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ARTICLES

# Determination of Bisphenol A in Foods as 2,2-bis-(4-(isopropoxycarbonyloxy)phenyl)propane by Gas Chromatography/Mass Spectrometry

**D. B. Feshin, P. V. Fimushkin, E. S. Brodskii, A. A. Shelepchikov, E. Ya. Mir-Kadyrova, and G. A. Kalinkevich**

*Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 119071 Russia*

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**Abstract**—A procedure has been developed for the determination of bisphenol A (**BPA**) in foods by gas chromatography/mass spectrometry as 2,2-bis-(4-(isopropoxycarbonyloxy)phenyl)propane formed in the reaction with isopropyl chloroformate. Optimal conditions have been found for BPA derivatization, providing its quantitative conversion into diether derivative in aqueous media. The concentration of BPA has been determined in some samples of canned foods and beverages (from 2.15 to 42.91 ng/g). The detection limit is 0.05–0.1 ng/g.

*Keywords:* bisphenol A, isopropyl chloroformate, gas chromatography, mass spectrometry.

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A special concern is raised by the contamination of the environment and foods by compounds with the properties of estrogenic hormones exerting negative effect onto human endocrine status. Similarly to reproductive hormone estrogen, very low concentrations (at the level of ng/g) of these compounds affect the reproductive functions of humans and block the expression of male hormones; they are characterized by mutagenic and carcinogenic properties and cause disorders of reproductive and endocrine systems [1–3].

One of these compounds is bisphenol A (**BPA**), 2,2-bis(4-hydroxyphenyl)propane, widely used as a monomer for the production of polycarbonates, epoxy resins, and others [4]. The area of application of BPA-based polymer products is very wide: from packing items for drinking water and inner coatings of metal cans for food and beverages to details for the automobile industry. The main source of BPA intake into human organism is the consumption of food packed into BPA-containing materials [5]. Nowadays in the European Union, tolerable daily intake level for BPA in food for human is 0.05 mg of BPA/kg of body weight per day [6]. In Russia the maximum permissible concentration of BPA in drinking water is 0.01 mg/L [7].

At the moment, the main method for BPA determination is gas chromatography/mass spectrometry (**GS/MS**), which is usually combined with derivatization by silylation or acetylation [8–10].

One of efficient methods for the determination of bisphenol A (as well as phenols) in aqueous media is its etherification with acetic anhydride in water followed by the extraction of the resultant derivatives [8]. However, acetic anhydride is included into list 4 of drugs, psychotropic substances, and their precursors [11] and is not actually available. It can be substituted by alkyl chloroformates, which are also used as derivatization agents for the determination of phenols in aqueous samples by GC/MS [12, 13]. Earlier we have obtained good results for the etherification of phenols by isopropyl chloroformate in aqueous media [14]. In this study we applied this reagent to the determination of BPA in foods and beverages by GC/MS as a corresponding diether derivative.

## EXPERIMENTAL

**Standards and materials.** BPA was purchased from Fluka. Isotope-labeled ($^{13}C_{12}$) standard of bisphenol A was purchased from Cambridge Isotope Laboratories (lot SCHJ-4325-S). All working solutions were prepared in acetonitrile and kept at –30°C. Methanol was purchased from Fluka; acetonitrile was purchased from Panreac; a 1.0 M solution of isopropyl chlorofor-

mate in toluene was purchased from Aldrich. High-purity acetone (Komponent-reaktiv), hexane (Kriokhrom) and reversed-phase C18 cartridges of high capacity produced by Altech (USA) were used.

**Glassware.** The glassware was heated at 250°C for at least 2 h before experiment.

**Sample preparation.** ***Water, beverages, pulpless juices.*** The sample (5–20 g) was placed into a 40 mL vial with a hermetic teflonated screw cap, a 100 ng portion of the internal standard in 100 μL of acetonitrile was added, and derivatization was carried out.

***Fruit and vegetable puree samples, canned fruit and vegetables. Milk, meat puree, and canned meat.*** 5–15 g of a homogenized sample was placed into a 50-mL extraction flask, 100 ng of the internal standard in 100 μL of acetonitrile was added, and the mixture was sonicated for 5 min. Then an aliquot of acetonitrile equal to the sample weight was added into the flask, the contents of the flask and stirred with a homogenizer for 10 min at 1500 rpm. Then the sample was centrifuged at 4000 rpm for 10 min. Water-acetonitrile phase was transferred by a Pasteur pipette into a pear-shaped flask. The solid residue remained after centrifugation was washed twice by 10 mL of acetonitrile, homogenized, and centrifuged at 4000 rpm for 10 min. The water–acetonitrile phase was transferred into the same pear-shaped flask.

