

A Novel Method for Quantification of 2-Methyl-3-furanthiol and 2-Furanmethanethiol in Wines Made from *Vitis vinifera* Grape Varieties

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A rapid, easy method has been developed for isolating and quantifying 2-methyl-3-furanthiol (2M3F) in wines. Until now, it was not possible to quantify this highly odoriferous compound, with a smell reminiscent of cooked meat, in wine. The original aspect of this method is the specific release of volatile thiols using a cysteamine solution applied in reverse flow to sample percolation on the basis of a *p*-hydroxymercuribenzoate (pHMB)–volatile thiol conjugate formed by the direct addition of pHMB to 50 mL of wine. Purification of volatile thiols in wines is much faster and easier than our previous method. This method may also be used to assay 2-furanmethanethiol in wine. This thiol's strong aroma of roasted coffee has been shown to contribute to the "roast coffee" aroma of certain wines. Assaying 2M3F by this method showed that it was present in the wines analyzed (red and white Bordeaux, Loire Valley Sauvignon blanc, white Burgundy, and Champagne) at concentrations up to 100 ng/L, i.e., significantly above the olfactory perception threshold for this compound in model dilute alcohol solution.

KEYWORDS: 2-Methyl-3-furanthiol; 2-furanmethanethiol; quantification; *Vitis vinifera*

INTRODUCTION

The sensory importance of 2-methyl-3-furanthiol (2M3F) and 2-furanmethanethiol (2FM), formed by Maillard reaction when food is cooked, is well-known (1–4). The formation mechanism of these compounds has been studied, particularly in cooked meat (5, 6), as well as in a model medium containing cysteine and pentose (7–9). The use of *p*-hydroxymercuribenzoate (pHMB), which has the property of forming reversible combinations with volatile thiols, together with deuterated analogues of the compounds to be assayed as internal standards, made it possible to assay these mercaptans accurately in cooked meats at concentrations around 10 µg/kg (6).

The presence of these volatile thiols has also been reported in certain wines over the years (10–13). The contribution of 2FM to the toasty aroma of wines aged in oak barrels and Champagnes has been clearly demonstrated (13, 14). However, the sensory impact of 2M3F on wines had never been proved with the exception of sensory reconstitution studies (15, 16), due to the difficulty of assaying it by the methods used until now to analyze volatile thiols in wine (13, 17). This article reports on a new specific extraction method for volatile thiols in wine that makes it possible to assay 2M3F in wine and estimate its organoleptic impact.

MATERIALS AND METHODS

Wines Analyzed. The dry white wines were made from Sauvignon blanc (vintage and appellation: 2001 Bordeaux, 1999 Sancerre, and 2001 Pouilly-Fumé) and Chardonnay (vintage and appellation: 2001 Puligny Montrachet, 1999 Chassagne Montrachet, and 1999 and 2001 Chablis). The red Bordeaux wines (blends of Merlot and Cabernet Sauvignon) were from the following appellations: Pauillac (1999), Saint Julien (1999), Saint Emilion (1996), and Pomerol (1998). The Champagnes were kindly donated by Laurent Perrier and Pol Roger (vintages: 1990–1982).

Column Preparation. The resin Dowex 1 (IX2–100) was reactivated by flushing it with a 0.1 M hydrochloric acid solution and then rinsed with ultrapure water until the supernatant had a pH of 5–6. The resin in suspension in water (approximately 70%) was loaded into a glass column, filling it completely up to the joint and draining off any excess water through the tap at the base of the column (Figure 1). The upper tap with a ground tip was then attached to the column using a SVL 22 connector. A percolation flask was fitted on the system. The column was rinsed with 50 mL of ultrapure water and was then ready for use. The resin was changed for each sample.

Specific Extraction of the Volatile Thiols from Wines. Five milliliters of a pHMB solution (2 mM in 0.1 M Tris) was added to 50 mL of wine containing 1.2 nmol of 4-methoxy-2-methyl-2-mercaptobutane (Oxford Chemicals, Elizabeth, NJ) as an internal standard and then brought to pH 7.0 using a sodium hydroxide solution (10 N) and subjected to magnetic stirring for 10 min. The wine was then percolated for 10 min on a Dowex 1 column prepared as mentioned above. The column was rinsed with 50 mL of sodium acetate buffer (0.1 M, pH 6) containing 0.02 mM *tert*-butyl-4-methoxyphenol as an antioxidant.

