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Automated analysis of 2-methyl-3-furanthiol and 3-mercaptohexyl acetate at ng L^{-1} level by headspace solid-phase microextracion with on-fibre derivatisation and gas chromatography–negative chemical ionization mass spectrometric determination☆

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Abstract

A fast and automated method for the analysis at ng L^{-1} level of aroma-powerful polyfunctional thiols has been developed and applied to wine. The sample is just poured in a 20 mL vial and its vapour extracted with a poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB) solid-phase microextraction fibre (65 μm thickness) previously exposed to vapours of the reactive (pentafluorobenzyl bromide) and of an alkali (tributylamine). The derivatised compounds are subsequently desorbed in the GC system and determined by negative chemical ionization mass spectrometry. The method is fully automated by using a Combi-Pal autosampler conveniently programmed. The analysis takes 50 min, which contrasts to the long and tedious methods previously proposed. The development of an optimal procedure is constrained by the aggressive character of the reagent (towards the fibre and the chromatographic column), its volatility and the quality of the blanks that can be obtained. Therefore, a critical step was fixing in the fibre a "safe" and repetitive amount of reagent. This was achieved by exposing the fibre (5 min) to the vapours of a water:acetone (9:1) solution containing 200 mg L^{-1} of reagent. Under these conditions, the extraction-derivatisation of analytes improves with time and temperature, and the best working conditions are dictated by a compromise between sensitivity, speed and chromatographic performance. Although analytes studied were 2-methyl-3-furanthiol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexanol, 2-furanmethanethiol and 3-mercaptohexyl acetate, a good analytical performance could be achieved only for these two last compounds. Both of them can be repetitively ($10\% < RSD < 20\%$) determined in wine at concentrations below 0.1 ng L^{-1}. Other aspects considered in the method setup were the oxidation of analytes during the process, and the electron-capture detection (ECD) and MS properties of the pentafluorobenzyl derivatives of different polyfunctional thiols.

Keywords: GC–MS–NCI; Electron capture; Thiols; Mercaptans; On-fibre derivatisation; SPME; Wine; Aroma; Flavour

1. Introduction

Mercaptans are compounds that have strong odours which very often are responsible for the sensory characteristics and quality of a product. Simple low-molecular-weight thiols, such as methanethiol or ethanethiol, have extremely unpleasant odours and its presence is most often linked to the existence of microbiological decomposition. On the other hand, polyfunctional mercaptans of higher molecular weight (4-methyl-4-mercapto-2-pentanone, 2-methyl-3-furanthiol, 3-mercaptohexanol, 3-mercaptohexyl acetate, 2-furanmethanethiol) have, at low concentrations, powerful and penetrating aromas that are responsible for the particular sensory characteristics of products [1,2] like mango, coffee [3], passion fruit and some wines [4–10].

The odour thresholds of these mercaptans are in the ng L^{-1} level, which implies that these compounds can be detected, or even can be key aroma compounds, at extremely low concentrations. The analytical determination of these compounds at these levels is particularly difficult, since different problems have to be overcome. A first problem is their poor "detectability". Most often, the mass spectra of these compounds lacks characteristic ions of high *m/z*. In addition, their chromatographic properties

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are often also poor because of the adsorptive characteristics of the thiol function, which causes intense tailing peaks. A second problem is their instability. These compounds are elusive and can react with oxygen and other oxidants [11] and, in addition, they form complexes and precipitates with a lot of metal ions.

It is not surprising, therefore, that not many analytical methods have been described for the quantitative analysis of these compounds at trace level. The most commonly used strategies for the analysis of these compounds make use of the complexing properties of the thiol group, particularly to certain forms of organic mercury. Darriet et al. [12] and Tominaga et al. [13], proposed a selective extraction of thiols from an organic solution with an aqueous solution of *p*-hydroxymercurybenzoate. Different versions of this idea have been also proposed in the recent literature [14]. Similarly, other authors use mercury covalently bonded to a certain resin or sorbent [15,16]. These strategies make it possible to get very good isolates containing almost exclusively the thiols present in the original sample, but do not solve the aforementioned problems of "detectability" and instability. As a consequence, a large sample has to be processed and the methods are very often long, expensive and complicated.

