Analysis of Benzothiazole in Italian Wines Using Headspace Solid-Phase Microextraction and Gas Chromatography−Mass Spectrometry

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Benzothiazoles are a part of the molecular structure of a large number of natural products, biocides, drugs, food flavors, and industrial chemicals. They also appear in the environment mainly as a result of their production and use as rubber vulcanization accelerators. A new headspace solid-phase microextraction (HS-SPME) method for analysis of benzothiazole (BTH) in wine is described. This method is fast, inexpensive, and does not require solvents. The detection limit of BTH in wine was 45 ppt with linearity up to 100 ppb. The quantification of BTH is performed by the standard additions method and does not require the use of an internal standard. We have analyzed 12 wines from different grape varieties grown in several regions, using SPME extraction and gas chromatography−mass spectrometry (GC-MS) detection. Under these experimental conditions, benzothiazole was found in all wines analyzed. Concentration levels in samples varied from 0.24 μg/L (Vermentino) to 1.09 μg/L (Franciacorta).

Keywords: *Wine analysis; benzothiazole; HS-SPME; GC-MS*

INTRODUCTION

Benzothiazoles are a group of xenobiotic, heterocyclic chemicals which contain a benzene ring fused with a thiazole ring. Benzothiazoles rarely occur as natural products, but they form part of the molecular structure of a large number of natural products, biocides, drugs, food flavors, and industrial chemicals. Major examples include vitamin B_1, the rubber accelerator 2-mercaptobenzothiazole, cambendazole, and thiabendazole (benzothiazole is closely related chemically to thiabendazole, which is the only postharvest fungicide registered for controlling dry rot on potatoes) (Zeringue, 1997). Benzothiazoles are used in a variety of industrial products and processes; 2-mercaptobenzothiazole (MBT) is a rubber additive chemical (e.g., vulcanization accelerators) used as a corrosion inhibitor and fungicide (Santodonato et al., 1976). Benzothiazole are used as slimicides in the paper and pulp industry (Meding et al., 1993), as fungicides (Schlor, 1970; Scheinpflug et al., 1977), as herbicides (Wegler and Eue, 1970; Cheng et al., 1975; Wegler and Eue, 1977), or as antialgal agents (Bujdakova et al., 1994). They enter the environment in a variety of ways, but particularly by routes associated with the manufacture and use of MBT and MBT-based rubber additives (Jungclaus et al., 1976). The parent compound benzothiazole is produced as a byproduct in the above processes and is used in a range of industrial effluents; thus it is commonly found in the acquatic environment (Brownlee et al., 1981). It is also produced as a result of wear on vehicle tires (Santodonato et al., 1976); the presence of BTH in river water has been proposed as an indicator of the presence of road runoff water in rivers (Spies et al., 1987). 2-Mercaptobenzothiazole can be subject to photodegradation in UV light, with BTH and 2-hydrobenzothiazole (BTOH) being among its byproducts (Brownlee et al., 1992). Benzothiazole has been seen in the aroma fraction of tea leaves (Vitzthum et al., 1975) and is also present in heated cow's milk (Friedrich and Acree, 1998). Benzothiazole is also found as a flavor compound produced by the fungi *Polyporus frondosus* and *Aspergillus clavatus* (Seifert and King, 1982; Gallois et al., 1990). Furthermore, benzothiazole is found in the volatile fraction of oak wood used for aging wines (Pérez-Coello et al., 1998).

Although BTH is so widely distributed in the environment and in food samples, it has not been studied and measured extensively in beverages. There is very limited research into the identification and quantitation of benzothiazole in Italian wines. We have developed an innovative, fast, inexpensive, and accurate method based on headspace solid-phase microextraction (HS-SPME) combined with GC-MS to selectively identify and quantify BTH in wine.

MATERIALS AND METHODS

Wines. Twelve wines, five red, six white, and one rosé produced in several Italian regions and derived from different grape varieties were purchased from local markets: Barbera del Monferrato from Piemonte; Sangiovese from Tuscany; Aglianico from Campania; Merlot and Sauvignon Feudi Carraresi from Veneto; Vermentino Aragosta from Sardegna; Muller Thürgau from Trentino; Terre di Franciacorta from Lombardy; Alcamo Racine from Sicily and Trebbiano, Montepulciano, and Cerasuolo from Abruzzo.

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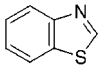

Figure 1. Structural formula of benzothiazole.

BTH and SPME. Benzothiazole (95%) was obtained from Fluka (Buchs, Switzerland). The SPME holder for manual sampling and the fiber partially cross-linked with Carbowax-divinylbenzene (65 mm) were purchased from Supelco Co. (Bellefonte, PA).

