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Quantitative Determination of Sulfur-Containing Wine Odorants at Sub Parts per Billion Levels. 2. Development and Application of a Stable Isotope Dilution Assay

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[$^2H_{10}$]-4-Mercapto-4-methylpentan-2-one (d_{10}-**1**), [$^2H_2$]-3-mercaptohexan-1-ol (d_2-**2**), and [$^2H_5$]-3-mercaptohex-1-yl acetate (d_5-**3**), deuterated analogues of impact odorants of wines, were used to determine quantitatively the natural compounds in white wines (Muscadet, Sauvignon, and Bacchus) with a stable isotope dilution assay using gas chromatography coupled either with ion trap tandem mass spectrometry (GC−ITMS-MS) or with atomic emission detection monitored on sulfur-selective acquisition (GC−AED). The thiol compounds were recovered from wines by liquid−liquid extraction, then purified from the wine extracts by covalent chromatography, and analyzed. The quantitative determination of 4-mercapto-4-methylpentan-2-one **1** in the wines that were analyzed was performed better with GC−AED than with GC−ITMS-MS under the conditions that were used. However, the detection limit of the method was higher than the odor threshold of 4-mercapto-4-methylpentan-2-one **1** in wine (5 vs 0.8 ng/L). The levels of this compound in the Sauvignon and Bacchus wines were much higher than its odor threshold, but it was not detectable in the Muscadet wines. On the contrary, GC−ITMS-MS was much more sensitive than GC−AED for detection of 3-mercaptohexan-1-ol **2** and 3-mercaptohex-1-yl acetate **3**, and the detection limits were much lower than their odor thresholds in wine. The former compound was detected in all of the Muscadet wines that were analyzed at levels always higher than its odor detection threshold, while the latter occurred at levels higher than its odor threshold in only one Muscadet wine.

KEYWORDS: Wine; aroma; stable isotope dilution assay; sulfur compounds; thiols; AED; ion trap tandem mass spectrometry

INTRODUCTION

Sulfur-containing compounds exhibit in general intense smelling properties due to their extremely low odor thresholds. Depending on their levels in beverages and foods, they contribute favorably to the aroma or to off-flavor. Thus, 4-mercapto-4-methylpentan-2-one **1** was described as having a pleasant box tree and black currant bud odor, but an unpleasant catty urine odor at higher levels (*1*, *2*). Its occurrence in Chenin blanc and Colombar wines was hypothesized in 1981 (*3*), but it was identified in Sauvignon wines only recently (*2*, *4*). In the same way, the sulfur compound hypothesized to be responsible for a black currant flavor in wines of two German grape varieties, Scheurebe and Bacchus (*5*), was demonstrated to be **1** in Scheurebe wine some years later (*6*).

3-Mercaptohexan-1-ol **2**, first identified in yellow passion fruits (*7*−*9*), has an odor reminiscent of grapefruit and passion fruit. It was recently reported in Sauvignon blanc wines (*10*) and in wines of other grape varieties (*11*−*13*). Its acetate, 3-mercaptohex-1-yl acetate **3**, reminiscent of tropical fruits, was also first identified in yellow passion fruits (*7*, *9*, *14*) and later in wines (*11*, *15*). These compounds have extremely low odor thresholds (*4*, *6*, *10*, *11*, *15*, *16*) and are impact odorants of wines of many grape varieties (*2*, *4*, *6*, *10*−*13*, *15*, *16*). In a wine model medium [12% (v/v) ethanol and 5 g/L tartaric acid], the odor thresholds of **1**−**3** were reported to be 0.8, 4.2, and 60 ng/L, respectively (*12*).

Thiol specific analytical methods have been developed to extract selectively and to determine quantitatively these ultra-trace compounds in wine (*11*, *16*, *17*). Although these methods were powerful in obtaining purified extracts of these compounds, their major drawback was that the internal standard, 4-methoxy-2-methyl-2-mercaptobutane, a tertiary aliphatic thiol and ether, was only partially functionally similar to the target compounds,

