Study on the migration of bisphenols from plastic food contact materials to food simulants

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Abstract. A rapid method for the detection of bisphenols was established by ultra performance convergence chromatography (UPC2). The migration rules of bisphenols in four different simulants including of pure water, acetic acid, ethanol and isooctane were first studied, and the effect of extraction solvent on recovery of bisphenols in aqueous simulants was also investigated. Furthermore, the influence of extraction solvent on recovery of bisphenols in aqueous simulants was discussed, and the relationship between migration amount of bisphenols and food simulants, migration time, migration temperature and other factors were explored. The experimental results showed that toluene exhibited the optimal extraction efficiency for bisphenols in aqueous simulants; bisphenols presented the most obvious migration in fatty food simulants; the migration amount of bisphenols increased with the extension of time; the migration rate and amount of bisphenols also enhanced with the increment of contact temperature.

1. Introduction

With the increasing public attention for food safety, higher requirements are put forward for food contact materials which are closely related to food quality. Bisphenols are a new class of hazardous migratory substances. These compounds are important raw materials for the production of polycarbonate, epoxy resin and other polymer materials [1-3]. Bisphenol A (BPA) is currently the largest amount of a kind of bisphenol substances, which is widely used in various of plastic food contact materials [4]. Studies showed that BPA has estrogen-like activity mutagenic, endocrine disrupting and carcinogenic effects [5-10]. Therefore, the European Union, United States, China and other countries have stipulated and restricted the use of BPA in food contact materials.

In recent years, the bisphenols which have similar structure with BPA were regarded as the substitutes for BPA, which have been gradually applied in the production of plastic food contact materials [11-14]. At present, bisphenol F (BPF) and bisphenol S (BPS) are commonly used to replace the use of bisphenol A, but researches have shown that BPF and BPS are also harmful to human health [15-17]. As an environmental hormone, bisphenol A-diglycidyl ether (BADGE) is destructive to endocrine system and immune system, as well as the genotoxicity and mutagenesis [18]. Tetrabromobisphenol A (TBBPA) and hexafluorobisphenol A (BPAF) also have reproductive toxicity which could lead to fetal dysplasia [19,20]. According to the national standard GB 9685-2016, the specific migration amount of BPA in food should not exceed 0.6 mg/kg, and that of bisphenol S
should not exceed 0.05 mg/kg. The EU stipulates that the total migration of BADGE and its hydrolyzed derivatives in food shall not exceed 9 mg/kg. However, relevant regulations on the migration of BPF, BPAF and other commonly used bisphenols have not been formulated.

Due to the similar structure and great variety of bisphenols, together with the complexity of food matrix, the detection interference of trace bisphenols is serious. Therefore, it is urgent to establish a detection method that can simultaneously analyze a variety of bisphenols and solve the problems of low extraction efficiency and large impurity interference in the sample pretreatment method. In this study, a detection method for four bisphenols (BPF, BPAF, BADGE and TBBPA) in plastic food contact materials was established by using the solid-phase extraction (SPE) and ultra performance convergence chromatography, and its migration behavior in food simulants was studied, which could provide data reference and technical support for testing and monitoring the migration of bisphenols in food contact materials.

2. Experimental and Methods

2.1. Materials and reagents

4,4'-dihydroxydiphenyl methane (BPF), 2,2-bis (4-hydroxyphenyl) hexafluoropropane (BPAF), 2,2-bis (2,6-dibromo-4-hydroxyphenyl) propane (BADGE), 2,2-bis (2,6-dibromo-4-hydroxyphenyl) propane (TBBPA), they were all supplied from Dr. Ehrenstorfer (Augsburg, Germany). HPLC grade methanol, isopropanol, acetonitrile, methylene chloride, ethyl acetate and n-hexane were purchased from Merck (Darmstadt, Germany).

