

Monitoring the Benzene Contents in Soft Drinks Using Headspace Gas Chromatography–Mass Spectrometry: A Survey of the Situation on the Belgian Market

CHRISTOF VAN POUCKE,^{*,†} CHRIST’L DETAVERNIER,[†] JAN F. VAN BOCXLAER,[‡]
RUDI VERMEYLEN,[§] AND CARLOS VAN PETEGHEM[†]

Laboratory of Food Analysis and Laboratory of Medical Biochemistry and Clinical Analysis, Faculty of Pharmaceutical Sciences, Vakgroep Bio-analysis, Harelbekestraat 72, B-9000 Ghent, Belgium; and Federal Agency for the Safety of the Food Chain, WTC III, 20th floor, Simon Bolivarlaan 30, B-1000 Brussels, Belgium

Whenever benzoic acid is combined with ascorbic acid in acidic beverages such as soft drinks, benzene can be formed. To determine the current situation on the Belgian market, a headspace gas chromatographic–mass spectrometric method was developed, which needs little to no sample preparation. This method was then used to analyze 134 soft drinks sampled on the Belgian market by the Federal Agency for the Safety of the Food Chain. Thirty-three percent of the samples contained no detectable benzene, whereas the majority of the samples (47%) contained trace amounts below the limit of quantification of the method ($0.3 \mu\text{g L}^{-1}$). Ten samples were above the European limit for benzene in drinking water of $1 \mu\text{g L}^{-1}$, and one sample had a concentration of $10.98 \mu\text{g L}^{-1}$, thereby exceeding the action limit for benzene in soft drinks of $10 \mu\text{g L}^{-1}$ discussed at the Standing Committee on the Food Chain and Animal Health of the European Commission. Statistical analyses revealed that besides benzoic acid, ascorbic acid, and acidity regulators, the packing may also play an important role in benzene formation.

KEYWORDS: Soft drink; benzene; headspace; GC-MS

INTRODUCTION

The daily intake of benzene, a molecule classified by the International Agency for Research on Cancer (IARC) as a human carcinogenic compound (group 1) (1), may vary significantly. For nonsmokers it is estimated at $200\text{--}450 \mu\text{g day}^{-1}$, whereas for smokers the intake levels are increased by a factor 2 or 3 (2). More than 99% of the intake of benzene is through the air (3), where it may originate from natural sources (e.g., forest fires) or from human activities such as smoking or exhaust fumes. Other sources of benzene are drinking water and food, both through contamination from the environment (2).

Already in 1993, Gardner et al. (4) described that the combination of sodium benzoate and ascorbic acid in food could lead to the formation of benzene, especially in acidic beverages such as soft drinks. Since that time, several surveys on the benzene contents in food (5) and soft drinks (6–9) have been published. In 1993, McNeal et al. (5) showed levels of benzene

ranging from <1 to $38 \mu\text{g kg}^{-1}$ in foods, including soft drinks with added benzoates and ascorbates. In their conclusion they stated that, although the detected levels were quite low in the analyzed soft drinks, several manufacturers were reformulating their drinks to reduce benzene formation. Because no legal limit for benzene in soft drinks exists, the limit in drinking water is mostly used as reference value. Both the report of the British Food Standard Agency (7) and the study by Fabietti et al. (6) compared the concentrations detected in soft drinks to the World Health Organization (WHO) limit for benzene in drinking water of $10 \mu\text{g L}^{-1}$ (2). In the former, 4 of 150 soft drinks contained $>10 \mu\text{g L}^{-1}$ benzene and 70% did not contain any detectable levels. The Italian study (6) analyzed 60 samples without any exceeding the WHO limits for drinking water. The U.S. Food and Drug Administration did a survey on more than 100 soft drinks and found that 5 samples exceeded the $5 \mu\text{g L}^{-1}$ U.S. Environmental Protection Agency maximum contaminant level for drinking water. In the most recent study, Cao et al. (9) analyzed 124 soft drinks from the Canadian market and found 60% of the samples to be negative; 6 products, however, were above the Canadian maximum acceptable concentration of $5 \mu\text{g L}^{-1}$ for benzene in drinking water and 2 were even higher than the WHO limit for benzene in drinking water of $10 \mu\text{g L}^{-1}$. An even more rigorous guideline is that of the European

* Corresponding author [telephone +32(0)9.264.81.34; fax +32(0)9 0.264.81.99; e-mail Christof.vanpoucke@UGent.be].

[†] Laboratory of Food Analysis, Ghent University.

[‡] Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University.

[§] Federal Agency for the Safety of the Food Chain.

