

Development of a Headspace-Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS-SPME-GC/MS) Method for the Determination of Benzene in Soft Drinks

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Received: 19 September 2012 / Accepted: 13 December 2012 / Published online: 28 December 2012
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Abstract A method based on headspace-solid phase microextraction and gas chromatography with mass spectrometry has been developed and validated for the determination of benzene in soft drinks. The extraction step was optimized using a rotatable central composite design including the following experimental variables: extraction temperature, extraction time, sample weight, and salt concentration. The optimized procedure, which was carried out at 30 °C during 30 min by using a 75 µm carboxen-polydimethylsiloxane fiber, showed good linearity within the concentration range 0–25 µgkg⁻¹ ($r^2 > 0.999$), mean recoveries from 97.5 to 103.1 %, and coefficients of variation from 1.5 to 13.4 % for repeatability and from 1.5 to 15.7 % for within-laboratory reproducibility. Limits of detection and quantification were calculated at 0.02 and 0.08 µgkg⁻¹, respectively. The method was applied to determine the concentrations of benzene in 77 samples of beverages from the Brazilian market. Levels from <0.08 to 10.84 µgkg⁻¹ were obtained, which are comparable to those verified in other countries. Most of the samples (72.2 %) contained benzene up to 1 µgkg⁻¹.

Keywords Benzene · Soft drinks · Solid phase microextraction · GC/MS

Introduction

Benzene, a chemical classified as a human carcinogen by the International Agency for Research on Cancer (IARC 2012), is present in the environment due to human activities and industrial processes, being also released from the burning of fossil fuels, cigarette smoke, and gasoline vapor. In foods, benzene may naturally occur at trace levels as a result of environmental contamination, through the migration of packaging materials or from process-induced changes such as cooking and ionizing radiation (Becalski and Nyman 2009).

Besides these potential sources of food contamination, it was found in the early 1990s that benzene could also be formed in certain beverages containing potassium or sodium benzoate and ascorbic acid (vitamin C), such as soft drinks, especially under exposure to heat and light conditions (Page et al. 1992; McNeal et al. 1993). These initial findings reported by Canada and United States showed that, for most of the analyzed beverages, benzene levels were lower than the maximum limit of 5 µg L⁻¹ established for the contaminant in drinking water by these countries (US EPA 1985; Health Canada 2003). However, concentrations above this value observed in a small number of samples led the beverage industry to reformulate the affected products in order to eliminate or minimize benzene formation.

In 2005, new findings of significant levels of benzene in some beverages from the United States (Mercer 2006) prompted global investigations in several countries such as the UK (UK FSA 2006), Australia and New Zealand (FSANZ 2006), South Korea (KFDA 2006), Canada (Cao et al. 2007), Ireland (FSAI 2006), Belgium (Van Poucke et al. 2008), and Germany (Lachenmeier et al. 2010). In

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general, the results reported in these surveys showed low microgram-per-liter levels of benzene in beverages containing benzoate salts and ascorbic acid, but concentrations as high as $263\text{ }\mu\text{g L}^{-1}$ were reported by KFDA (2006).

Methods described in the literature for the determination of benzene in soft drinks usually apply gas chromatography/mass spectrometry (GC/MS) preceded by headspace sampling (Page et al. 1992; Cao et al. 2007; Lachenmeier et al. 2010; Nyman et al. 2008; Van Poucke et al. 2008; Vinci et al. 2010). Limits of detection and quantification in the ranges of 0.016–0.26 and 0.05–1.0 $\mu\text{g L}^{-1}$, respectively, have been reported for many published procedures (CCCF 2009). In addition to static headspace as a means of sampling, some researchers have used purge and trap analysis (McNeal et al. 1993; Fabietti et al. 2001).

To date, only few studies have proposed the use of solid phase microextraction (SPME) for the analysis of benzene in beverages. Cardeal et al. (2005) applied this technique in a qualitative study of volatile organic compounds (VOCs) in typical Brazilian fruits and fruit juices in which the presence of benzene was identified in guava and mango juice. In 2006, a SPME method employing a 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber was used to analyze the benzene content of soft drinks in a survey carried out in the UK, but no other analytical condition was provided (UK FSA 2006). This technique was also used for comparison purposes during the development of a method based on proton transfer reaction-mass spectrometry (Aprea et al. 2008). Other authors reported the use of a solid phase microextraction-gas chromatography/mass spectrometry method for the determination of benzene in soft drinks, including an optimization of some SPME parameters (incubation time, extraction time, and sample volume) (Tang et al. 2009).

