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ORIGINAL PAPER

Determination of bisphenol A in Iranian packaged milk by solid-phase extraction and HPLC

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Abstract A solid-phase extraction (SPE) method for sample cleanup and preconcentration of bisphenol A in Iranian milk packages and its determination by HPLC–UV is presented. Optimum conditions for SPE were 10 cm^3 water/methanol (80:20, v/v) at pH 7 as washing solvent and 4 cm^3 methanol for elution, providing good cleanup and high recovery (>97%) for the analyte. Maximum loading capacity and breakthrough volume for the SPE were 600 μg and 750 cm^3 of 5 ng/ml for bisphenol A, respectively. The best mobile phase used for HPLC was water/methanol (30:70, v/v) at pH 6.5 (adjusted with phosphate buffer) at 25 °C. The limit of detection, linear range, and repeatability of retention time and peak height were 0.039, 0.50–50.0 μg/cm^3 ($r^2 > 0.99$), 3.2, and 2.4%, respectively. Recoveries of bisphenol A ranged between 97 and 102%. Precision of the overall analytical procedure was estimated by five replicate measurements for bisphenol A in milk samples and ranged from 0.472 to 1.014 mg/dm^3.

Keywords Bisphenol A · Milk · Solid-phase extraction · HPLC–UV

Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA, Fig. 1) is a member of the class of diphenols in which two phenolic rings are joined together through a bridging group, in this case isopropylidene [1]. BPA is a primary raw material used for the production of polycarbonates, epoxy resins, phenolic resins, polyesters, and polyacrylates [2]. Bisphenol A is also used as a stabilizing material or antioxidant for many types of plastics, such as poly(vinyl chloride) [3]. Polycarbonates (PC) are characterized by great strength, stability, elasticity, and low density. For these reasons, they have widely been used for the production of food and pharmaceutical packaging, bottles for infants, kitchen utensils, medical equipment, computers, electronic devices, and in the production of dental fillings [4–7]. BPA residues were detected in food products stored in packages made of PC [8].

Recent research indicates that BPA and its derivatives have high potential as endocrine disruptors in humans and wildlife [9]. In addition, it was reported that BPA exhibits estrogenic activity in vitro at concentrations of 10–25 nM (2–5 ng/cm^3), competing with [^{3}H]estradiol for binding to estrogen receptors from rat uterus [10]. It was established that BPA can be liberated from PC containers and migrate into the food products kept in them [11]. Hence, the potential adverse effect of BPA on human health through beverage and food consumption has generated great concern during recent years.

Several analytical techniques were developed to allow HPLC-based determination of BPA, e.g., microwave-assisted extraction and centrifugation [12], liquid–liquid extraction [13], soxhlet [14], and alkaline digestion followed by SPE [15]. The SPE strategy comprises the isolation and preconcentration of the analyte from a complex matrix by adsorption onto an appropriate sorbent, removal of interfering impurities by washing with a suitable solvent system, and selective recovery of the retained analyte with a suitable solvent. BPA has usually been measured by means of chromatographic techniques, mainly HPLC [16–18] with MS [19–21], more frequently, fluorescence [22–26] and UV detection [27].

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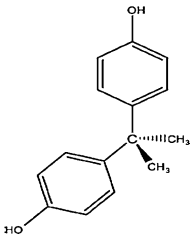

Fig. 1 Structure of BPA (MW 228.29)

Although fluorescence is one of the more frequent detection methods used for determination of BPA, it usually requires a tedious sample preparation to increase the selectivity and sensitivity to complex matrices. Moreover, derivatization of BPA has been proposed to increase sensitivity with fluorescence detection, but this adds further extraction and reaction steps [28]. In contrast, GC–MS allows identification with a high degree of confidence, but a time-consuming derivatization step is required. Because of its thermal instability, bisphenol A should be trimethylsilylated before GC analysis, but derivatization reactions can cause problems, especially in the handling of natural products. MS detection is complicated and it is difficult to maintain high stability of analytical results, especially for low concentrations of analyte [29, 30]. Additionally, this detection method is not available everywhere and is expensive.