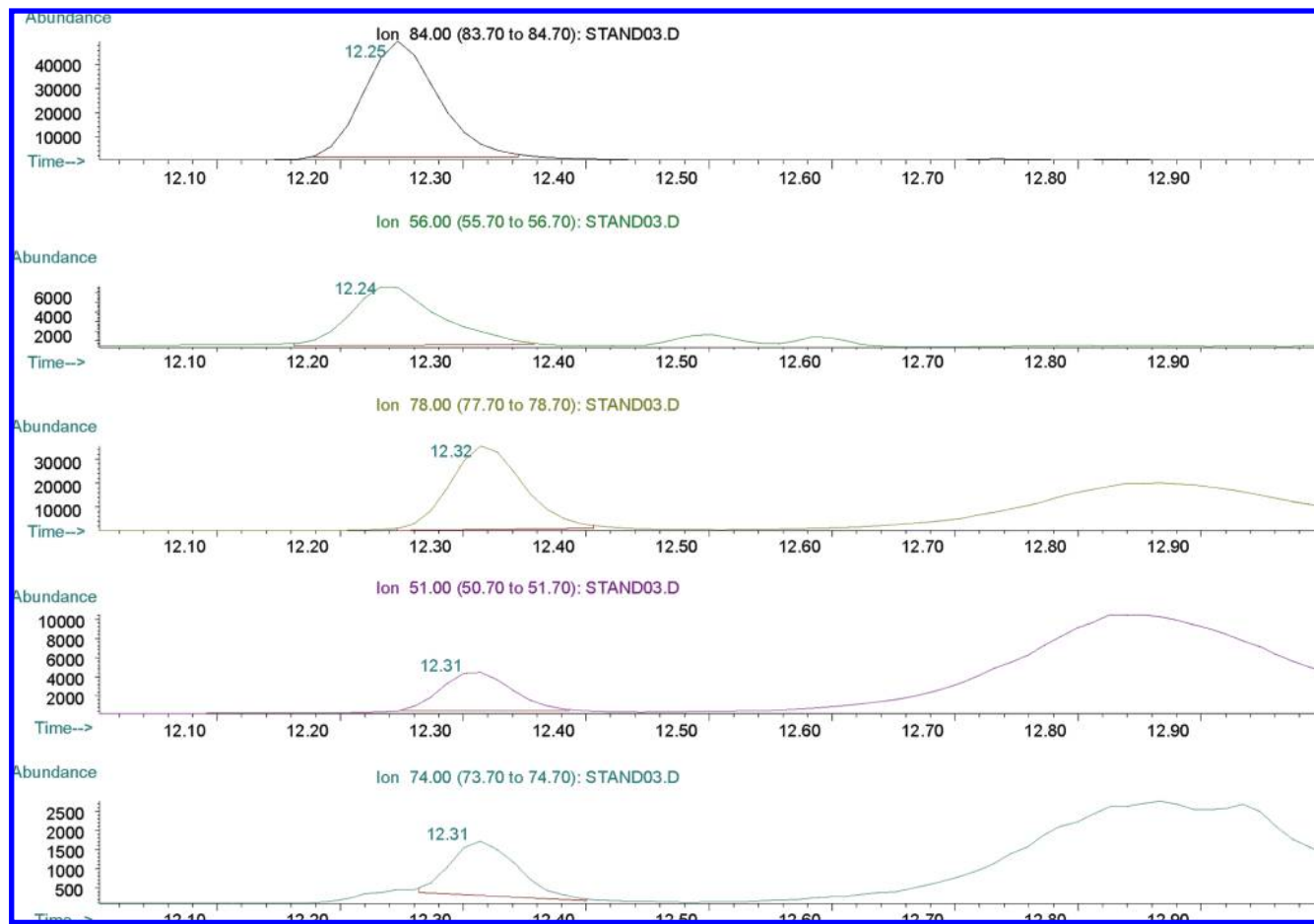


Figure 1. Chromatogram of a standard solution of $0.3 \mu\text{g L}^{-1}$ benzene showing the diagnostic ions of the internal standard benzene- d_6 (84, 56) and benzene (78, 51, 74).

Council, which has fixed the limit for benzene in drinking water at $1 \mu\text{g L}^{-1}$ (10). This limit was suggested as an acceptable reference point for benzene in soft drinks by the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain (11). In view of the lack of legal limits in soft drinks and the different reference points used in the various studies, a uniform evaluation of the data remained rather difficult. Currently, on a European level, the Standing Committee on the Food Chain and Animal Health of DG Health and Consumer Protection has suggested an action level for benzene in soft drinks of $10 \mu\text{g L}^{-1}$ (12). However, due to its carcinogenic properties, it has to be emphasized that no risk-free action level for benzene can be fixed.

All studies mentioned use some form of headspace gas chromatography. The purge and trap approach (5, 6) is most often used, but the use of SPME has also been mentioned (7), all with a limit of quantification of $1 \mu\text{g L}^{-1}$ (5, 7, 8). Cao et al. (9) used a static headspace approach resulting in a detection limit of $0.26 \mu\text{g L}^{-1}$. The aim of this survey was to examine the benzene content in soft drinks on the Belgian market. Whereas several surveys on the occurrence of benzene in soft drinks have been conducted in different countries (5–9), this is, to our knowledge, the first study that uses a statistical evaluation of the data presented to identify some factors affecting benzene formation.