An interesting alternative for improving detectability and stability is forming derivatives, but again not many references for the derivatization of volatile thiols can be found in the scientific literature. Hoffman et al. [11] proposed 4-vinylpyridine as derivatizating reagent, and recently, Ortín et al. [17,18], have developed a method using this reagent for the quantitative analysis of these compounds in wine. However, there are some other reagents able to produce derivatives with much enhanced detectability. By using 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) as derivatisation reagent, the derivatives formed show excellent electron-capturing properties. These derivatives could be then determined by means of negative ion chemical ionisation mass spectrometry (NCI–MS). NCI shares with the electron-capture detection (ECD) a selective and sensitive response to electrophilic atoms (halogens), but adds molecule-specific information, which makes this technique one of the most selective and sensitive.

On the other hand, one of the most interesting and promising approaches for carrying out an analytical derivatisation reaction, is directly in the solid-phase microextraction (SPME) fibre, before or after the extraction of analytes. Several interesting examples of these strategies have been recently published [19–23], although no one using pentafluorobenzyl bromide as the reagent. In this paper, we explore the possibilities of headspace in fibre derivatisation SPME for the automated analysis of ultratrace aromatic thiols in wine and other natural products.

2. Materials and methods

2.1. Reagents and standards

n-Hexane for organic trace analysis (UniSolv) and ethanol, gradient grade for liquid chromatographie (LiChrosolv) were from Merck (Darmstadt, Germany). Acetonitrile was supragradient HPLC grade from Scharlau (Barcelona, Spain), ethyl acetate was for instrumental analysis quality from Panreac (Barcelona, Spain). Anhydrous sodium sulphate and L(+)-tartaric acid were for analysis ACS-ISO quality from Panreac (Barcelona, Spain). Tributyl-, tripropyl- and triethylamine and ethylenediaminetetraacetic acid disodium salt 2-hydrate (EDTA) were from Aldrich (Steinheim, Germany). 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) and BHA (3-*tert*-butyl-4-hydroxyanisole) were from Fluka (Buchs, Switzerland). 4-Methyl-4-mercapto-2-pentanone 1% PG and 3-mercaptohexyl acetate were from Oxford Chemicals (Hartlepool, UK), 3-mercaptohexanol was supplied by Interchim (Monluçon, France), 2-furanmethanethiol and 1-hexanethiol were from Lancaster (Strasbourg, France) and 2-methyl-3-furanthiol was from Aldrich.

PDMS–DVB (65 μm) SPME fibres were purchased from Supelco (Bellefonte, PA, USA).

2.2. Proposed method

Two standard SPME 20 mL vials containing the reagent (10 mL of 200 mg L^{-1} PFBBr in water with 10% of acetone) and the secondary reagent (10 mL of tribuytilamine) were prepared and left in positions 2 and 3 of the autosampler incubator tray, programmed at 55 °C (see all the setup parameters of the autosampler in Table 1). Samples of wine (10 mL) were also pipetted into 20 mL vials. To each sample, 0.05 g of ethylenediaminetetraacetic acid disodium salt 2-hydrate (EDTA) and 10 μL of a 100 μg L^{-1} surrogate solution were also added. Before sealing the vial, the samples were bubbled for 2 min with a 5 mL min^{-1} stream of nitrogen and left in the autosampler tray at 4 °C.

The injection cycle begins by exposing the PDMS–DVB fibre 5 min to the tributylamine vapours (agitation speed 250 rpm), followed by other 5 min to the reagent solution vapours, and finally 10 min to the vapours of the sample (previously equilibrated at 55 °C for 5 min). After this, the fibre containing the derivatised analytes is desorbed (2 min at 275 °C) in the GC–MS system.

