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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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GC oven temperature was initially maintained at 150 °C for 2 min; then increased at a rate of 12 °C/min to reach 230 °C for 3 min; and finally increased at a rate of 50 °C/min to reach 280 °C for 7 min. The GC–MS/MS quadrupole, interface, MS source, and ion source temperatures were set at 150 °C, 290 °C, 230 °C, and 220 °C, respectively. The mass spectrometer was used in the positive electron impact ionization (+EI) mode at an electron acceleration energy of 70 eV and a solvent delay of 6 min was set. An aliquot of 3 μL of the sample with 3 μL of BSTFA + TMCS (99:1, v/v) was injected in the splitless mode. Initially, to confirm the derivatization and retention time of all bisphenols, the full scan mode at m/z of 50–550 amu was set. After confirmation of retention time and the most abundant precursor ions of each bisphenols, product ions were scanned. Then MS/MS acquisition was operated in selected reaction monitoring (SRM) mode and product ions were set as SRM transitions, which were further used for quantification of bisphenols.

2.5. Method validation and quantification

The proposed analytical method was validated with respect to linearity, limit of detection (LOD), and limit of quantification (LOQ) as per ICH Q2(R1), 2005 recommendations [35]. The calibration curve was plotted by the serial dilution of mixed standard solution of bisphenols ranging from 0.097–100 ng mL^{-1} (i.e. 0.097, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 ng mL^{-1}) and regression coefficients, slopes, and intercepts were calculated of each compound (n = 3). LOD and LOQ of each bisphenol were calculated from the equation of standard deviation of the response and the slope as mentioned in ICH guideline: $LOD = 3 \times \sigma/s$ and $LOQ = 10 \times \sigma/s$, where σ and S are the standard deviation of the response and slope of the calibration curve, respectively. The precision was done using three different concentrations (i.e. low- 1 ng mL^{-1}, medium- 50 ng mL^{-1} and high- 100 ng mL^{-1}) of mixed bisphenols in five number of repeats (n = 5) within a day (intra-day precision). While inter day precision of the method was measured with the above mentioned concentrations of mixed bisphenols (n = 5) in two consecutive days. The precision values were expressed as percent relative standard deviation (% RSD).

2.6. Enrichment factor

The enrichment factor (EF) was calculated by following equation [36]:

$$EF = C_{Floated}/C_o$$

Where, the $C_{Floated}$ and C_O are the concentration of the analytes in the floated phase (i.e. extraction solvent), and initial concentration of the analytes in the spiked aqueous sample, respectively.

3. Results and discussion

3.1. Optimization of IPS parameters

Optimization of the efficient extraction procedure signifies the maximum extraction and good pre-concentration factor. Apart from this, instrumental response is a greater concern in analytical chemistry, in order to obtain best results in the form of good chromatographic resolution, sensitivity of the detector, and rapid determination of the compounds of interest. Specially, assay development time should be shorter without sacrificing the above mentioned attributes. Traditionally, the common way used for optimization of analytical procedure for GC–MS analysis is in-vial silylation for the detection and enhancement of sensitivity of polar compounds. However, derivatization is the process which enhances the volatility, thermal stability, and sensitivity of the analytes to confer their detection in gas chromatographic (GC) analysis. Instead, IPS is one of the substitute of derivatization process, which is non-destructive and require minimal detection time for the determination of a wide spectrum of the compounds and comparatively advantageous than in-vial silylation. Since, IPS takes place inside the injector port of the GC–MS, it requires the maintenance of some parameters like collision energy, injection port temperature, volume of sample, derivatization reagent, and the effect of the solvent in which analytes are dissolved, therefore; these parameters were determined accordingly.

3.1.1. Selection of collision energy

In tandem mass spectrometry, collision-induced dissociation (CID) is an important factor, which required to generate the fragmented ions by the collision of electrons having a definite energy with analytes carried by neutral gas molecules (i.e. helium) [37]. Thus, collision energy could be optimized to generate the most abundant fragments. Different collision energies varied from 5 to 30 eV (at an interval of 5 eV) were optimized for MS/MS to induce fragmentation of precursor ions in the gas phase for the transition of bisphenols. The result shows that most bisphenols derivatives dissociated into their fragments with the collision energy of 15 eV, whereas, few derivatives required collision energy of 20 and 30 eV. The electron impact resulted to the loss of valuable stable fragment peaks of the respective bisphenols. Therefore, these collision energies were selected for appropriate bisphenol derivatives in the method (Table 1).