MATERIALS AND METHODS

Reagents and Chemicals. Benzene stock solutions of $5000 \mu\text{g mL}^{-1}$ (Supelco) and benzene- d_6 stock solutions of $2000 \mu\text{g mL}^{-1}$ (Supelco)

were both supplied by Sigma-Aldrich (Bornem, Belgium). Double-deionized water (Milli-Q; Millipore Corp., Billerica, MA) of $18.2 \text{ M}\Omega \text{ cm}^{-1}$ resistivity was used throughout.

Apparatus and Materials. Samples were injected on the GC-MS system using a Gerstel MultiPurposeSampler MPS-1 injector (Gerstel, Mülheim an der Ruhr, Germany) in the headspace sampler mode. The analysis was performed on an Agilent 6890 gas chromatograph equipped with a CIS-4 Cooled Injection System (Gerstel) and coupled to an Agilent 5973 mass selective detector (Agilent, Palo Alto, CA). The CIS injector liner was cooled using a pressurized vessel containing liquid nitrogen (Air-Liquide, Liege, Belgium). Full automation was achieved using Gerstel Master software in conjunction with Agilent Chemstation data acquisition and data handling software.

The column used was a CP-Select 624 custom-made capillary column ($41 \text{ m} \times 0.25 \text{ mm}$ (i.d.); $2.1 \mu\text{m}$ film thickness; Chrompack, Middelburg, The Netherlands). The oven temperature was programmed from $45 \text{ }^\circ\text{C}$ (held for 3 min) to 90 at $5 \text{ }^\circ\text{C min}^{-1}$ and then to 150 at $40 \text{ }^\circ\text{C min}^{-1}$, which was held for 4.5 min. High-purity helium (Air-Liquide) was used as carrier gas at a flow rate of 1 mL min^{-1} .

To enhance sensitivity, cryofocusing in the injector liner was applied. To that end, the deactivated liner was packed with Tenax TA (20/35 mesh, Alltech, Lokeren, Belgium). After incubation of the sample at $70 \text{ }^\circ\text{C}$ (25 min), 1 mL of the vapor phase was injected onto the Tenax bed in the liner and benzene was trapped by cryocooling at $-40 \text{ }^\circ\text{C}$. Excess nontrapped vapor was evacuated from the injector using the CIS solvent purge mode (1/8 split), after which the injector temperature was quickly ($12.0 \text{ }^\circ\text{C/s}$) raised to $180 \text{ }^\circ\text{C}$, flash desorbing benzene to the GC column.

The mass spectrometer was operated in the selected ion monitoring (SIM) mode recording the m/z values of 84 and 56 for benzene- d_6 and 78, 74, and 51 for benzene (100 ms dwell times).