General HS-SPME Procedure. For headspace sampling, 4 mL of wine was placed in a 10 mL vial capped with a silicon septa, giving a headspace volume equal to 6 mL. One gram of sodium chloride (25% w/v) was then added to the sample. During extraction, the fused silica fiber was not immersed in the liquid phase; the fiber was exposed to the vapor phase generated by the liquid sample, while being kept at 50 °C for 15 min. Immediately after completion of the SPME step, the analytes absorbed in the SPME fiber were analyzed by GC-MS. For thermal desorption, the SPME fiber was kept in the injector for 10 min.

Instrumental Analysis. HS-SPME analysis was performed with a Hewlett-Packard (Palo Alto, CA) HP-5890 gas chromatograph coupled to an HP-5971 mass-selective detector. A 30 m, 0.25 mm i.d., 0.25 mm film thickness MDN-5S (5% Phenyl Methyl Siloxane) column (Supelco) was used. A split/splitless injector was used in the splitless injection mode; the injector temperature was set at 250 °C. Ultrahigh-purity helium was used as carrier gas at a flow rate of 1 mL/min, column head pressure was 7.6 psi. The detector (transfer line) was at 300 °C; oven equilibration time was 1 min. The GC oven temperature was programmed as follows: 50 °C held for 1 min, increased to 150 °C at a rate of 20 °C/min, 150 °C held for 3 min, increased to 280 °C at a rate of 4 °C/min.

MS information: acquisition mode selected ion monitoring (SIM), solvent delay 3.0 min; electron multiplier voltage 2035 V.

Quantitative Analysis. Quantitative analysis was performed using the standard addition method: different volumes of a BTH standard solution in ethanol were added to wines to give sample spikes of 0, 1, 2 and 4 ppb. BTH in each wine sample was quantitated in single using a standard addition curve made with four calibration points; each point was in single. For each wine sample, four 10 mL aliquots were spiked with different BTH amounts (0, 1, 2, and 4 ppb), each spiking concentration was made in single.

RESULTS AND DISCUSSION

To establish the retention time and the characteristic spectrum of the compound of interest, 1 mL of standard solution containing benzothiazole (Figure 1) at the concentration of 0.1 μg/mL was injected into the GC-MS with the instrument on the full scan mode. The mass spectrum of benzothiazole (Figure 2) shows the molecular ion at *m*/*z* 135 as main peak, and a less abundant peak at *m*/*z* 108, derived from the molecular ion by the loss of HCN. The selected ion monitoring (SIM) mode focuses the mass selective detector on *m*/*z* 135 (target ion) and *m*/*z* 108 (qualifier ion) for benzothiazole (retention time of 7.38 min). Peak detection was based on the retention times (±0.1 min), the presence of the qualifier ion, and the predetermined ratio between the target ion and the qualifier ion.

Wine samples were analyzed by HS-SPME using the GC-MS detector. A 65 mm Carbowax-divinylbenzene coating fiber was chosen because its sensitivity was more than three times greater than that of a 100 mm poly(dimethylsiloxane) when 0.1 mg/mL of benzothiazole in ethanol (4 mL) was extracted for 15 min at 50 °C (data not shown). The extraction time is the result of a compromise between the time required to perform the analysis and an acceptable precision for the BTH determinations. Thus, as shown in Figure 3, even if a better reproducibility of the method was achieved after 30 min extraction (Table 1), a 15 min extraction time was chosen in order to increase the yield of the method, because precision in these HS-SPME conditions was found to be acceptable (less than 15%).

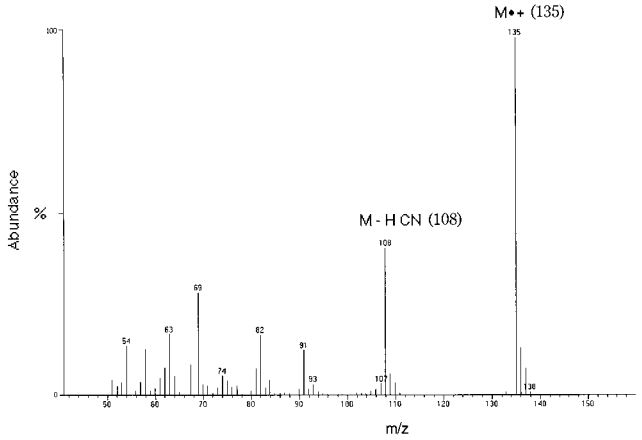

Figure 2. Mass spectrum of benzothiazole.

