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Measurement of bisphenol A in human serum by gas chromatography/mass spectrometry

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Abstract

The purpose of this study is to establish an easy and accurate method for the determination of bisphenol A (BPA) in the human serum. The samples were applied to the C_{18} solid phase extraction (SPE) column for clean up of samples. The BPA is conjugated with tetrabutylammonium hydrogen sulfate as the counter ion in alkali solution. The ion paired BPA is moves from the aqueous phase to the organic phase as an ion paired extraction. BPA extracted from human serum were derivatized with pentafluorobenzyl bromide (PFBBr). The derivative was analyzed by gas chromatography (GC)/mass spectrometry (MS) using negative chemical ionization (NCI). The instrumental detection limit of BPA was 5 pg/ml (10 fg). The instrumental response between 0.01 and 100 pg/ml of BPA standards was linear ($r^2 = 0.998$). The recovery of BPA spiked into human serum was 101.0 ± 4.63 (1 pg/ml) and 100.9 ± 3.75 (10 pg/ml), respectively. The concentration of BPA in the human serum from 20 individuals was 0.54 pg/ml.

Keywords: Bisphenol A; Human serum; Gas chromatography/mass spectrometry; Pentafluorobenzyl bromide

1. Introduction

Bisphenol A (BPA) is used in the manufacture of polycarbonate and epoxy and phenoxy-resins, and used as stabilizing materials of plastics such as polyvinyl chloride [1,2]. Although in 1938, Dodds noted oestrogenic activity of BPA, it is only in the last few years this compound has received extensive attention [3]. Krishnan also reported that the BPA extracted from polycarbonate produced breast cancer cell proliferation in vitro [4]. BPA has also been reported to decrease the prostate weight of mice born to dams administrated BPA during pregnancy [5].

The analysis of BPA has previously been accomplished by paper chromatography [6,7], gas chromatography (GC)/mass spectrometry (MS) [8–10], or HPLC with conventional ultraviolet detection [11], fluorescence [12,13] or electrochemical detection [14–16]. Pentafluorobenzyl bromide (PFBBr) has been used with electron capture detection for derivatization of detection of amount of carboxylic acid, mercaptans, and phenols compounds [17–19]. However, it is difficult to detect trace level of BPA in biological materials using GC/ECD or GC/MS with EI mode due to high background interferences [20]. Therefore, it is considered to be obtained high sensitivity in

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GC/MS with negative CI mode by use of BPA–PFB, which has fluorine group by reaction BPA with PFBBr, resulted in negative ion by the chemical ionization.

In this paper, a GC/MS with negative CI mode for determination of BPA derivatized with PFBBr in the human serum is reported.

2. Experimental

2.1. Reagents and materials

All reagents and solvents are analytical grade unless specified otherwise (Burdick and Jackson, Muskegon, MI). All glassware was rinsed with hexane followed by acetone.

$^{13}C_{12}$-ring-labeled BPA as internal standard and standard BPA were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Tetrabutylammonium hydrogen sulfate was obtained from Eastman Kodak Inc. (Rochester, NY, USA), and formic acid and sodium hydroxide were obtained from Mallinckrodt Baker Inc. (Paris, KY, USA).

BPA free-Water was obtained by purification with C_{18} SPE column (500 mg, Mallionkrodt Baker Inc., Paris, KY, USA) pre-treated with methanol 5 ml. PFBBr was purchased from Supelco Inc. (Belletonte, PA, USA).

Serum specimens were collected anonymously and then polled from at least five persons.

2.2. Instruments

All chromatographic measurements were performed with a GC fitted with an Autosampler, a split–splitless injector for capillary columns and a Mass Spectrometer with as CI of 180 eV as ionization source and quadruple mass filter (GC:HP6890, MSD:HP 5973, Auto sampler:HP7686, Hewlett Packard, USA). The column was a DB-5 (30 m × 0.25 μm i.d., 0.25 μm d_f J&W Scientific USA) was used. The oven temperature was initially 60 °C, and was raised to 200 °C at a rate of 15 °C/min, 220 °C at a rate of 10, and 270 °C at a rate of 15 °C/min, then held at that temperature for 13 min. The methane was used as the reaction gas for CI. The MS transfer line temperature was 280 °C For qualitative analysis, the MS was operated in scan mode. For quantitative analysis, the MS was operated in the selected ion-monitoring (SIM) mode.