This work presents a method for the determination of BPA in Iranian milk packages by isocratic HPLC with UV detection using solid-phase extraction. Appropriate conditions for SPE (optimization of washing solution, selection of a suitable solvent and its volume for elution of BPA, maximum loading capacity, and breakthrough volume) and HPLC (percentage of organic modifier, pH of mobile phase, and temperature) were examined and used for determination of BPA in packaged milk samples manufactured in Iran.

Results and discussion

Determination of breakthrough volume and maximum loading capacity of solid-phase cartridge for BPA

The breakthrough volume of the cartridges was determined by Hennion's procedure [31]. Thus, 5 μg of a BPA standard was dissolved in 50–1,500 cm^3 of doubly distilled deionized water (adjusted to pH 3 with concentrated HCl) and passed through the cartridge. The maximum loading capacity of solid-phase cartridges for adsorption of BPA was determined by passing different volumes (10–100 cm^3) of 10 $\mu g/cm^3$ of each aqueous standard solution (adjusted to pH 3 with concentrated HCl) through the cartridge. In both cases, the retained analyte was eluted with 4 cm^3 methanol and dried by evaporation at 50 °C in vacuo. The residue was reconstituted in 1 cm^3 methanol and injected into the HPLC system. Figure 2 shows that the breakthrough volume was 750 cm^3. According to Fig. 3 the maximum loading capacity for BPA was 600 μg.

Optimization of solid-phase extraction

Isolation of BPA from the milk matrix is a prerequisite to any chromatographic determination. Solid-phase extraction using C_{18} is a simple technique which can be employed for extraction of BPA from milk. Use of a carefully chosen washing solution in the SPE process to provide a milk extract free from interfering matrix components leads to enhanced selectivity in the separation step and more accurate determination. To achieve this purpose, the washing solution must contain the appropriate amount of organic solvent and highest possible pH (in the recommended range for stability of the sorbent) to remove interfering matrix components (fatty acids and other polar compounds) without eluting the analyte. SPE parameters

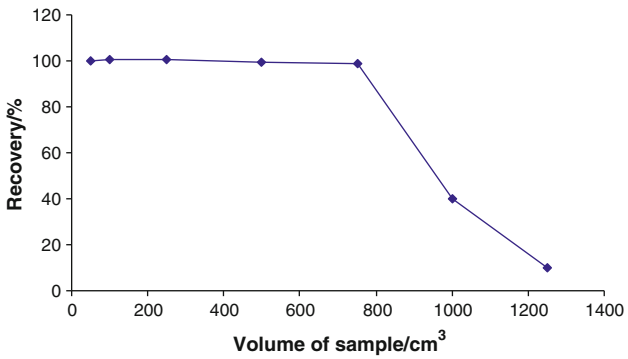

Fig. 2 Breakthrough volume of the cartridge. Conditions: sample loaded 100–1,250 cm^3 of standard solutions containing 5 μg of BPA; flow rate 2.4 cm^3/min; cartridge RESPREP C_{18}. HPLC: mobile phase water/methanol (30:70, v/v); pH 6.5; flow rate 1 cm^3/min; column C_{18} (250 × 4.6 mm, 10 μm); λ 282 nm; room temperature

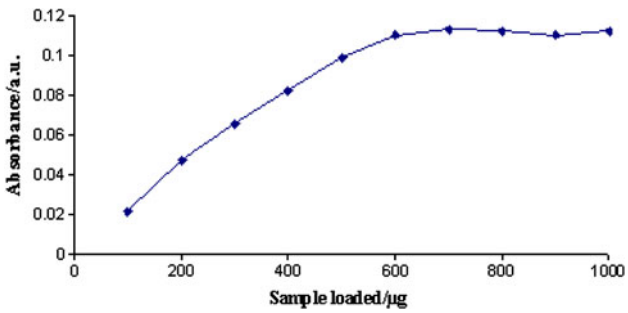

Fig. 3 Maximum loading capacity of RESPREP C_{18} cartridge for adsorption of BPA. Conditions: sample loaded 10–100 cm^3 of 10 ppm BPA solution; flow rate 2.4 cm^3/min; elution solvent 4 cm^3 of methanol; other conditions similar to Fig. 2

such as percentage of methanol in the washing solution and its pH, type of elution solvent and its volume were investigated.