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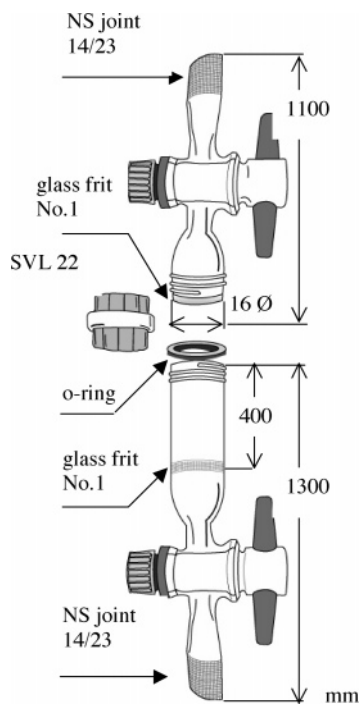


Figure 1. Illustration of the column specifically designed for elution in the opposite direction to percolation.

The volatile thiols were released from the thiol-*p*HMB conjugate fixed on the column by turning it upside down and eluting it using a cysteamine solution (500 mg/50 mL in the rinsing buffer) adjusted to pH 6, for 15 min, in the opposite direction from the way the wine percolated. The eluate containing the volatile thiols released from the column by the cysteamine was collected in a 100 mL flask. To improve the extraction, ethyl acetate (0.5 mL) was added and the solution was extracted using 4 and 2 mL of dichloromethane for 5 min each with magnetic stirring. The organic phases were collected, dried on anhydrous sodium sulfate, and then concentrated to approximately 500 μ L under a nitrogen flux in a 10 mL graduated tube. The concentrate was then transferred to a 1 mL vial, concentrated to 25 μ L, and injected into a gas chromatography/mass spectrometry (GC/MS) within 24 h.

Impact of the Method of Eluting the Column with Cysteamine on the Thiol Recovery Rate. Two methods of eluting volatile thiols from the Dowex 1 column were compared as follows: in the same direction the wine percolated through or the opposite direction.

Stability of the Volatile Thiols. An extract sample containing the volatile thiols purified by the method described above was injected into the GC/MS after 24, 48, and 72 h.

Calibration. Calibration standards were prepared by adding increasing quantities (50–300 ng/L) of the two volatile thiol reference compounds to a wine. The concentrations of volatile thiol standards were previously determined by the Ellman's method (18) using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). For each concentration, the volatile thiols were extracted from the wine using the method described above. The wine selected for purpose of calibration was a red Bordeaux (2003 vintage). The analysis of this wine presented a slight amount of the volatile thiols. The calibration of these compounds was therefore corrected by subtracting the blank ratios (height of peak formed by a selected ion of these compounds contained naturally in this wine/that of internal standard).

Repeatability of the Assay. The same wine was analyzed five times (extraction and assay) to determine the variation coefficient for assaying each compound.

Spiking Experiments. To determine the impact of the matrix on the assay, spiking experiments were conducted on different types of wines (dry white and red) before and after spiking known quantities (25 and 100 ng/L) of volatile thiols (2M3F and 2FM).

Recovery Rate. The recovery rate during purification for 2M3F and 2FM, as well as the internal standard, was calculated as follows. The

volatile thiols were selectively extracted from a red wine according to the method described above. The final organic phase obtained was added to a 100 ng amount of octan-3-ol as an external standard and concentrated to 25 μ L. The following two ratios were calculated from these assay results:

ratio (A) =
height of internal standard peak/height of external standard peak

(thiol initial) ratio =
height of peaks for 2M3F or 2FM/height of internal standard peak

Then, 25 or 100 ng/L 2M3F and 2FM was spiked to the red wine, together with the internal standard, and extracted in the same way. Analysis of the thiols in these two samples made it possible to determine the thiol-spiked ratios.

thiol-spiked ratios =
height of peaks for 2M3F or 2FM/height of internal standard peak

Finally, internal standard was added to the solution (50 mL) used as a flushing buffer in the purification column, together with 2M3F and 2FM (25 or 100 ng/L), and then extracted directly with dichloromethane (4 and 2 mL). The organic phases were collected together, 100 ng of octan-3-ol (external standard) was added, and the resulting solution was concentrated to 25 μ L.