While analyzing of fruit and vegetable purees, canned fruits and vegetables, acetonitrile was rotary evaporated from the flask with the extract. The aqueous phase remained after evaporation was transferred into the 40-mL reaction vial with a hermetic teflonated screw cap; the flask was washed with 5 mL of distilled water. Water wash was also transferred into the vial. If necessary, the level of the aqueous phase in the reaction vial was adjusted to 25 mL. Then derivatization was carried out.

While analyzing milk, meat puree, and canned meat samples, the combined water–acetonitrile phase was collected into 40 mL vials and fat residues were removed by extraction with 3 mL of hexane. The hexane layer was separated; the water–acetonitrile phase was transferred into the flask and diluted with distilled water (10 mL of water per 1 mL of acetonitrile). Then the resultant solution was purified on a C18 cartridge.

***Dry milk mixtures.*** A sample (5–15 g) was placed into a 50-mL vial with a screw cap and dissolved in distilled water of the weight equal to the weight of the sample under vigorous stirring and sonication. Then the resultant solution was treated as the samples of fruit and vegetable puree, canned fruits and vegetables, milk, meat puree, and canned meat.

***Purification on C18 cartridge.*** The cartridge was washed sequentially with 20 mL of methanol and 20 mL of water; then the extract of the sample was passed through. The cartridge was then washed with 10 mL of distilled water and 20 mL of 30% methanol in distilled water. BPA was eluted by 10-mL of a 50% solution of acetonitrile in distilled water and 30 mL of methanol. The eluate was collected into a pear-shaped flask and organic solvents were rotary evaporated. The resultant aqueous solution was transferred into a 40-mL reaction vial with a screw hermetic teflonated cap; the flask was washed with 5 mL of distilled water. Water wash was also transferred into the vial. The level of the aqueous phase in the reaction vial was adjusted to 25 mL. Then the derivatization reaction was carried out.

*Derivatization and extraction of the reaction products.* 1-g portions of $K_2CO_3$ were placed into the reaction vials with the samples or their purified extracts; the salt was dissolved, and the pH of the resultant solution measured using universal pH indicator paper. If the pH was below 9, 1 g of $K_2CO_3$ was additionally introduced into the reaction vial and the pH of the solution was checked once more. Then 1 mL of a 1.0 M solution of isopropyl chloroformate in toluene was added into the reaction flask. The reaction vials were vigorously shaken for 15 min, then 1 mL of the reagent was additionally introduced and the mixture was shaken for 15 min more. When the reaction was completed, the flasks were centrifuged for 5 min at 1500 rpm. The upper organic phase was collected by a Pasteur pipette and passed through a microcolumn with anhydrous $MgSO_4$. The microcolumn was made of a Pasteur pipette. For this purpose, a small plug made of quartz wool was placed into the pipette and tightened in the narrow part of the pipette; a 1.5–2-cm layer of anhydrous $MgSO_4$ was placed above the plug. The reaction products were additionally twice extracted by 1.5 portions of hexane. The hexane layer was also passed through the same microcolumn with anhydrous $MgSO_4$. The combined extract was collected into test tubes with conical bottom, 10 μL of tridecane were added, and the mixture was evaporated to 10 μL in an air flow at the temperature 40°C or below. The sample then was analyzed by GC/MS.

*Instrument and analysis conditions.* The analysis was made on a gas chromatograph/mass spectrometer, which included a Finnigan Trace GC Ultra gas chromatograph and a Finnigan PolarisQ (ion trap) mass spectrometer. Capillary quartz column (length 20 m and inner diameter 0.18 mm with the DB-5ms stationary phase (0.18 μm-thick film)) was used. The conditions of the chromatographic separation were as follows: injector temperature 220°C, flow rate of the

carrier gas (helium) 1 mL/min. The temperature program: start temperature 80°C, 2 min hold, heating at the rate of 10°C/min to 160°C, then 8°C/min to 300°C, hold 6 min. Mass spectra were scanned at: electron ionization, electron energy 70 eV, ionization chamber temperature 220°C, scanning range 41–550 Da, scanning time 0.53 sec, cathode turn on delay 15 min. 2 μL of the sample solution was injected into the gas chromatograph, flow split was 1 : 20.