3.1.2. Selection of injection port temperature

Injection port temperature is necessary to be optimized during GC–MS analysis to establish a significant yield of IPS. Thus, the temperature of the injection port was investigated in the range of 50–350 °C (at an interval of 50 °C). From Fig. 1A, it has been clearly noted that, when the temperature of the injector port increases from 50 to 200 °C, the peak areas of most bisphenols also increased and beyond 200 °C, a notable decrease in their peak response was observed. The possible reason of this phenomenon could be the degradation of trimethylsilyl (TMS) derivative of bisphenols beyond 200 °C. Therefore, 200 °C was selected as the optimum injector port temperature.

3.1.3. Selection of derivatization reagent and sample injection volume

The analytes were derivatized initially with BSTFA through IPS and subsequently vaporized during the gas phase reaction into the injection port. Thus, the amount of sample and BSTFA at a ratio of 1:1 were varied from 0.5 to 3 μL at an interval of 0.5 μL to get the optimum injection volume of both, which is needed for complete derivatization of bisphenols. Results showed that 3 μL of sample volume as well as BSTFA exhibited maximum peak response for all TMS derivatives of bisphenols.

3.1.4. Selection of organic solvent

The detection capacity of an instrument varies with the maximum affinity of organic solvent to different analytes. Therefore, the solvent optimization is an important factor while performing the IPS. Hence, six different solvents (acetonitrile, acetone, ethyl acetate, *n*-hexane, trichloroethylene, and ethanol) were selected and optimized to dissolve the bisphenol samples. The standard stock solution of acetonitrile was taken in 0.1 μg mL^{-1} concentration in different aliquots as 500 μL and dried in a centrifugal vacuum concentrator, and these aliquots were later reconstituted with 500 μL volume of aforesaid solvents. Fig. 1B shows that ethyl acetate gave the maximum derivatization yield of all bisphenols. Therefore, ethyl acetate was selected and optimized to obtain the

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Table 1
GC–MS/MS parameters of bisphenols.

Compound	Retention time (min)	Precursor ions (amu)	Product ions (amu)	SRM Collision energy (V)	Time window (min)	MW before derivatization	MW after derivatization
BPAF	9.07	465 480	73, 315 411	15	1	336	480
BPF	10.68	329 344	73, 179 73, 163, 179	20	1	200	344
BPE	11.04	343 358	73 343	30	1	214	343
BPA	11.50	357 372	73, 191 357	20	1	228	357
BPC	12.24	385 400	73, 205 385	20	1	256	385
BPG	12.43	441 456	73, 233 441	30	1	312	441
BPZ	14.28	397 412	179, 247 203, 369	15	1	268	412
BPS	14.91	379 394	73, 165 73, 229, 379	15	1	250	394
BPM	16.86	475 490	207, 309 475	20	1	346	475

MW-molecular weight.
SRM- selected reaction monitoring.