2.2.1. GC–NCI–MS analysis

The apparatus was a Shimadzu QP-2010 gas chromatograph with a quadrupole mass spectrometric detection system. The

Table 1
Autosampler method parameters

Parameter	Value
Agitator temperature	55 °C
Pre-extraction equilibration time in agitator (for loading and extraction steps)	1 min
Agitation speed	250 rpm (5 s clockwise, 2 s pause, 5 s anti-clockwise, 2 s pause)
Tributylamine loading time	5 min
Derivatization reagent loading time	5 min
Extraction time	10 min
Desorption time	2 min
Fibre conditioning temperature	250 °C
Bake-out time in conditioning unit	5 min

injector was a standard split/splitless operated in splitless mode and containing a SPME glass insert of 0.8 mm I.D. The carrier gas was He programmed to flow at a constant linear speed of 45 cm s^{-1} during all the run (flow 1.2 mL min^{-1}), except during the splitless time (2 min) which was at 2.47 mL min^{-1}. The column was a Factor Four capillary column VF-5MS from Varian, 20 m × 0.15 mm I.D., with 0.15 μm film thickness. The chromatographic oven was held at 60 °C for 2 min, then raised to 130 °C at 10 °C min^{-1}, then to 180 °C at 5 °C min^{-1} and finally to 300 °C at 30 °C min^{-1}; the oven was held at 300 °C for 2 min. The ionisation source was able to work in NCI, positive ion chemical ionisation (PCI) or normal electron impact ionization (EI) modes. In quantitative analysis, it was operated in NCI using methane at 2 bar as reagent gas. The mass analyser was operated in single ion monitoring (SIM) mode. The solvent cut window was 10 min.

2.2.2. Autosampler

Total automation of the procedure was achieved using a CTC CombiPal autosampler (Zwingen, Switzerland), which was programmed using the CycleComposer with macro editor software and equipped with sample trays, a temperature controlled agitator tray and a fibre-conditioning device.

2.3. Chemical synthesis of derivatives used as standards

Derivatives were obtained with the following procedure, adapted from [24]. Two microliters of analyte were added over 600 μL of acetonitrile. To these solutions, 100 μL of PFBBr and 100 μL of triethylamine were then added. The derivatisation reaction took place during 15 min at room temperature. After this, 5 mL of ethyl acetate were added and mixed, followed by 5 mL of hexane. At this point a white precipitate was formed (triethylamine bromide). After 15 min at room temperature, the mixture was collected and washed (15 min of stirring at room temperature) with two fractions of 10 mL of 0.5 M HCl. The organic phase is finally dried by the addition of anhydrous sodium sulphate, sealed and stored in the freezer. The purity and chemical identity of the derivatives was assessed by GC–ECD and GC–MS analysis.

2.4. Method optimization

2.4.1. Fixing the reagent in the fibre

In order to fix a given and reproducible amount of reagent in the SPME fibre different strategies were assessed:

(a) Direct exposition at different times and temperatures of the fibre to the vapours of reagent.
(b) Direct exposition of the fibre at different times and temperatures to the vapours of solutions of reagent in different solvent systems [poly(ethylene glycol) 200 and 300, pentadecane, heptadecane, water/acetone solutions].

In all cases, the fibre containing the reagent was desorbed in a GC injector and the amount of reagent determined by measuring its flame ionisation detection (FID) area under the following conditions. A ThermoQuest from CEI Instruments Trace gas chromatograph with FID system was used. The column was a MFE-73 from Análisis Vínicos (Spain), 25 m × 0.25 mm I.D., with 0.25 μm film thickness. The carrier gas was H_2 at 1 mL min^{-1}. The injection was carried out in the splitless mode, with a pulse pressure of 20 psi for 1.5 min. The splitless time was also 1.5 min. The chromatographic oven was held at 80 °C for 10 min. A calibration graph, area versus mass of reagent was built by the analysis of hexane solutions containing known amounts of reagent.