*Identification of the analytes.* BPA and $^{13}C_{12}$-BPA were identified as diether derivatives by the following characteristics:

(i) the presence of synchronous chromatographic peaks with corresponding retention time on the ion mass chromatograms for characteristic ions of both compounds (*m/z* 213, 228, and 255 for diether derivative of BPA and *m/z* 225, 240, and 267 for diether derivative of $^{13}C_{12}$-BPA); deviation of the retention time from the reference value by no more than 2 sec;

(ii) 10 : 1 : 3 ratio of the peak areas on the ion mass chromatograms for ions *m/z* 213, 228, and 255 for diether derivative of BPA and *m/z* 225, 240, and 267 for diether derivative of $^{13}C_{12}$-BPA; the deviation of the values of peak area ratios from the reference values not exceeding 10%. The reference values were the retention time and peak intensity ratios for the characteristic ions of BPA in calibration solution.

*Calibration and quality control.* Calibration was made to estimate linear analytical range and stability of the calibration factor in this range. To obtain calibration solutions, from 5 to 1200 ng of BPA was added to 20 mL of distilled water and derivatizated. The analysis of the calibration solutions was made under the same conditions as the analysis of samples. The calibration factor $k_i$ was calculated using the equation:

$$k_i = (S_{is}m_{\mathrm{BPA}})/(S_{\mathrm{BPA}}m_{is}),$$

where $S_{\mathrm{BPA}}$ is the peak area of diether derivative of BPA on the mass chromatogram restored for sum of the ion peaks with *m/z* 213 and 228; $S_{is}$ is the peak area of the diether derivative of internal standard $^{13}C_{12}$-BPA on the ion mass chromatogram for sum of the ion peaks with *m/z* 225 and 240; $m_{\mathrm{BPA}}$ is the weight of the introduced BPA; $m_{is}$ is the weight of the introduced internal standard $^{13}C_{12}$-BPA. The average value of the calibrating factor $k_i$ was calculated from the data of at least eight experiments. The detection and quantification limits were determined from the dependence of the calibrating factor on BPA concentration in the calibration solutions. The chromatograms were registered on the total ion current. For the determination of BPA, ion mass chromatograms for the ions with *m/z* 213 and 228 for BPA, *m/z* 225 and 240 for $^{13}C_{12}$-BPA were used. Each group of samples analyzed according to this procedure included one blank sample (20 mL of distilled water), one control sample spiked by 30 ng/g BPA, one of the tested samples spiked by 30 ng/g BPA.

*Quantitative determination of BPA.* The concentration of BPA in the sample was calculated using the equation:

$$c_{\mathrm{BPA}} = (k_i S_{\mathrm{BPA}}m_{is})/(S_{is}m_{\mathrm{Sample}}),$$

where $S_{\mathrm{BPA}}$ was summarised peak area of diether derivative of BPA for the ions with *m/z* 213 and 228; $k_i$ was calibration factor, $m_{is}$ was the weight of added internal standard; $S_{is}$ was summarized peak area of diether derivative of the internal standard for the ions with *m/z* 225 and 240; $m_{\mathrm{Sample}}$ was the weight of the sample.

## RESULTS AND DISCUSSION

The general scheme of BPA determination in different foods is presented in Fig. 1. The determination of BPA in water, beverages (including soda beverages), and pulpless juice did not require special sample preparation. Derivatization could be carried out without the preliminary extraction of BPA from the aqueous matrix. For analysis of canned fruit, vegetables, meat, milk, and dry milk mixtures, BPA was extracted three times with acetonitrile. Water–acetonitrile extracts of the fat-containing foods were additionally purified from fat by hexane extraction followed by purification on C18 cartridges. This simple and all-purpose procedure provided sufficient removal of fatty acids and other components which could overload chromatographic column, contaminate injector, or overlap with BPA peak [15, 16].

**Derivatization.** For derivatization of BPA, we used a 1.0 M solution of isopropyl chloroformate in toluene. Our early experiments on the determination of the phenols in water using this reagent have demonstrated quantitative yields of the corresponding isopropyloxycarbonyl derivatives [14].