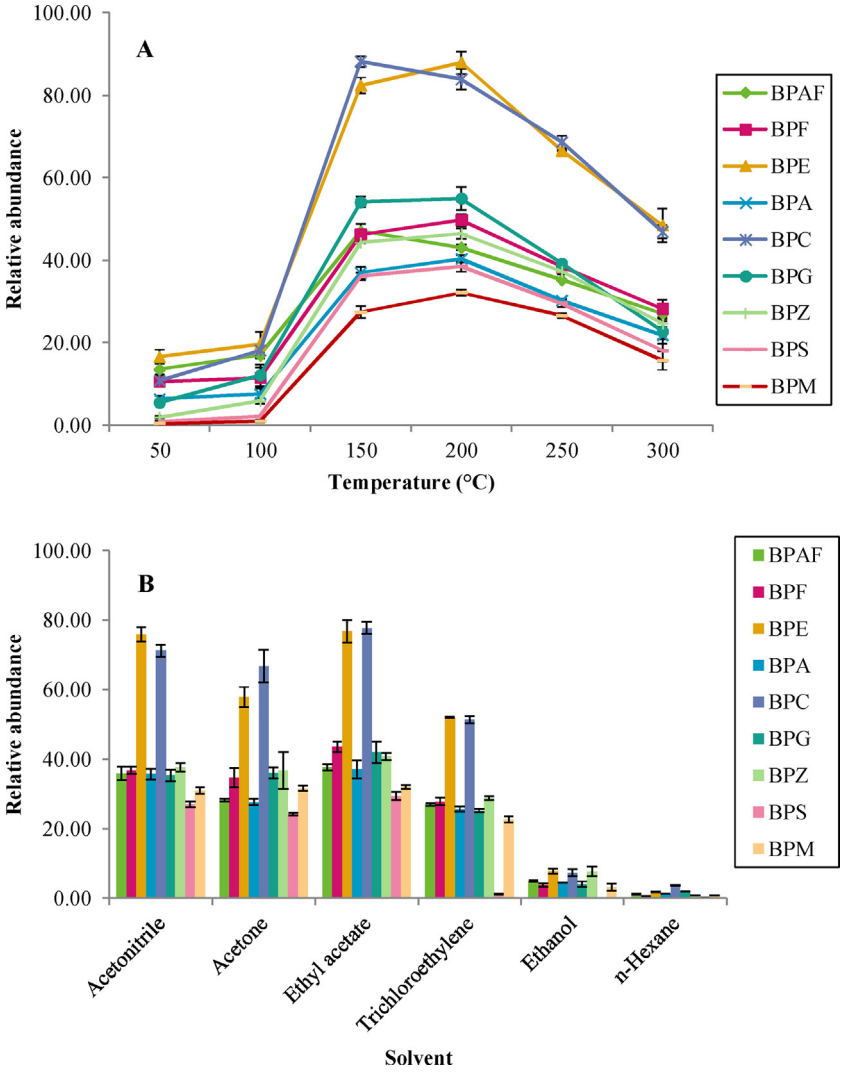

Fig. 1. Optimization of IPS parameters; (A) effect of injection port temperature, (B) effect of organic solvent in which bisphenols were dissolved.

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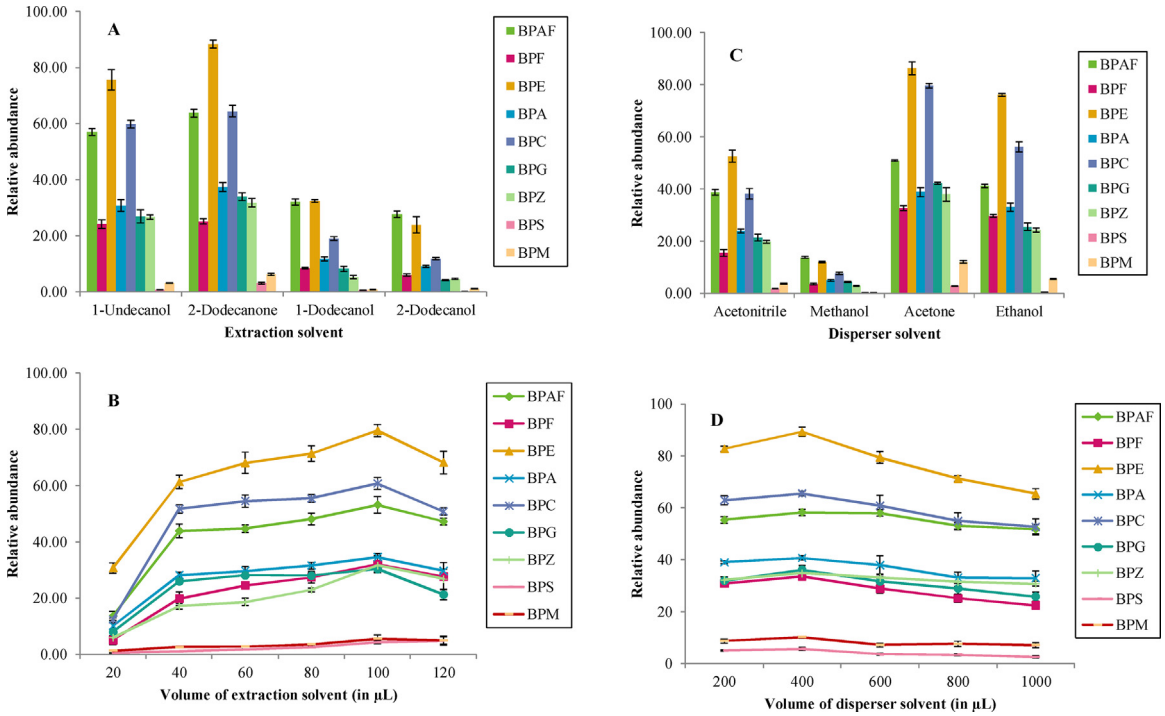