The other GC/ECD system was performed with a Hewlett Packard HP 5890A, with Auto sampler HP767. The column was a DB-5 (60 m × 0.25 μm i.d., 0.25 μm d_f J&W Scientific USA). The oven temperature was initially 60 °C, and was raised to 200 °C at a rate of 10 °C/min, 220 °C at a rate of 2 °C/min, and 270 °C at a rate of 10 °C, then held at that temperature for 30 min. The helium was used as the carrier gas.

2.3. Purification of BPA from serum

A 10 ml of 1 pg/ml $^{13}C_{12}$-ring-labeled BPA was added to 1 ml serum sample and stirred until completely mixed. Formic acid (1 ml, 32%) was added to the solutions, and then was sonicated for 5 min. Acidification using formic acid in the sample act as prevention for ionization of BPA and deproteinization of the sample. The samples were applied to the C_{18} solid phase extraction (SPE) column previously treated with 15 ml of methanol and 3 ml of water. After washing SPE column with 5 ml of water/methanol (9:1), the BPA was eluted with 8 ml of methanol. Re-filtration with the C_{18} SPE column was done for purification of sample. The solvent was concentrated to 0.5 ml under nitrogen at 55 °C (Turbovap; Zymark, Framingham, MA, USA).

2.4. PFB derivatization of bisphenol A

Dichloromethane (0.5 ml), 0.5 ml of 0.1 M tetrabutylammonium hydrogen sulfate, 0.5 ml of 0.2 M sodium hydroxide and 20 ml of PFBBr were added to 0.5 ml serum sample containing an adequate amount of BPA. After the emulsion mixture was shaken for 20 min at 25 °C, 1 ml of isooctane was added, and then the solution was evaporated to dryness under nitrogen for 20 min at 55 °C. BPA is extracted into dichloromethane solution as ion pair form by use of tetrabutylammonium hydrogen sulfate, and alkylated with PFBBr in the dichloromethane. Finally, residue was dissolved in 0.5 ml of isooctane. The isooctane solution was transferred into clean GC vials.

2.5. GC/MS analysis

A 2 ml aliquot of the extract was injected by the autosampler in the injector using splitless mode. The selected ions of the compound for SIM mode operation were 407 *m*/*z*. The concentration of the serum were calculated by the internal standard method.