In addition, a second sample of reagent-adsorbed in the fibre, was desorbed in a GC–ECD system to assess the number and level of electron-capturing impurities. The conditions used were those that follow. A Varian CP-3800 gas chromatograph with an electron-capture detector was used. The column was a CP-Sil 5 CB from Varian, 15 m × 0.25 mm I.D., with 0.25 μm film thickness. The carrier gas was He at 1 mL min^{-1}. The injection was carried out in the splitless mode (a 0.8 mm I.D. SPME glass liner was used), with a pulse pressure of 20 psi for 1.5 min. The splitless time was also 1.5 min. The chromatographic oven was held at 60 °C for 1.5 min, then raised to 120 °C at 10 °C min^{-1}, then to 250 °C at 30 °C min^{-1} and finally was held at 250 °C for 5 min.

2.4.2. Extraction conditions: effect of time and temperature

Two different experiments were carried out. In the first one, the reagent-containing fibre was exposed to the vapours of 10 mL of a synthetic solution (12%, v/v, ethanol, 5.0 g L^{-1} tartaric acid, pH adjusted to 3.5 with 0.1 M NaOH) containing 10 μg L^{-1} of each analyte at nine different temperature–time conditions (35, 45 or 55 °C and 10, 20 and 40 min). The effect of both parameters was measured by desorbing the reagent contained in the fibre in the GC–ECD system previously described. In the second experiment, the fibre was exposed to the vapours of 10 mL of a wine spiked with 100 ng L^{-1} each of 2-methyl-3-furanthiol, 2-furanmethanethiol and 3-mercaptohexyl acetate and with 1 μg L^{-1} each of 4-methyl-4-mercapto-2-pentanone and 3-mercaptohexanol. In this case, six different temperature–time conditions were used (35 and 55 °C and 5, 10 and 20 min). Desorption and analysis took place in the GC–NCI–MS conditions described in Section 2.2.

2.4.3. Extraction conditions: addition of salt

A saturated NaCl solution was mixed with the synthetic solution described before in different proportions: 1:2, 1:3, 1:5 and 1:10. Ten milliliters aliquots from such mixtures were then spiked with analytes to adjust their level to 10 μg L^{-1}. The reagent-containing fibre was then exposed to the vapours of these solutions and desorbed and analysed in the GC–ECD system previously described.

2.4.4. Extraction and reaction conditions: addition of amine

Different ways of fixing an alkali on the fibre were studied. Two different volatile and lipophilic amines, triethyl and tributylamine, were assessed. In the first experiment, 2 or 4 μL of different pentane solutions (0.03 or 0.06 M in one of the amines)

Table 2
Negative ion chemical ionisation mass spectrometric (NCI) properties of the derivatives: mass spectra and detection limits

		MW	MW + PFB	Ret. time	NCI ions	GC–ECD LOD ($\mu g\,L^{-1}$)	MS–NCI–SIM LOD ($ng\,L^{-1}$)
	Analytes						
1	2-Methyl-3-furanthiol	114	294	11.8	274 (100), 113 (60)	0.3	0.11
2	2-Furanmethanethiol	114	294	14	274 (100), 162 (35)	0.2	0.05
3	4-Methyl-4-mercapto-2-pentanone	132	312	15.2	131 (100), 194 (73)	0.5	0.5
4	3-Mercaptohexanol	134	314	17.3	133 (100), 194 (37)	0.2	0.8
5	3-Mercaptohexylacetate	176	356	18.9	194 (100), 213 (39)	0.1	0.03
	Standard						
6	1-Hexanethiol	118	298	14.16	213 (100), 194 (42), 115 (37), 278 (24)	–	2
7	Benzylthiol	124	304	17.15	284 (100), 162 (15), 213 (17)	–	0.9

Detection limits obtained in GC–ECD analysis.

were directly deposited in the reagent-containing fibre. The fibres were then exposed to the vapours of analyte-containing samples, desorbed and analysed in the GC–ECD system. In the second experiment, the reagent-containing fibre was directly exposed to the vapours of the pure amines, and then exposed to the vapours of analyte-containing samples (synthetic solutions or spiked wines), desorbed and analysed in the GC–ECD (synthetic solutions) or in the GC–NCI–MS (spiked wines) systems.