Fig. 2. Optimization of DLLME-SFO method in the spiked aqueous sample at a concentration of 50 ng mL^{-1} of bisphenols; (A) the effect of type of extraction solvent, (B) selection of volume of extraction solvent, (C) the effect of type of disperser solvent, and (D) selection of volume of disperser solvent.

better sensitivity, maximum quantification via linearity, and precision of all bisphenols in this method.

3.2. Optimization of DLLME-SFO parameters

A quantitative assay should be precise, specific, accurate, and highly sensitive while performing a potential extraction procedure. In order to achieve the above said aspects, a series of experiments were carried out to optimize important DLLME-SFO parameters that affect the extraction efficiency including, selection and volume of extraction and disperser solvents, effect of sample volume, pH, and salt effect.

3.2.1. Selection of extraction solvent

The extraction solvent should fulfill the special characteristics following DLLME-SFO procedure; it should have lower density than water, high efficiency for the maximum yield of the interested compounds in extraction, low solubility in water, low melting point (i.e. 10–30 °C), and good chromatographic behavior. Therefore, in order to determine best extraction solvent, four low density based solvents were investigated namely 1-undecanol, 2-dodecanone, 1-dodecanol, and 2-dodecanol. To select the best out of these extraction solvents, a set of experiment was performed. Premix of four above mentioned extraction and disperser solvent (acetonitrile) were prepared at a ratio of 1:4, respectively. Thus, the premix solutions (400 µL) were rapidly injected into 5 mL of milli-Q water fortified with 50 ng mL^{-1} of bisphenols. Fig. 2A shows that, among the tested extraction solvents, 2-dodecanone exhibited the best extraction efficiency. Thus, 2-dodecanone was chosen as the extraction solvent for further experiments.

3.2.2. Selection of volume of extraction solvent

The influence of the volume of extraction solvent is presented in Fig. 2B. To examine the effect of volume of selected extraction solvent (2-dodecanone), different amounts (20, 40, 60, 80, 100, and 120 µL) were taken with a fixed volume (320 µL) of acetonitrile. The result showed that the recovery of extraction increased with the volume of 2-dodecanone from 20 to 100 µL and subsequently decreased beyond this volume. This lessening of the recovery with 120 µL extraction solvent could be attributed by the fact that the extraction efficiency decreases, perhaps by increment of volume of floating phase leads to incompetent beyond a limit resulting in the loss of analytes.

3.2.3. Selection of disperser solvent

The disperser solvent plays an important role in DLLME-SFO method; furthermore, dispersion promotes to achieve phenomenal equilibrium between extraction solvent and aqueous phase by the formation of cloudy solution, which exerts the larger contact surface of extraction solvent to withdraw the analytes of interest. Therefore, several disperser solvents including acetonitrile, methanol, acetone, and ethanol were screened to find out best disperser solvent. A premix of above mentioned disperser solvents (320 µL) with 2-dodecanone (100 µL) were mixed to 5 mL of milli-Q water fortified with 50 ng mL^{-1} of bisphenols. The results showed that acetone exhibited the higher dispersion capacity compared to other tested disperser solvents; therefore, it was selected as disperser solvent (Fig. 2C).

3.2.4. Selection of volume of disperser solvent

In order to achieve a steady volume of disperser solvent, different volumes of acetone was examined through a set of experiment conducted by fixing the volume of 2-dodecanone at its optimized value (i.e., 100 µL) and varying the volume of acetone in the range of 200–1000 µL at an interval of 200 µL. The extraction recovery of all bisphenols was increased from 200 to 400 µL and subsequently decreased beyond this volume (Fig. 2D). It was also noted, the cloudy state was not formed satisfactorily with low volume (i.e. 200 µL) of the disperser solvent. Thus, 400 µL of acetone was selected as the optimum volume of disperser solvent for further study.