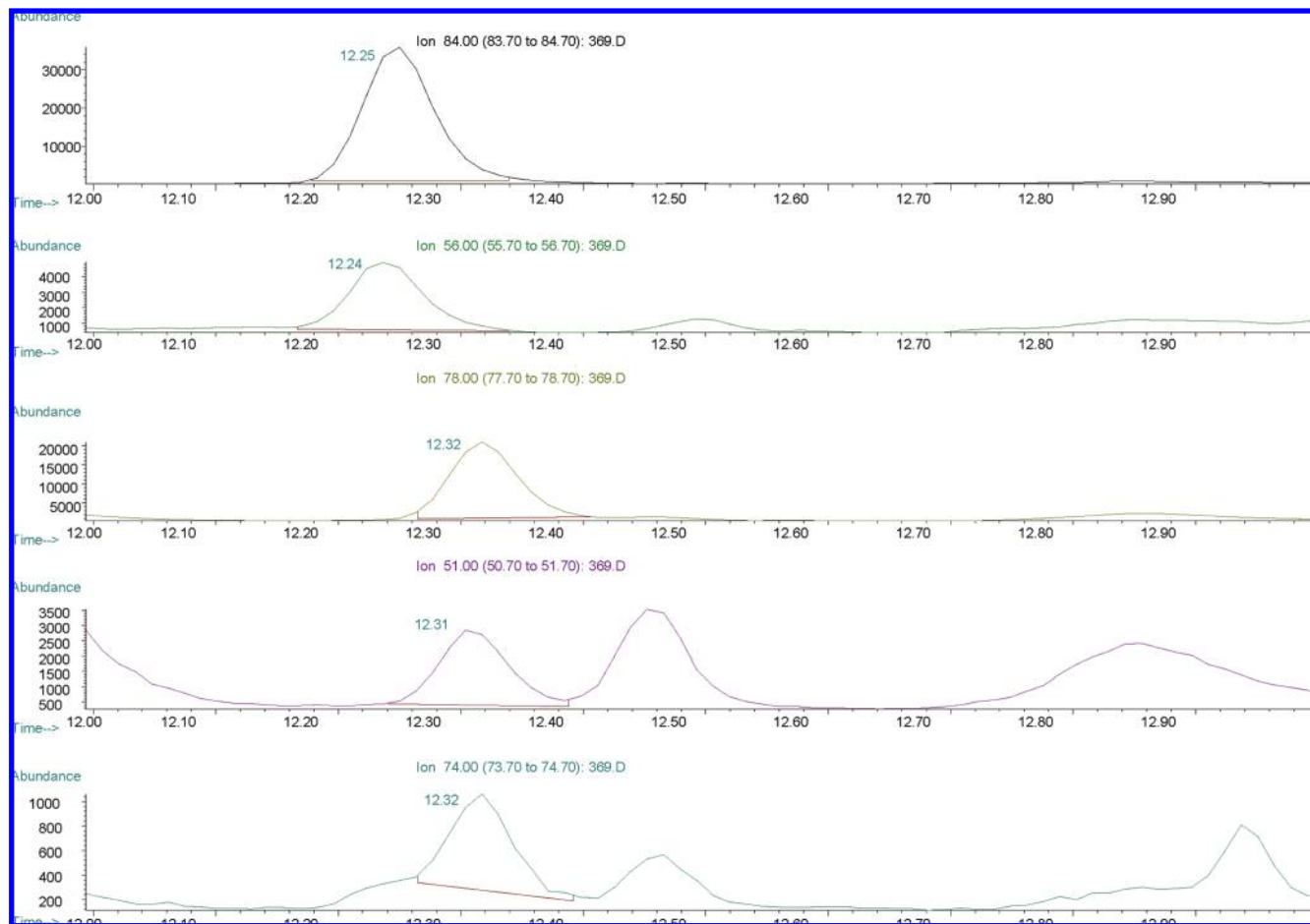


Figure 2. Chromatogram of a soft drink sample containing $0.32 \mu\text{g L}^{-1}$ benzene showing the diagnostic ions of the internal standard benzene- d_6 (84, 56) and benzene (78, 51, 74).

Sample Preparation. All samples were gently homogenized by shaking for 30 s. Carbonaceous drinks were degassed by sonicating about 5 mL of the soft drink for about 1.5 min until all carbon dioxide bubbles had disappeared. Of each sample, 3 mL was transferred to a headspace vial, and the internal standard benzene- d_6 was added at a concentration of $0.6 \mu\text{g L}^{-1}$.

For quantification and quality control purposes each batch of analysis was accompanied by a water sample (blank), two water samples spiked at $1 \mu\text{g L}^{-1}$ (control sample), to be analyzed at the beginning and the end of each batch of samples, and calibration samples spiked at 0.3, 0.6, 1, 2, and $3 \mu\text{g L}^{-1}$.

Statistical Analysis. To identify the factors with a significant contribution ($\alpha < 0.05$) to the detected benzene content an analysis of variance (ANOVA) was done using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). For this evaluation, all data below the limit of quantification (LOQ) were replaced by half the determined LOQ. Post hoc comparisons between different groups from a significant factor were done with a Sheffé test, in which the mean difference between two groups was tested at the 0.05 level.

RESULTS AND DISCUSSION

For the determination of benzene in soft drinks a very simple and rapid analysis was developed. The sample pretreatment consisted only of elimination of CO_2 in carbonaceous samples. During a preliminary experiment no difference was noted in the detected benzene concentration between the addition of the internal standard before or after elimination of the CO_2 content. Consequently, it was decided to add the internal standard after sonication to facilitate the sample preparation. After addition of the internal standard, the sample was placed in the automated headspace sampler, and prior to injection, each sample was, in

turn, incubated during exactly 25 min at 70°C . This brings the total analysis time to 43 min for each sample with an 18 min overlap between two samples.

This method was in-house validated for the following parameters: specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and intralaboratory reproducibility. To determine the specificity, 10 blank samples (water) and 4 samples spiked with other volatile substances, such as hexane, ethanol, ether, and ethyl acetate, were analyzed as described above. As no interfering peaks within the 0.5% margin of the relative retention time of benzene could be detected, it was concluded that the method was specific for the determination of benzene. For the determination of the LOD and LOQ of the method, a calibration graph with the following concentrations was constructed: 0.15, 0.3, 0.6, 0.75, and $1 \mu\text{g L}^{-1}$. This curve was analyzed on three different days, and linearity was evaluated with a lack-of-fit test, by which a significance of 0.426 was obtained and the hypothesis of a linear correlation was accepted. The LOD was calculated as 3 times the residual standard deviation of the y intercept divided by the slope of the curve. The LOQ was calculated as 3 times the limit of detection. This resulted in LOD and LOQ values of 0.1 and $0.3 \mu\text{g L}^{-1}$, respectively, which are far below the reference points for benzene in soft drinks as suggested by the Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain (11). Although it is generally accepted that a dynamic headspace approach such as purge and trap is more sensitive than static headspace chromatography, the approach used in this study resulted in detection and quantification limits below those of