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which does not allow an accurate quantification (*18*−*20*), especially in the case of such reactive compounds (*21*−*23*). Indeed, **2** and **3** were secondary thiols and, in addition, primary alcohol and acetate, respectively, while the tertiary thiol **1** was also a ketone; as a result, their physicochemical properties were different from those of the internal standard. Conversely, a stable isotopomer of the analyte is widely recognized to be preferable to even a close homologue, as the physicochemical properties of the labeled analogue are very close to those of the analyte. Thus, a stable isotope dilution assay (SIDA) is the method that provides the closest approach yet to a definitive method, as discussed previously in review articles (*19*, *20*), and would be the most accurate method for the quantification of these trace odorants. Such a method, using [$^{13}C_4$]-**1** and GC−MS in the chemical ionization mode with methane, was developed for the quantification of **1** in Scheurebe and Gewürztraminer wines, but its limitations were not explicitly discussed (*24*). Hence, we reported in this paper the use of [$^2H_{10}$]-4-mercapto-4-methylpentan-2-one (d_{10}-**1**), of [$^2H_2$]-3-mercaptohexan-1-ol (d_2-**2**), and of [$^2H_5$]-3-mercaptohex-1-yl acetate (d_5-**3**), synthesized as reported previously (*21*), in the development of an analytical SIDA method for quantification of their natural analogues in wines.

MATERIALS AND METHODS

Reagents and Other Materials. Pentane and dichloromethane (ultrapure grade) were obtained from Riedel de Häen (St. Quentin Fallavier, France). 2-Ethoxythiazol was purchased from Sigma-Aldrich (St. Quentin Fallavier, France), 1,4-Dithio-DL-threitol was purchased from Fluka Chemie (St. Quentin Fallavier, France). **2** (97 wt %) and **3** (97 wt %) were purchased from Interchim (Montlucon, France). Affi-Gel 501 was purchased from Bio-Rad S.A. (Ivry sur Seine, France); it is no longer manufactured by Bio-Rad, but can be prepared easily by reacting Affi-Gel 10 (Bio-Rad) with *p*-aminophenylmercuric acetate according to a procedure obtained on request from Bio-Rad. The deuterated thiols (d_{10}-**1**, d_2-**2**, and d_5-**3**) were synthesized as reported in the preceding paper (*21*). All glassware was cleaned by washing with alcohol, followed by oven baking at 100 °C prior to use.

Wines. The 10 Muscadet wines were produced according to standard wine making by ITV France (Institut Technique du Vin, Unité de Nantes, France) in 1998 and 1999. These wines were stored at 4 °C until analysis. The two Sauvignon and the Bacchus wines were commercial wines.

Gas Chromatography Coupled with Tandem Ion Trap Mass Spectrometry (GC−ITMS-MS). GC−ITMS-MS analysis was carried out using a Varian 3800 gas chromatograph fitted with a 30 m fused-silica column (0.25 mm inside diameter and 0.5 μm film thickness), coated with DB WAX (J&W Scientific). The injection of the extracts (2 μL) was on-column at 20 °C. The temperature of the injector was increased at a rate of 180 °C/min to 250 °C and held there throughout the analysis. The carrier gas was helium 6.0 (Linde gaz, Marseille, France), with a flow rate of 1 mL/min. The oven temperature program was as follows: 3 min at 60 °C and then the temperature increased at a rate of 3 °C/min to 245 °C and held at this temperature for a further 20 min. The GC instrument was coupled to a Varian Saturn 2000 mass spectrometer. The trap and transfer line temperatures were set at 150 and 170 °C, respectively.

The detection of **2** and **3** was performed by chemical ionization (methane, 0.35 bar) and multiple-reaction monitoring in nonresonant mode. The parent ions *m*/*z* 83 and 117 were chosen for **3** and **2**, respectively, and the parent ion *m*/*z* 119 was chosen for both deuterated analogues. The isolation windows was 3 amu, the isolation time 5 ms, the excitation amplitude 30 V, the excitation time 20 ms, the scan range *m*/*z* 50−150, and the analytical scan 1 s. The quantification was performed using the ions *m*/*z* 55 and 56 for the natural and labeled compounds, respectively.

The detection and quantification of **1** and its deuterated analogue were performed similarly, with the following modifications: isobutane was used as the reactant gas, the scan range was *m*/*z* 40−110, the parent ions were *m*/*z* 99 and 108, and the ions used as quantifiers were *m*/*z* 43 and 46 for the natural and deuterated compounds, respectively.