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Table 2
Analytical performance through validated method parameters.

Compound	Linear equation	R^2	Precision[a] (n = 5; Spiked levels[b])									LOD[b]	LOQ[b]
			Intra-day			Inter day 1st			Inter day 2nd				
			1	50	100	1	50	100	1	50	100		
BPAF	y = 134589x – 4396	0.999	5.50	2.66	2.38	7.98	3.63	3.19	5.72	6.69	5.41	0.034	0.112
BPF	y = 125513x + 285.3	0.999	8.54	4.83	3.90	3.74	5.41	5.46	6.53	8.17	7.69	0.034	0.112
BPE	y = 295843x + 16654	0.995	5.68	3.28	1.49	7.73	4.40	4.07	7.47	4.96	4.78	0.104	0.343
BPA	y = 150279x + 6717	0.998	3.71	3.19	3.11	3.85	4.43	3.15	5.97	6.45	5.63	0.070	0.230
BPC	y = 268712x + 199.4	0.999	3.14	4.22	3.96	8.28	5.47	5.34	10.95	8.05	7.65	0.026	0.086
BPG	y = 177666x – 417.6	0.999	4.90	3.36	2.75	7.91	5.11	5.07	6.11	7.08	6.86	0.029	0.096
BPZ	y = 108820x – 2540	0.999	6.41	2.81	1.64	5.68	4.01	3.74	4.56	7.33	6.60	0.022	0.072
BPS	y = 47977x + 1131	0.999	4.22	4.19	2.48	1.57	3.94	2.79	6.51	8.04	7.01	0.021	0.070
BPM	y = 63959x + 2656	0.997	3.69	2.84	3.05	1.04	4.77	4.06	2.46	9.96	9.18	0.092	0.302

[a] %RSD.
[b] Expressed in ng mL^{-1}.

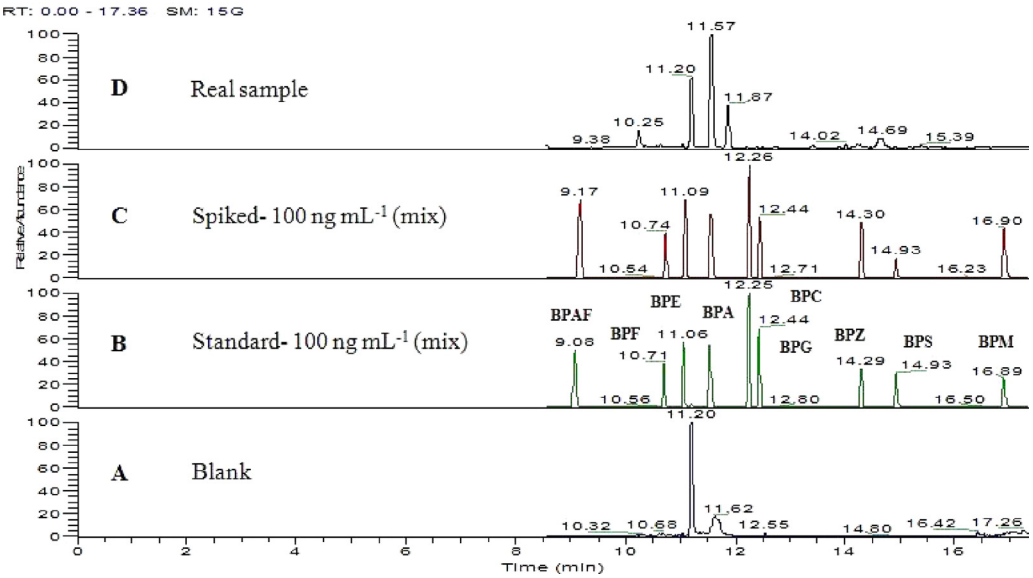

Fig. 3. GC–MS/MS chromatograms of; (A) blank, (B) mixed bisphenols standard at concentration of 100 ng mL^{-1}, (C) spiked sample of mixed bisphenol at concentration of 100 ng mL^{-1}, and (D) real sample {beverage 1} after extraction at optimized conditions.