2.2. Instruments and equipment

ACQUITY UPC² ultra performance convergence chromatography (Waters, USA); N-EVAP-112 nitrogen blower (Organomation, USA); BILON-2000CT ultrasonic cleaner (Bilang, Shanghai); BSXT-06 soxhlet extractor (Lanyi, Shanghai).

2.3. Methods

2.3.1. Chromatographic conditions

Column: ACQUITY UPC² Torus DIOL column (150 mm×30 mm, 1.7 μm); Column temperature: 35°C; Mobile phase: supercritical CO₂- methanol; Detection wavelength: 220 nm; Back pressure: 1800 psi; Injection volume: 3 μL. Elution gradient: 0~1 min, 5%~9% B; 1~2 min, 9%~10% B; 2~2.5 min, 10%~20% B; 2.5-5 min, 20% B; 5~5.5 min, 20%~5% B. The flow rate was maintained at 1.3 mL/min.

2.3.2. Migration experiment

(1) Soaking treatment

The plastic food contact materials samples were sealed and soaked at in food simulants (water, 3% acetic acid, 10% ethanol and isooctane) at temperature of 4, 20, 40, 60 or 80°C, and the simulated fluid were taken at the time points of 2, 4, 8, 12, 24, 48, 120 and 240 h, respectively. After the temperature was restored to room temperature, the pretreatment was carried out according to the method as follows.

(2) Pretreatment of simulants

Aqueous simulant: 10 mL aqueous simulant and 10 mL organic solvent (toluene, n-hexane, dichloromethane or ethyl acetate) were taken into the separating funnel, oscillated for extraction and repeated twice. After that, combined the organic phase and dried with nitrogen flow at 45°C, then dissolved the residue with methanol and diluted to volume of 1.0 mL for UPC² analysis.

Isooctane simulant: 10 mL isoocctane simulant was directly dried with nitrogen flow at 45°C, the residue was dissolved with methanol and diluted to volume of 1.0 mL for UPC² analysis.
3. Results and Discussion

3.1. Linearity, LODs, and LOQs
Standard mixture solutions with concentrations of 0.2, 1.0, 5.0, 20.0, 50.0 and 100.0 mg/L were prepared for constructing the calibration curves. External standard method was used for quantitative analysis. The chromatographic peak area $Y$ of each substance was used for linear regression of mass concentration $X$. The results showed that the correlation coefficients of the four bisphenols were all greater than 0.999. In the blank food simulants without bisphenols, standard mixture solutions of different concentrations were successively added, and the detection limits (LODs) were calculated according to three times signal-to-noise ratio ($S/N=3$). The LODs of the four bisphenols in the aqueous simulants (water, 3% acetic acid and 10% ethanol) was 0.10 mg/L, and the LODs in the isooctane simulants was 0.05 mg/L.

3.2. Migration experiment

3.2.1. The Selection of extraction solvent for food simulants
In the experiment, the extraction solvent for the four bisphenols in aqueous food simulants (water, 3% acetic acid and 10% ethanol) were firstly investigated. The blank aqueous simulants were added with high, medium and low concentrations of standard mixture, respectively. Toluene, n-hexane, dichloromethane and ethyl acetate were used as extraction solvents. The extraction efficiency in acidic and alcoholic food simulants were similar to those in pure water substrate. As shown in Table 1, the recoveries of bisphenols extracted by n-hexane were the lower than 65.0%, while the recoveries extracted by toluene were the highest (>85.0%). Under the condition of toluene extraction, the recoveries of the four bisphenols in the pure water simulant were ranged from 80.8% to 94.5%, and the relative standard deviations (RSDs) were in the range of 2.69%~6.17%, indicating that the toluene extraction method for food simulants in this experiment can meet the actual test requirements. Therefore, toluene was selected as the optimal solvent for extraction of bisphenols in aqueous simulants.