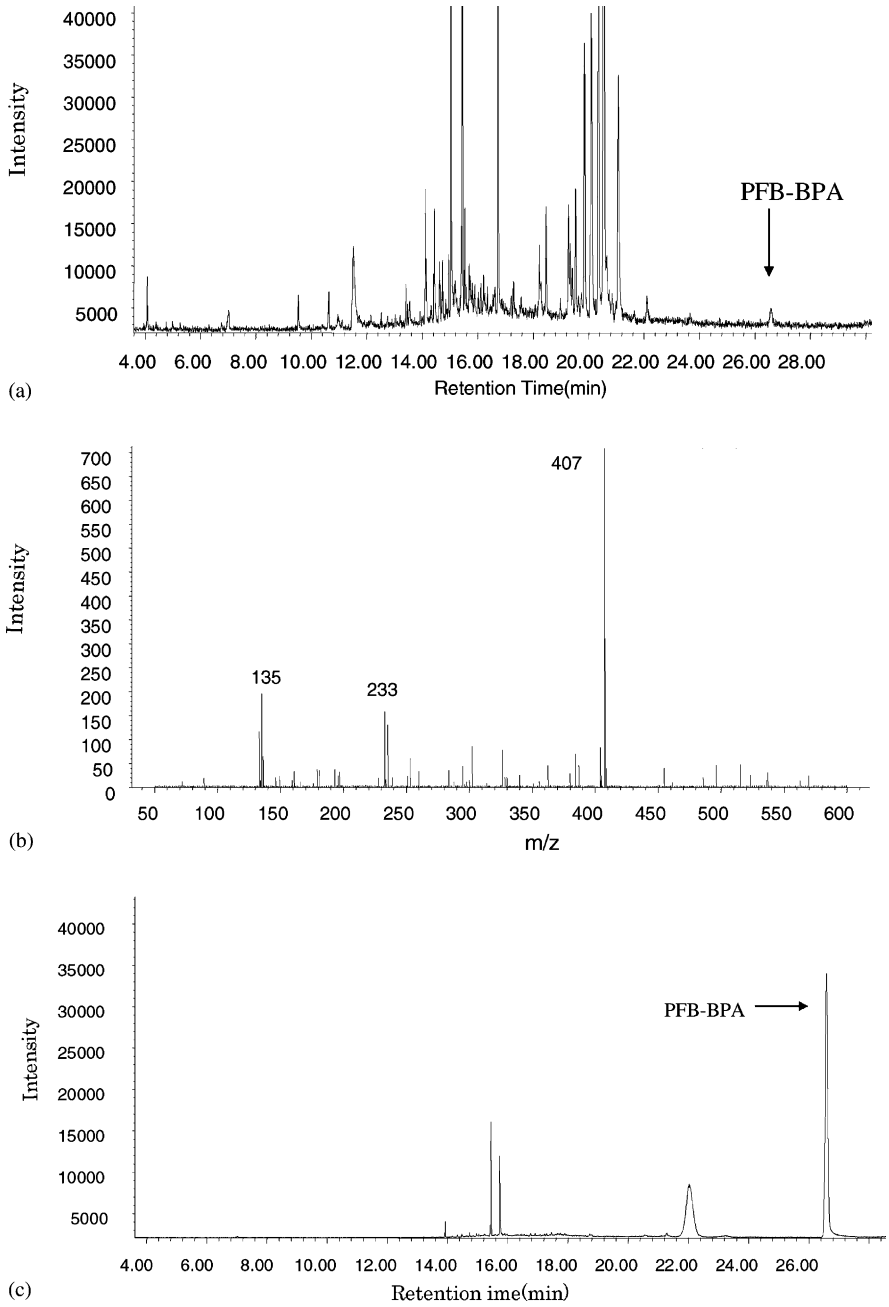

Fig. 1. TIC chromatogram (a), mass spectrum (b), SIM chromatogram (c) of PFB-bisphenol A.

3. Results and discussion

3.1. Identification of PFB–BPA

A typical TIC chromatogram obtained with 10 pg/ml BPA standard solution by using of GC/MS NCI mode is shown in Fig. 1a. A peak of PFB–BPA was identified at 26.59 min by the mass spectrum which showed 407 *m/z* as molecular peak as shown in Fig. 1b. Therefore, it was found that BPA react with PFBBr by the ratio of 1:1 shown in Fig. 2. Typical SIM chromatogram of PFB–BPA obtained with 10 pg/ml BPA standard solution is shown in Fig. 1c. Good separation of BPA and impurities was observed by using SIM mode. The 419 *m/z* as molecular peak was used as internal standard of PFB–^{13}C-ring-labeled BPA.

3.2. Effect of solvent for extraction of ionpaired BPA and PFB–BPA

First, BPA is conjugated with tetrabutylammonium hydrogen sulfate as the counter ion in alkali solution. The ion paired BPA is moves from the aqueous phase to the organic phase (methylene chloride/isooctane) as an ion paired extraction. Then, BPA is alkylated with PFBBr in the organic solvents to give the PFB–BPA. After evaporation of organic solvent, the residue is dissolved in adequate organic solvent. In this PFB derivatization of BPA, the effect of extraction of ionpaired BPA and PFB–BPA were examined by use of various organic solvents. In the solvents for extraction of ion paired BPA and PFB–BPA, isooctane was most effective solvent as shown in Fig. 3. An optimum volume of isooctane for extraction of ionpaired BPA was obtained as shown in Fig. 4, 1 ml of isooctane was used as extraction volume.

