Benzene Determination in Soft Drinks

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Objectives: Benzene is a known human carcinogen. We are exposed to benzene mainly through inhalation and a lesser extent through the ingestion of food and water. Benzene is formed in beverages when ascorbic acid coexists with benzoate salts. Certain additional conditions (heat, UV light, metallic ions) are required for detectable levels of benzene to be formed. The aim of this study was to investigate soft drinks for the presence of benzene.

Methods: A GC method was developed to identify and quantify benzene in beverages. Fifteen samples containing benzoate sodium and ascorbic acid were analyzed.

Results: The benzene concentration ranged between the limit of quantification and 8.81 µg/l. The EU guideline limits the benzene concentration at 1 µg/l in drinking water; 6 of the products tested were above this admitted level.

Conclusion: Based on the presented data, the safety of benzene as a preservative agent should be reconsidered.

Keywords: benzene, gas chromatography, soft drinks, benzoate

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Introduction

Benzene is a known human carcinogen by all routes of exposure. Studies have shown that even low levels of benzene exposure can cause haematological malignancies (myelogenous leukemia, lymphocytic leukemia, aplastic anemia). In vitro studies and animal experiments demonstrated low birth weight, increased skeletal variants when pregnant mice and rats breathed benzene [1,2]. Sources of human exposure are tobacco smoke, industrial emissions, automobile exhaust, drinking water and food [3,4].

Since 1990 it is known that some types of beverages had benzene contamination. Studies showed that benzene is formed by decarboxylation of benzoate salts (commonly found food preservatives, E210–E213) in the presence of ascorbic acid (E330) or erythorbic acid. Elevated temperatures, light and metallic ions can stimulate, while EDTA salts and sugar inhibit benzene formation, therefore higher amounts of benzene are found in light beverages [3,5,6,7,8].

There are no regulatory limits for benzene in soft drinks, various authorities have set limits of benzene content in drinking water and bottled water. The World Health Organization (WHO) has set a guideline level for benzene in drinking-water of 10 µg/l [9]; the limit set by the United States Environmental Protection Agency (EPA) is 5 µg/l [10] and the limit set by the EU is 1 µg/l [11].

There are many methods for benzene determination in soft drinks. Headspace gas chromatography/mass spectrometry (GC-MS) [8,12] with a limit of quantification of 0.3 µg/l, and it's variations: isotope dilution GC with detection limit of 0.01 µg/l [13], and capillary GC [14]. In these studies benzene levels ranged between none detected to 23 µg/l [13], none detected to 10.98 µg/l [8], and none detected to 1.1 µg/l [12]. These methods require high-cost equipment, thus inhibiting their routine use.

The aim of our study was to develop a low-cost, very simple GC method, which is sensitive enough to detect amounts of benzene lower than the EU limit of 1 µg/l in soft drinks. Furthermore, this method was used to assess benzene concentration in 15 samples of soft drink products.

Material and method

Instrumentation and reagents

- centrifuge: Sigma 2K15, Sigma Aldrich, Germany
- gas chromatograph: GC-8A with FID detector, Shimadzu Corp., Japan;
- column: Propach N, 1.2 m;
- hydrogen: 4.5, Linde Gas, Romania;
- argon: 4.7, Romsif Impex SRL, Romania;
- benzene: gradient grade, 99.9%, Merck KgaA, Germany;
- toluen: GC quality, Merck KgaA, Germany;

Analytical parameters

- column and FID temperature: 170°C;
- range: 1;
- attenuation: 4;
- argon pressure: 2.5 kg/cm^2;
- hydrogen pressure: 0.5 kg/cm^2;
- air pressure: 0.5 kg/cm^2.
- injected amount 5 µL;

Standard solutions

A 1 µg/l solution of benzene in water was prepared by serial dilution of benzene with distilled water. Two-hundred fifty ml of standard solution was extracted with 2.5 ml toluene in a separating funnel, realizing in this manner a 100-fold concentration.