| Extracting solvent | Spiked level (mg/L) | R% | RSD% |
|--------------------|---------------------|----|------|
|                    |                     | BPF | BPAF | BADGE | TBBPA | BPF | BPAF | BADGE | TBBPA |
| toluene            | 1                   | 84.3 | 87.6 | 80.8 | 81.1 | 6.07 | 5.19 | 6.17 | 4.68 |
|                    | 50                  | 89.4 | 90.8 | 86.4 | 84.0 | 4.29 | 5.03 | 4.81 | 3.15 |
|                    | 100                 | 94.5 | 91.6 | 89.7 | 90.2 | 2.94 | 3.58 | 3.10 | 2.69 |
| n-hexane           | 1                   | 41.3 | 46.4 | 51.8 | 49.6 | 8.16 | 7.49 | 6.40 | 6.84 |
|                    | 50                  | 50.5 | 56.7 | 59.4 | 60.1 | 6.08 | 4.28 | 5.64 | 4.36 |
|                    | 100                 | 61.9 | 59.2 | 64.5 | 63.4 | 4.15 | 3.16 | 3.49 | 3.30 |
|                    | 1                   | 66.8 | 71.0 | 73.6 | 69.4 | 7.11 | 6.18 | 5.66 | 5.94 |
| dichloromethane    | 50                  | 75.9 | 78.4 | 80.2 | 73.1 | 5.02 | 5.34 | 4.09 | 3.27 |
|                    | 100                 | 84.9 | 82.2 | 85.1 | 80.6 | 3.55 | 2.99 | 4.18 | 3.05 |
| ethyl acetate      | 1                   | 70.8 | 75.1 | 77.4 | 69.2 | 6.22 | 6.67 | 5.61 | 5.06 |
|                    | 50                  | 73.5 | 76.0 | 80.7 | 78.8 | 3.58 | 5.44 | 5.03 | 4.39 |
|                    | 100                 | 81.9 | 83.3 | 85.1 | 82.6 | 2.87 | 4.21 | 3.47 | 3.77 |
3.2.2. The effect of simulants on the migration amount of bisphenols

In this experiment, plastic food contact materials samples were immersed in food simulants at 40°C for 24 h, respectively, the migration amount of bisphenols in different food simulants were shown in Figure 1. The results showed that only BPAF migrated in the pure water simulant, while BPF, BADGE and TBBPA were not dissolved. In the ethanol and isooctane food simulants, all the four bisphenols were dissolved in different degrees, and the maximum migration was obtained in isooctane. Therefore, it is not recommended to add bisphenols to plastic food contact materials with ethanol and fatty food.

![Figure 1](image1.png)

Figure 1. Migration of bisphenols in different simulants (40°C, 24 h).

3.2.3. The effect of time on migration amount of bisphenols

Because the migration behavior of bisphenols in isooctane was the most obvious, isooctane was chosen as the simulant in this experiment. Under the contact temperature of 40°C, the migration amount of four bisphenols in the contact materials of plastic food was investigated from 1 to 48 h. As shown in Figure 2, with the extension of contact time, the migration amount of bisphenols also increased. BPAF reached the migration balance at 36 h, while BPF, BADGE and TBBPA all reached the migration balance at 24 h.

![Figure 2](image2.png)

Figure 2. The effect of time on migration amount of bisphenols

4. Conclusions

UPC² was used to establish the detection methods of four bisphenols (BPF, BPAF, BADGE and TBBPA) in plastic food contact materials. The influence of extraction solvent on recoveries of bisphenols in aqueous simulants was investigated. It was found that the extraction efficiency of
toluene was the most satisfactory. The effects of simulants, migration time and migration temperature on the migration amount of bisphenols were investigated. Experimental results showed that the migration behaviors of the four bisphenols were the most obvious in isooctane. At the same temperature, the migration of bisphenols increased with the extension of time. The migration speed and migration amount of bisphenols increased significantly with the increase of migration temperature.