Although SPME has been little applied for the quantitative determination of benzene in soft drinks, this technique could represent a simple and solvent-free alternative method to traditional headspace, which justifies a better investigation. Since benzene occurs at trace levels in beverages, SPME could provide enough selectivity and sensitivity for the analysis of this contaminant with low limits of detection and minimum interferences of matrix compounds. Moreover, a detailed optimization and validation of an isotope dilution headspace-solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS) method has not been described so far.

Therefore, the aim of this work was to develop and validate a simple method for the selective and sensitive analysis of benzene in soft drinks based on HS-SPME-GC/MS, and apply the proposed procedure to determine the levels of this contaminant in beverages from the Brazilian market. Whereas several surveys on the occurrence of benzene in soft drinks have been conducted in different

countries, this is, to our knowledge, the first comprehensive study in beverages containing potential benzene precursors conducted in Brazil.

Experimental

Standards and Chemicals

Benzene (Fluka, purity >99.9 %) and benzene- d_6 (Aldrich, purity >99.9 %) were obtained from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA). Methanol was of HPLC-grade (Tedia Company Inc., Fairfield, OH, USA) and water was purified by reverse osmosis (Gehaka, São Paulo, SP, Brazil). Individual stock solutions of both standards at ca 2 mg mL^{-1} were prepared every 2 weeks by dissolving the compounds in methanol. For that, a 5-mL glass vial was filled with methanol and sealed, and the exact weight of methanol was recorded. The headspace in the vials might be kept as small as possible, in order to keep partitioning of benzene into the gas phase low. About 10 μL of the standard was injected in the vial through a silicone-PTFE septum with a syringe, holding the vial in an inverted position. The exact weight of added standard was recorded, and the concentration was expressed in milligrams per milliliter, taking into account the density of methanol. Intermediate (ca $4\text{ }\mu\text{g mL}^{-1}$, every week) and work solutions (ca $0.04\text{ }\mu\text{g mL}^{-1}$, daily) were prepared in water by using the same procedure described previously and taking appropriate volumes of stock and intermediate solutions, respectively. All solutions were stored at $4\text{ }^\circ\text{C}$.

SPME Optimization

Initial experiments were conducted with 1.0 g of an orange-flavored soft drink. Five types of fiber (CAR/PDMS 75 μm , DVB/CAR/PDMS 50/30 μm , PDMS/DVB 65 μm , PDMS 100 μm , and PDMS 30 μm) acquired from Supelco (Supelco, Bellefonte, PA, USA) were assessed in order to obtain the best sensitivity and selectivity for the extraction of benzene. The fibers were previously conditioned according to the manufacturer instructions and tested at two temperatures (25 and $40\text{ }^\circ\text{C}$) during an incubation time of 30 min and stirring rate of approximately 1,200 rpm.

To evaluate the effects of extraction temperature (T), extraction time (t), sample weight (w) and ionic strength (NaCl addition), as well as their interactions on the SPME procedure, a 2^4 rotatable central composite design (RCCD) with three central points and two axial points for each independent variable was employed (Rodrigues and Lemma 2005). The investigated ranges were $T=25\text{--}55\text{ }^\circ\text{C}$, $t=10\text{--}60\text{ min}$, $w=1\text{--}10\text{ g}$, and NaCl addition= $0\text{--}30\text{ }\%$. The

used design is presented in Table 1. All statistical analyses were carried out by using the software STATISTICA 5.5.

SPME Procedure

The SPME was carried out taking into account the optimized conditions. A portion of 5.0 g of sample was weighed in a 40 mL glass vial containing a 15×5 mm PTFE-coated stir bar. After the addition of 125 μ L of the benzene- d_6 internal standard working solution, the vial was immediately closed with a screw-cap fitted with a silicone–PTFE septum. A CAR/PDMS 75 μ m fiber was exposed to the headspace of the vial by using a manual holder (Supelco) during 30 min at 30 °C, under a constant magnetic agitation rate of 1,200 rpm, approximately. After the extraction, the fiber was removed from the headspace of the vial and immediately inserted into the injector port of the GC/MS system for desorption of the compounds, where it remained exposed for 6 min.

GC/MS Analysis

The GC/MS system consisted of a HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a MSD 5973 mass spectrometer (Agilent Technologies). Helium was used as the carrier gas at a flow rate of 0.7 mL min⁻¹. The programmable temperature vaporizing injector was operated in the splitless mode under the following temperature program 40 °C (held for 0.1 min), 700 °C min⁻¹ to 230 °C (held until the end of the run). The split valve remained closed for 0.7 min. The separation was performed on a 60 m×0.25 mm, *df*0.25 μ m HP-INNOWAX capillary column (Agilent Technologies), and the oven temperature program was 30 °C (held for 0.1 min), 2 °C min⁻¹ to 40 °C (held for 3 min), and 12 °C min⁻¹ to 200 °C (held for 2 min). The mass spectrometer was operated in positive electron impact ionization mode (+EI) with 70 eV of electron energy. The quadrupole and the ionization source were maintained at 150 °C and 230 °C, respectively. Selected ion monitoring was applied for the detection of benzene and benzene- d_6 , using three characteristic ions for benzene (m/z 78 as quantifier, m/z 77, and m/z 51 as qualifiers) and two

characteristic ions for benzene- d_6 (m/z 84 as quantifier and m/z 82 as qualifier).