Table 1. Overview of the Results, the Package Used, and the Presence of an Acidity Regulator, Benzoic Acid, and Ascorbic Acid (As Mentioned on the Label) of the Analyzed Soft Drinks

| sample | result ^a ($\mu\text{g L}^{-1}$) | packing material | benzoic acid | ascorbic acid | citric acid | phosphoric acid | tartaric acid | malic acid |
|--------|--|------------------|--------------|---------------|-------------|-----------------|---------------|------------|
| 1 | <LOQ | plastic | | | x | x | | |
| 2 | <LOQ | plastic | | | | x | | |
| 3 | <LOQ | can | | | x | | | |
| 4 | <LOQ | can | | x | x | | | |
| 5 | <LOQ | can | x | | x | x | | |
| 6 | 0.32 | can | | | x | x | x | |
| 7 | ND | can | | | x | x | | |
| 8 | <LOQ | plastic | x | | x | x | | |
| 9 | <LOQ | glass | x | | x | x | | |
| 10 | <LOQ | glass | | | x | x | | |
| 11 | <LOQ | glass | x | x | x | | | |
| 12 | <LOQ | glass | x | | x | x | | |
| 13 | ND | plastic | | | x | x | | |
| 14 | <LOQ | plastic | | x | x | | | |
| 15 | ND | plastic | | | x | X | | |
| 16 | <LOQ | can | x | | x | X | | |
| 17 | 0.40 | plastic | x | | x | X | x | |
| 18 | <LOQ | plastic | | | x | X | | |
| 19 | 5.3 | plastic | x | x | x | | | |
| 20 | ND | plastic | | | x | X | | |
| 21 | 3.10 | glass | x | | x | X | | |
| 22 | <LOQ | can | | x | x | | | |
| 23 | 0.80 | can | x | | x | X | | |
| 24 | <LOQ | can | | | | x | | |
| 25 | ND | can | | | x | | | |
| 26 | <LOQ | glass | | | | | | |
| 27 | ND | glass | x | | x | x | | |
| 28 | 0.30 | glass | x | | x | x | | |
| 29 | <LOQ | glass | x | | x | x | | |
| 30 | 2.31 | plastic | x | | | | | |
| 31 | 10.98 | plastic | x | x | x | | | |
| 32 | <LOQ | plastic | x | | x | | | |
| 33 | <LOQ | can | x | | x | x | x | |
| 34 | 0.38 | can | | x | x | | | x |
| 35 | 0.48 | can | x | | x | x | x | |
| 36 | <LOQ | can | x | x | x | | | |
| 37 | <LOQ | can | x | | x | | | |
| 38 | 0.93 | can | x | | x | x | | |
| 39 | <LOQ | can | x | | x | x | x | |
| 40 | <LOQ | can | x | | x | x | | |
| 41 | <LOQ | can | x | | x | | | |
| 42 | ND | can | | x | x | | | |
| 43 | <LOQ | glass | x | | x | x | x | |
| 44 | <LOQ | glass | | x | | | | |
| 45 | <LOQ | glass | x | | x | x | x | |
| 46 | <LOQ | glass | x | | x | x | | |
| 47 | ND | glass | | | x | x | | |
| 48 | <LOQ | glass | | x | x | | | |
| 49 | <LOQ | glass | x | | x | x | x | |
| 50 | 7.51 | plastic | x | x | x | | | |
| 51 | <LOQ | plastic | x | | x | x | | |
| 52 | 0.78 | plastic | x | x | x | | | |
| 53 | <LOQ | plastic | x | | x | | | |
| 54 | 1.66 | plastic | x | | x | | | |
| 55 | ND | glass | | | | | | |
| 56 | <LOQ | glass | | | | | | |
| 57 | ND | glass | | | | | | |
| 58 | <LOQ | can | | | x | x | | |
| 59 | 0.60 | can | x | | x | x | x | |
| 60 | ND | can | | x | x | | | |
| 61 | ND | plastic | x | | x | x | x | |
| 62 | ND | plastic | x | | x | x | x | |
| 63 | 0.52 | plastic | x | | x | x | x | |
| 64 | ND | plastic | x | | x | x | | |
| 65 | ND | plastic | | x | x | | | |
| 66 | <LOQ | glass | | | | x | | |
| 67 | ND | glass | | x | x | | | |
| 68 | <LOQ | glass | x | x | x | | | |
| 69 | <LOQ | glass | x | | x | x | | |
| 70 | <LOQ | glass | x | | x | x | x | |
| 71 | <LOQ | can | | | x | | | |
| 72 | ND | can | | | x | x | | |
| 73 | 0.30 | can | x | | | | | |

