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Journal of Chromatography A, 963 (2002) 179–183

JOURNAL OF CHROMATOGRAPHY A

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Application of solid-phase microextraction combined with gas chromatography–mass spectrometry to the determination of butylated hydroxytoluene in bottled drinking water

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Abstract

Butylated hydroxytoluene (BHT) is an antioxidant utilized as additive in foods and packaging plastic. Its presence in drinking water is possible if it is used as an antioxidant in the packaging plastic because it may migrate into the package's contents. A method for the determination of BHT in water by means of solid-phase microextraction and gas chromatography–mass spectrometry has been developed and evaluated with respect to the time of fiber exposure, limits of detection and quantitation, linearity and precision. Finally, the method was applied to evaluate the presence of this substance in samples of mineral and mineralized bottled drinking water, and it appeared to be present in seven out of a total of fifteen commercial brands.

Keywords: Water analysis; Packaging materials; Hydroxytoluene, butylated

1. Introduction

Butylated hydroxytoluene (BHT) has been used since 1947 as a common antioxidant in rubber and petroleum products and, more recently, in plastics. It has been added since 1949 as an antioxidant to many fat-containing foods, to edible fats and oils and to cosmetics [1]. Chemical migration from plastic packaging into the package's contents was shown [2,3]. Hence, BHT consumption by ingestion can result both from its use as an additive in food and beverages and from its use as additives in plastic containers that finally migrate to its content.

This antioxidant has exhibited contradictory actions depending on the study and on the experimental animal studied. Cancer growth has shown to be inhibited in some studies, and increased in others [4–8], since its toxicological implication is in permanent revision. The maximum levels of use for BHT in foods widely vary in different countries [9]. The acceptable daily intake (ADI) was established at 0–0.3 mg/kg body weight (bw) by the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) [10]. On the other hand, the WHO, did not include BHT in the list of drinking water contaminants [11].

Nowadays, a lot of people living in urban areas are increasingly consuming bottled water as a means of reaching some or all of their daily requirements. This study was motivated due to a particular regional problem. The city of Bahía Blanca, located to the southeast of the Buenos Aries Province, has a growing population of about 300 000 inhabitants (extrapolated from [12]). Due to problems with the

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00217-0

tap water supply in the Bahía Blanca city region, the consumption of bottled drinking water and soda has risen by 50% during 2000 [13]. Therefore, there is a risk of potential increase of the daily ingestion of BHT due to its migration from the plastic package into the water. All drinking water samples analyzed were bottled in a plastic material consisting of polyethylene terephthalate (PET).

Solid phase micro extraction (SPME) was implemented and applied for the extraction of BHT from water samples and further determination by capillary gas chromatography–mass spectrometry (GC–MS). Originally developed and extensively studied by Pawliszyn and co-workers [14–16], SPME was further develped over the last decade [17]. Applications of SPME to evaluate the quality of the drinking water in relation to the presence of diverse organic compounds have been reported [18–25]. In these works the most studied compounds are those that are related to the presence of pesticides and other organic substances that can affect the quality of drinking water not only because they are present in natural water supplies but also because it can originate during the different treatment systems. In this study, the developed method was applied to the determination of an organic substance (BHT) in samples of mineral and mineralized bottled drinking water as the BHT is potentially used as an additive antioxidant in the plastic package.

2. Experimental

2.1. Preparation of solutions

BHT (>99% purity) was purchased form Merck–Schuchardt, Germany. A standard solution was prepared by dissolving 2.0027 g in 50 ml of methanol (HPLC grade). An intermediate standard solution was prepared by diluting 200 μl to 25 ml with methanol. A working standard was prepared by diluting 200 μl of intermediate solution to 25 ml of methanol, and this solution (500, 1000, 1500, 2000 and 2500 μl) was used to spike 100 ml of deionized water to produce water standards containing 12.8, 25.5, 38.3, 51.1 and 63.8 μg l^{-1} of BHT. Mineral drinking water solutions of BHT were prepared by spiking 100 ml of mineral drinking water with 1000, 1500 and 2000 μl of working standard. In this way, solutions containing 25.5, 38.3 and 51.1 μg l^{-1} were obtained. The main characteristics of the mineral drinking water composition were: calcium 30 mg l^{-1}, magnesium 3 mg l^{-1}, sodium 10 mg l^{-1}, potassium 4 mg l^{-1}, chloride 4 mg l^{-1}, hydrogencarbonate 79 mg l^{-1}, sulfate 44 mg l^{-1}, nitrate<3 mg l^{-1} and total dissolved solids 176 mg l^{-1}.

2.2. SPME procedure

To perform our study we used an SPME manual holder and fiber assembly with a 100 μm polydimethylsiloxane film, and an amber screw top vial with white PTFE–silicone septa (Supelco, Bellefonte, PA, USA). The fiber was exposed to 15 ml aliquots (maximum capacity). A 10×3 mm stir bar was included and the maximum magnetic stirring velocity of 2200 rpm with conventional equipment (Heidolph MR1000) was chosen in a previous test. Extractions were performed at room temperature (20–25 °C). The exposition time was 30 min, except for the particular experience shown in Fig. 1.

