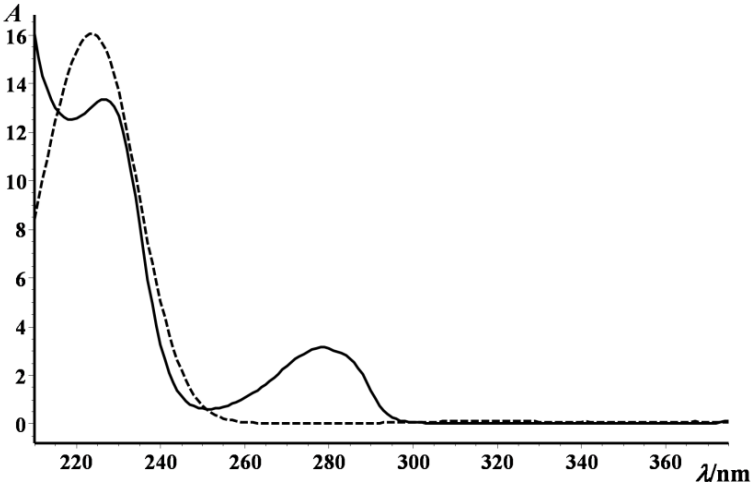

Figure 7: Overlapped UV spectra of pure analytical standard of BPA and unknown compound with retention time of 0.654 min of sample A, recorded at 200 nm

CONCLUSIONS

A method of RP-HPLC with UV-DAD detection was applied for qualitative and quantitative determination of BPA in beverages. The best results were obtained using Purospher® STAR RP-18 endcapped (30 mm x 4 mm; 3 μm) analytical column, mobile phase of acetonitrile/water 50/50 (*v*/*v*), flow rate of 1 mL/min, injection volume of 5 μL, UV detection at 200 nm and temperature of 25°C. Under these experimental conditions the retention time of BPA was 0.627 min, dead time of the column was 0.215 min and the retention factor was 1.916. Validation of the method was tested by following parameters: linearity, sensitivity, selectivity, precision and accuracy. Linearity of the method was tested over the concentration region from 300 to 900 ng/mL. Under used chromatographic conditions BPA was not detected in the analyzed samples of beverages.

REFERENCES

AOAC Peer-verified methods program manual on policies and procedures, AOAC International, 1998.

Almeida S, Raposo, A, Almeida-Gonzalez, M, Carrascosa, C. 2018. Bisphenol A: Food Exposure and Impact on Human Health, Compr. Rev. Food SCI F, 17; 1503-1517.

Aristiawan Y, Aryana N, Ramadhaningtyas D. P, Styarini D. 2015. Analytical Method Development for Bisphenol a in Tuna by Using High Performance Liquid Chromatography-UV, Procedia Chem., 16; 202-208.

Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. 2009. Analytical methods for the determination of bisphenol A in food, J. Chromatogr. A., 1216(3); 449-469.

Barens, K. A.; Sinclar, C. R.; Watson, D. H. 2007. Chemical migration and food contact materials, Woodhead Publishing Limited, Cambrage, England, CRC Press, Boca Raton Boston, New York, Washington DC.

Barbalas M. P, Garland W. A. 1991. A Computer Program for the Deconvolution of Mass Spectral Peak Abundance Data from Experiments Experiments Using Stable Isotopes, J. Pharm. Sci., 80; 922-927.

Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. 2009. Analytical methods for the determination of bisphenol A in food, Journal of Chromatography A, 1216(3); 449-69.

Brede C, Fjeldal P, Skjevrak I, Herikstad H. 2003. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing, Food Addit Contam., 20; 684-689.

Braunrath R, Podlipna D, Padlesak S, Cichna-Markl M. 2005. Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection, J. Agric. Food Chem., 53; 8911-8917.

Cao X. L, Corriveau J, Popovic S. 2010. Bisphenol a in canned food products from Canadian markets, J. Food Prot., 73(6); 10851089.

Commission Regulation (EU) No 10/2011, plastic materials and articles intended to come into contact with food, 2011.

Commission Regulation (EU) 2018/213, the use of bisphenol A in varnishes and coatings intended to come into contact with food, 2018.

Matuszewski B. K. 2006. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS Bioanalysis, J. Chromatogr. B, 830; 293-300.

Meyer, V., Practical High-Performance Liquid Chromatography, Fifth Edition, John Wiley & Sons, Ltd., 2010.

Niessen W. M. A, Manini P, Andreoli R. 2006. Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry, Mass Spectrom. Rev., 25; 881-889.

Nerín C, Aznar M, Carrizo D. 2016. Food contamination during food process, Trends Food Sci. Technol., 48; 63-68.

Rykowska I, Szymanski A, Waslak W. 2004. Method Based on Solid Phase Extraction, LC and GC for Analysis of Bisphenol A in Drinking Water, Chem. Pap., 58(6); 382-385.

Silvestro, L.; Tarcomnicu, I.; Rizea Savu, S. Tandem Mass Spectrometry - Molecular Characterization, Open access peer-reviewed chapter, INTECH, pp. 1-38, 2013.

Struckhofova Z. B, Marki M. C. 2006. Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection, Food Addit. Contam., 23(11); 1227-1235.

Sadeghi M, Nematifar Z, Fattahi N, Pirsaheb M, Shamsipur M. 2016. Determination of Bisphenol A in Food and Environmental Samples Using Combined Solid-Phase Extraction–Dispersive Liquid–Liquid Microextraction with Solidification of Floating Organic Drop Followed by HPLC, Food Anal. Methods, 9; 1814-1824.

Tanigawa, T.; Watabe, Y.; Kubo, T.; Hosoya, K. 2011. Determination of bisphenol a with effective pretreatment medium using automated column-switching HPLC with fluorescence detection J. Sep. Sci., 34, 2840.

Taskeen A, Naeem I. 2010. Analysis of Bisphenol A in Canned Food: A Mini Review, Asian J. Chem., 22(5); 4133-4135.

Xu-Liang, C. J. Liq. Chromatogr. R T, 2012, 35(19), 2795.

Yoshida T, Horie M, Hoshino Y, Nakazawa H, Horie M, Nakazawa H. 2010. Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography, Food Addit. Contam., 18(1); 69-75.