Gas Chromatography Coupled with Atomic Emission Detection (GC−AED). The system consisted of a Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with an HP 7673A automatic sampler and coupled to an HP 5921A atomic emission detector. The gas chromatograph was fitted with a megabore 30 m fused-silica column (0.53 mm inside diameter and 0.5 μm film thickness), coated with DB WAX (J&W Scientific). The injection of the extracts (up to 10 μL) was on-column at 35 °C. The temperature of the injector was increased at a rate of 180 °C/min to 250 °C and held there throughout the analysis. The carrier gas was helium 6.0 (Linde gaz), with a flow rate of 5 mL/min. The oven temperature program was as follows: 35 °C, increased at a rate of 3 °C/min to 170 °C, and then increased at a rate of 6 °C/min to 245 °C. The temperatures of the AED were as follows: inlet at 250 °C, transfer line at 250 °C, and cavity block at 290 °C. Element-selective chromatograms were obtained for carbon- and sulfur-containing compounds (emission wavelengths of 193.03 and 181.40 nm, respectively). Helium was used for the plasma at 4.16 bar. The reagent gas was oxygen at 1.73 bar and hydrogen at 4.85 bar. The spectrometer was purged using ultrapure nitrogen 5.0 Norme Aga at 1.4 bar. The discharge tube was cooled by water at 65 °C.

Calibration Curves. 1−3 and their deuterated analogues were diluted with pentane in 50 mL volumetric flasks, and the levels of each compound were quantitated by GC−AED (sulfur-selective acquisition) using a solution of 2-ethoxythiazole (1.765 g/L) in pentane as internal standard.

Calibration curves were plotted for target compounds **1**−**3**. Serial dilutions of these compounds were made in the solvent used for the extraction of the volatile compounds followed by addition of the labeled internal standards.

*4-Mercapto-4-methylpentan-2-one **1** Using GC−AED*. Integrated peak area ratios were plotted against the concentration ratios (nanograms of **1** per 110 ng of d_{10}-**1**) for a **1** concentration range of 1.4−200 ng/mL. The resultant curve was linear [response ratio = (1.15 × concentration ratio) + 0.02; $R^2 = 0.996$].

*4-Mercapto-4-methylpentan-2-one **1** Using GC−ITMS-MS*. Integrated peak area ratios (peak area of the ion *m*/*z* 43 divided by peak area of the ion *m*/*z* 46) were plotted against the concentration ratios (nanograms of **1** per 43.6 ng of d_{10}-**1**) for a **1** concentration range of 1.4−52.9 ng/mL. The resultant curve was linear [response ratio = (1.99 × concentration ratio) − 0.004; $R^2 = 0.99$].

*3-Mercaptohexan-1-ol **2** Using GC−ITMS-MS*. Integrated peak area ratios (peak area of the ion *m*/*z* 55 divided by peak area of the ion *m*/*z* 56) were calculated and plotted against the concentration ratios (nanograms of **2** per 1432.5 ng of d_2-**2**) for a **2** concentration range of 7.8−1500 ng/mL. The resultant curve was linear [response ratio = (0.97 × concentration ratio) − 0.01; $R^2 = 0.996$].

*3-Mercaptohex-1-yl Acetate **3** Using GC−ITMS-MS*. Integrated peak area ratios (peak area of the ion *m*/*z* 55 divided by peak area of the ion *m*/*z* 56) were plotted against the concentration ratios (nanograms of of **3** per 183.5 ng of d_5-**3**) for a **3** concentration range of 1.5−61 ng/mL. The resultant curve was linear [response ratio = (1.39 × concentration ratio) − 0.004; $R^2 = 0.999$].

*3-Mercaptohex-1-yl Acetate **3** Using GC−AED (Sulfur Detection)*. Integrated peak area ratios were calculated and plotted against the concentration ratios (nanograms of **3** per 33.7 ng of d_5-**3**) for a **3** concentration range of 3−48 ng/mL. The resultant curve was linear [response ratio = (1.28 × concentration ratio) + 0.054; $R^2 = 0.996$].

All these calibration parameters are given in **Table 1**.