It was thus possible to determine ratio B and the thiol control ratios.

ratio B =
height of internal standard peak/ height of external standard peak

thiol control ratios =
height of 2M3F or 2FM peaks/height of internal standard peak

The recovery rate (E %) for the internal standard was $A/B \times 100$ (%). The recovery rate (T %) for the volatile thiols was (thiol spiked – thiol initial)/thiol control $\times E$ (%).

GC/MS. The chromatographic conditions were identical to those described by Tominaga et al. (13) using a DB-XLB column (HP, 60 m \times 0.25 mm, 0.25 μ m) instead of the BPX-35. The analysis was carried out on a gas chromatograph (Hewlett-Packard 5890-II) coupled with a Hewlett-Packard 5972 series mass selective detector (MSD). The extract (2 μ L) was injected. The operating conditions were as follows: temperature program: isothermal at 40 $^{\circ}$ C for 1 min, then raised to 230 $^{\circ}$ C at 3 $^{\circ}$ C/min; injector temperature, 250 $^{\circ}$ C; injection mode, splitless (splitless time 1 min). Helium 55 (Air Liquide, France) was the vector gas used (135 Kpa). 2M3F and 2FM were detected in SIM mode selecting the following ions, $m/z = 71, 85,$ and 114 for 2M3F and $m/z = 53, 81,$ and 114 for 2FM, and quantified with $m/z = 114$ for both compounds. The internal standard was detected with the ion $m/z = 134$.

RESULTS AND DISCUSSION

Detecting the Purified Volatile Thiols. The method developed for purifying volatile thiols makes it possible to isolate volatile thiols easily and rapidly from conjugate mixtures, such as wine. In fact, the formation of the *p*HMB–thiol conjugate by adding *p*HMB directly to wine (50 mL) instead of preparing an organic extract of *p*HMB from 500 mL of wine (13, 14, 17, 19–21) simplifies the method, thus minimizing any loss of volatile thiols. The conjugate thus obtained is fixed on an anion exchange column. The volatile thiols fixed on the column were released by percolating with a cysteamine solution in the opposite direction to the sample. The volatile thiols purified by the above method were detected by GC/MS in SIM mode with the three selected ions. In view of the mass spectra of the reference materials in scan mode (Figure 2a,b), ions 71, 85, and 114 and ions 53, 81, and 114 were selected for 2M3F and 2FM, respectively. These selected ions were superimposed on the retention times of the reference compounds (Figure 3). The

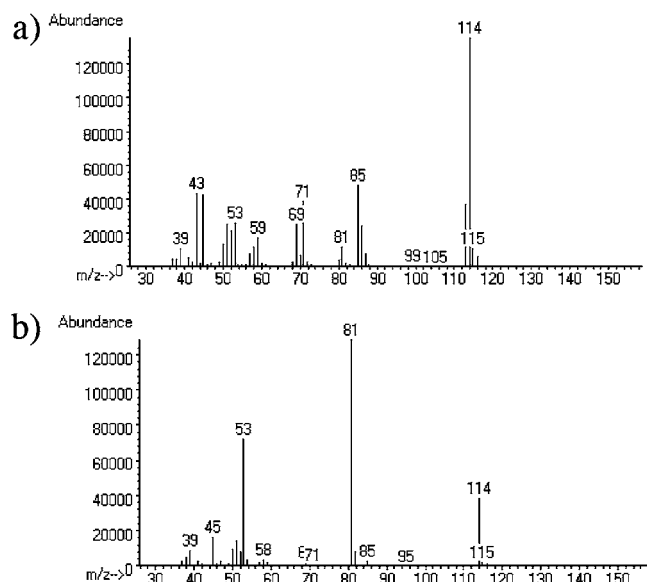


Figure 2. Mass spectra of 2-methyl-3-furanthiol (a) and 2-furanmethanethiol (b).