2.3. GC

The chromatographic determinations were carried out using a Hewlett-Packard (HP6890 GC system and a HP5972 MS system). Helium at 1 ml/min (36 cm/s) was used as a carrier gas. A HP5 column (30

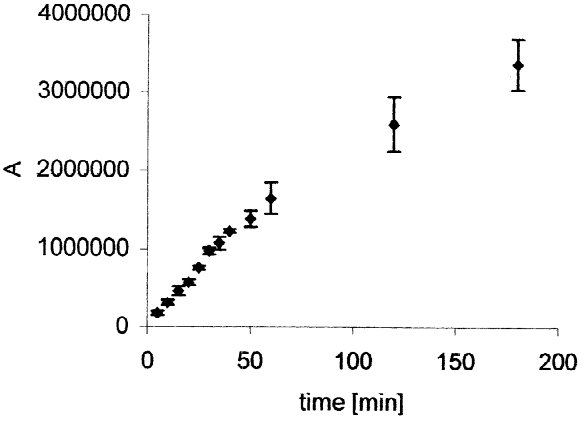

Fig. 1. Peak area versus extraction time of BHT of mineralized drinking water (28.8 μg l^{-1}). Points are averages from triplicates and error bars are ±SD.

m×0.25 mm, 0.25 μm) was used with the following oven temperature program: 40 °C for 5 min, increased to 250 °C at 20 °C min^{-1} and held for 5 min (total time: 20.5 min). All sample injections were performed manually. In all cases, immediately after exposure the fiber was desorbed for 4 min in an isothermal injector port at 250 °C (previously verified to avoid memory effects) operated in splitless mode. The mass spectrometer was operated in the scan mode (mass range m/z 35–500). The ionization mode was by electron impact at electron energy of 70 eV. Peaks were identified by comparison with the mass spectra library of Hewlett-Packard (NBS75K) and with the retention time of the standard solution.

3. Results and discussion

3.1. Extraction time

Fig. 1 represents the study carried out on the optimization of the extraction time. The graph shows the results of peak area versus extraction time up 3 h ($n=3$) on a sample of commercial mineralized drinking water with BHT's concentration of 28.8 μg l^{-1}. The adsorption time of 30 min showed a reasonable compromise between a good peak area, an acceptable time of analysis and a low relative standard deviation (4.3%). In view of these results, and as for the routine analysis it is not necessary to reach the equilibrium if the fiber exposure time is maintained, an exposition time of 30 min was chosen. This allows that during a chromatographic run we can be extracting the next sample.

3.2. Performance of the method

The calibration curve was drawn using five points at a concentration range 12.8–64.0 μg l^{-1}. For each point of the calibration curve three extractions were carried out. The individual peak area–concentration values had been used to apply linear regression by the minimum least squares method. The obtained linear regression equation was $y = 67\,905\ (\pm 43\,910)x + 31\,618\ (\pm 10\,370)$—in parenthesis the standard deviation—with a correlation coefficient (r) of 0.998 and r^2 statistic of 0.98. Analysis of variance (ANOVA) was used to detect the lack of fit in linear regression [26–28] in order to verify whether the model chosen is the correct one (Table 1). The lack of fit term calculated ($F=0.53$) is lower than the tabulated F at 95% confidence level, so the null hypothesis (the straight line model describes the relationship between signal and BHT concentration) cannot be rejected. The limit of detection (LOD) (4.2 μg l^{-1}) was calculated by 3 times the standard deviation of the intercept divided by the slope and the limit of quantitation (LOQ) (13.9 μg l^{-1}) was calculated by using 10 times the standard deviation of the intercept divided the slope. The LOQ value being higher than the first point of the calibration curve, the range from 4.2 μg l^{-1} (LOD) to 13.9 μg l^{-1} (LOQ) was considered as detection zone, and 13.9 μg l^{-1} (LOQ) to 64.0 μg l^{-1} as quantitation zone.

3.3. Analytical application

This method was applied to the determination of BHT in mineral drinking water spiked at 25.5, 38.3 and 51.1 μg l^{-1}. Table 2 shows the recoveries and RSDs. Chromatograms of mineral drinking water free of BHT (a), the same water spiked with a standard solution of BHT at 38.3 μg l^{-1} (b), standard solution of BHT of 38.3 μg l^{-1} (c) and a real sample with a BHT concentration of 21.5 μg l^{-1} (d) are shown in Fig. 2, where no prominent

Table 1
Analysis of variance to lack of fit test

Source	df	Sum of squares	Mean square	F-ratio	P-value
Regression	1	$4.886\cdot10^{12}$	$4.886\cdot10^{12}$	553.68	$4.8\cdot10^{12}$
Residual	13	$1.147\cdot10^{11}$	$8.825\cdot10^{9}$		
Lack of fit	3	$1.577\cdot10^{10}$	$5.257\cdot10^{9}$	0.53*	0.67
Pure Error	10	$9.895\cdot10^{10}$	$9.895\cdot10^{9}$		

df: Degree of freedom; *: Tabulated $F(3,10)=3.71$ ($\alpha=0.05$).