For optimizing the washing solution, different percentages of methanol in water at different pH ranging from 2.5 to 7.5 were examined. According to Fig. 4 the optimum conditions for the washing solution were 20% methanol, pH 7, and 10 cm^3 washing solution volume.

To obtain a suitable solvent for elution of BPA from the cartridge, different solvents such as methanol, acetonitrile, ethyl acetate, diethyl ether, and acetone were examined. The best elution solvent for BPA was found by using 5 cm^3 of each solvent (Fig. 5a). The best recovery of BPA was achieved by methanol. For determination of a suitable volume of the elution solvent, different volumes (2, 4, 6, 8, and 10 cm^3) of methanol were used for elution of retained BPA from the cartridge. The most suitable volume of elution was 4 cm^3 (Fig. 5b).

The optimum SPE conditions were evaluated in the analysis of real samples. The recovery percentage of the analyte in milk samples was measured by means of spiking. The milk sample was spiked with 0.5 and 5.0 μg/cm^3 of BPA. The recoveries of BPA in the milk samples are shown in Table 1.

Optimized conditions for determination of BPA in milk

In order to select a suitable mobile phase for determination of BPA, methanol and acetonitrile were examined as organic modifier. Methanol provided better results than acetonitrile owing to the much more effective interaction between methanol and analyte rather than acetonitrile. Therefore, methanol was chosen as organic modifier in the mobile phase. According to the nature of the analyte, phosphate buffer (pH 3.0–7.5) was used. The best peak shape was obtained by a mobile phase with phosphate buffer at pH 6.5.

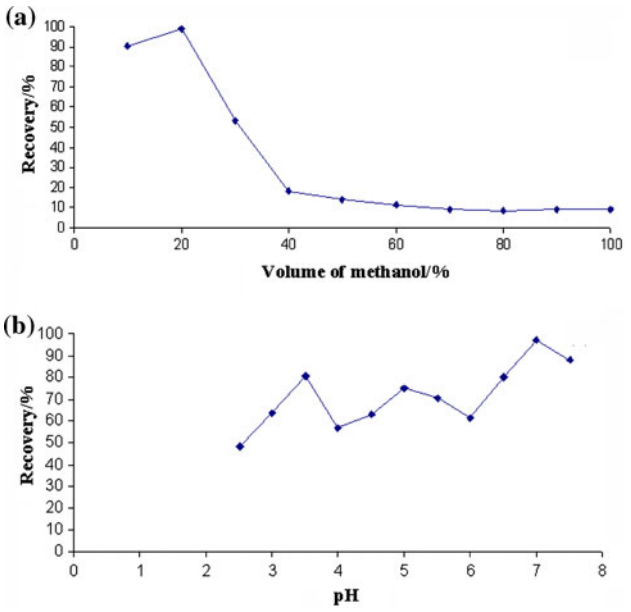

Fig. 4 Selection of volume and pH of washing solvent for SPE using HPLC. **a** Recovery against volume percentages of organic solvent; **b** recovery against pH of washing solvent. Conditions: sample loaded 20 cm^3 of 2 ppm BPA solution; other conditions similar to Fig. 2

