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A dispersive liquid-liquid microextraction based on solidification of floating organic droplet followed by injector port silylation coupled with gas chromatography–tandem mass spectrometry for the determination of nine bisphenols in bottled carbonated beverages

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ABSTRACT

In the present study, a method has been efficiently developed for the first time to determine nine bisphenol analogues [bisphenol A (BPA), bisphenol C (BPC), bisphenol AF (BPAF), bisphenol E (BPE), bisphenol F (BPF), bisphenol G (BPG), bisphenol M (BPM), bisphenol S (BPS), and bisphenol Z (BPZ)] together in bottled carbonated beverages (collected from the local market of Lucknow, India) using dispersive liquid-liquid microextraction process. This is based on solidification of floating organic droplet (DLLME-SFO) followed by injector port silylation coupled with gas chromatography–tandem mass spectrometry. The process investigated parameters of DLLME-SFO (including the type of extraction and disperser solvents with their volumes, effect of pH, ionic strength, and the sample volume), factors influencing to injection port derivatization like, collision energy, injector port temperature, derivatizing reagent with sample injection volume, and type of organic solvent. BPA, BPF, BPZ, and BPS were detected in each sample; whereas, other bisphenols were also detected in some carbonated beverage samples. After optimizing the required conditions, good linearity of analytes was achieved in the range of 0.097–100 ng mL^{-1} with coefficients of determination (R^2) $\geq$ 0.995. Intra-day and inter day precision of the method was good, with relative standard deviation (% RSD) $\leq$ 10.95%. The limits of detection (*LOD*) and limits of quantification (*LOQ*) values of all bisphenols were ranged from 0.021 to 0.104 ng mL^{-1} and 0.070 to 0.343 ng mL^{-1}, respectively. The recovery of extraction was good (73.15–95.08%) in carbonated beverage samples and good enrichment factors (96.36–117.33) were found. Thus, the developed method of microextraction was highly precise, fast, and reproducible to determine the level of contaminants in bottled carbonated beverages.

1. Introduction

The extensive use of bisphenol based polymers for the packaging of food and beverages have increasingly accelerated the chances of bisphenol contamination and thereby cause hazards to human health. Among several analogues of bisphenol, bisphenol A (BPA; 2,2-Bis(4-hydroxyphenyl)propane) is being used extensively to make polymers like, polycarbonates and epoxy resins [1,2]. It is also used in the formation of dental sealants, adhesives, thermal paper, and printing inks [3–5]. The occurrence of BPA in environment has driven through widespread use of consumer products such as polycarbonate tableware, epoxy resin based food, and beverage cans, thereby leaching into contact materials through hydrolysis of epoxy groups of BPA based polymers [6]. In previous studies, BPA migration from polycarbonate containers, baby feeding bottles, drinking bottles and plastic films into water, and food have been well reported [7–11]. The anti-estrogenic behavior of BPA suggests that it affects the human endocrine system and acts as most prominent endocrine disrupting chemical (EDC) [12,13]. Moreover, EDC causes hormonal dysfunction resulting uneven fate of hormone biosynthesis and innate metabolic action leads to deviation in reproduction and normal homeostatic controls [14]. Evidences from earlier studies in animals suggested that BPA triggered genotoxic and mutagenic pathways [15], testicular mito-

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chondrial dysfunction [16], carcinogenesis [17–19], reproductive dysfunction [20,21], neurotoxic alterations [22,23], and nephrotoxicity [24].

Apart from the predominant use of BPA in polymer production, other concordant analogues of bisphenols includes, bisphenol F (BPF; Bis(4-hydroxyphenyl)methane), bisphenol AF (BPAF; 2,2-Bis(4-hydroxyphenyl) hexafluoropropane), bisphenol E (1,1-Bis(4-hydroxyphenyl)ethane), bisphenol C (2,2-Bis(4-hydroxy-3-methylphenyl)propane), bisphenol G (2,2-Bis(4-hydroxy-3-isopropylphenyl)propan), bisphenol Z (4,4′-Cyclohexylidenebisphenol), bisphenol S (Bis(4-hydroxyphenyl) sulfone), and bisphenol M (4,4′-(1,3-Phenylenediisopropylidene)bisphenol), which are also causes similar health hazards as described above [25–27]. Hence, there is an unmet need to monitor these toxic chemicals in consumer products.