As compared with silylating agents and trifluoroacetic acid anhydride, isopropyl chloroformate showed moderate reactivity, which allowed us to exclude BPA extraction step before derivatization and made it possible to carry out the reaction by adding reagent to the aqueous solution of the analyte.

Quantitative conversion of BPA to its diether derivative occurred as a result of the two-step addition of the reagent at a 15 min interval (total reaction time being 30 min) at room temperature. Carrying out the reaction under heterophase conditions provided a combination of derivatization and extraction of the reaction products.

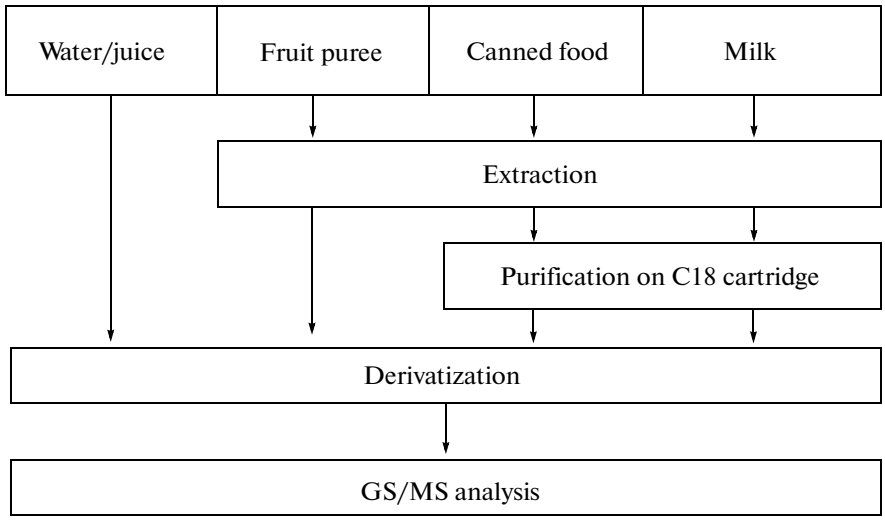


**Fig. 1.** Scheme of sample preparation for determination of BPA in various foods.

**Identification of reaction products.** The fragments of the mass spectra of the diether derivatives of BPA and $^{13}C_{12}$-BPA are presented in Fig. 2. Obviously, the main peaks in the spectrum of the corresponding derivative of BPA are the ion peaks with *m/z* 213, 228, and 255 (intensity ratio 10 : 1 : 3) and *m/z* 225, 240, and 267 for the derivative of $^{13}C_{12}$-BPA (intensity ratio 10 : 1 : 3).

Figure 3 presents a fragment of the mass chromatogram of canned meat sample.

**Accuracy parameters.** In the range 5–1200 ng, the dependence of the signal on the concentration of BPA was linear (Fig. 4). The detection limit for BPA on total ion current (signal-to-noise ratio 3 : 1) was 5 ng, the quantification limit (signal-to-noise ratio 10 : 1) was 10 ng. To estimate the accuracy of the analysis, we made the experiments using the standard addition method (Table 1), by adding certain amounts of BPA to a sample of apple juice. Deviation of found values from added amounts did not exceed 5%. BPA concentrations obtained as a result of repeated analyses of vegetable–meat puree were 1.28, 1.32, and 1.38 ng/g, the average value was equal to 1.33 ng/g, relative standard deviation was 0.005.

Table 2 shows the results of BPA determination in some canned food samples. The concentration of BPA varied from 2.15 to 42.91 ng/g, the detection limits were from 0.05 to 0.1 ng/g.

Hereby, BPA was quantitatively converted into 2,2-bis(4-(isopropoxycarbonyloxy)phenyl)propane by the reaction with isopropyl chloroformate in aqueous media. A procedure was developed for the determination of BPA in environmental samples and foods by the reaction with isopropyl chloroformate followed by GC/MS analysis.

