Food Chemistry 258 (2018) 206–213

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Solid phase extraction with high polarity Carb/PSA as composite fillers prior to UPLC-MS/MS to determine six bisphenols and alkylphenols in trace level hotpot seasoning

Hao Dong[a,b,*], Xiaofang Zeng[a,*], Weidong Bai[a,*]

[a] College of Light Industry and Food Sciences, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong 510225, China
[b] School of Food Science and Technology, South China University of Technology, Guangzhou 510640, China

ARTICLE INFO

Keywords:
Hotpot seasoning
Bisphenol
Alkylphenol
Solid phase extraction
Ultra high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

ABSTRACT

The present study reports an ultra high-performance liquid chromatography tandem mass spectrometry method for the simultaneous determination of six bisphenols (bisphenol A, bisphenol B and bisphenol F) and alkylphenols (4-nonylphenol, 4-*n*-nonylphenol and octylphenol) in hotpot seasoning. Samples were dispersed in *n*-hexane after addition of internal standards bisphenol A-d_4 and 4-*n*-nonylphenol-d_4. Sample solutions were then centrifuged, and the supernatants purified using solid phase extraction with high polarity Carb/PSA composite fillers. Six target analytes were separated on a Waters ACQUITY BEH C18 column by gradient elution with methanol and 0.05% ammonium hydroxide in water as the mobile phase, and determined under multiple reactions monitoring mode. The limits of detection and quantitation, matrix effect, recovery and precision of the method were investigated. Results were linear in the concentration range 0.1–250 μg/L for all compounds of interest, with $R^2 > 0.9950$. Limits of detection were in the range 0.1–0.4 μg/kg, and limits of quantitation were between 0.5 μg/kg and 1.0 μg/kg. The mean recoveries for negative samples at three spiked concentrations were in the range 87.9%–102.4%, and the intra-day precision and inter-day precision were in the ranges 2.1–8.2% and 4.8–11.2%, respectively. This method is accurate and sensitive, and had good clean-up characteristics, which might apply to screening and quantitation of target bisphenols and alkylphenols in hotpot seasoning.

1. Introduction

The presence of bisphenols and alkylphenols in foods, such as bisphenol A (BPA), 4-*n*-nonylphenol (4-*n*-NP) and octylphenol (OP), are of increasing concern because of their endocrine disrupting properties (Niu, Zhang, Wu, & Shao, 2011; Pastor-Belda, Viñas, Campillo, & Hernández-Córdoba, 2017; Pernica, Poloucká, Seifertová, & Šimek, 2015; Xian, Wu, Dong, Guo, Wang, & Wang, 2017). BPA is one of the most important monomers used worldwide in the manufacture of polycarbonates, phenol resins, polyesters, epoxy resins and also in polyester resin intermediates and flame retardants (Choi, Cho, Lee, Luu, & Guo, 2012; Gallo et al., 2017b; Matozzo, Gagné, Marin, Ricciardi, & Blaise, 2008). Some alternatives with physical and chemical properties similar to BPA have been adopted but also show high estrogenic activity (Gallart-Ayala, Moyano, & Galceran, 2011; Jiang et al., 2017; Regueiro & Wenzl, 2015). Among these structural analogues, bisphenol F (BPF) and bisphenol B (BPB) are used most commonly (Xian et al., 2017).

Alkylphenols are a family of compounds formed by a substituted phenolic ring and an alkyl chain. Among all alkylphenols, 4-NP and OP are the alkylphenols used most extensively in the production of textiles, plastic products, paper and agricultural chemical products (Salgueiro-González et al., 2012b; Salgueiro-González, Muniategui-Lorenzo, López-Mahía, & Prada-Rodríguez, 2017). With respect to 4-*n*-NP, although it is scarcely used for industrial purpose, it is present in aquatic systems and can easily be bio-accumulated (Lisboa et al., 2013).

The specific migration limit in foods for BPA is 0.6 mg/kg and the use of BPF is prohibited in the Commission Directive 2004/19/EC. As for alkylphenols, OP and 4-NP have been included in a list of 45 priority substances in European water legislation (Directive 2013/39/EU) in order to preserve the environment and guarantee public safety. Unfortunately, bisphenols and alkylphenols are already ubiquitous in the environment and foods due to their extensive use (Maragou, Lampi, Thomaidis, & Koupparis, 2006; Niu et al., 2011; Noonan, Ackerman, & Begley, 2011; Regueiro & Wenzl, 2015; Salgueiro-González et al., 2012b; Yang et al., 2014). Foods are the main source of overall exposure to bisphenols and alkylphenols, as these compounds can enter the food chain via environmental pollution and migration from food containers during processing and preservation (Liu et al., 2013).