3.2.5. Selection of sample volume

The effect of sample volume was studied to find the fact, whether the extraction efficiency was affected or not with the variable sample volumes. However, it is quite possible that the increased sample volume could enhance the pre-concentration (enrichment) factor. Thus, the sample volume was optimized with 2, 5, 7, and 10 mL. From the results (Fig. S-1), it was found that 7 mL of the sample volume gave the best extraction efficiency of all bisphenols and selected for further study.

3.2.6. Effect of pH and ionic strength

It is important to maintain the optimal pH for the conversion of analytes into their neutral forms, which could enhance the extraction efficiency. Therefore, the effect of pH was investigated in the range of 2–12 in the spiked aqueous medium. The pH of the samples was maintained with the help of 5 N HCl and 5 N NaOH. Results (Fig. S-2A) showed that the best extraction recovery was found at pH of about 6 for all bisphenols; whereas, further increase in the pH resulted to decline extraction recovery. Hence, a pH 6.0 was selected as the optimum pH.

Salt addition to sample is usually enhances the ionic strength of the analytes (due to salting out effect) resulting to enhanced extraction efficiency. Therefore, to check the salt effect a set of experiment was conducted by adding the amount of NaCl in the range of 0–5% (w/v). The results (Fig. S-2B) suggested that extraction recovery was slightly enhanced by 1% NaCl and peak area of bisphenols remains constant. Eventually, the amount of salt was selected as 1% for extraction procedure.

3.3. Method validation

In order to validate the method, linearity and precision test were conducted.% RSD of intra-day and inter-day was calculated to check the in-house repeatability and inter day precision of the developed analytical method. The data shown in Table 2 indicated that, the LOD and LOQ were found in the range of 0.021–0.104 ng mL^{-1} and 0.070–0.343 ng mL^{-1}, respectively, whereas; good precision was found with% RSD ≤ 10.95%. The GC–MS/MS chromatograms presented in Fig. 3A–C are shown as the TMS derivative of bisphenols in a processed blank, a standard solution of 100 ng mL^{-1} and spiked sample at a concentration of 100 ng mL^{-1} respectively, after application of the developed DLLME-SFO and IPS-GC–MS/MS method condition.

3.4. Recovery

Recovery study was conducted by spiking the standard bisphenols in the carbonated beverage sample at three different

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Table 3
The relative recovery in spiked carbonated beverage samples and enrichment factors.

Compound	% Relative recovery ± SD(%RSD) (n = 3)[b] Spiked levels[a]			EF
	1	50	100	
BPAF	73.15 ± 6.88(9.41)	79.88 ± 7.94(9.95)	81.24 ± 5.29(6.51)	117.33
BPF	82.28 ± 7.04(8.56)	85.98 ± 4.75(5.53)	88.21 ± 9.06(10.27)	106.19
BPE	75.22 ± 3.36(4.47)	80.52 ± 9.60(11.92)	89.15 ± 10.46(11.73)	115.40
BPA	82.24 ± 7.35(8.94)	88.95 ± 13.43(15.10)	93.61 ± 10.03(10.71)	110.02
BPC	81.20 ± 7.66(9.43)	84.06 ± 10.41(12.38)	90.66 ± 10.17(11.22)	100.48
BPG	87.86 ± 3.64(4.15)	95.08 ± 8.22(8.64)	93.76 ± 11.87(12.66)	100.78
BPZ	81.66 ± 9.38(11.48)	89.13 ± 6.16(6.91)	88.61 ± 8.91(10.06)	99.00
BPS	77.36 ± 4.91(6.34)	78.84 ± 5.50(6.98)	80.52 ± 4.73(5.87)	111.15
BPM	77.41 ± 3.90(5.04)	84.96 ± 10.00(11.76)	90.67 ± 4.95(5.46)	96.36

EF- enrichment factor.
[a] Mix bisphenols concentration spiked carbonated beverage samples; expressed in ng mL^{-1}.
[b] Three numbers of replicates.

Table 4
Determination of bisphenols in different bottled carbonated beverage samples.