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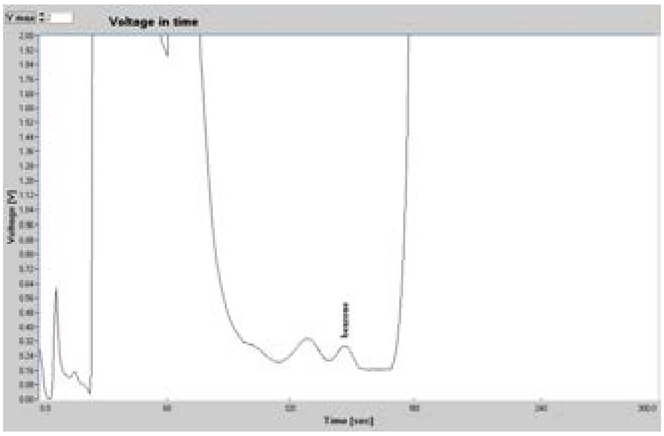

Fig. 1. Typical chromatogram obtained for beverage samples

Sample preparation

Two-hundred fifty ml of sample was extracted with 2.5 ml toluene in a separating funnel; toluene layer was centrifuged at 10000 rpm for 10 minutes. Five µl of the toluene phase was injected into the GC.

Results

Method parameters

Specificity: soft drink sample without benzoic acid shows no interference peak in the interested zone (Figure 1).

Linearity: was determined at 5 concentration levels ranging from 0.5 µg/l to 2 µg/l. The coefficient of correlation was 0.9996 with a slope of 0.6799. The residuals are randomly scattered above and below the abscissa when represented as a function of concentration, and their value was below 5.5%.

Recovery: was determined by spiked samples (soft drink without benzoate) at four concentration levels (0.5, 1, 1.5, 2 µg/l). The recovery levels ranged from 87.2% to 111%, with a coefficient of correlation of 4.95%. Recoveries for spiked samples were calculated by height ratio of the samples and the standard solution.

Limit of detection (LOD) and limit of quantification (LOQ): the LOD is the concentration of benzene yielding a peak with a signal to noise ratio of 3:1, and LOQ was considered the concentration of benzene yielding a signal to noise ratio of 10:1. The LOD for benzene was found to be 0.1 µg/l; the LOQ was 0.5 µg/l.

In order to measure benzene concentration in soft drinks, samples containing benzoic acid and citric acid were purchased from local stores. The identification of benzene in soft drinks was made using the retention time 150±5 sec. The obtained results are presented in Table I.

The obtained benzene concentration ranged between LOD and the value of 8.81 µg/l.

Discussions

In all samples, except one soft drink type, benzene was found at levels below the EPA guideline of 5 µg/l. Samples of 6 products contained benzene at levels higher than the EU guideline of 1 µg/l. The obtained results are in concordance with those obtained by Health Canada [7] when 118 products were analyzed and amounts of benzene in soft drinks between BLQ and 23 µg/l were found. The products with higher amount of benzene than the WHO guideline were reformulated and reanalyzed, and it was concluded that in the reformulated products, benzene was either not detected or was present at such low levels that it could not be measured [7]. We suggest that products in which benzene levels were found to be in excess of the EU guideline of 1 µg/l should be reformulated.

Table I. Compounds identified at HPLC analysis of SEZ1 solution

Tested product	Benzene concentration (µg/l)
Lemon taste soft drink	0.67
Cola taste soft drink (very cheap)	1.55
Lemon-lime taste soft drink*	1.42
Sour cherry taste soft drink	1.22
Multi fruit soft drink	1.15
Orange taste soft drink	3.25
Citrus flavored soft drink	0.40
Grape-apple taste soft drink concentrate	0.27
Peach taste soft drink	8.81
Pear taste soft drink	0.47
Multivitamin soft drink	0.84
Orange taste soft drink (light version)	0.33
Orange flavored soft drink*	0.23
Ginger flavored soft drink	0.10
Pineapple taste soft drink	below LOQ

*internationally sold product

The potential exposure to benzene from soft drinks constitutes a small part of the total benzene exposure, but considering that children are consuming many of these products, the benzene level in soft drinks must be limited.

Conclusions

Our measurements show that important amounts of benzene could be present in soft drinks, especially when in their formulation benzoate salts coexist with ascorbic acid. Based on these data and other published works, benzoate utility as a preservative in soft drinks should be reconsidered.

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