Identification and Quantification

Identification of benzene was made by the relative retention time (RRT) and the presence of the selected ions. For confirmatory purposes, a comparison with a standard solution was performed using an acceptable deviation of ± 0.5 % for RRT, ± 15 % for ionic relative abundance considering m/z 77/78, and ± 20 % for ionic relative abundance considering m/z 51/78, according to the acceptance criteria as stipulated in European Commission Decision 2002/657 (EC 2002). The quantification of benzene in the samples proceeded by extrapolation from a linear analytical curve (0–25 μ g kg⁻¹), using benzene- d_6 as internal standard at a final concentration of 1 μ g kg⁻¹.

Method Validation

The method was validated in terms of linearity, selectivity, limit of detection (LOD), limit of quantitation (LOQ), trueness (recovery), and precision (repeatability and within-laboratory reproducibility) according to the guidelines laid down by the Brazilian Institute of Metrology, Standardization and Industrial Quality (Instituto Nacional de Metrologia, Normalização e Qualidade Industrial 2007). Linearity was evaluated by using eight calibration points in the concentration range from 0 to 25 μ g kg⁻¹. Selectivity was determined by comparison between curves set on solvent (water) and on matrix by applying the F-test (Snedecor) and *t*-test (Student). LOD and LOQ were estimated by seven independent replicates of the matrix and calculated as three- and ten-fold the standard deviation of the obtained responses, respectively. Recovery, repeatability, and within-laboratory reproducibility were evaluated by spiking the sample with benzene at 0.1, 1, and 10 μ g kg⁻¹ (seven replicates for each concentration level). An orange-flavored soft drink containing 0.46 μ g kg⁻¹ of benzene was used in the experiments.

Samples

The content of benzene was evaluated by the in-house validated method in 59 samples of soft drinks of different flavors (lemon, orange, grape, cola, *guaraná*, and tonic water), comprising carbonated beverages and flavored waters. The method was also applied to analyze 18 samples of various concentrated fruit juices (orange, passion fruit, guava, cashew, *acerola*, mango, *cupuaçu*, grape, pineapple, and peach). The sampling was carried out based on the presence of benzoate salts in the formulation (with or without added ascorbic acid), according to information provided on the label, and included drinks sold in cans, glass bottles,

Table 1 Experimental design for optimization of solid phase micro-extraction (SPME) conditions

Variables	−2	−1	0	+1	+2
Temperature (°C)	25	32.5	40	47.5	55
Time (min)	10	22.5	35	47.5	60
Sample weight (g)	1	3.25	5.5	7.75	10
NaCl (%)	0	7.5	15	22.5	30

and PET bottles, in the traditional and low-calorie versions. Samples were collected in supermarkets from the city of Campinas, SP, Brazil, between October 2011 and January 2012, stored in the absence of excessive light and heat, and chilled at 4 °C for at least 4 h prior to analysis in order to prevent loss of volatile compounds in the opening of the product.

Results

The first step in the development of the proposed HS-SPME-GC/MS method for the analysis of benzene in soft drinks was to define the most appropriate chromatographic conditions. Considering the similarities in many chemical and physical properties of benzene and other VOCs, initial tests were performed by using the same conditions established in our previous work for the determination of furan in baby foods (Arisseto et al. 2010). Preliminary results obtained for standard solutions showed a symmetric and well-resolved peak of benzene at 11.7 min. Experiments conducted with an orange-flavored soft drink showed a chromatogram free of apparent interferences, indicating the suitability of the chromatographic conditions for the analysis of benzene in this matrix (Fig. 1a).

The next objective of this study was to choose the most appropriate SPME fiber in order to obtain the higher sensitivity and selectivity for the determination of benzene in soft drinks. The fibers were pre-selected considering the molecular weight, polarity, and volatility of the analyte, according to the fiber selection guide provided by the fabricant. As can be seen in Fig. 2, the results obtained by using the CAR/PDMS 75 µm fiber were approximately two- and eight-fold higher than those verified for DVB/CAR/PDMS 50/30 µm and PDMS/DVB 65 µm fibers at 25 °C, respectively. At 40 °C, the best results were also obtained with the CAR/PDMS 75 µm fiber. No peak of benzene was observed in the chromatogram when PDMS fibers were used. As the CAR/PDMS 75 µm fiber provided the best extraction yield in comparison to the other coatings, this fiber was used in all subsequent experiments.