3.3. Analytical parameters

Calibration curve for standard samples treated according to the analytical procedure described above were made using SIM mode. There were linear for the concentration range 0.01–100 ng/ml of BPA (Fig. 5). Good linearity of 0.998 as correlation coefficient at

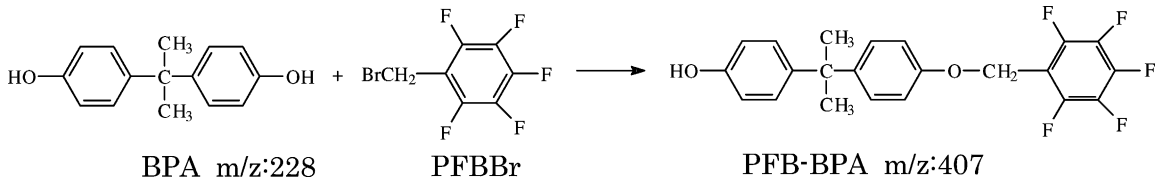

Fig. 2. PFB derivatization of bisphenol A.

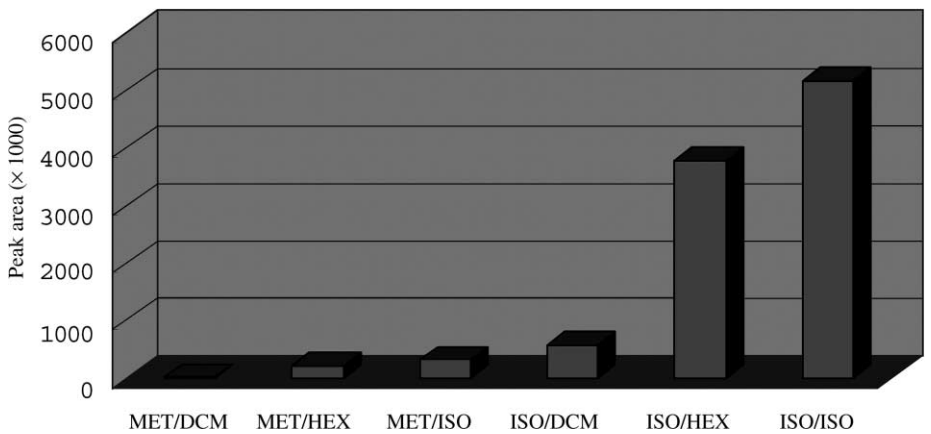

Fig. 3. Effect of various solvents on the extraction of the ion paired bisphnol A and PFB-bisphnol A: MET, methanol; DCM, dichloromethane; HEX, hexane, and ISO, isooctane.

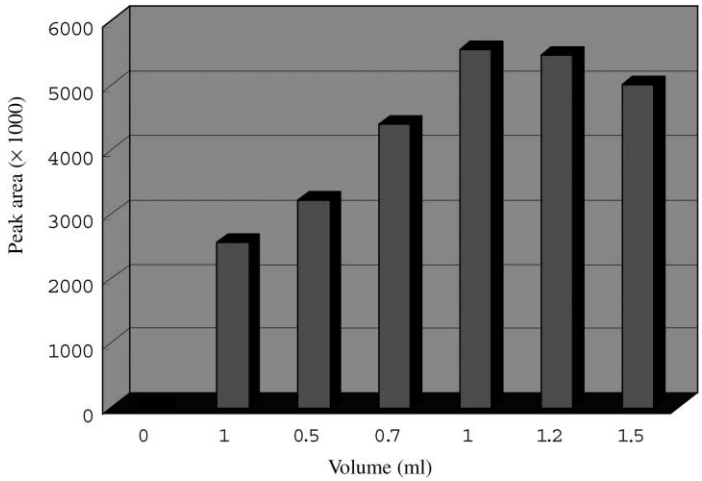

Fig. 4. Effect of volume of isooctane on the extraction of PFB-bisphenol A.

the range of 0.01–100 pg/ml of BPA was obtained. Detection limit and quantification limit for BPA were 5 pg/ml (10 fg BPA) as $s/n = 3$ and 15 pg/ml as $s/n = 10$, respectively. Reproducibility of this analytical method using 1 and 0.1 pg/ml of BPA were 4.76 and 5.42% as R.S.D. ($n = 6$), respectively.

In the other hand, a peak of PFB–BPA by using of GC/ECD was observed at 54.33 min. The detection limit for BPA was 0.15 pg/ml as $s/n = 3$. Therefore, it was found that sensitivity of this analytical method using GC/MS with SIM was 30 times as that of GC/ECD. Sajiki reported that the detection limit of BPA by use of HPLC/ECD and HPLC/MS were 0.2 ng/ml and 0.1 pg/ml, respectively.