Isolation of Volatile Compounds from Wines and Purification of the Extracts by Covalent Chromatography on Affi-Gel 501 (*25*). Five hundred milliliters of wine was placed in a 1 L erlenmeyer, cooled to 1 °C with an ice bath under nitrogen, and then spiked with 50 μL of a d_{10}-**1** solution in pentane (2.2 μg/mL), 50 μL of a d_2-**2** solution in pentane (28.65 μg/mL), and 50 μL of a d_5-**3** solution in pentane (3.67 μg/mL). One hundred milliliters of dichloromethane was added, and the mixture was stirred for 15 min at 700 rpm. Then, the mixture was supplemented with 100 mL of dichloromethane, and stirring was continued for 15 min. The organic phase was separated in a separatory

Table 1. Calibration Parameters, Repeatability, and Detection Limit in the SIDA Quantification of 4-Mercapto-4-methylpentan-2-one **1**, 3-Mercaptohexan-1-ol **2**, and 3-Mercaptohex-1-yl Acetate **3** in Wines Using GC–AED (**1**) and GC–ITMS-MS (**2** and **3**)

		calibration parameters			repeatability	detection limit[c]
	method of detection	linear regression[a]	concentration range (ng/mL)	R^2	(%CV)[b,c] ($n = 3$)	(ng/L)
1	AED	$y = 1.15x + 0.02$	1.4–200	0.996	12%	5
	ITMS-MS	$y = 1.99x - 0.004$	1.4–52.9	0.990	–	15
3	AED	$y = 1.28x - 0.054$	3–48	0.996	–	5
	ITMS-MS	$y = 1.39x - 0.004$	1.5–61	0.999	3%	0.7
2	ITMS-MS	$y = 0.97x - 0.01$	7.8–1500	0.996	9%	1

[a] y is the area ratio and x the concentration ratio relative to the natural thiol and the corresponding labeled standard, respectively. [b] %CV is the variation coefficient. [c] Determined using the Bacchus wine for **1** and the La Haie Fouassière 1999 Muscadet wine for **2** and **3** (see the text and **Table 2**).

funnel, centrifuged for 5 min at 9000*g* (4 °C), dried over sodium sulfate, and concentrated to ~5 mL under vacuum at 30 °C, then to 1 mL using a Dufton column. Five hundred microliters of Affi-Gel 501 was loaded into a Pasteur pipet (glass wool at the bottom) and conditioned with 5 mL of isopropyl alcohol and 5 mL of a pentane/dichloromethane mixture (2:1, v:v). The wine extract (diluted in 2 mL of pentane) was passed through the column, which was then washed with 25 mL of a pentane/dichloromethane mixture (2:1, v:v). The thiols were finally eluted with 5 mL of a 1,4-dithio-DL-threitol solution [5 mM in a pentane/dichloromethane mixture (2:1, v:v)]. The extract was washed with 1 mL of Millipore water, dried over sodium sulfate, and concentrated to ~500 μL using a Dufton column. Then the extract was concentrated down to ~100 μL under a nitrogen flow and analyzed by GC−ITMS-MS or GC−AED (sulfur detection). The final concentration factor was 5000.

RESULTS AND DISCUSSION

Isolation of Thiols by Liquid−Liquid Extraction and Cleanup of Wine Extracts. Since only traces of the thiols that were analyzed, particularly **1** and **3**, were present in wine, their enrichment by extraction and removal of interfering constituents from the extracts was necessary for their quantitative determination.

Methods for obtaining purified extracts of **1**−**3** from wine were reported recently (*11*, *15*−*17*, *24*). All these methods began with the nonselective extraction of the target thiols from wine, either by liquid−liquid extraction or by headspace trapping (vacuum distillation or dynamic headspace). Then, one or more steps were used to obtain a purified concentrated extract of these thiols. The procedure reported by Guth (*24*) used a separation in neutral and acidic fractions, followed by fractionation of the neutral fraction by column chromatography on silica gel. The drawback of this process was that this purification was not selective for thiol compounds. The other procedures (*11*, *15*−*17*) involved the use of a *p*-hydroxymercuribenzoic acid solution to selectively trap the thiol compounds extracted in the first step. However, as discussed previously (*16*), this sole extraction was not sufficiently thiol-selective to measure efficiently the concentration of certain thiols in the last GC step. Thus, additional purification in an anion exchange column was developed (*16*). Although very efficient, this procedure was time-consuming. Thus, the procedure we used was the technique reported for the extraction and cleanup of 8-mercapto-*p*-menthan-3-one from some essential oils, which seemed more convenient (*25*).