Considering the SPME variables evaluated through the RCCD (extraction temperature, extraction time, sample weight, and ionic strength), only extraction temperature and sample weight showed significant linear effects ($p < 0.05$) on the peak area of benzene and benzene- d_6 (Table 2). Quadratic and interaction effects were not significant. The regression coefficients obtained for extraction temperature and sample weight were $-3,693,857$ and $7,337,999$, respectively, indicating that the response increases at lower temperatures and

higher sample amounts. The analysis of variance (ANOVA) demonstrated the validity of the model ($F_{calc} = 22.58 > F_{tab} = 3.03$; $R^2 = 0.75$).

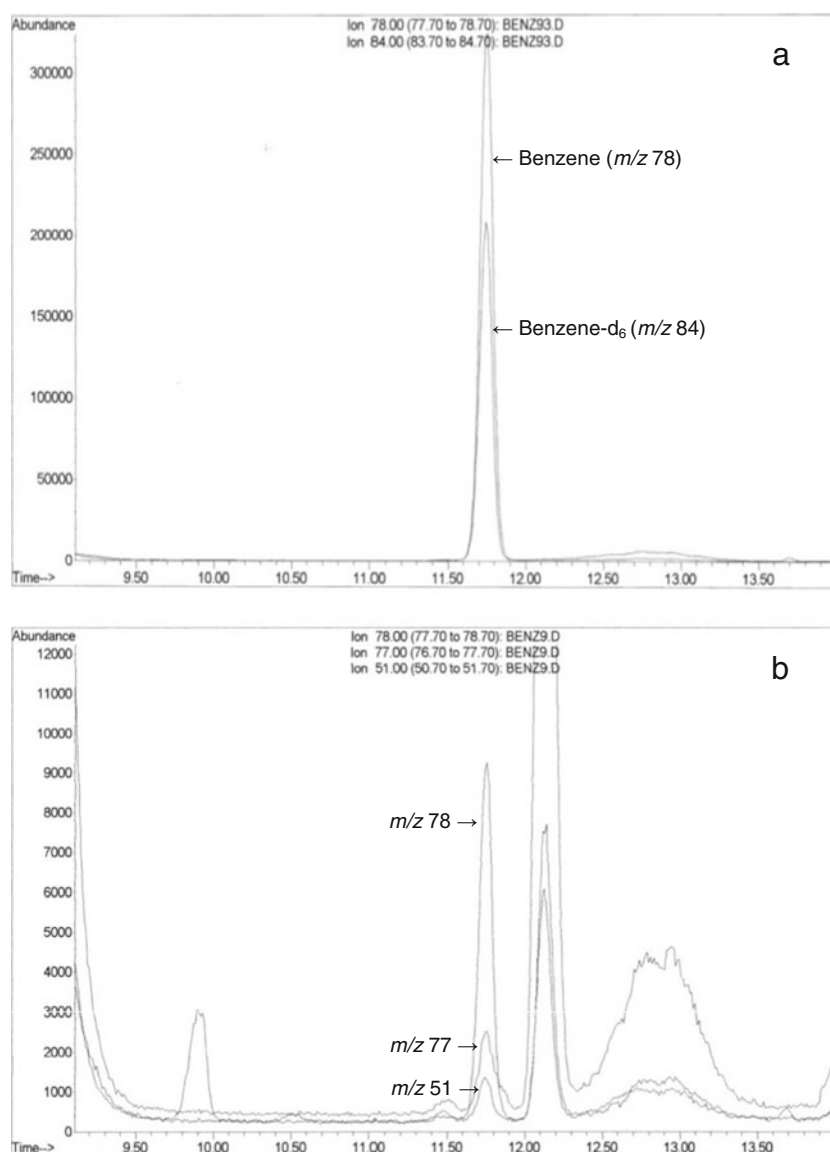
Taking into account the lack of significant effects of extraction time and NaCl amount, these variables were fixed at 30 min (due to the convenience in relation to the run total time) and no salt addition, respectively. The response surface and contour plot obtained for extraction temperature versus sample weight considering linear effects are shown in Fig. 3. As can be seen, the optimum value for these parameters is situated outside the tested range, indicating the need of a further optimization. However, no additional experiments were carried out because temperatures lower than 25 °C were not feasible to achieve due to instrumental and operational limits when using manual SPME device and because higher amounts of sample could lead to fiber saturation (in the case of highly contaminated samples). In order to define the best values for extraction temperature and sample weight, a response surface considering quadratic effects was used, although they were not statistically significant. It was verified that an extraction temperature of 30 °C and a sample weight of 5 g were appropriate to achieved good results. At these conditions, very similar results were observed for equilibration times of 0, 5, 10, and 15 min before the extraction.