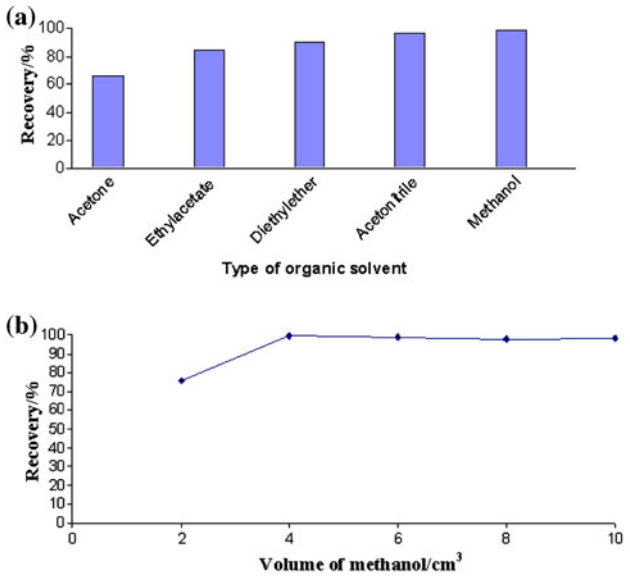

Fig. 5 Selection of type and volume of elution solvent for SPE using HPLC. **a** Recovery against type of organic solvent; **b** recovery against volume of methanol. Conditions: sample loaded 20 cm^3 of 2 ppm BPA solution; elution volume for **a** = 5 cm^3; other conditions similar to Fig. 2

Table 1 Recovery of BPA after SPE extraction

Aqueous standard 2.0 μg/cm^3 ($n = 5$)	Milk sample spiked at 0.5 μg/g ($n = 3$)	Milk sample spiked at 5.0 μg/g ($n = 3$)
98.5 ± 2.1	97.8 ± 2.8	101.4 ± 3.1

Conditions: mobile phase water/methanol (30:70, v/v); pH 6.5; flow rate 1 cm^3/min; column C_{18} (250 × 4.6 mm, 10 μm); injection volume 10 mm^3; λ 282 nm; temperature 25 °C

Values are % recovery ± SD

Determination of BPA in milk

Four different brands of packaged milk (purchased from local supermarkets in Babolsar in November and December 2010) were used. The concentration of BPA in milk samples was determined by five replicate measurements using the standard addition method ($n = 5$). Table 2 presents the coating type of the food contact surface of the packaged milk and the concentration of BPA found in the

Table 2 Type of package and the concentration of BPA found in the milk

Milk	Body lacquer	Concentration of BPA (mg/dm^3)
Brand A	Epoxy resin	0.472 ± 0.043
Brand B	Epoxy resin	0.617 ± 0.059
Brand C	Epoxy resin	1.014 ± 0.066
Brand D	Epoxy resin	0.702 ± 0.053

Conditions: mobile phase water/methanol (30:70, v/v); pH 6.5; flow rate 1 cm^3/min; column C_{18} (250 × 4.6 mm, 10 μm); injection volume 10 mm^3; λ 282 nm; temperature 25 °C

milk. BPA was identified by spiking the sample with standard BPA and comparing its retention time with that of the standard. Typical chromatograms of standard BPA, milk sample, and spiked milk sample at optimum mobile phase conditions are presented in Fig. 6.

There was a considerable difference between the obtained results in comparison with previous studies that report BPA concentrations in powdered milk and milk between less than 1.7 and 15.2 ng/g [20] and 0.28 and 2.93 ng/g [32]. The results suggest that the levels of BPA in Iran are likely to be of concern, according to the recently established specific migration limit of 0.6 mg/dm^3. However, considering that the estrogenic activity of BPA at low levels is under discussion [33] and that milk is the main nourishment of babies, determination of BPA in milk samples is essential.

Figures of merit

Limit of detection (LOD), linear range (LR), and repeatability of retention time and peak height (eight injections) for BPA were determined. The limit of detection was calculated on the basis of $3S_b/m$, where S_b is the standard deviation of blank and is equal to P–P noise when only mobile phase was passed through the column for 45 min, and m is the slope of the calibration curve. The calibration curve was obtained by triplicate injection of standard solution with a correlation coefficient of $r^2 > 0.99$. The values of LOD, LR, and repeatability of retention time and peak height for BPA were 0.039, 0.50–50.0 μg/cm^3 ($r^2 > 0.99$), 3.2, and 2.4%, respectively.