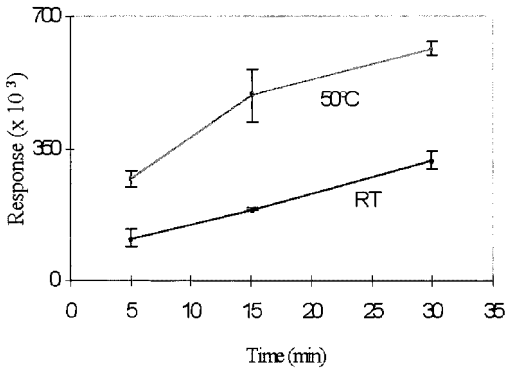

Figure 3. Solid-phase microextraction kinetics for benzothiazole using headspace sampling at two different temperatures (50 °C and room temperature). Each value is the mean ± RSD.

Table 1. Precision of the SPME/GC-MS Analysis of a Red Wine Spiked with 100 ppb of BTH

time (min)	CV%[a] at room temperature	CV%[a] at 50 °C
5	20.8	7.2
15	2.3	14.4
30	7.8	3.0

[a] Coefficient of variation on three replicates.

For thermal desorption, the SPME fiber was kept in the injector for 10 min, a time period sufficient to avoid carry-over phenomena. The carry-over of BTH in this fiber phase was evaluated by fiber blank analysis after extraction of a BTH-containing wine sample. In this blank analysis, BTH was less than 3% of the concentration found in the wine sample. Consequently, after the analysis of a highly contaminated wine, the fiber was further exposed in the GC injector, to make a blank run and avoid possible contamination of the subsequently extracted sample.

Table 2 lists the main characteristic of the wines. It is important to point out that each wine is different and comes from various geographic areas, thus representing almost all the Italian regions. Benzothiazole was identified by adding different amounts of a BTH solution in ethanol to the wine sample, to give BTH spike concentrations of 0, 1, 2, and 4 ppb. With this procedure, a