The proposed method was in-house validated and showed excellent linearity in the concentration range of 0–25 µgkg⁻¹ ($r^2 = 0.999$). A comparison between the calibration curves prepared with and without the sample by applying the F-test and *t*-test showed no significant matrix effect ($F_{calc} = 1.00 < F_{tab} = 3.79$; $t_{calc} = 0.17 < t_{tab} = 2.15$). The LOD and LOQ were calculated at 0.02 and 0.08 µgkg⁻¹, respectively. Figure 1b shows a chromatogram of all diagnostic ions of a sample of lemon-flavored water containing a level of benzene close to the LOD, indicating its feasibility. Trueness and precision results are presented in Table 3. As can be seen, mean recoveries varied from 97.5 to 103.1 %, and the coefficients of variation ranged from 1.5 to 13.4 % for repeatability and from 1.5 to 15.7 % for within-laboratory reproducibility.

Tables 4 and 5 shows the results obtained in relation to the occurrence of benzene in soft drinks and juices, respectively, by applying the validated HS-SPME-GC/MS method. The concentrations of the contaminant ranged from <0.08 to 10.84 µgkg⁻¹, and all results were above the LOD. A total of 72 samples (93.5 %) presented levels above the LOQ, and most of the quantifiable samples (72.2 %) had concentrations of benzene up to 1 µgkg⁻¹. The highest levels were found in a sample of diet *guaraná* and in the samples of peach juice.

Considering the samples of soft drinks, no significant differences were observed between beverages of different

Fig. 1 Chromatogram of an orange-flavored soft drink containing $1.57 \mu\text{g kg}^{-1}$ of benzene (a) and a lemon-flavored water containing a level of benzene close to the limit of detection (b)



flavors, material packages, and traditional/low-calorie versions. In relation to the samples of fruit juices, the concentrations found in peach juice appear to be higher than those observed in juices from other fruits.

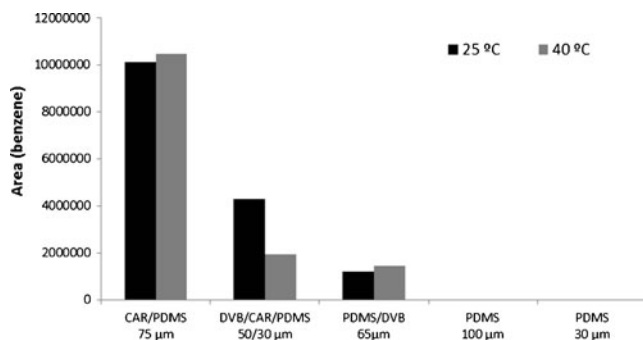


Fig. 2 Peak areas of benzene for each fiber evaluated

Discussion

Method Optimization and Validation

According to the methods available in the literature, several columns and different chromatographic conditions have been proposed for the determination of benzene in soft drinks. Stationary phases containing 5 % phenyl–95 % dimethylpolysiloxane (Fabiatti et al. 2001; Cardeal et al. 2005; Tang et al. 2009), 6 % cyanopropyl/phenyl–94 % dimethylpolysiloxane (Cao et al. 2007; Lachenmeier et al. 2010; Van Poucke et al. 2008; Vinci et al. 2010), and polystyrene–divinylbenzene (Nyman et al. 2008) have been employed for chromatographic separation in most of published methods. In the present study, the analyses were performed by using a polar stationary phase containing polyethylene glycol, in which the elution of the analyte

Table 2 Effect estimates

Factors	Effect	Standard error	<i>t</i> (12)	<i>p</i> -value
Mean	27,201,974	4,226,608	6.435888	3.23E-05
(1) Temperature (L)	−7,387,713	2,988,663	−2.47191	0.02939
Temperature (Q)	−2,107,399	3,169,956	−0.6648	0.518748
(2) Time (L)	2,272,901	2,988,663	0.760508	0.461633
Time (Q)	−1,179,528	3,169,956	−0.3721	0.716309
(3) Sample weight (L)	1,467,598	2,988,663	4.910556	0.000359
Sample weight (Q)	−1,597,618	3,169,956	−0.50399	0.623395
(4) % NaCl (L)	1,977,831	2,988,663	0.661778	0.520619
% NaCl (Q)	−2,098,903	3,169,956	−0.66212	0.520405
1 L by 2 L	5,539,143	3,660,350	1.513282	0.156092
1 L by 3 L	241,109.9	3,660,350	0.065871	0.948565
1 L by 4 L	−570,762	3,660,350	−0.15593	0.87868
2 L by 3 L	157,713.9	3,660,350	0.043087	0.966341
2 L by 4 L	3,120,340	3,660,350	0.85247	0.410645
3 L by 4 L	2,171,130	3,660,350	0.593148	0.564094