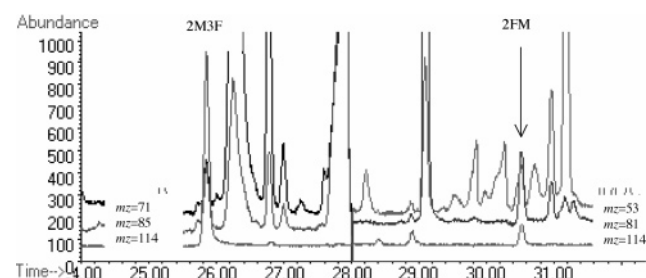


Figure 3. Detection of 2M3F and 2FM from a red wine in SIM mode by overlapping the ions selected (m/z 71, 85, and 114 for 2M3F and m/z 53, 81, and 114 for 2FM).

number of peaks formed by the three ions increased, while maintaining the same ratio, after coinjection of the reference materials in quantities equivalent to those present in wines (results not shown). We were, therefore, sure that these volatile thiols could be detected by selecting these ions. Ion 114 was used to quantify these volatile thiols as the baseline formed by this ion had very little background noise.

Impact of the Method of Eluting the Column with Cysteamine on the Thiol Recovery Rate. The release of volatile thiols by percolating a cysteamine solution in the opposite direction to that taken by the sample is a key stage in this method. **Figure 4** compares the recovery rates for 2M3F and 2FM using the two elution methods with cysteamine. Reverse elution improves recovery of these two volatile thiols, especially 2M3F, which is very poor and not reproducible when the column is eluted in the “usual” direction. It is probable that reverse eluting the volatile thiols releases them from the column more rapidly, thus minimizing losses by oxidation and nonspecific interaction with the resin.

Stability of the Volatile Thiols. Repeated injections of the same sample demonstrated the stability of the volatile thiols preserved in dichloromethane at $-10\text{ }^{\circ}\text{C}$ over a 24 h period (**Figure 5**). 2FM remained stable for 48 h under these conditions, while 2M3F decreased clearly after 24 h. All of the organic thiol extracts used in this study were, therefore, injected into the GC/MS within 24 h.

Standard Curves, Sensitivity, and Repeatability. The ratio of the height of the peaks corresponding to the selected ion of the compounds to be assayed and the peak for the internal

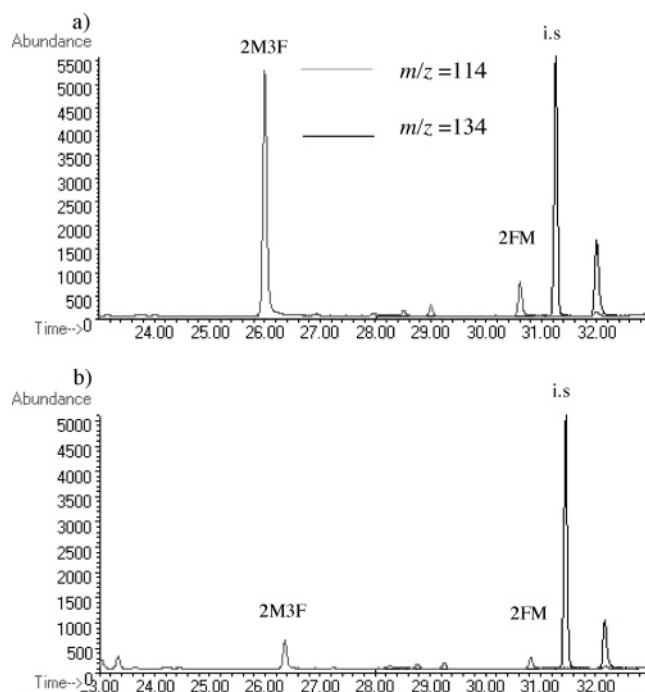


Figure 4. Comparison of the recovery rates of 2M3F and 2FM from the Dowex column by elution in (a) the opposite direction or (b) the same direction as the percolation of the sample; i.s., internal standard.

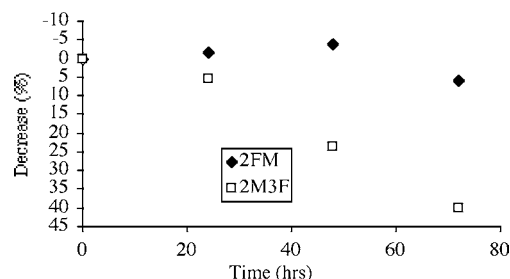


Figure 5. Stability of 2M3F and 2FM purified from a white wine and kept in dichloromethane at $-10\text{ }^{\circ}\text{C}$.