Thus, the isolation of the thiols, their deuterated analogues (added previously to the wine), and other volatiles from wine was achieved by liquid−liquid extraction with dichloromethane in an ice bath under nitrogen. This method was similar to that reported for analysis of wine aroma by gas chromatography−olfactometry (*26*). It had the advantage of being less prone to oxidation of thiols, which were shown to be partly oxidized during their synthesis (*21*) and during some isolation processes from food (*22*).

Covalent chromatography on Affi-Gel 501, a cross-linked agarose gel containing phenylmercurium chloride, was then used for the enrichment of the thiols from the wine extracts, according to the procedure reported previously (*25*). The recovery yields for this cleanup procedure were 38, 44, and 86% for d_{10}-**1**, d_5-**3**, and d_2-**2**, respectively. The recovery yield reported previously for the cleanup procedure of thiols using *p*-hydroxymercuribenzoate and an anion exchange column was better (16). However, as a SIDA method was used, the less time-consuming Affi-Gel procedure was chosen. The extracts containing the target thiol compounds and their deuterated analogues were analyzed using either GC−AED or GC−ITMS-MS.

Stable Isotope Dilution Assay Using GC−AED. GC−AED allowed the selective and sensitive detection of each element found in a gas chromatographic effluent (*27*, *28*). This type of detection was used generally in qualitative analysis of heteroatom-containing volatiles and in determination of empirical formulas (*27*−*31*). GC−AED was also used for the quantitative analysis of volatiles. Indeed, calibration with the authentic analyte was not necessary as no pronounced structural influences for the compounds of interest were to be expected (*32*). Thus, the advantage of GC−AED (sulfur detection) in SIDA of sulfur-containing compounds was that they were selectively and sensitively detected and that the chromatographic responses of the labeled and unlabeled compounds were equal as both contained the same number of sulfur atoms. Therefore, this system was used, on one hand, for the quantitative determination of the concentration of the synthesized labeled compound solutions and, on the other hand, for the quantitative determination of the natural thiols in wines.

Our first goal was to selectively detect the labeled and unlabeled thiols using both deuterium and sulfur detections. However, deuterium detection gave no reliable results under our conditions. Thus, the use of this system with sulfur detection only was dependent on the chromatographic separation of the labeled and unlabeled compounds, as they were detected concomitantly.

GC−AED, monitored on sulfur-selective acquisition, seemed to be adequate for analyzing **1**, as d_{10}-**1** and **1** gave two well-resolved chromatographic peaks, due to the labeling with 10 deuterium atoms (**Figure 1**). The use of a megabore capillary chromatographic column allowed injection of a greater volume (up to 10 μL) of the thiol extract. Using extracts obtained as described above, the coefficient of variation for three replicates of the Bacchus wine was 11.7% and the detection limit was 5 ng/L with an estimated signal-to-noise ratio of 3:1 (**Table 1**). That was much better than the detection limit obtained using GC−ITMS-MS with isobutane chemical (CI) ionization (~15

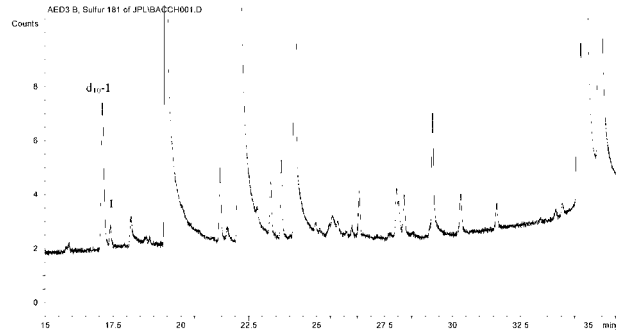

Figure 1. GC–AED (sulfur-selective detection) chromatogram obtained in the quantitation of 4-mercapto-4-methylpentan-2-one **1** in the Bacchus wine.

ng/L). Indeed, **1** did not generate under these conditions any selective ion sufficiently abundant to match the GC−AED sensitivity, but these detection limits were high relative to the odor threshold of **1** in a model wine (0.8 ng/L; *12*). Thus, the GC−AED system was selected for the quantitation of **1** in wine. As this compound was reported to be absent in Muscadet wines (*16*, *17*), its occurrence was investigated not only in the 10 Muscadet wines that were analyzed but also in three other wines of Sauvignon and Bacchus, in which its levels should be above the detection limit of our method (*5*, *16*, *17*), To our knowledge, the application of the GC−AED system for the quantitative determination of trace volatiles using SIDA has not been reported previously. However, the quantification of the two other thiol compounds was achieved better using the GC−ITMS-MS system.