Conclusions

The combination of SPE and HPLC–UV analysis permitted a sensitive, reliable, and rapid determination of trace BPA in packaged milks. SPE parameters (selection of a suitable solvent and its volume for elution of BPA, maximum loading capacity of C_{18} cartridge, and breakthrough volume) and HPLC conditions (percentage of organic modifier, pH of mobile phase, and temperature) were optimized. The recoveries of analyte were over 97%. Good resolution and the analysis time (approximately 6 min) as well as the simplicity of HPLC and the low-cost, fast, and simple sample preparation made this method a useful tool for the routine analysis of BPA in milk samples.

Materials and methods

Chemicals and stock solutions

Bisphenol A was purchased from Sigma–Aldrich (Steinheim, Germany). HPLC-grade acetonitrile and methanol used were from Fluka (Buchs, Switzerland). Glacial acetic acid, NaOH, NaH_2PO_4, and HCl were obtained from Merck (Darmstadt, Germany). Water used was doubly distilled and deionized. All mobile phases were filtered by a 0.45-μm membrane (Millipore, Bedford, MA, USA).

The stock solutions of BPA (500 mg/dm^3) were prepared in methanol and stored in the dark at 4 °C. These solutions were stable for at least 3 months. All solutions were filtered through 0.45-μm membranes (Millipore) prior to use. Milk samples were provided from local supermarkets from Babolsar in the north of Iran.

Apparatus and conditions

The chromatographic measurements were carried out with an HPLC system equipped with a series 10 LC pump, UV detector model LC-95 set at 282 nm, and a model 7125 manual injector (PerkinElmer, Norwalk, CT, USA). Adjustment of pH of the HPLC mobile phase was carried out by a model 3030 Jenway pH meter (Leeds, UK). The column used was a C_{18} (250 × 4.6 mm, 10 μm) from Waters (Milfold, MA, USA). The SPE cartridge was a RESPREP C_{18} (6 cm^3, 500 mg) from Restek (Bellefonte, PA, USA).

Sample preparation and solid-phase extraction

Milk samples (5 cm^3) were diluted with 20 cm^3 of water/methanol (17:3, v/v), adjusted to pH 3 with concentrated HCl and stirred with a magnetic stirrer at room temperature for 10 min. The addition of water reduces the viscosity of the sample, thus a better flow is achieved during SPE. Adjustment of solution to pH 3 prevents ionization of BPA. The addition of methanol aims to destabilize the milk's emulsion [31]. The solution was filtered through a 0.45-μm membrane. The solid-phase C_{18} cartridge was sequentially conditioned with 5 cm^3 of *n*-hexane, 5 cm^3 of methanol, and 10 cm^3 of doubly distilled deionized water without allowing the cartridge to dry. The filtrate was passed