2.4.5. Metal-complex and oxidation-related problems

The effect on the analyte signals of the addition to the samples of $5\,g\,L^{-1}$ EDTA and of $5\,mg\,L^{-1}$ BHA to samples was studied. The oxidation problems were diagnosed by monitoring the signals of two surrogated standards: 1-hexanethiol and benzylthiol added to a wine (containing $5\,g\,L^{-1}$ EDTA) at $100\,ng\,L^{-1}$. A set of 14 samples was left in the autosampler tray kept at room temperature, and were analysed following the proposed procedure in an automated operation which allowed one sample per hour. The same experiment was then repeated, but in this occasion the vials were carefully bubbled with nitrogen before sealing and the samples were kept at 4 °C until the moment of being analysed.

2.5. Method validation

Precision was evaluated by means of triplicate analysis of a white wine spiked with the analytes at two levels of concentration such as can be seen in Table 3. Detection limits were calculated as the concentration giving a peak height three times the signal-to-noise ratio. In order to evaluate the existence of matrix effects, three calibration curves in three different wines: synthetic (aqueous solution containing $5\,g\,L^{-1}$ of tartaric acid, 12% of ethanol and pH 3.5), young white and young red wine, were built. The slopes were then statistically compared. In addition, a standard recovery experiment was carried out by analysing five different wines spiked or not with known amounts of the two analytes.

3. Results and discussion

The main goal of the present paper is to explore the possibilities of on-fibre derivatisation with PFBBr to quantitatively determine some important volatile thiols of wine and other natural products.

3.1. Spectrometric characteristics of PFBBr derivatives (NCI spectra)

The most prominent ions obtained from the derivatives of several volatile thiols by NCI are shown in Table 2. It can be observed that there is a remarkable amount of fragmentation in the conditions used and in no case was it possible to isolate the $[MD]^-$ ion. In some cases a strong fragment corresponding to the $[MD\text{-}HF]^-$ ion was observed (compounds 1, 2, 6, 7). The existence of fragmentation has some advantages, namely that it is possible to work in SIM mode with at least two ions: one quantifying ion (the most abundant and selective) and one qualifying ion (the next most abundant).

Approximated limits of detection (LODs) in GC–NCI–MS and in GC–ECD can also be found in the table. For ECD, the sensitivities were in the 0.1–0.5 pg level, while for the NCI–MS system, they range from 0.03 fg (5) to 0.8 fg (4). The much higher sensitivity of the NCI–MS must be attributed to the absence of noise. The lower sensitivity of compound 4 must be attributed to the tailing of this compound, as it can be seen in Fig. 1, while the lower sensitivity of compound 3 is due to a lower yield in the derivatisation. Whatever the case, sensitivities are extremely high (2 or 4 magnitude orders better than by EI of the underivatised analytes) which should, a priori, make the analysis of these compounds possible, even at very low levels.

3.2. In fibre derivatisation

The formation of derivatives can be carried out in different ways, such as direct reaction of analytes in the aqueous phase, simultaneous extraction-derivatisation [25,26], directly in a SPE bed previously wetted by the reactive [27] or even directly by HS-on-fibre derivatisation SPME [22,23,28]. This last strategy, to our knowledge, has not been applied to form the derivatives of any compound using PFBBr as reagent due to the high volatility of the reactive [28]. However, and given the advantages of this strategy (cleanliness, easiness, easy automation, less potential interferences) it will be our technique of choice.