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**Table 1.** Results of BPA determination by standard addition method

| Experiment no. | Added BPA (ng) | Found BPA (ng) | Δ % |
|---|---|---|---|
| 1 | 300 | 315 | 5 |
| 2 | 300 | 302 | 0.7 |
| 3 | 300 | 312 | 4 |
| 4 | 600 | 623 | 4 |

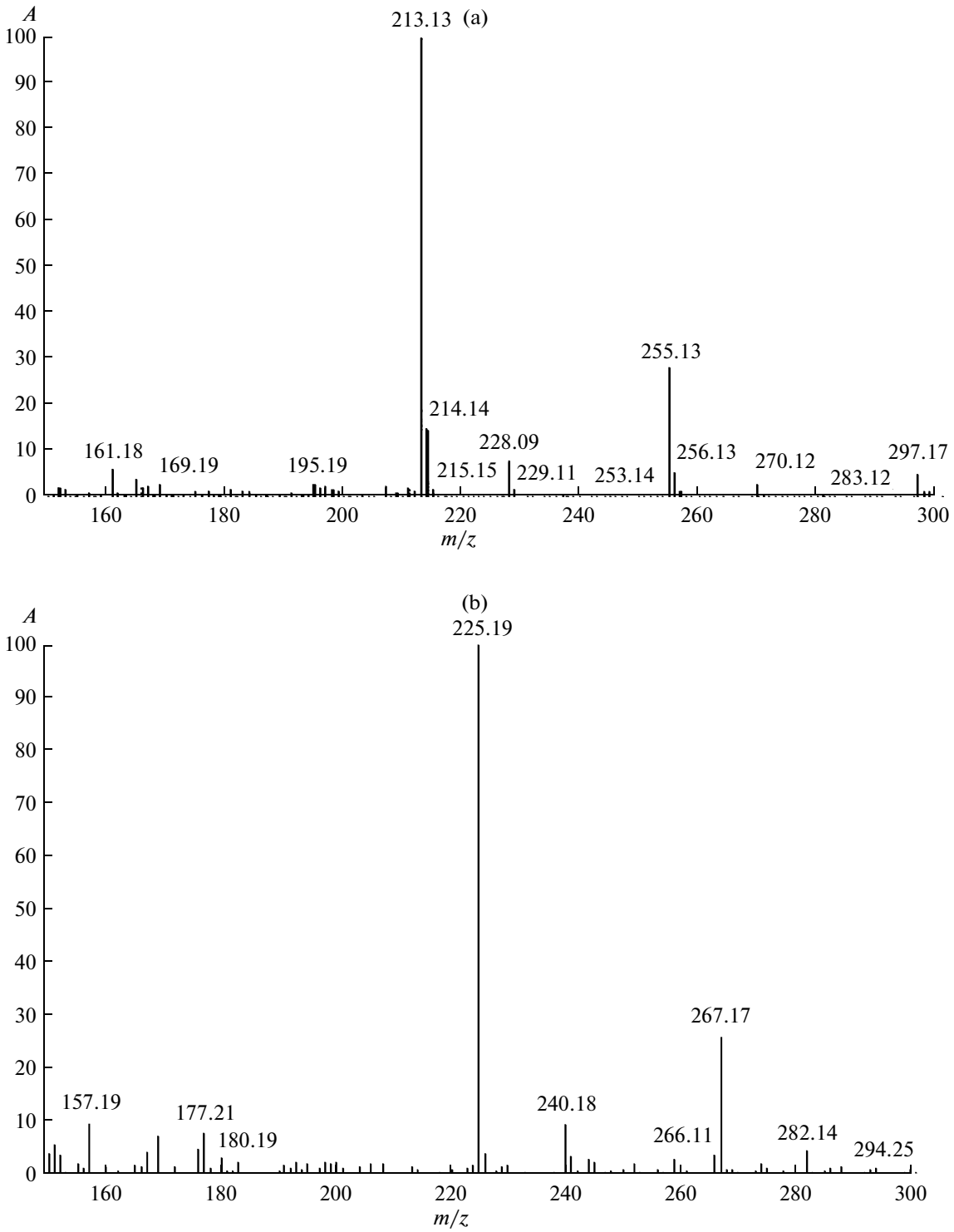