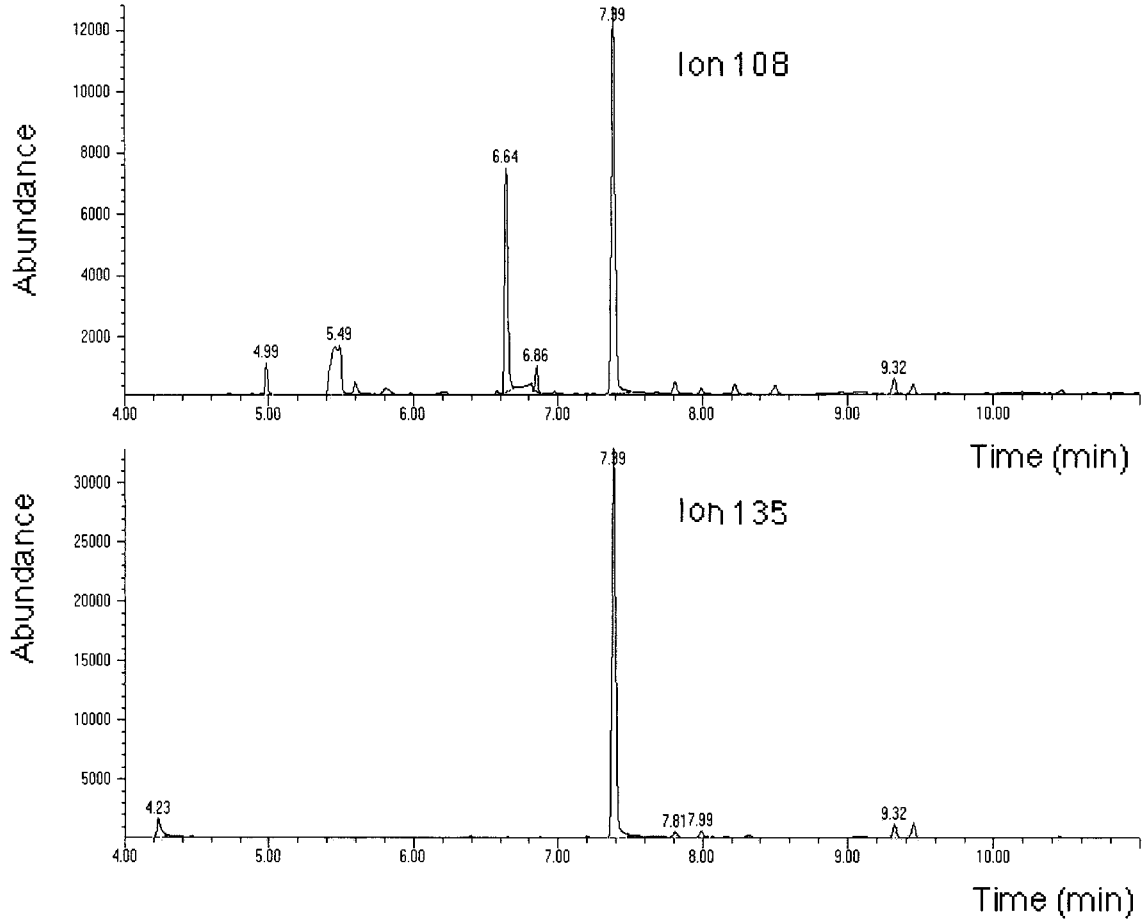

Figure 4. Chromatogram relative to the GC-MS analysis of a red wine sample with addition of 100 ppb BTH.

Table 2. Characteristics of the Wine Samples

wine	type	year	geographic origin	alcohol degree (%)
Barbera del Monferrato	red	1998	Piemonte	12
Montepulciano d'Abruzzo	red	1998	Abruzzo	12
Feudi Carraresi-Merlot	red	1997	Veneto	12
Sangiovese	red	1997	Toscana	12
Aglianico	red	1998	Campania	12
Terre di Franciacorta	white	1998	Lombardia	12
Feudi Carraresi-Sauvignon	white	1998	Veneto	12
Muller Thürgau	white	1998	Trentino	12
Vermentino-Aragosta	white	1998	Sardegna	11.5
Trebbianod'Abruzzo	white	1996	Abruzzo	11.5
Alcamo Racine	white	1998	Sicilia	11.5
Cerasuolo d'Abruzzo	rose′	1996	Abruzzo	11.5

linear detector response was obtained in the 1−100 ppb range for the concentration of added BTH (correlation coefficient: $r = 0.99991$). A typical SIM chromatogram obtained from a red wine is shown in Figure 4. The GC-MS analysis gave a limit of detection (LOD) of 45 ppt, based on a signal-to-noise ratio of 3:1 for the BTH peak in the wine sample.

Benzothiazole was found in all wines analyzed. BTH levels in the different wines analyzed ranged from 0.24 to 1.09 ppb, and here, the CV% of 14.4 can be considered acceptable for all measurements, in that it was obtained at the condition of 50 °C and 15 min. Our study shows that BTH levels were not correlated to wine types and their geographical origin among the red wines (Table 3), Montepulciano d'Abruzzo contained the highest amount of benzothiazole, whereas Terre di Franciacorta (Bianco del Castelletto) was highest among the white wines.

Table 3. Concentrations of Benzothiazole in Twelve Commercial Italian Wines

wine	benzothiazole (μg/L)
Barbera del Monferrato[a]	0.73
Montepulciano d'Abruzzo[a]	1.01
Feudi Carraresi-Merlot[a]	0.53
Sangiovese[a]	0.67
Aglianico[a]	0.58
Terre di Franciacorta[b]	1.09
Feudi Carraresi-Sauvignon[b]	0.42
Muller Thürgau[b]	0.58
Vermentino −Aragosta[b]	0.24
Trebbiano d'Abruzzo[b]	0.45
Alcamo Racine[b]	0.45
Cerasuolo d'Abruzzo[c]	0.53

[a] Red wines. [b] White wines. [c] Rosè wine.

CONCLUSIONS

This study describing the presence of benzothiazole in wines gives new information on wine components, contributing to better characterization of beverages. The source of this molecule is still unclear. The hypothesis that benzothiazole could derive from oak wood has been discarded in that the wines here analyzed are not aged. As benzothiazole is part of several natural molecular structures, it may be formed during the fermentation process. Furthermore, benzothiazole can enter the environment by routes associated with synthesis of xenobiotics, thus being considered a food contaminant. HS-SPME combined with GC-MS is an efficient method of analysis of benzothiazole in Italian wines for several reasons: low LOD, wide linearity range, short analysis

time, low cost, and high volatility of the analyte, which reduces interference from other constituents of the matrix.

ABBREVIATIONS USED

SPME, solid-phase microextraction; GC, gas chromatography; MS, mass spectrometry; BTH, benzothiazole; MBT, mercaptobenzothiazole; BTOH, hydroxybenzothiazole; HS, headspace; HP, Hewlett-Packard; SIM, selected ion monitoring; LOD, limit of detection.

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