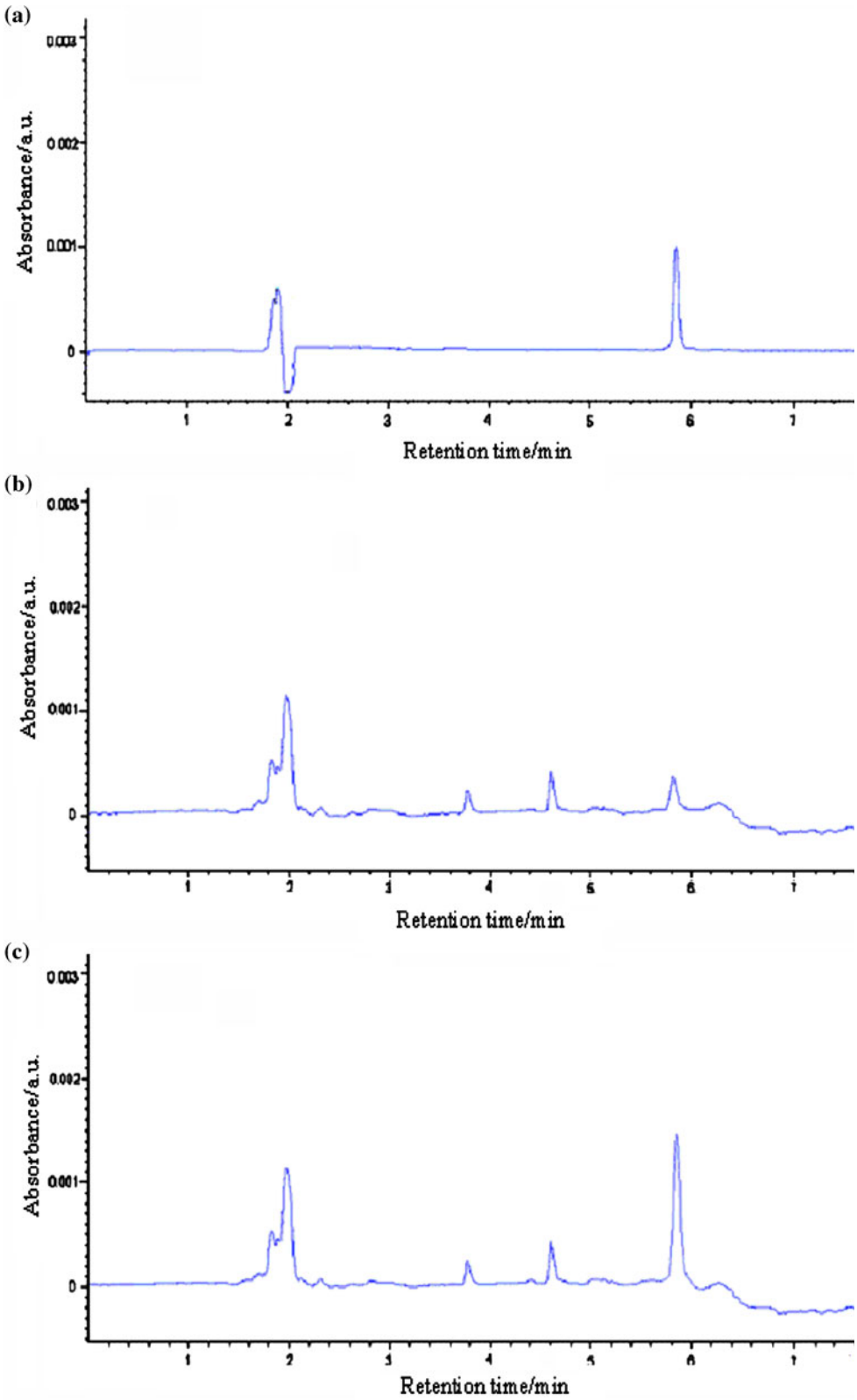

Fig. 6 Typical chromatograms: **a** standard (5 μg/cm^3), **b** unspiked milk sample (brand A), and **c** spiked milk sample (brand A) with 5 μg/cm^3. Conditions: mobile phase water/methanol (30:70, v/v); pH 6.5; flow rate 1 cm^3/min; column C_{18} (250 × 4.6 mm, 10 μm); injection volume 10 mm^3; λ 282 nm; temperature 25 °C

through the cartridge, rinsed with 20 cm^3 of water to remove all sugars and other polar constituents of milk, washed with 10 cm^3 water/methanol (80:20, v/v) to remove interferences, and eluted with 4 cm^3 HPLC-grade methanol. The eluate was dried under reduced pressure in a rotary evaporator at 50 °C and reconstituted in 1 cm^3

methanol, filtered through a 0.45-μm syringe filter, and injected into the HPLC system. Under these conditions the BPA peak separated well from interferences and had a retention time of about 5.6 min.