**Fig. 2.** Fragments of mass spectra of diisopropyloxycarbonyl derivatives of BPA (a) and $^{13}C_{12}$-BPA (b).

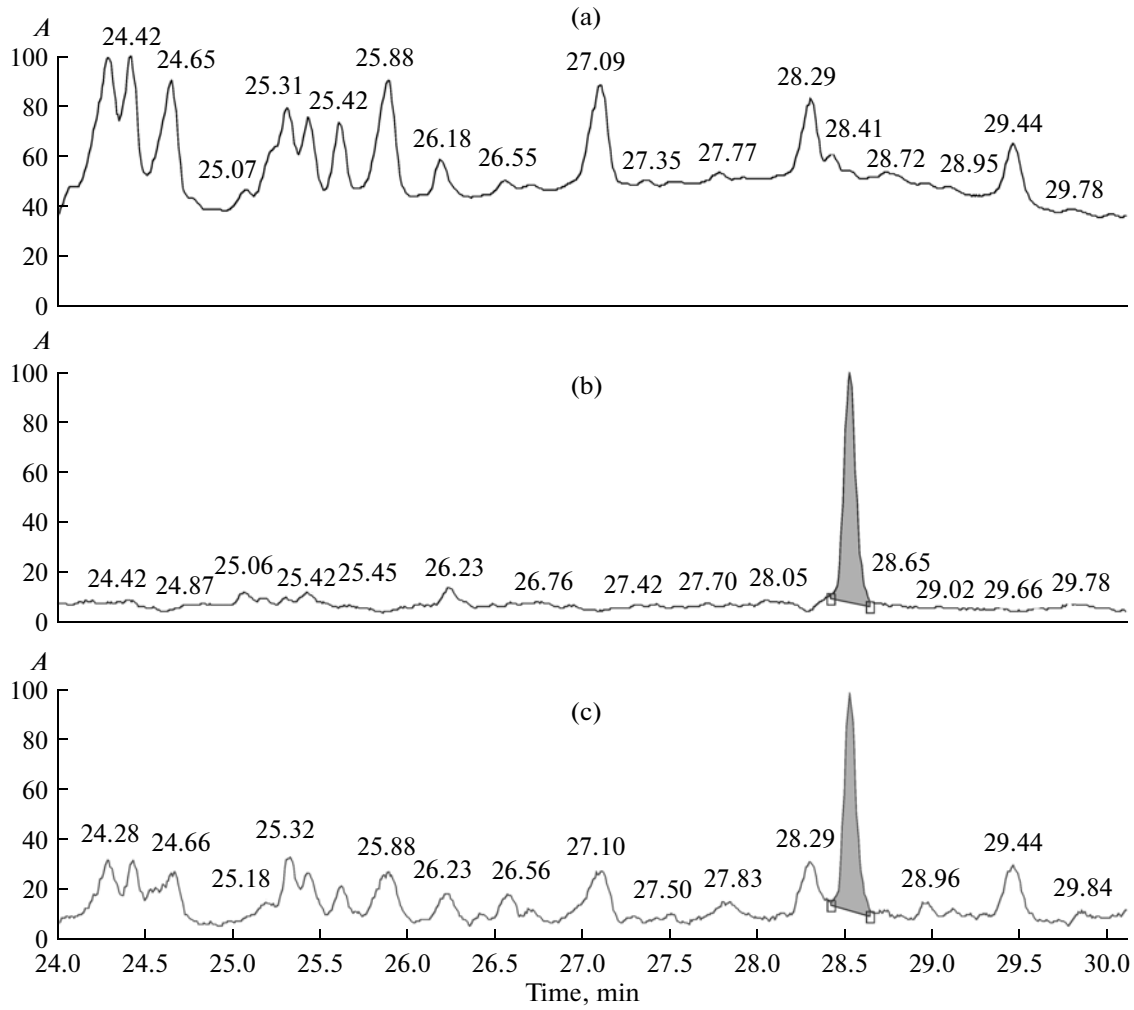


**Fig. 3** Fragments of a mass chromatogram of the extract of a canned meat sample: (a), mass chromatogram for total ion current; (b), chromatogram for sum of ion peaks with $m/z$ 213 and 228 for BPA; (c), mass chromatogram for sum of ion peaks with $m/z$ 225 and 240 for $^{13}C_{12}$-BPA.

