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Development of a routine analysis of 4-mercapto-4-methylpentan-2-one in wine by stable isotope dilution assay and mass tandem spectrometry

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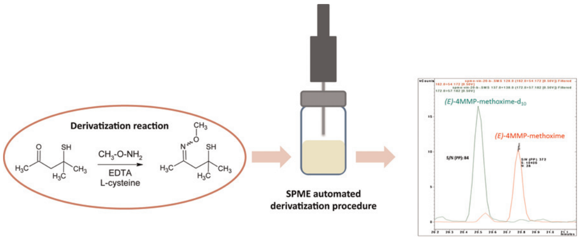
HIGHLIGHTS

- We proposed a routine method to analyze the 4-methyl-4-mercapto-pentan-2-one in wine.
- We developed the first method with sensitivity below the 4MMP perception threshold.
- We obtained an accurate method by using the stable isotope dilution assay approach.

GRAPHICAL ABSTRACT



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ABSTRACT

The 4-mercapto-4-methylpentan-2-one (4MMP) is a key aroma compound in wines, especially in Sauvignon Blanc ones. Its accurate quantification is quite difficult due to its traces levels and its reactivity in wine conferred by the thiol function. In this paper, we proposed a new method for its quantification in wine without any sample preparation, based on automated derivatization procedure by methoximation and SIDA–SPME–GC–MS/MS analysis. The derivatization procedure was adapted from a previously published method in order to decrease the amount of reagents and the volume of wine (only 3 mL are required). The use of SPME and the detection conditions have also been optimized to reach the best sensitivity as possible. The method was then validated according to the International Organization of Vine and Wine recommendations and exhibited excellent performances. Indeed, this method allowed us to quantify the 4MMP in wine at traces levels (LOD = 0.19 ng L^{-1}) with reproducible results (RSD < 15%) and a very good accuracy (recovery = 102%).

1. Introduction

The 4-mercapto-4-methylpentan-2-one (4MMP), first identified in Sauvignon Blanc [1] and Colombard [2] wines, is a potent odoriferous varietal thiol responsible for box-tree and blackcurrant bud notes in young white wines. Occurring at traces level, it has been later identified as a key odorant compound of white wines such as Scheurebe [3], Maccabeo [4], Gewurztraminer, Riesling, Muscat, Colombard, Petit Manseng and Tokay [5,6] and more recently in red wines [7]. The 4MMP exhibited a very low perception threshold equal to 0.8 ng L^{-1} in model medium [5]. Due to the presence of the thiol function, the 4MMP is highly reactive from a chemical point of view and requires the use of sophisticated quantification methods to obtain reliable results.

Few articles report the 4MMP quantification in wine [8,9] and most of them are particularly time-consuming, not sensitive enough and therefore, not usable as a routine method.

Abbreviations: 4MMP, 4-mercapto-4-methylpentan-2-one; SIDA, stable isotope dilution assay; SPME, solid phase micro-extraction; LOD, limit of detection; LOQ, limit of quantification; RSD, residual standard deviation.

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The main difficulty of the analysis lay in the 4MMP extraction from wine matrix and several methodologies has been proposed. The original method suggested by Tominaga et al. [10] was based upon a selective extraction of 4MMP from a wine dichloromethane extract using the reversible chelation of the thiol function by the sodium *p*-hydroxymercuribenzoate. Nevertheless, this method presents a major drawback concerning the 4MMP specificity since detection by GC–MS was performed only on one ion used both as quantifier and qualifier. Similar extraction procedures were later reported [11–13] and few of them had a limit of detection equal to the 4MMP perception threshold [12]. These methods were highly time consuming and not much safer for the analyst. Some improvements were later reported and consisted on derivatizing the 4MMP with pentaflurobenzyl bromide with [14,15] or without [16] a preliminary oximation step. Under these conditions, analysis sensitivity was increased since the reported limits of detection were below the 4MMP perception threshold. Recently, the ethyl propiolate was presented as a new derivatizing agent to quantify the 4MMP in wines [17]. Even if the sample preparation is much easier than those reported, the limit of detection equal to 24.5 ng L^{-1} was not sufficient to analyze the 4MMP in wines.

Concerning quantification procedures, few methods were based on stable isotope dilution assay (SIDA). Due to the non-commercial availability of labeled 4MMP, most of published methods used internal standards such as the 4-methoxy-2-methylbutan-2-thiol [10,13] or the 2-octanol [12]. Those standards are obviously not convenient to overcome the analyte losses during sample preparation since they do not have the same reactivity as4MMP. Thus, the best quantification procedure remains SIDA in term of accuracy.

Until now, there is no method allowing the quantification of 4MMP in wines with sensitivity below its perception threshold, an easy sample preparation and a quantification method base upon SIDA approach. For this purpose, we proposed for the first time a high throughput and fully validated method to quantify 4MMP in wine by SIDA–SPME–GC–MS/MS.