In the proposed method, PFB–BPA has five fluorine in the molecular, resulted in negative ion by chemical

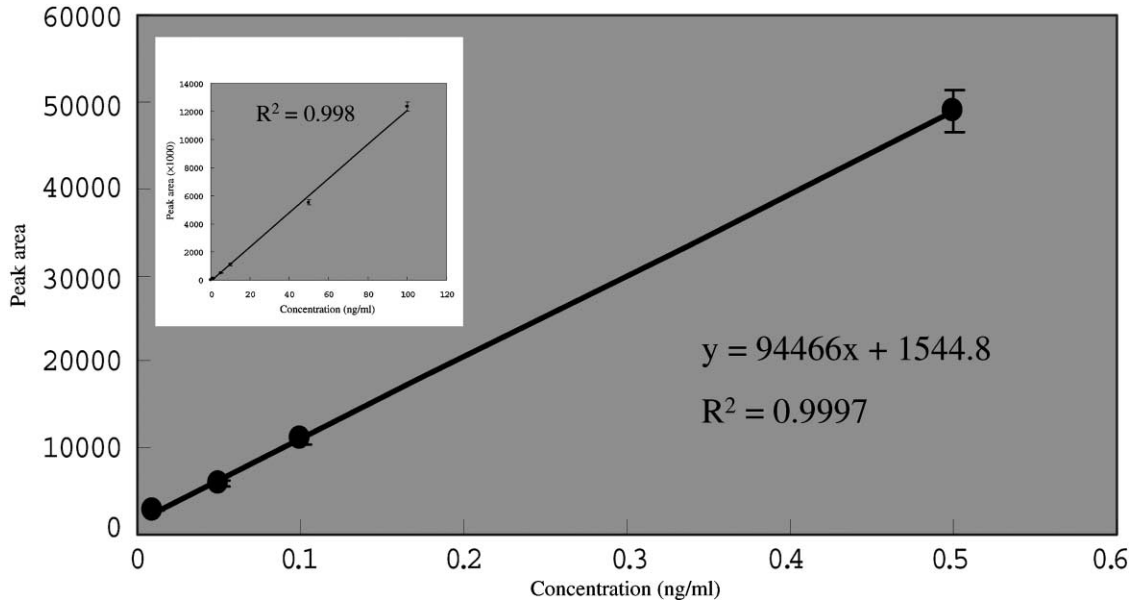

Fig. 5. Calibration curve of PFB-bisphnol A between 0.01 and 100 pg/ml.

Table 1
Content of bisphenol A in unspiked human sera

Pool	Bisphenol A (ng/ml)	Pool	Bisphnol A (ng/ml)
1	0.43	11	0.47
2	0.45	12	0.49
3	0.46	13	0.60
4	0.43	14	0.43
5	0.41	15	0.84
6	0.39	16	0.46
7	0.41	17	0.92
8	0.79	18	0.57
9	0.44	19	0.79
10	0.45	20	0.66

Average: 0.54 pg/ml. Serum specimens were collected anonymously and then polled from at least five persons.

ionization. Therefore, it is obtained high sensitivity by GC/MS with NCI mode.

3.4. Determination of BPA in the serum

Calibration curve of spiked sample added BPA in the range of 0.1–10 pg/ml using $^{13}C_{12}$-ring-labeled BPA as internal standard was examined. The good relationship of 0.999 was observed in this range. The recovery tests and R.S.D. for the human serum showed 101.0, 5.4 (1 pg/ml), and 100.9, 3.7% (10 pg/ml), respectively. Therefore, the proposal method using spiked serum sample could be determined without the matrix effect, had a straight the origin which passes through the origin. As actual samples, concentration of BPA in the human serum collected anonymously and then polled from at least five persons were 0.39–0.92 and 0.54 pg/ml ($n = 5$, R.S.D. 5.0%) as average of 20 human serum as shown in Table 1.

4. Conclusion

This paper provides development of a high sensitive method by GC/MS with NIC mode for the determination of BPA. The sample treatment was a simple by using C_{18} column as SPE, followed by derivatization of BPA with PFBBr. This method was applied to human serum with good recoveries and precision. It is suggested that this method is very useful for determination of the trace BPA in the human serum.

Acknowledgements

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