Table 1. Continued

| sample | result ^a ($\mu\text{g L}^{-1}$) | packing material | benzoic acid | ascorbic acid | citric acid | phosphoric acid | tartaric acid | malic acid |
|--------|--|------------------|--------------|---------------|-------------|-----------------|---------------|------------|
| 74 | <LOQ | plastic | x | | x | | | |
| 75 | ND | can | x | | | | | |
| 76 | <LOQ | can | | | x | | | |
| 77 | <LOQ | can | | | x | x | | |
| 78 | <LOQ | glass | | | | | | |
| 79 | ND | glass | | | | | | |
| 80 | <LOQ | glass | | | | | | |
| 81 | 0.34 | can | | | x | | | |
| 82 | ND | glass | | | | | | |
| 83 | <LOQ | glass | | x | x | | | |
| 84 | ND | glass | x | | x | | | |
| 85 | 0.79 | plastic | x | x | x | | | |
| 86 | ND | plastic | x | | x | | | |
| 87 | ND | plastic | | x | x | | | |
| 88 | ND | glass | | x | x | | | |
| 89 | 0.33 | glass | x | x | x | | | |
| 90 | ND | glass | | | x | | | |
| 91 | 0.60 | glass | x | x | x | | | |
| 92 | ND | glass | x | | x | x | | |
| 93 | 4.19 | plastic | x | x | x | | | |
| 94 | 4.15 | glass | | | x | | | |
| 95 | ND | glass | | x | | | | |
| 96 | 0.49 | glass | x | x | x | | | |
| 97 | <LOQ | glass | x | | x | | | |
| 98 | 0.46 | carton | x | x | x | | | |
| 99 | 2.77 | carton | x | | x | | | |
| 100 | <LOQ | carton | | x | x | | | |
| 101 | <LOQ | can | | x | x | | | |
| 102 | ND | plastic | | | x | x | | |
| 103 | ND | glass | | x | | | | |
| 104 | ND | glass | | | x | x | | |
| 105 | ND | glass | | x | x | | | |
| 106 | ND | can | x | | | | | |
| 107 | <LOQ | can | x | | x | | | |
| 108 | N.D. | can | x | | | | | |
| 109 | <LOQ | can | | x | x | | | x |
| 110 | ND | plastic | x | x | | | | |
| 111 | <LOQ | carton | | | | | | |
| 112 | <LOQ | glass | | x | | | | |
| 113 | <LOQ | carton | | | | | | |
| 114 | <LOQ | carton | | | | | | |
| 115 | N.D. | carton | | | | | | |
| 116 | <LOQ | glass | x | | x | x | | |
| 117 | <LOQ | glass | x | | x | x | | |
| 118 | <LOQ | glass | | x | | | | |
| 119 | ND | can | | x | x | | | |
| 120 | <LOQ | glass | | x | x | | | |
| 121 | ND | plastic | | x | x | | | |
| 122 | <LOQ | plastic | | x | x | | | |
| 123 | ND | glass | x | | x | x | | |
| 124 | <LOQ | glass | | x | | | | |
| 125 | ND | plastic | | | | x | | |
| 126 | <LOQ | plastic | x | | x | x | x | |
| 127 | <LOQ | can | x | | x | x | x | |
| 128 | 4.05 | glass | | | x | | | |
| 129 | ND | glass | | x | x | | | |
| 130 | ND | carton | | x | | | | |
| 131 | ND | plastic | | x | | | | |
| 132 | ND | carton | | x | | | | |
| 133 | <LOQ | carton | | x | | | | |
| 134 | ND | carton | | | | | | |

^a LOQ, 0.3 $\mu\text{g L}^{-1}$; ND, not detected.

the studies using such a dynamic headspace approach (5, 7, 8) and comparable to those of the only other study using static headspace chromatography (9). **Figure 1** shows a chromatogram of a standard of 0.3 $\mu\text{g L}^{-1}$ and **Figure 2** a real soft drink sample containing 0.32 $\mu\text{g L}^{-1}$, indicating that the calculated LOQ is a realistic value. For the determination of the repeatability, a sample containing 2 $\mu\text{g L}^{-1}$ was analyzed six times, and this was repeated on a different day to determine the intralaboratory

reproducibility. The obtained repeatability and intrareproducibility were 2.5 and 8.2%, respectively.

Each batch of samples was analyzed under strict quality control criteria. The correlation coefficient of each calibration graph had to be at least 0.995, and the apparent recovery (the observed value derived from an analytical procedure by means of a calibration graph) of the control samples had to be within 90–110%. A sample was found to be positive for the presence

Table 2. Factors Making a Significant ($\alpha < 0.05$) Contribution to the Measured Benzene Content^a

| factor | significance |
|---|--------------|
| acidity regulator | 0.023 |
| ascorbic acid \times acidity regulator | 0.001 |
| benzoic acid \times acidity regulator | 0.002 |
| benzoic acid \times ascorbic acid | 0.000 |
| packing material | 0.004 |
| packing material \times ascorbic acid | 0.000 |
| packing material \times benzoic acid | 0.000 |
| packing material \times benzoic acid \times ascorbic acid | 0.001 |

^a Tested factors in the four-way ANOVA: packing material, benzoic acid, ascorbic acid, and acidity regulator.

of benzene only if the identification criteria based on those mentioned in Commission Decision 2002/657/EC (13) were fulfilled. These criteria include that the relative retention time of all three diagnostic ions is within a 0.5% margin of the relative retention time as determined in the control samples and that the relative ion ratio is within 10, 20, and 50% margins for ions 78 (quantification ion), 51, and 74, respectively. These criteria were fulfilled with the sample shown in **Figure 2**, resulting in a positive sample containing $0.32 \mu\text{g L}^{-1}$ benzene.