Stable Isotope Dilution Assay Using GC−ITMS-MS. As discussed previously (*33*), GC−ITMS-MS, with the practical and technical advantages of ion-trap technology, can be considered an upgrade for numerous SIDA methods using full-scan or selected ion monitoring (SIM) detection. Combining its strengths, ITMS-MS offered identity confirmation like full-scan MS, excellent sensitivity (baseline noise and interference were minimized) like SIM, and selectivity exceeding that of both full-scan and SIM modes, gained by monitoring one or more product ions of the collision-induced dissociation (CID) process. Thus, this method should be adequate for detecting small traces of the target thiol compounds in the Affi-Gel-purified extracts (down to ∼1 ng in 100 μL). As the thiol compounds had low molecular weights, chemical ionization was chosen to obtain the most selective parent ions. Nonresonant excitation was chosen in the CID process, as this method resulted in reproducible product ion spectra unaffected by changes in the trapping conditions and sample concentration.

The quantitative determination of **2** in the wines was performed with the GC−ITMS-MS system only. Indeed, the use of the GC−AED system was not possible, due to the coelution of d_2-**2** and **2**, despite the optimization of their resolution by GC−AED using a wide-bore column. As this compound was much more abundant than the other two target thiols, it was easily detected using GC−ITMS-MS with methane chemical ionization which gave the major ions *m*/*z* 117 (water loss from the pseudomolecular ion) for the natural thiol (**Figure 2**) and *m*/*z* 119 for its deuterated analogue. The product ions *m*/*z* 55 and 56 obtained by refragmentation of the respective parents ions were used as quantifiers (**Figure 2**). For the La Haie Fouassière 1999 Muscadet wine, the coefficient of variation for three replicates was 9% and the detection limit was 1 ng/L, with an estimated signal-to-noise ratio of 3:1 (**Table 1**).

The GC−AED system could be used in the SIDA quantification of **3**, as the separation of d_5-**3** and **3** was almost complete

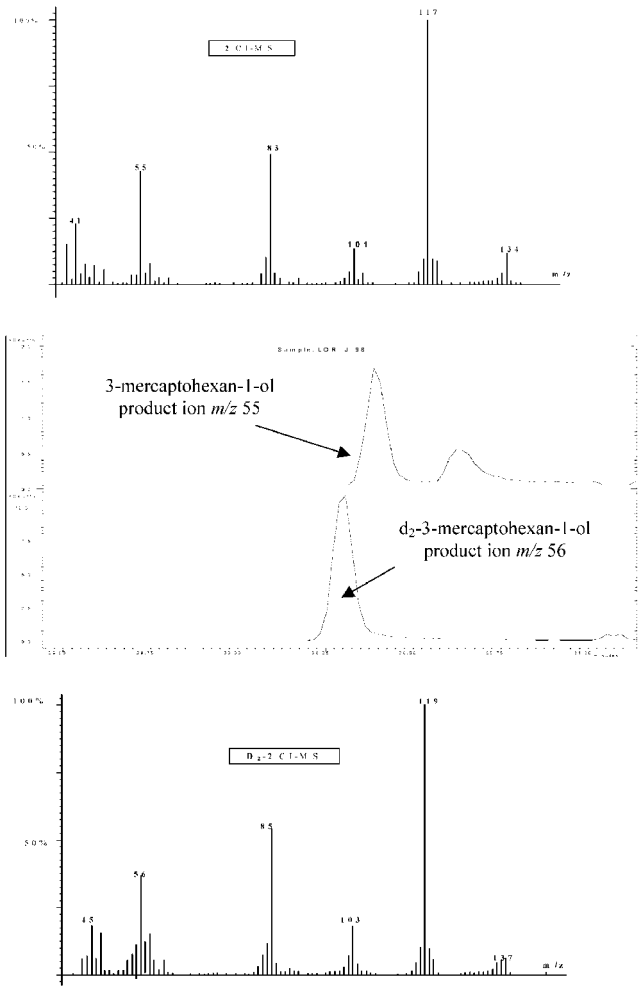

Figure 2. Methane CI-MS of **2** and d_2-**2** and chromatographic plots of the quantifier product ions *m*/*z* 55 (**2**) and 56 (d_2-**2**) obtained in the quantitation of 3-mercaptohexan-1-ol **2** in the 1998 Muscadet wine of Le Loroux Bottereaux.