L linear, *Q* quadratic

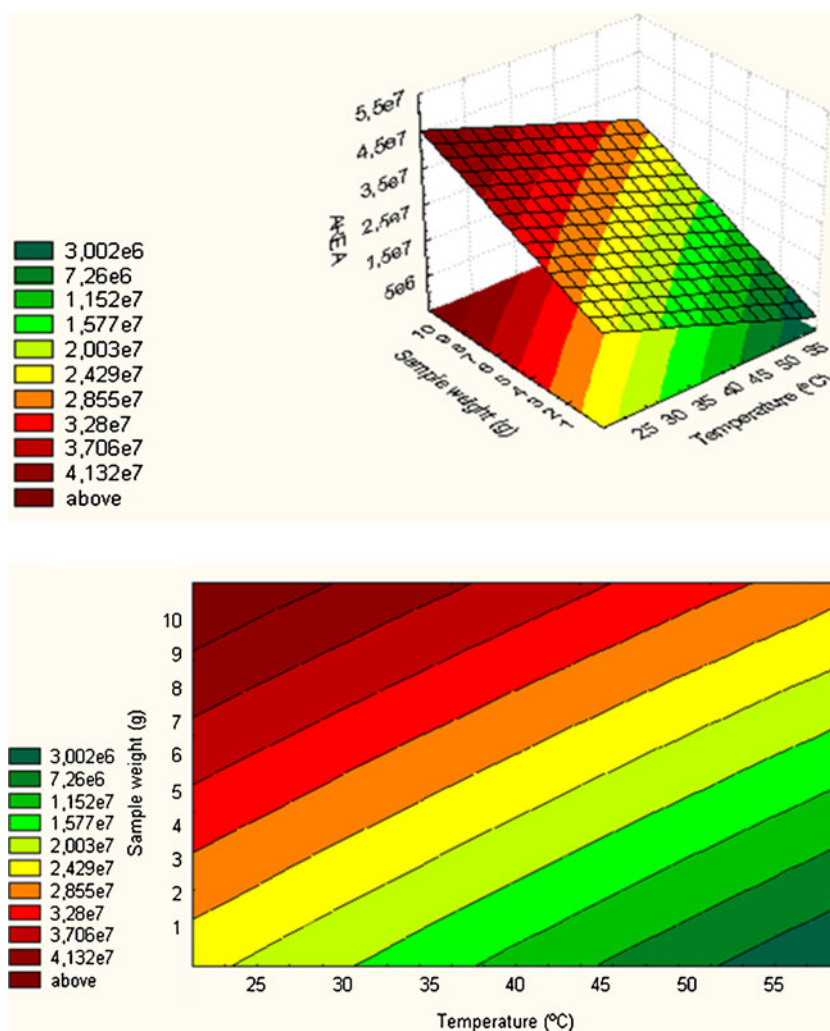
Fig. 3 Response surface and contour plot (extraction temperature×sample weight)

Table 3 Trueness and precision

Spike level ($\mu\text{g kg}^{-1}$)	<i>R</i> (%)	<i>CV_r</i> (%)	<i>CV_R</i> (%)
0.1	97.5	13.4	15.7
1	99.4	1.5	1.5
10	103.1	3.0	3.4

R recovery ($n=14$ for each level), *CV_r* coefficient of variation under repeatability conditions (same day, $n=7$ for each level), *CV_R* coefficient of variation under within-laboratory reproducibility conditions (same laboratory, two different days, $n=14$ for each level)

was achieved at an appropriate retention time (11.7 min). Other authors have also reported the use of polyethylene glycol stationary phase for analysis of benzene in soft drinks (Aprea et al. 2008).

CAR/PDMS 75 μm fiber provided the best extraction yield in comparison to the other evaluated SPME fibers (Fig. 2). The higher sensitivity observed for mixed phases containing carboxen (CAR) may be due to the strong retention of the analyte into the small pores of this coating layer. The lower capacity of the DVB/CAR/PDMS compared with CAR/PDMS is probably due to the higher mean micropore diameter of the former, which confirms the applicability of CAR/PDMS fibers for the SPME analysis of small molecules in the C_2 – C_6 range, such as benzene. The use of CAR/PDMS fibers for the analysis of benzene in soft drinks was reported in other available studies (UK FSA 2006; Aprea et al. 2008; Tang et al. 2009).