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References
[1] Biedermann-Brem S., Grob K., Fjeldal P. (2008) Release of bisphenol A from polycarbonate baby bottles: mechanisms of formation and investigation of worst case scenarios. Eur. Food Res. Technol., 227: 1053-1060.
[2] Shao B., Han H., Li D., et al (2007) Analysis of alkylphenol and bisphenol A in meat by accelerated solvent extraction and liquid chromatography with tandem mass spectrometry. Food Chem., 105: 1236-1241.
[3] Sun X., Peng J., Wang M., et al (2018) Determination of nine bisphenols in sewage and sludge using dummy molecularly imprinted solid-phase extraction coupled with liquid chromatography tandem mass spectrometry. J. Chromatogr. A, 1552: 10-16.
[4] Cacho J. I., Campillo N., Viñas P., et al (2012) Stir bar sorptive extraction coupled to gas chromatography–mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables. J. Chromatogr. A, 1247: 146-153.
[5] Vandenbergh L. N., Colborn T., Hayes T. B., et al (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrin. Rev., 33: 378-455.
[6] Huang Y. Q., Wong C. K., Zheng J. S., et al (2012) Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. Environ. Int., 42: 91-99.
[7] Chen D., Kannan K., Tan H., et al (2016) Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-a review. Environ. Sci. Technol., 50: 5438-5453.
[8] Clayton E. M., Todd M., Dowd J. B., et al (2011) The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ. Health Perspect., 119: 390-396.
[9] Zeng J., Kuang H., Hu C., et al (2013) Effect of bisphenol A on rat metabolic profiling studied by using capillary electrophoresis time-of-flight mass spectrometry. Environ. Sci. Technol., 47: 7457-7465.
[10] Matsuda S., Matsuzawa D., Ishii D., et al (2013) Perinatal exposure to bisphenol A enhances contextual fear memory and affects the serotonergic system in juvenile female mice. Horm. Behav., 63: 709-716.
[11] Chen M., Ike M., Fujita M. (2002) Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. Environ. Toxicol., 17: 80-86.
[12] Li Y., Burns K. A., Arao Y., et al (2012) Differential estrogenic actions of endocrine-disrupting chemicals bisphenol A, bisphenol AF, and zearalenone through estrogen receptor alpha and beta in vitro. Environ. Health Perspect., 120: 1029-1035.
[13] Cunha S. C., Fernandes J. O. (2010) Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquidliquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta, 83: 117-125.
[14] Pozdnyakov I. P., Guo L., Glebov M., et al (2010) Aqueous photochemistry of bisphenol E in the presence of beta-cyclodextrin. High Energy Chem., 45: 214-221.
[15] Helies-Toussaint C., Peyre L., Costanzo C., et al (2014) Is bisphenol S a safe substitute for bisphenol A in terms of metabolic function? An in vitro study. Toxicol. Appl. Pharmacol., 280: 224-235.

[16] Kinch C. D., Ibhaeziebo K., Jeong J. H., et al (2015) Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish. Proc. Natl. Acad. Sci., 112: 1475-1480.

[17] Eladak S., Grisin T., Moison D., et al (2015) A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril., 103: 11-21.

[18] Munguia-Lopez E. M., Soto-Valdez H. (2001) Effect of heat processing and storage time on migration of bisphenol A (BPA) and bisphenol A-diglycidyl ether (BADGE) to aqueous food simulant from Mexican can coatings. J. Agri. Food Chem., 49: 3666-3671.

[19] Li Y., Burns K. A., Arao Y., et al (2012) Differential estrogenic actions of endocrine-disrupting chemicals bisphenol A, bisphenol AF, and zearalenone through estrogen receptor alpha and beta in vitro. Environ. Health Perspect., 120: 1029-1035.

[20] Wang J., Xiao X., Chen T., et al (2016) High-performance liquid chromatography-ultraviolet method for the determination of total specific migration of nine ultraviolet absorbers in food simulants based on 1,1,3,3-tetramethylguanidine and organic phase anion exchange solid phase extraction to remove glyceride. J. Chromatogr. A, 1451: 58-66.