due to the labeling with five deuterium atoms instead of two for **2**. But the detection limit was much higher than that obtained with the GC−ITMS-MS system, i.e., 5 vs 0.7 ng/L with an estimated signal-to-noise ratio of 3:1 for the La Haie Fouassière 1999 Muscadet wine (**Table 1**). As the levels of **3** in most Muscadet wines that were analyzed were below 5 ng/L (**Table 2**), its quantitative determination in these wines was performed with the GC−ITMS-MS system only, under the same selected reaction monitoring conditions as described for **2**. Indeed, **3** gave under these conditions the same precursor ions (*m*/*z* 117 and 119 for the natural and deuterated thiols, respectively) by acetic acid loss from the pseudomolecular ions (**Figure 3**). However, the quantification of **3** in some wine extracts suffered from the disadvantage that the spectrum of the product ions of the parent ion *m*/*z* 117 showed the coelution of a compound giving rise partly to the quantifier ion *m*/*z* 55. This problem was solved by selecting the parent ion *m*/*z* 83 (loss of hydrogen sulfide from the ion *m*/*z* 117), which gave a pure product ion *m*/*z* 55, used as a quantifier (**Figure 3**). Under these conditions, the coefficient of variation for three replicates of the La Haie Fouassière 1999 Muscadet wine was 3% (**Table 1**). That showed limitations to the use of AED, less selective than tandem MS, for quantification of ultratraces in complex extracts.

Levels of the Thiols in Wines. *4-Mercapto-4-methylpentan-2-one **1**.* As expected (*16*, *17*), this compound could not be detected in any Muscadet wine that was analyzed (**Table 2**).

Table 2. Levels of 4-Mercapto-4-methylpentan-2-one **1**, 3-Mercaptohexan-1-ol **2**, and 3-Mercaptohex-1-yl Acetate **3** Determined in Wines Using GC–AED (**1**) and GC–ITMS-MS (**2** and **3**)

wine	vineyard	[**2**] (ng/L)	[**3**] (ng/L)	[**1**] (ng/L)
1998 Muscadet	La Haie Fouassière	63	nd[a]	nd[a]
	Le Loroux Bottereaux	309	3	nd[a]
	La Limouzinière	445	2	nd[a]
	Drain	279	nd[a]	nd[a]
	Monnières	115	3	nd[a]
1999 Muscadet	La Haie Fouassière	120	6	nd[a]
	Le Loroux Bottereaux	126	2	nd[a]
	La Limouzinière	143	3	nd[a]
	Drain	271	nd[a]	nd[a]
	Monnières	69	2	nd[a]
1998 Sauvignon	Languedoc	–[b]	–[b]	8
1998 Sauvignon	Bordeaux	–[b]	–[b]	17
1998 Bacchus	Germany	–[b]	–[b]	25

[a] Not detectable. The detection limits of **3** and **1** were 0.7 and 5 ng/L, respectively. [b] Not quantitated. The Sauvignon and Bacchus wines were analyzed using GC–AED only.