Samples	BPAF	BPF	BPE	BPA	BPC	BPG	BPZ	BPS	BPM
Beverage 1	0.28	0.68	0.39	2.97	0.12	*<LOQ*	0.42	3.42	0.42
Beverage 2	0.28	0.86	*<LOQ*	1.81	0.25	0.16	0.68	4.08	0.58
Beverage 3	0.37	1.37	*<LOQ*	2.30	0.41	0.19	1.38	4.79	0.70
Beverage 4	0.20	1.08	*<LOQ*	1.23	0.14	*<LOQ*	0.63	4.32	*<LOQ*
Beverage 5	*<LOQ*	1.42	0.41	1.05	*<LOQ*	0.10	0.15	11.75	*<LOQ*
Beverage 6	0.30	0.69	*<LOQ*	2.83	0.26	0.13	0.82	4.43	0.47
Beverage 7	0.14	1.39	*<LOQ*	0.71	0.22	0.10	0.37	5.03	*<LOQ*
Beverage 8	*<LOQ*	0.43	*<LOQ*	0.86	0.15	*<LOQ*	2.41	6.68	*<LOQ*
Beverage 9	*<LOQ*	0.66	*<LOQ*	1.01	0.16	*<LOQ*	0.17	5.56	*<LOQ*
Beverage 10	0.13	0.24	*<LOQ*	0.51	0.13	*<LOQ*	0.13	5.97	*<LOQ*
Beverage 11	*<LOQ*	0.37	*<LOQ*	1.25	0.21	*<LOQ*	0.12	4.76	*<LOQ*
Beverage 12	*<LOQ*	0.27	*<LOQ*	0.98	0.21	*<LOQ*	0.53	13.43	*<LOQ*
Beverage 13	*<LOQ*	1.44	*<LOQ*	0.75	0.21	*<LOQ*	0.43	5.77	*<LOQ*
Beverage 14	*<LOQ*	0.83	0.80	1.02	0.11	*<LOQ*	0.26	3.21	*<LOQ*

Amount of bisphenols are expressed in ng mL^{-1}.
LOQ- limit of quantification.

concentrations (low- 1 ng mL^{-1}, medium- 50 ng mL^{-1} and high- 100 ng mL^{-1}) in three replicates (n = 3). The results for the relative recovery are presented in Table 3, which were calculated on the basis of the obtained concentration in spiked sample and non-spiked samples. The results showed that the present method exhibited good recoveries (73.15–95.08%). Thus, the matrix, i.e. carbonated beverage samples have no significant obstruction on the extraction efficiency and the developed procedure was reliable, and accurate for the determination of all bisphenols in these samples. However, the calculated enrichment factors were expressed in Table 3, the range of an enrichment factor of all bisphenols was found in between 96.36–117.33.

3.5. Real sample analysis

The applicability and performance of the developed DLLME-SFO method using GC–MS/MS was applicable for the determination of bisphenols in bottled carbonated beverages. The concentrations were determined of each bisphenol derivatives by interpolation from the standard calibration curve within their linear dynamic range. The results showed that some beverage samples were contaminated by four bisphenols (BPG, BPE, BPM, and BPAF) at ≥LOQ levels while some samples were found uncontaminated (<LOQ). Whereas, BPA, BPF, BPZ, BPC and BPS were found in almost all beverage samples at ≥LOQ levels; in which BPS possess to be the most abundant contaminant in carbonated beverages (Table 4). The chromatogram (Fig. 3D) depicts the contamination levels of real (carbonated beverage) samples from bisphenols.

4. Conclusion

In this study, a novel DLLME-SFO procedure combined with GC–MS/MS and IPS was developed for the rapid determination of bisphenols in bottled carbonated beverage samples. The sample pretreatment and extraction procedure was simple, rapid, economic, and environment friendly. The process involving dispersed fine droplets of extraction solvent (2-dodecanone) into the sample solutions exhibited the enhanced and enriched mass-transfer of the target analytes with extraction solvent. Use of low toxic extraction solvent and simple collection procedure after solidification in floating phase increased the extraction efficiency in the procedure. The direct application of melt extraction solvent for injection port derivatization reduced the time and extra steps involving in in-vial silylation accurately. Moreover, this method was proven to be the better microextraction method for simultaneous determination of bisphenol analogues and qualifies as the good alternatives to maintain the mandatory aspects of green chemistry. The performance of the developed method for extraction and quantification of bisphenols from carbonated beverage samples was satisfactorily applied and suggested the effective extraction and accurate determination of bisphenols. Finally, it has been concluded that, the developed method would be recognized as useful for food industries for the routine analysis and toxicological evaluation of bisphenol analogues in beverages.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2017.10.071.

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