* Corresponding authors at: College of Light Industry and Food Sciences, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong 510225, China.
E-mail addresses: xiaofang_zeng2015@163.com (X. Zeng), whitebai2002@163.com (W. Bai).

https://doi.org/10.1016/j.foodchem.2018.03.074
Received 29 April 2017; Received in revised form 4 March 2018; Accepted 17 March 2018
Available online 19 March 2018
0308-8146/

A number of techniques including solid phase extraction (SPE) (Gallart-Ayala et al., 2011; Maragou et al., 2006; Regueiro & Wenzl, 2015), stir bar sorptive extraction (SBSE) (Nguyen, Scapolla, Di Carro, & Magi, 2011), solid phase microextraction (SPME) (Salgueiro-González et al., 2012a), dispersive liquid-liquid microextraction (DLLME) (Cunha & Fernandes, 2013; Haeri, 2016; Luo et al., 2010), and dispersive micro solid phase extraction (DMSPE) (Jiang et al., 2017; Xian et al., 2017) have been used for the extraction of bisphenols and alkylphenols from different matrices. Among these techniques, SPE, which is based on the partition equilibrium of analytes between sorbent and samples, is used most frequently due to its enrichment and cleaning up capacities (Lisboa et al., 2013).

The main analytical techniques reported for determination of bisphenols and alkylphenols are gas chromatography-mass spectrometry (GC–MS) (Luo et al., 2010; Pastor-Belda et al., 2017; Sánchez-Brunete, Miguel, & Tadeo, 2009) or liquid chromatography (LC) coupled to an ultraviolet (UV), photodiode array (PDA) or fluorescence (FLD) detector (Alabi, Caballero-Casero, & Rubio, 2014; Gallo et al., 2017a; Haeri, 2016; Wu et al., 2014). However, a time-consuming derivatization step during sample preparation is required for GC–MS analysis and the risk of contamination of the samples is not insignificant. The accuracy and sensitivity of LC depends mainly on the detector, some of which may not meet anlytical requirements (Maragou et al., 2006). LC-MS/MS has been used widely in trace analysis of harmful compounds in foods (Dong & Xiao, 2017; Xian et al., 2016; Xian et al., 2017; Dong, Xiao, Xian, & Wu, 2018) due to its selectivity, sensitivity and tolerance for interference. It has also been adopted for the determination of bisphenols and alkylphenols in food matrices (Niu et al., 2011; Pernica et al., 2015; Regueiro & Wenzl, 2015; Salgueiro-González et al., 2012a).

As a part of Chinese culinary culture, hotpots have gained popularity among Chinese communities. Hotpot seasoning prepared with oil and spices are added in order to improve taste and aroma (Wu, Guo, Tsui, Chen, & Zhao, 2012). To the best of our knowledge, few methods have been developed for the analysis of bisphenols and alkylphenols in hotpot seasoning. Hence, the objective of this study was to develop a sensitive and simple analytical method for the routine detection of six bisphenols and alkylphenols in hotpot seasoning using SPE followed by ultra high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The method developed was validated and applied to determine the presence of bisphenols and alkylphenols in 45 hotpot seasoning samples from Guangzhou, Chengdu and Beijing, China.

2. Materials and methods

2.1. Chemicals and reagents

Standard BPB (98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of BPA (98.5% purity), BPF (> 99.0% purity), 4-NP (≥98.0% purity), 4-*n*-NP (99.0% purity) and OP (99.0% purity) were all purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Chemical structures of 6 bisphenols and alkylphenols are presented in Supplemental Material Fig. S1. Internal standards BPA-d_4 (> 99.0% purity) and 4-*n*-NP-d_4 of 97.8% purity were purchased from CDN (Quebec, Canada). HPLC grade methanol (MeOH) and acetonitrile (ACN) were supplied by Merck (Darmstadt, Germany). Acetone (AC), *n*-hexane and dichloromethane of high-purity grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ammonia water (mass concentration, 25%) was of analytical pure and obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China). Florisil SPE cartridge (1000 mg, 6 mL, Waters Corp., Milford, LA, USA), Carb/PSA SPE cartridge, Carb/NH_2 SPE cartridge and ENVI-Carb SPE cartridge (500 mg, 6 mL, Supelco Corp., Bellefonte, PA, USA) were used in the sample extraction and clean-up, and their impact on extraction of target analytes compared. Ultra pure water was obtained by the Milli-Q ultrapure system (Millipore, Bedford, MA, USA) and used in the entire experiment.