Chromatographic conditions for determination of BPA

The HPLC determination of BPA was performed using a mobile phase consisting of water/methanol (30:70, v/v) with a flow rate of 1.0 cm^3/min at room temperature. Before use, all mobile phases were passed through a 0.45-μm membrane filter and degassed under vacuum. The sample injection volume was 10 mm^3, and the analyte was monitored at 282 nm.

References

1. Rykowska I, Wasiak W (2006) Acta Chromatogr 16:7
2. Perez P, Pulgar R, Olea-Serrano F, Villalobos M, Rivas A, Metzler M, Pedraza V, Olea N (1998) Environ Health Perspect 106:167
3. Yamamoto T, Yasubara A (1999) Environ Health Perspect 38:2569
4. Olea N, Pulgar R, Perez P, Olea-Serrano MF, Rivas A, Novillo-Fertell A, Pedraza V, Soto AM, Sonnenschein C (1996) Environ Health Perspect 104:298
5. Staples CA, Dorn PB, Klecka GM, Branson DR, O'Block ST, Harris LR (1998) Chemosphere 36:2149
6. Pulgar R, Olea-Serrano MF, Novillo-Fertell A, Rivas A, Pazos P, Pedraza V, Navajas J-M, Olea N (2000) Environ Health Perspect 108:21
7. Kuo HW, Ding WH (2004) J Chromatogr A 1027:67
8. Summerfield W, Goodson A, Cooper I (2004) Food Addit Contam 21:1015
9. Birn Baun LS, Staskal DF (2004) Environ Health Perspect 112:9
10. Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D (1993) Endocrinology 132:2279
11. Szymansky A, Rykowska I, Wasiak W (2006) Acta Chromatogr 17:161
12. Pedersen SN, Lindholst C (1999) J Chromatogr A 864:17
13. Toyo'oka T, Oshige Y (2000) Anal Sci 16:1071
14. Meester RJW, Schröder HF (2002) J Anal Chem 74:3566
15. Yasuhara A, Otaka H, Morita M (2003) Anal Sci 19:1663
16. Sajiki J, Takahashi K, Yonebuko J (1999) J Chromatogr B 736:255
17. Inoue K (2000) Prog Polym Sci 25:453
18. Uematsu Y, Hirata K, Suziki K, Iida K, Saito K (2001) Food Addit Contam 18:177
19. Berger U, Oehme M, Giradin L (2001) Fresenius J Anal Chem 369:115
20. Thomaidis NS, Maragou NC, Lampi EN, Kouparis MA (2006) J Chromatogr A 1129:165
21. Wei Y, Ying L, Lixia Z, Jig-Ming L (2009) J Chromatogr A 1216:7539
22. Losado PP, Lamela CP, Fabel MFL, Fenollera PS, Lozano JS (1997) J Agric Food Chem 45:3493
23. Lintschinger J, Rauter W (2000) Eur Food Res Technol 211:211
24. Theobald A, Simoneau C, Hannaert P, Roncari A, Rudolph T, Anklam E (2000) Food Addit Contam 17:881
25. Kang J-H, Kondo F (2003) J Food Protect 66:1439
26. Xiaoyan L, Yongsheng J, Haixia Z, Mancang L (2008) Food Addit Contam 25:772
27. Moon HJ, Lim BS, Lee YK, Kim COW (2000) J Korean Chem Soc 21:1115
28. Sun Y, Trie M, Kishikava N, Wada M, Kurod N, Nakashima K (2004) Biomed Chromatogr 18:501
29. Del Olmo M, Gonzalez CA, Navas NA, Vilchez JL (1997) Anal Chim Acta 346:87
30. Hammarling L, Gustavsson H, Svensson K, Oskarsson A (2000) Food Addit Contam 17:937
31. Hennion MC (2000) Sample handling and trace analysis of pollutants techniques, application and quality assurance. Elsevier, Amsterdam, p 4
32. Casajuana N, Lacorte S (2004) J Agric Food Chem 52:3702
33. Scientific Committee on Food (2002) Opinion of the Scientific Committee on Food on bisphenol A, SCF/CS/PM3936. European Commission, Brussels