From October 2006 until May 2007, 134 low-calorie soft drinks were sampled by the Belgian Federal Agency for the Safety of the Food Chain and submitted to our laboratory for the determination of the benzene content. The results obtained for these soft drinks are summarized in **Table 1**. In only 44 samples (33%) could no benzene could be identified, either because the molecule was simply not detected or because the identification criteria mentioned above were not fulfilled. In approximately half of the samples (47%) trace amounts of benzene were identified at concentrations below the LOQ of the method ($0.3 \mu\text{g L}^{-1}$). Twenty-seven samples (20%) contained quantifiable concentrations of benzene, of which 10 were above the European limit for benzene in drinking water of $1 \mu\text{g L}^{-1}$ (10) and 3 were above the $5 \mu\text{g L}^{-1}$ U.S. Environmental Protection Agency maximum contaminant level for drinking water. One of the samples even had a concentration of $10.98 \mu\text{g L}^{-1}$, thereby exceeding the action limit for benzene in soft drinks of $10 \mu\text{g L}^{-1}$ proposed at the Standing Committee on the Food Chain and Animal Health (12).

To identify the factors that have an influence on the benzene formation, the listing of benzoic acid, ascorbic acid, and an acidity regulator (e.g., citric acid, phosphoric acid) on the labels of the different low-calorie soft drinks was evaluated. Also, the type of packing material was taken into consideration, and an overview of all these data is included in **Table 1**. The obtained data were analyzed using a four-way ANOVA to test the influence of the different factors (packing material, benzoic acid, ascorbic acid, and acidity regulator) and the interaction between these individual factors on the benzene formation. This evaluation identified eight factors that make a significant contribution ($\alpha < 0.05$) to the amount of benzene that was detected in the different diet soft drinks (**Table 2**). As could be expected, the interaction between the preservative benzoic acid and the antioxidant ascorbic acid had a significant effect on the benzene content, but also the interaction between benzoic acid and the acidity regulator and ascorbic acid and the acidity regulator had an influence. Because only one sample containing the combination of benzoic acid and ascorbic acid without the presence of an acidity regulator was analyzed, no statistical evaluation could be made on the influence of the acidity regulator on the combination of the above-mentioned interaction between the preservative and the antioxidant.

Table 3. Results of the Post Hoc Scheffé Test on the Packing Material

| packing material (I) | packing material (J) | mean difference ^a (I - J) | standard error | significance |
|----------------------|----------------------|--------------------------------------|----------------|--------------|
| plastic | glass | 0.6964* | 0.22262 | 0.024 |
| | can | 0.8379* | 0.23915 | 0.009 |
| | carton | 0.6693 | 0.35057 | 0.308 |
| glass | plastic | -0.6964* | 0.22262 | 0.024 |
| | can | 0.1415 | 0.21902 | 0.936 |
| | carton | -0.0271 | 0.33717 | 1.000 |
| can | plastic | -0.8379* | 0.23915 | 0.009 |
| | glass | -0.1415 | 0.21902 | 0.936 |
| | carton | -0.1686 | 0.34830 | 0.972 |
| carton | plastic | -0.6693 | 0.35057 | 0.308 |
| | glass | 0.0271 | 0.33717 | 1.000 |
| | carton | 0.1686 | 0.34830 | 0.972 |

^a *, the mean difference is significant at the 0.05 level.

Table 4. Overview of the Results of 12 Identical Soft Drinks with Different Batch Numbers Containing Benzoic Acid and Two Acidity Regulators

| sample | date of analysis | result ($\mu\text{g L}^{-1}$) | expiration date | days before expiration |
|--------|------------------|---------------------------------|-----------------|------------------------|
| 9 | Nov 7, 2006 | <LOQ | March 31, 2007 | 144 |
| 12 | Nov 14, 2006 | <LOQ | March 31, 2007 | 137 |
| 21 | Oct 27, 2006 | 3.1 | Nov 30, 2006 | 34 |
| 27 | Nov 7, 2006 | | April 30, 2007 | 174 |
| 28 | Nov 7, 2006 | 0.3 | Feb 1, 2007 | 86 |
| 29 | Nov 7, 2006 | <LOQ | March 1, 2007 | 114 |
| 46 | Nov 14, 2006 | <LOQ | Feb 1, 2007 | 79 |
| 69 | Nov 7, 2006 | <LOQ | April 30, 2007 | 174 |
| 92 | April 17, 2007 | | Sept 30, 2007 | 166 |
| 116 | May 7, 2007 | <LOQ | June 30, 2007 | 54 |
| 117 | May 7, 2007 | <LOQ | July 31, 2007 | 85 |
| 123 | April 30, 2007 | | July 31, 2007 | 92 |

As shown in **Table 2**, the type of packing material also played an important role in the benzene formation, either as an individual factor or due to interaction with other factors. To identify the type of packing that contained soft drinks with significantly higher benzene concentrations, a post hoc comparison was made using a Scheffé test. This test (**Table 3**) indicates that significantly higher concentrations of benzene were detected in soft drinks sold in plastic bottles than in canned soft drinks or those commercialized in glass bottles.