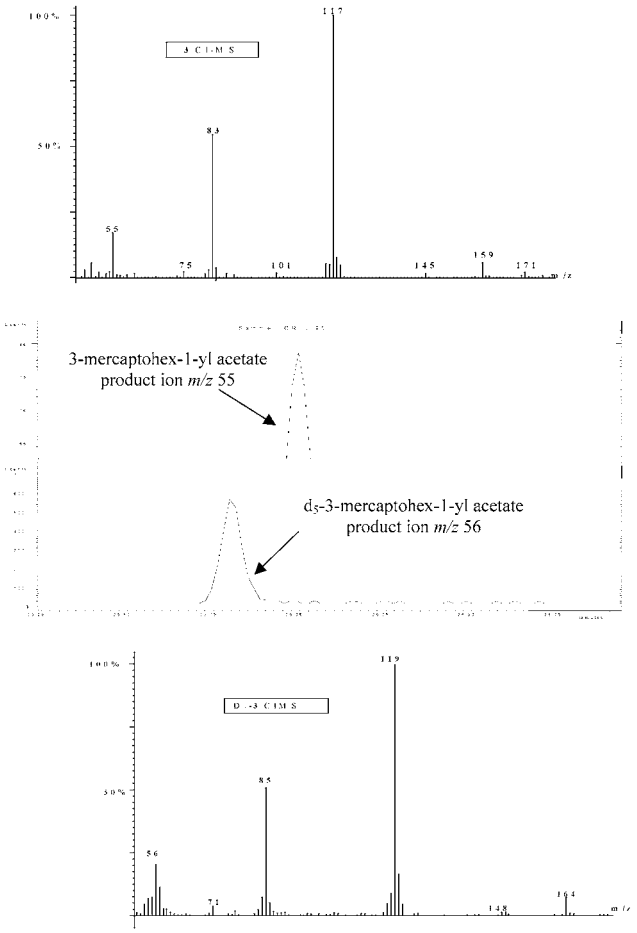

Figure 3. Methane CI-MS of **3** and d_5-**3** and chromatographic plots of the quantifier product ions *m/z* 55 (**3**) and 56 (d_5-**3**) obtained in the quantitation of 3-mercaptohexan-1-yl acetate **3** in the 1998 Muscadet wine of Le Loroux Bottereaux.

However, as the detection limit of the method that was used (5 ng/L) was higher than the odor threshold of this compound in a model wine (0.8 ng/L; *12*), the question of its impact on the aroma of these wines could not be answered. On the contrary, the two Sauvignon blanc wines and the Bacchus wine contained levels of **1** higher than its odor threshold, in agreement with previous studies which reported its importance in the aroma of Sauvignon wines (*2*, *4*, *16*, *17*) and with the hypothesis of its occurrence in Bacchus wine (*5*).

*3-Mercaptohexan-1-ol **2** and 3-Mercaptohex-1-yl Acetate **3***. The same Muscadet wines as above were analyzed (**Table 2**). The levels of **2** in all of these wines were higher than its odor threshold (60 ng/L), i.e., between 1- and 10-fold higher. Thus, **2** was an impact odorant of the aroma of the wines that were analyzed. The levels of **3** found in these wines were much lower than their levels of **2**; they reached the odor threshold in only one sample.

CONCLUSION

Covalent chromatography on Affi-Gel 501 proved to be a convenient method for enrichment of **1**−**3** in wine extracts. The SIDA method that was developed was efficient for their quantification because of their chemical reactivity (*21*−*23*) and their occurrence in small trace levels in wines. GC−AED, monitored on sulfur-selective acquisition, proved to be an effective detection mode for SIDA, provided that the extraction and chromatography conditions allowed the separation of the labeled and natural thiols without any interference of a detectable contaminant. Under these conditions, the GC−AED detection of **1** in the wine extracts was more sensitive than GC−ITMS-MS detection. However, as the detection limit was much higher than its odor threshold (5 vs 0.8 ng/L), this method was not diagnostic for estimating the sensory contribution of this compound to the aroma of wine samples. Furthermore, the limitations of the GC−AED system for the quantification of **2** and **3** were not only related to their detection limits. On one hand, the chromatography conditions did not allow the separation of d_2-**2** and **2**; on the other hand, AED did not have the power of identity confirmation offered by ITMS-MS, which could lead to false results, as shown in the case of **3**. Thus, GC−ITMS-MS seemed more promising for reaching levels of **1** in wine, down to its odor threshold, while ascertaining its identity, but the method will have to be refined. In particular, more appropriate conditions could be found by optimization of mass spectrometric conditions such as ionization mode (*19*). Such conditions were already reached to estimate the sensory contribution of thiols **2** and **3** to the aroma of Muscadet wine. However, more work will be necessary to evaluate if the method can really be applied to every kind of wine, which could lead to optimization of the analysis conditions even for **2** and **3**.

ABBREVIATIONS USED

GC, gas chromatography; MS, mass spectrometry; IT, ion trap; EI, electronic impact; CI, chemical ionization; SIDA, stable isotope dilution assay; AED, atomic emission detection; SIM, selected ion monitoring.

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