**Table 2.** Results of BPA determination in foods

| Sample | Concentration of BPA (ng/g) | Detection limit (ng/g) |
|---|---|---|
| Energetic beverage | 2.81 | <0.05 |
| Infant meat puree | 35.22 | <0.1 |
| Infant formula feed | 2.15 | <0.1 |
| Canned meat | 19.39 | <0.1 |
| Canned vegetables | 42.91 | <0.1 |

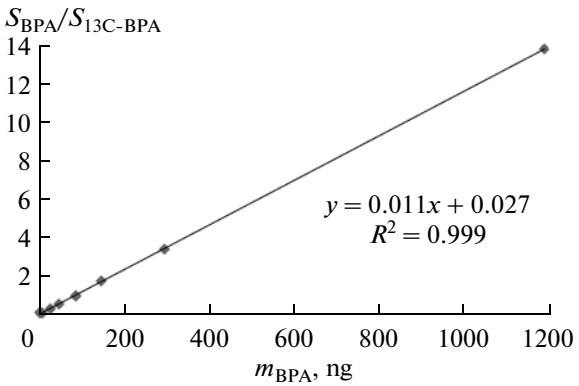


**Fig. 4.** Ratio of the peak areas for BPA and the internal standard ($S_{BPA}/S_{13C\text{-}BPA}$) as a function of BPA concentration.

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## REFERENCES


1. Zoeller, R.T., Bansal, R., and Parris, C., *Endocrinology*, 2005, vol. 146, p. 607.
2. Wetherill, Y.B., Petre, C.E., Monk, K.R., Puga, A., and Knudsen, K.E., *Mol. Cancer Ther.*, 2002, vol. 7, p. 515.
3. Akingbemi, B.T., Sottas, C.M., Koulova, A.I., Klinefelter, G.R., and Hardy, M.P., *Endocrinology*, 2004, vol. 145, p. 592.
4. Han-Wen Kuo and Wang-Hsien Ding, *J. Chromatogr., A*, 2004, vol. 1027, p. 67.
5. Maragou, N.C., Lampi, E.N., Thomaidis, N.S., and Koupparis, M.A., *J. Chromatogr., A*, 2006, vol. 1129, p. 165.
6. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (ACF) on Request from the Commission Related to 2,2-bis(4-Hydroxyphenyl)Propane (Bisphenol A), *EFSA J.*, 2006, vol. 428, p. 1. doi:10.2903/j.efsa.2007.428, http://www.efsa.europa.eu/en/efsajournal/pub/428.htm
7. *GN* (Hygienic Standards) *2.1.5.1315-03: Maximum Permissible Concentrations (MPCs) of Chemicals in the Water of Water Objects Used for Drinking and Domestic-Recreation Purposes*, Moscow, 2003.
8. Ballesteros-Gomez, A., Rubio, S., and Perez-Bendito, D., *J. Chromatogr., A*, 2009, vol. 1216, p. 449.
9. Gyong, Y., Shin, J.H., Kim, H.-Y., Khim, J., Lee, M.-K., and Hong, J., *Anal. Chim. Acta*, 2007, vol. 603, p. 67.
10. Szyrwińska, K., Kołodziejczak, A., Rykowska, I., Wasiak, W., and Lulek, J., *Acta Chromatogr.*, 2007, vol. 18, p. 161.
11. *Perechen' narkoticheskikh sredstv, psikhotropnykh veshchestv i ikh prekursorov, podlezhaschikh kontrolyu v RF* (List of Narcotic Drugs, Psychotropic Substances, and Their Precursors Controlled in the Russian Federation), List IV. http://base.garant.ru/12112176.htm
12. Kyoung-Rae Kim and Hyub Kim, *J. Chromatogr., A*, 2000, vol. 866, p. 87.
13. Henriksen, T., Svensmark, B., Lindhardt, B., and Juhler, R.K., *Chemosphere*, 2001, vol. 44, p. 1531.
14. Feshin, D.B., Pavlovets, V.V., Shelepchikov, A.A., and Brodskii, E.C., Abstracts of Papers, *IV s"ezda VMSO i III Vserossiiskoi konferentsii "Mass-spektrometriya i ee prikladnye problemy"* (IV Conf. of All-Russian Soc. of Mass Spectrometry and III All-Russian Conf. "Mass Spectrometry and Its Application Problems"), Moscow, 2009, p. 62.
15. Mungía-López, E.M. and Soto-Valdez, H., *J. Agric. Food Chem.*, 2001, vol. 49, p. 3666.
16. Wingender, R.J., Niketas, P., and Switala, C.K., *J. Coat. Technol.*, 1998, vol. 70, p. 75.