A further evaluation of the data revealed that 12 samples were the same type of soft drink, from the same manufacturer and all bottled in glass but with different batch numbers and sampled by different people on different days and locations. These samples contained the combination of benzoic acid and both citric and phosphoric acid, two acidity regulators, a combination that came out of the above-mentioned statistical analysis as a significant factor in the benzene formation. As can be seen from the results of these 12 samples (**Table 4**), a high variability, ranging from absence to $3.1 \mu\text{g L}^{-1}$, in the detected benzene concentration was found. Although the sample with the highest benzene concentration was closest to the expiration date (34 days) of all 12 samples, too few data are available to make a reliable conclusion about the correlation of the expiration date and the detected benzene content. Other factors, such as storage conditions, may also play an important role and need to be further investigated.

All of the above data indicate that the problem of benzene formation in soft drinks is still a valid topic. Statistical analyses revealed that besides benzoic acid, ascorbic acid, and acidity regulators, the packing may also play an important role in

benzene formation. Also, other factors such as storage conditions may contribute. According to the International Council of Beverages Association (<http://www.icba-net.org/>) “. . . the industry has taken a responsible approach to prevent/minimize the presence of benzene in its beverages” (14). Already in 1993 McNeal et al. (5) stated that several manufacturers were re-evaluating their formulations and processes to minimize benzene formation; however, many soft drinks in this study still contained benzoic acid as a preservative.

ACKNOWLEDGMENT

The Federal Agency for the Safety of the Food Chain (Brussels, Belgium) is thanked for making available the benzene data.

LITERATURE CITED

- (1) IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; Proceedings for the Advisory Group To Plan Volume 100: A Review of Human Carcinogens, IARC: Lyon, France, 1998.
- (2) WHO. *Benzene in Drinking-Water*; Background Document for Development of WHO Guidelines for Drinking-Water Quality; World Health Organization: Geneva, Switzerland, 1996.
- (3) *Benzene*; WHO Regional Office for Europe: Copenhagen, Denmark, 2000.
- (4) Gardner, L. K.; Lawrence, G. D. Benzene production from decarboxylation of benzoic-acid in the presence of ascorbic-acid and a transition-metal catalyst. *J. Agric. Food Chem.* **1993**, *41*, 693–695.
- (5) McNeal, T. P.; Nyman, P. J.; Diachenko, G. W.; Hollifield, H. C. Survey of benzene in foods by using headspace concentration techniques and capillary gas-chromatography. *J. AOAC Int.* **1993**, *76* (6), 1213–1219.
- (6) Fabietti, F.; Delise, M.; Bocca, A. P. Investigation into the benzene and toluene content of soft drinks. *Food Control* **2001**, *12* (8), 505–509.
- (7) Survey of benzene in soft drinks; <http://www.food.gov.uk/multimedia/pdfs/fsis0606.pdf> (Feb 15, 2007).
- (8) Data on benzene in soft drinks and other beverages; <http://www.cfsan.fda.gov/~dms/benzdata.html> (Feb 15, 2007).
- (9) Cao, X. L.; Casey, V.; Seaman, S.; Tague, B.; Becalski, A. Determination of benzene in soft drinks and other beverages by isotope dilution headspace gas chromatography/mass spectrometry. *J. AOAC Int.* **2007**, *90* (2), 479–484.
- (10) Council Directive 98/83/EC. *Off. J. Eur. Communities* **1998**, *L330*, 32–54.
- (11) *Gebruik van de geldende norm voor benzeen in water voor het vastleggen van een actielimiet voor benzeen in frisdrank (dossier Sci Com 2006/18)*; Scientific Committee of the Belgian Federal Agency for the Safety of the Food Chain; 2006.
- (12) *Summary Record of The Standing Committee on The Food Chain and Animal Health, Brussels, Belgium, 31 March 2006*; DG Health and Consumer Protection; 2006.
- (13) Commission Decision 2002/657/EC. *Off. J. Eur. Communities* **2002**, *L221*, 8.
- (14) *ICBA Guidance Document to Mitigate the Potential for Benzene Formation in Beverages*; International Council of Beverages Association: Washington, DC, 2006.

Received for review August 29, 2007. Revised manuscript received January 22, 2008. Accepted March 21, 2008.

JF072580Q