The RCCD results showed that the response increases at lower temperatures, which may be due to an increase in the distribution constant of benzene between the headspace and the fiber coating. Similar results were reported by Tang et al. (2009). As expected, an increase in sample weight also enhanced the obtained response. On the other hand, no significant effect was observed for ionic strength, although

Table 4 Benzene levels in soft drinks ($N=59$)

Soft drink	<i>N/N>LOQ</i>	Benzene ($\mu\text{g kg}^{-1}$)	
		Mean	Min–max
Tonic water	9/6	0.32	<0.08–1.11
Orange	8/8	0.95	0.18–2.51
Grape	6/6	1.00	0.33–3.23
Lemon	17/15	0.40	<0.08–1.92
Citrus	4/4	0.80	0.26–1.19
Guaraná	10/10	1.83	0.20–10.84
Cola	5/5	1.08	0.34–2.75

N number of samples, *LOQ* limit of quantification ($0.08 \mu\text{g kg}^{-1}$)

To calculate the mean, values below LOQ were treated according to the Instructions for electronic submission of data on chemical contaminants in foods to GEMS/Food (WHO 2002)

Table 5 Benzene levels in fruit juices ($N=18$)

Fruit juices	<i>N/N>LOQ</i>	Benzene ($\mu\text{g kg}^{-1}$)	
		Mean	Min–max
Orange	1/1	0.19	–
Passion fruit	2/2	0.34	0.26–0.42
Guava	2/2	0.99	0.44–1.54
Cashew	3/3	0.19	0.11–0.28
Acerola	1/1	0.08	–
Mango	2/2	0.53	0.52–0.54
Cupuaçu	1/1	3.51	–
Grape	2/2	0.64	0.53–0.74
Pineapple	2/2	2.19	1.29–3.09
Peach	2/2	7.18	6.82–7.54

To calculate the mean, values below LOQ were treated according to the Instructions for electronic submission of data on chemical contaminants in foods to GEMS/Food (WHO 2002)

N number of samples, *LOQ* limit of quantification ($0.08 \mu\text{g kg}^{-1}$)

many organic molecules show a decrease in aqueous solubility in the presence of salt, improving the partitioning to the headspace. The effect of extraction time was also not significant, although Tang et al. (2009) observed a significant effect when considering extraction times from 6 to 24 min.

The in-house validation results showed that the proposed method generates a linear response in the tested calibration range, is selective, and can be applied to determine very low levels of benzene in soft drinks. The limits of detection and quantification can be compared with those reported for the most sensitive methods based on headspace extraction (CCCF 2009). The obtained data have also shown that the method is reliable and reproducible, since trueness and precision results were within the tolerances considered acceptable (EC 2002).

Benzene Levels in Commercial Beverages

Benzene levels obtained in the present study are in accordance to those reported in other countries. For comparison purposes, Table 6 shows the results of international surveys on benzene content in soft drinks carried out from 2005. As can be seen, the large majority of the samples have also shown concentrations below $10 \mu\text{g kg}^{-1}$.

Because there are no legal limits for benzene in soft drinks, the maximum level permitted in drinking water is mostly used as reference value. This concentration is limited to $10 \mu\text{g L}^{-1}$ by the World Health Organization (WHO 2008), $5 \mu\text{g L}^{-1}$ by the U.S. Environmental Protection Agency (US EPA 1985), and $1 \mu\text{g L}^{-1}$ by the European Union (EC 1998). In Brazil, the National Health

Table 6 Benzene levels in soft drinks reported in international surveys from 2005

Country (reference)	N	Min–max ($\mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$)	Comments
UK (UK FSA 2006)	150	<1–28	107 samples below LOQ ($1 \mu\text{g kg}^{-1}$). Four products contained average levels of benzene above $10 \mu\text{g L}^{-1}$.
Australia and New Zealand (FSANZ 2006)	68	<1–40	30 samples below LOQ ($1 \mu\text{g kg}^{-1}$). More than 90 % of all beverages surveyed had levels of benzene below $10 \mu\text{g L}^{-1}$.
Ireland (FSAI 2006)	76	<1–91	69 samples below LOQ ($1 \mu\text{g kg}^{-1}$). Seven beverages contained benzene above the LOQ and of those, 2 were above the $10 \mu\text{g L}^{-1}$.
Canada (Cao et al. 2007)	124	nd–23	75 samples below LOD (0.16 and $0.26 \mu\text{g L}^{-1}$). Six samples above $5 \mu\text{g L}^{-1}$ and 2 (out of 6) above $10 \mu\text{g L}^{-1}$.
United States (Nyman et al. 2008)	199	nd–88.9	74 samples below LOD (0.05 ng g^{-1}). Seventy-one percent contained less than 1 ng g^{-1} benzene and 18 samples were found to contain greater than 5 ng g^{-1} benzene.
Belgium (Van Poucke et al. 2008)	134	nd–10.98	44 samples below LOD ($0.1 \mu\text{g L}^{-1}$). Forty-seven percent contained trace amounts below the LOQ ($0.3 \mu\text{g L}^{-1}$). Ten samples were above $1 \mu\text{g L}^{-1}$, and one sample above $10 \mu\text{g L}^{-1}$.
Germany (Lachenmeier et al. 2010)	451	<0.13–41.8	259 samples below LOQ ($0.13 \mu\text{g L}^{-1}$). Out of 313 samples of soft drinks, seven samples were above $1 \mu\text{g L}^{-1}$ and only one sample exceeded $10 \mu\text{g L}^{-1}$.