2. Materials and methods

2.1. Chemical and standards

The 4-mercapto-4-methylpentan-2-one (4MMP) was obtained from Sigma–Aldrich (Saint Quentin en Fallavier, France) and its labeled analogue (4MMP-d_{10}) has been synthesized according to the procedure reported by Kotseridis et al. [18]. Ethylenediaminetetraacetic acid (EDTA), L-Cysteine hydrochloride monohydrate and *o*-methylhydroxylamine hydrochloride were of analytical pure grade and were from Sigma–Aldrich (Saint Quentin en Fallavier, France). SPME fiber (Divinylbenzene/Carboxen/Polydimethylsiloxane) (DVB/CAR/PDMS) was obtained from Supelco.

2.2. Model wine preparation

Model wine was composed of water (800 mL), ethanol (120 mL) and tartaric acid (3,5 g L^{-1}). The pH of both was adjusted to 3.5 using potassium carbonate.

2.3. Analysis of 4MMP

2.3.1. Derivatization procedure

To an aliquot of wine (3 mL), we added successively deionized water (7 mL), the internal standard (4MMP-d_{10}) at 52.3 ng L^{-1} then sodium chloride (4 g). Then, derivatizing agents were successively added to the SPME vial as follow: EDTA (25 mg), L-cystein hydrochloride monohydrate (16 mg) and *o*-methylhydroxylamine hydrochloride (10 mg). The derivatization reaction was then carried out using an automatic CombiPal system (CTC Analytics, Zwingen, Switzerland). In practice, vials were stirred for 45 min at 55 °C, then SPME extraction on a DVB/CAR/PDMS fiber, previously conditioned at 250 °C for 12 min, was conducted for 30 min at 55 °C. Finally, the compounds were desorbed into the GC inlet at 250 °C for 3 min.

2.3.2. GC–MS/MS conditions

Analyses were performed using a GC–MS/MS system that consisted of a GC 3800 (Varian) coupled with an ion trap MS4000 (Varian). Analytes were separated on a DB WAX capillary column (60 m × 0.25 mm × 0.25 μm) from Supelco. The oven temperature program was as follow: 35 °C for 3 min, increased to 80 °C at 15 °C min^{-1}, increased to 130 °C at 3 °C min^{-1} then increased to 245 °C at 20 °C min^{-1} for 10 min. The carrier gas was helium with a constant flow rate of 1 mL min^{-1} and the injector temperature was set at 250 °C. Injection was performed in splitless mode for 3 min then a split of 1/20 was operated. The trap and transfer line temperatures were set at 150 °C and 170 °C respectively. Ionization was performed in positive chemical ionization using isobutane (pressure 1.5 bar). Detection was performed in MS/MS conditions in resonant mode. The *m/z* 163 and *m/z* 173 ions were chosen for 4MMP and 4MMP-d_{10} as parent ions respectively with an isolation window of 3 amu. The excitation amplitude was set at 0.6 V and the excitation storage level was adjusted to *m/z* 53.5 ($q = 0.3$) and *m/z* 56.8 ($q = 0.3$) for *m/z* 163 and *m/z* 173 respectively. The scan ranged from *m/z* 57 to *m/z* 182. The quantification was performed using *m/z* 128 and 138 as daughter ions for the natural and labeled compounds respectively.

2.4. Validation procedure

The analytical method validation was performed by evaluating the following parameters: matrix effect, linearity, accuracy, repeatability, intermediate reproducibility, limits of detection (LOD) and quantification (LOQ).

Matrix effect was measured by comparing the signal obtained in a model and real samples (red, white and rosé wines) spiked with 4MMP at the same concentration. Real samples were spiked with 4MMP at 0 and 20 ng L^{-1} in triplicate and the signal corresponding to the enrichment was compared with those obtained for a model wine spiked at 20 ng L^{-1}.

Linearity was calculated by spiking model wine with 4MMP at different levels and by plotting the 4MMP to 4MMP-d_{10} ratios against the corresponding area ratios. Linearity was evaluated by a lack of fit test. For this purpose, 9 samples (model wine spiked with 4MMP at 9 levels of concentrations (0–100 ng L^{-1}) and always 52.3 ng L^{-1} of 4MMP-d_{10}) were analyzed in triplicate.

Accuracy, repeatability and intermediate reproducibility were measured by spiking real samples with 4MMP at 5 levels and results were expressed as the recovery (concentrations calculated from calibration compared with theoritical concentrations, expressed in %) and mean RSD (%) respectively. In practice, real samples spiked with 4MMP at 5 levels (10, 20, 46, 70 and 100 ng L^{-1}) were analyzed in triplicate and under repeatability conditions. For intermediate reproducibility, spiked samples with 4MMP at 20 ng L^{-1} were prepared and analyzed in triplicate on 3 different days and by 2 different operators.

LODs and LOQs were evaluated as the lowest concentration at which a signal-to-noise (S/N) ratio greater than 3 and 10, respectively, could be consistently measured in spiked wine ($n = 3$) samples.