Table 1. Calibration Line Parameters

analyte	calibration line	R^2	linear range (ng/L)	limit of quantification (ng/L)	validation range (ng/L)
2M3F	$y = 206.39x$	0.9668	0–300	0.52	0.52–300
2FM	$y = 658.88x$	0.9998	0–300	2.2	2.2–300

standard is expressed on the graph in relation to the concentrations mentioned previously. The correlation between the two parameters is linear for both volatile thiols to be assayed (**Table 1**). The limit of quantification was calculated as the minimum concentration that generated a peak signal 10 times higher than the signal from adjacent noise. The limit of quantification was 0.52 ng/L for 2M3F and 2.2 ng/L for 2FM, respectively (**Table 1**). Repeatability of the assay was confirmed by a series of five extractions. **Table 2** shows the variation coefficients obtained for these two compounds. It was in the vicinity of 3.5% for the 2M3F assay and less than 5.0% for 2FM.

Spiking Experiments and Recovery Rates of the Volatile Thiols. The two volatile thiols spiked (25 or 100 ng/L) to the various types of wines were recovered independently of type of wine used (**Table 3**). Thus, the type of matrix had no impact on the assay. The recovery rate for these two volatile thiols

Table 2. Repeatability of the 2M3F and 2FM Assay in a Champagne (1990)

samples	2M3F (ng/L)	2FM (ng/L)
1	77.3	22.7
2	76.9	22.3
3	76.2	20.9
4	71.6	20.9
5	78.4	20.2
average ($n = 5$)	76.08	21.4
SD	2.63	1.05
CV (5%)	3.0	4.2

Table 3. Quantity of Added 2M3F (25 or 100 ng/L) or 2FM (25 or 100 ng/L) Recovered from Different Types of Wine

2M3F					
	initial 2M3F concn (ng/L)	2M3F spiked			
		25 ng/L		100 ng/L	
		observed concn (ng/L)	recovery (ng/L)	observed concn (ng/L)	recovery (ng/L)
white wine 1	96	120	24	206	110
white wine 2	49	75	26	153	104
red wine 1	99	124	25	209	110
red wine 2	93	116	23	200	107

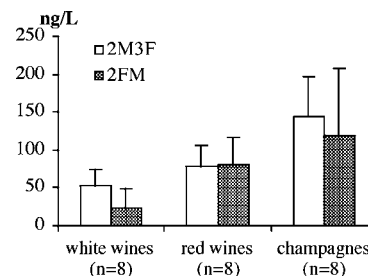
2FM					
	initial 2FM concn (ng/L)	2FM spiked			
		25 ng/L		100 ng/L	
		observed concn (ng/L)	recovery (ng/L)	observed concn (ng/L)	recovery (ng/L)
white wine 1	49	75	26	149	101
white wine 2	14	37	23	112	98
red wine 1	52	79	27	158	106
red wine 2	33	61	28	140	108

was approximately 75–85%, irrespective of the quantity added. The recovery rate for the internal standard was over 85%.

Quantification of the Volatile Thiols in Wine. Because of highly oxidizable compounds (22), quantification of the two volatile thiols normally demands the use of a deuterated analogue as an internal standard. The original aspect of the method described in this article is purification of thiols as a conjugate *p*HMB–thiol by percolation through a strongly basic anion exchanger column [Dowex (1X2–100)]. During purification, the SH group of the target compounds is protected by *p*HMB. Although the internal standard used here is not quite similar in behavior to the target compounds, the method developed has a good sensibility and repeatability (**Table 1**).

The 2M3F and 2FM quantifications were carried out using several white Sauvignon blanc and Chardonnay wines from different French appellations, Champagnes, and several red Bordeaux wines made from the Cabernet and Merlot grape varieties. The average concentrations of these compounds (**Figure 6**) in the wines analyzed varied from 50 to 145 ng/L for 2M3F and from 25 to 140 ng/L for 2FM. The highest values were found in Champagnes. In view of the perception threshold of 2M3F in model dilute alcohol solution (4 ng/L), it would partially contribute to the overall “toasty” aroma in wines.

The 2FM contents of both red and white wines were significantly above the perception threshold (0.4 ng/L) (20). We

**Figure 6.** 2M3F and 2FM concentrations (ng/L) in certain red and white wines and Champagnes.

can estimate that this highly odoriferous thiol would have a certain contribution to the roasted coffee aroma found in some wines (13, 14, 23).

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