2.2. Preparation of standard solutions

Individual standard stock solutions (100 mg/L) were prepared by dissolving an appropriate amount of each standard and internal standard in MeOH. A mixed standard stock solution (10 mg/L) was prepared by combining appropriate amounts of each individual standard stock solution in MeOH. All the stock solutions were stored at −20 °C in amber glass vessels. 1 mg/L of mixed internal standard working solution was obtained by dilution and mixture of each internal standard stock solutions with methanol. A series of mixed standard working solutions containing 20 μg/L BPA-d_4 and 20 μg/L 4-*n*-NP-d_4 were prepared by diluting the mixed standard and mixed internal standard solutions with MeOH before use.

2.3. Sample and sample preparation

A total of 45 hotpot seasoning samples, including the main brands consumed in China, were purchased from local supermarkets in three major cities, namely Chengdu, Beijing and Guangzhou. All of them were packaged in plastic bags or containers.

Information about these samples is summarized in Supplemental Material Table S1.

Approximately, 1.0 g of hotpot seasoning was weighed accurately into a clean 10 mL centrifuge glass tube. Samples were then dispersed in 5 mL *n*-hexane with a MS3 basic vortex mixer (IKA GmbH, Germany), after spiking with 20 μL mixed internal standard working solution. The dispersed sample solution was centrifuged with a 5418 high speed centrifuge (Eppendorf Corp., Germany) at 4000 rpm for 5 min. Then, the supernatant was transferred to a different SPE cartridges, which was activated previously with 10 mL of MeOH-dichloromethane (v/v = 1/3) and 10 mL of *n*-hexane. After the sample solution had passed through the column, 10 mL of *n*-hexane and 5 mL of MeOH-AC (v/v = 1/1) were used for leaching. Subsequently, 5 mL of MeOH-dichloromethane (v/v = 1/3) was used for elution and the eluant dried under nitrogen stream in a 40 °C water-bath. Finally, the residue was dissolved in 1.0 mL MeOH and passed through a 0.22 μm polytetrafluoroethene (PTFE) syringe filter (Waters Corp., Beverly, MA) before UPLC-MS/MS analysis.

2.4. UPLC-MS/MS conditions

The UPLC-MS/MS apparatus consists of a Waters ACQUITY™ UPLC system and a Waters Xevo™ TQ tandem triple quadrupole mass spectrometer (Waters Corp., Beverly, MA). UPLC analysis was performed with a Waters ACQUITY™ UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm) with the column temperature set at 30 °C. The mobile phase consisted of water with 0.05% ammonia (A) and methanol (B). The gradient elution program used was as follows: 0–2.0 min, 40% B (flow rate, 0.2 mL/min); 2.0–2.5 min, 40–70% B (flow rate, 0.2–0.3 mL/min); 2.5–4.5 min,70–100% B (flow rate, 0.3 mL/min); 4.5–5.0 min, 100–40% B (flow rate, 0.3–0.2 mL/min); 5.0–8.0 min, 40% B (flow rate, 0.2 mL/min). The injection volume was 20 μL. MS/MS acquisition was conducted on the Waters Xevo™ TQ MS triple quadrupole mass pectrometer equipped with an ESI interface operating in negative ion mode (ESI^-). The capillary voltage was 1.5 kV. The ion source and desolvation temperatures were held at 150 °C and 400 °C, respectively. Nitrogen (purity, 99.9%) was used as the cone gas and desolvation gas at a flow rate of 50 L/h and 800 L/h, respectively. Argon (purity > 99.999%) was used as the collision gas at a flow rate of 0.2 mL/min. For each target analyte, two characteristic ions were selected for identification and the corresponding cone voltage and collision energy were optimized for maximum intensity. Only one characteristic ion was monitored for the internal standards. The optimum cone voltage, collision energy and the characteristic ions for 6 bisphenols and alkylphenols,