N number of samples, LOD limit of detection, LOQ limit of quantification, nd not detected

Surveillance Agency establishes a maximum level of $5 \mu\text{g L}^{-1}$ of benzene in drinking water (BRASIL 2004). According to the results obtained in the present study, three samples showed concentrations above $5 \mu\text{g L}^{-1}$, which exceeds the maximum limit established for drinking water in Brazil. One out of these three samples showed a benzene level above $10 \mu\text{g kg}^{-1}$.

It should be noted that variations from one production lot to another were not fully evaluated in this survey since samples of only two products (tonic water and mango juice) were collected from two different lots while the rest of products were collected from a single lot. Although no significant difference was observed in benzene levels between different lots of these products, certain conditions of transportation and storage, especially elevated temperature and light exposure, can stimulate benzene formation from benzoates and ascorbic acid. Thus, even the lots with the same initial benzene concentration at the time of production might have a different composition when consumed, depending on the conditions of transportation and storage (Cao et al. 2007).

Due to the unequivocal evidence of the carcinogenicity of benzene in humans and laboratory animals (IARC 2012), it is important to evaluate if the consumption of beverages containing this substance could represent a potential concern to human health. For that, a preliminary exposure assessment was carried out by combining the levels of benzene obtained here with data on the consumption of soft drinks and juices provided by the *Analysis of Personal Food Consumption in Brazil* (IBGE 2011). Using a point estimate

calculation (Kroes et al. 2002), intakes of 5 and $21 \text{ ng kg body weight (bw)}^{-1} \text{ day}^{-1}$ were estimated for average and high consumers, respectively.

The margin of exposure (MOE) approach, which establishes the margin between a dose causing cancer in animal studies and the estimated human exposure to the substance (EFSA 2005), was then applied to evaluate the potential risks to human health. Considering a BMDL₁₀ (the lower bound of a 95th confidence interval on a benchmark dose corresponding to a 10 % tumor incidence in laboratory animals) of $17.6 \text{ mg kg bw}^{-1} \text{ day}^{-1}$ (Smith et al. 2010) and the intakes estimated in the present study, MOEs of 3,400,906 and 841,192 were obtained for average and high consumers, respectively. As it has been considered that a MOE of 10,000 or higher would be of low concern from a public health point of view (EFSA 2005), it can be suggested that the exposure of Brazilians to benzene through the consumption of soft drinks does not represent a potential risk. Similar results have been obtained in other countries, and, as concluded by some international food regulatory agencies (CCCF 2009), the exposure to benzene from the consumption of soft drinks and other beverages is generally low, representing a minor contribution compared with total benzene exposure from other sources (e.g., indoor/outdoor air, vehicular emissions, and the consumption of drinking water and foods in which benzene may occur as an environmental contaminant).

In conclusion, the SPME proved to be a simple, convenient, and highly sensitive technique for the analysis of benzene in soft drinks. The in-house validation experiments

showed good linearity, trueness, and precision, indicating the suitability of the proposed method as an alternative to traditional headspace used in several studies. The procedure was successfully applied to determine benzene levels in samples of soft drinks and concentrated juices collected in the Brazilian market. The concentrations ranged from <0.08 to 10.84 $\mu\text{g kg}^{-1}$, which are comparable to those verified in other countries. Although the occurrence of benzene in soft drinks in Brazil does not appear to be an immediate concern, it is important that the concentration of this contaminant in beverages containing benzoate salts and ascorbic acid be as low as reasonably achievable. Changes in products formulations should be considered in some specific cases.

Acknowledgments Financial support was provided by the National Council for Scientific and Technological Development, Ministry of Agriculture, Livestock and Food Supply, and Secretariat of Animal and Plant Health and Inspection (CPNq/MAPA/SDA Proc. 578381/2008-7).

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