The gas chromatography coupled to mass spectrometry (GC–MS) could be useful for simultaneous determination of bisphenols. Although GC–MS provides high selectivity and good sensitivity with high separation efficiency at low concentration of analyte, nevertheless, it has some drawbacks, such as, susceptibility to gain moisture, requirement of high temperature, longer reaction time, and derivatization steps with silylation reagents. To overcome these problems, we have used injector port silylation. However, the selection of a reliable and rapid sample preparation technique have much importance in account to achieve sensitive determination of bisphenols even in lower concentrations. Furthermore, the sample enrichment and extraction techniques should be environmental friendly, cost effective, use less organic solvents, and involve less time consumption. Previously, many sample extraction procedures have been developed to quantify bisphenols in different matrices [28–31]. Recently, dispersive liquid-liquid micro extraction (DLLME) method has been used for the determination of bisphenol analogues in urine samples by Rocha et al. [32]. Although the DLLME method has its own advantages, but it has some concerns regarding the extraction solvents, which are chlorinated, toxic in nature, and possess environmental hazards [33]. To overcome these challenges, DLLME based on solidification of floating organic droplet (DLLME-SFO) an upgraded technique was introduced by Zanjani et al. [34]. This technique apply similar principle of DLLME and uses novel extraction solvents (non chlorinated) having low density than water rather. Consequently, in this study, we used the novel combination of DLLME-SFO followed by injector port silylation coupled with gas chromatography–tandem mass spectrometry (IPS-GC–MS/MS) for simultaneous determination of nine bisphenols in bottled carbonated beverages to achieve better pre-concentration and good derivatization condition.

2. Experimental

2.1. Standards and reagents

All bisphenol standards BPA (purity >99.0%), BPC (purity >99.0%), BPAF (purity 99.0%), BPE (purity >98.0%), BPF (purity >98.0%), and BPG (purity 98.0%), BPM (purity 99.0%), BPS (purity 98.0%), BPZ (purity 99.0%), Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + TMCS; 99:1; v/v), extraction solvents (1-dodecanol, 2-dodecanol, 2-dodecanone, and 1-undecanol), and trichloroethylene were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, acetone, methanol, and ethanol were purchased from Merck Specialities Pvt. Ltd. HPLC grade ethyl acetate and *n*-hexane were purchased from Sisco Research Laboratories Pvt. Ltd. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Standard stock solutions (0.5 mg mL^{-1}) were individually prepared by weighing 5.0 mg of each bisphenol into a 10.0 mL volumetric flask and dissolved in acetonitrile. A mixture of working solution was further prepared by combining individual stock solutions with acetonitrile and stored at 4 °C until analysis. Initially, the method was optimized for injection port silylation parameters selection with this standard solution. Later on, standard stock solutions in above same concentration were prepared with ethyl acetate for further quantification.

2.2. Sample collection and pretreatment

Bottled carbonated beverage samples of fourteen brands (having polycarbonate bottle packaging) were collected from the local market of Lucknow, India and stored at 4 °C until analysis. Before analysis, samples were allowed to gentle shaking by hand, about 30 mL of each sample was taken in glass tube, degassed in an ultrasonic bath for 10 min, then 7.0 mL of each sample was transferred in a 15 mL centrifuge tube containing 70 mg NaCl (1% w/v) and mixed thoroughly by vortex and the pH was adjusted at about 6.0 by adding 5 M NaOH solution. All sample solutions were used for the determination of bisphenols.

2.3. DLLME-SFO and IPS procedure

Initially, the process was optimized by 7 mL of milli-Q water fortified with each bisphenol standard to make a concentration of 50 ng mL^{-1}. Then each fortified water sample was placed in a 15 mL screw cap centrifuge tube. Further, a premix solution of extraction solvent (100 μL) and disperser solvent (400 μL) was prepared and rapidly injected by a 2 mL syringe in order to form a cloudy solution into the sample and the mixture was gently shaken. In this step, all bisphenols in water sample were thoroughly extracted into the fine droplets of extraction solvent. The resultant mixture was then centrifuged for 5 min at 6000 rpm in order to corpus analytes into extraction solvent from the cloudy solution and to extractant phase. Incidentally, centrifugation leads to the separation of aqueous phase and organic solvent due to the difference in their density, the fine droplets of extraction solvent floated in the form of a ring at the top level of the centrifuge tube. The tube was then placed to 0 °C in the refrigerator and allowed to solidification of the extraction solvent. After refrigeration of the extraction solvent, the aqueous phase was decanted; the remaining extraction solvent melts quickly at room temperature, which was transferred into a 300 μL glass insert contained in a GC vial. An aliquot of 3 μL of the enriched extract was injected into the GC–MS for quantification of bisphenols.

The enriched extract was subjected to auto derivatization process through injector port silylation and the relevant parameters essential to derivatization procedure such as, collision energy, temperature of the injection port; derivatizing reagent, sample volume, and the type of organic solvent were optimized for the better quantification of bisphenols.

2.4. GC–MS/MS analysis

Analysis of the bisphenols was carried out on an Ultra GC equipped with a Tri Plus auto-sampler (used for auto-IPS) and connected to a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, FL, USA). The separation was conducted on an Agilent DB-5 MS capillary column (having phase composition of 5% Phenyl and 95% dimethyl arylene siloxane) fitted with injection port of GC–MS/MS. The Tri-Plus auto sampler was set to the internal standard mode and a vial containing BSTFA + TMCS (99:1, v/v) was kept at internal standard position in auto sampler tray. Helium gas was used as a carrier at a constant flow of 1.1 mL/min. The

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