Table 2
Recoveries of BHT from spiked mineral drinking water samples (number of replicates = 3)

Added ($\mu g\ l^{-1}$)	Found ($\mu g\ l^{-1}$)	Recovery[a] (%)	RSD (%)
25.5	21.3	84	7
38.3	38.1	99	16
51.1	60.7	119	14

[a] RSD: Relative standard deviation.

impurity peaks are observed. A mass spectrum of BHT obtained from the real water sample (corresponding to d in Fig. 2), is presented in Fig. 3 showing a match quality of 95% against the BHT library spectrum. Finally, the method was applied to evaluate fifteen samples of commercial mineral and mineralized drinking water (Table 3). BHT was present in seven out of a total of fifteen commercial trademarks with quantifiable concentrations in five of them. The maximum observed value was 38.0 $\mu g\ l^{-1}$. Considering an average body mass of 60 kg and 2 l of daily ingestion, this value implies a contribu-

Table 3
BHT concentrations in commercial mineral and mineralized drinking water samples

Sample	Volume bottle (l)	BHT ($\mu g\ l^{-1}$)	SD ($\mu g\ l^{-1}$)	*n*
A	0.5	38.0	1.9	4
	1.5	21.5	1.1	3
	2	28.8	1.1	4
B	1.5	n.s.		
C	1.5	n.s.		
D	0.5	<LOD		
	1.5	n.s.		
	2	n.s.		
E	1.5	n.s.		
F	1.5	28.0	2.2	3
G	1.5	n.s.		
H	1.5	n.s.		
I	2	n.s.		
J	1.5	<LOQ		
K	1.5	34.3	6.1	3
L	2	n.s.		
M	1.5	n.s.		
N	1.5	n.s.		
O	1.5	n.s.		

SD: Standard deviation; n.s.: No signal; *n*: Number of replicates.

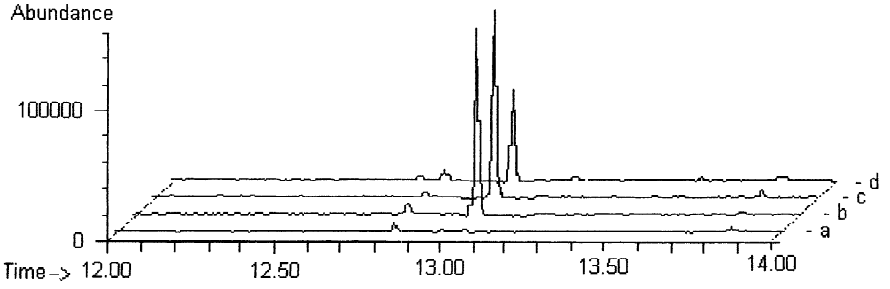

Fig. 2. Chromatograms of mineral drinking water free of BHT (a), the same water spiked with a standard solution of BHT at 38.3 $\mu g\ l^{-1}$ (b), standard solution of BHT of 38.3 $\mu g\ l^{-1}$ (c) and a real sample with a BHT concentration of 21.5 $\mu g\ l^{-1}$ (d). Time scale in min.

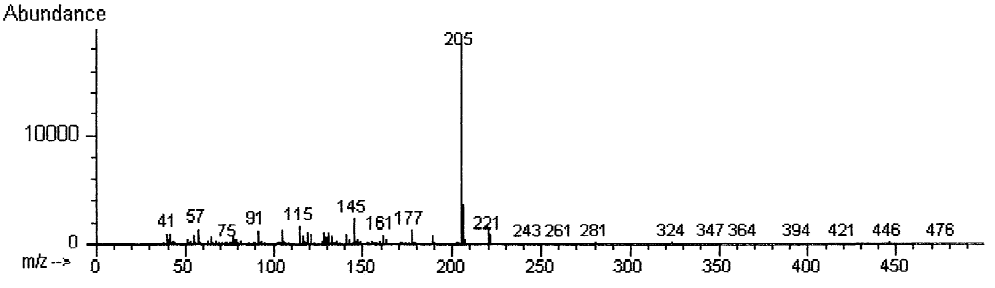

Fig. 3. Mass spectrum of BHT obtained from an actual water sample (corresponding to c in Fig. 2).

tion of the 1.3% on the 0.3 mg/kg of body mass/day fixed as ADI by the WHO [10]. The values found are higher than those found in the study of contamination of drinking water via carbon dioxide enrichment devices in Bavaria, Germany [29].

4. Conclusions

Though this method needs further optimization, it appears to be a rapid and inexpensive alternative to the qualitative and quantitative analysis of butylated hydroxytoluene from commercial mineral drinking water samples. Effects of ionic strength, pH and temperature, and election of a more appropriated fiber coating would be tested. Even with those considerations, this method yields an analyte recovery (84 to 119%) and a precision (RSD between 7 and 16%) that were acceptable considering the measured magnitudes [30], and allows the measurement of real samples. The obtained results show that BHT is present in 46% of the analyzed samples of commercially bottled drinking water. BHT's contribution to the ADI is not very big (1.3% at worst), but maybe it should not be despised.

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