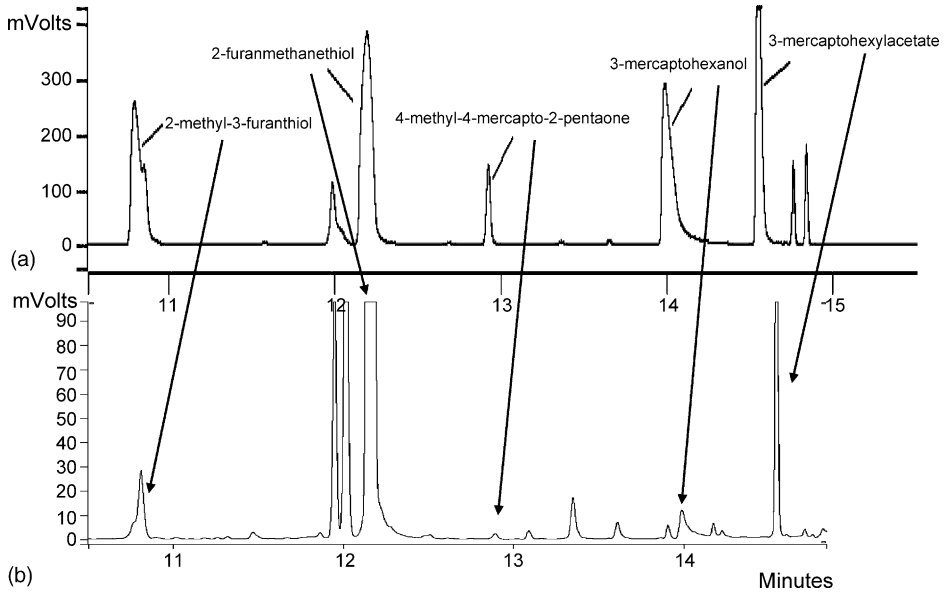

Fig. 1. GC–ECD chromatograms of (a) a splitless injection of a solution containing approximately 200 μg L^{-1} of the derivatives, and (b) a typical SPME chromatogram obtained from the analysis of a synthetic wine containing 10 μg L^{-1} of analytes.

Some basic decisions were taken in order to simplify the optimization procedure:

- The fibre of choice was a PDMS–DVB of 65 μm thickness, since DVB has a high affinity to the reagent [25,29] and its stability and reproducibility are better than those shown by the DVB fibre.
- Standard 20 mL headspace vials filled with 10 mL of sample were used to ensure a good automatic operation.
- For the same reason, the temperatures used to load in the fibre the reagent, any secondary reagent (alkali) or to extract the sample will be the same.
- The first task of the optimization process was the search of a robust, safe and clean procedure for the dose of known amounts of derivatisation reagent to the fibre. This was accomplished by monitoring in GC–FID and GC–ECD systems the amount of reagent loaded in the fibre, the general profile of the chromatogram, and the number of electron-capturing impurities also loaded and released from the fibre.

3.2.1. *Fixing the derivatisation reagent in the fibre*

In other studies the derivatisation reagent is fixed in the fibre by simply exposing the latter to the vapours of the reagent [22,23]. This simple procedure resulted impractical because the fibre can be very easily destroyed using this strategy (apparently, the bonds between the sorbent phase and the syringe are broken, which makes the coating to come off and fall down). Even if this risk lessens at lower temperatures (0–20 °C), the reproducibility obtained was quite deficient, which made us to consider other possibilities. The simplest and more efficient way to transfer a known and reproducible amount of derivatisation reagent to the fibre was by using solutions of the reagent in solvents with a high boiling point. The exact amount of reagent fixed in a short time at a given temperature can be controlled simply by adjusting the concentration of reagent in the solvent. As an example, the exposure of the fibre (5 min at 55 °C) to a 200 g L^{-1} solution of the reagent in PEG 200 will deliver 60 nmol of reagent, being the amount fixed proportional to the concentration. However, the use of these solvents was not practical because of the large number of impurities also transferred (most of them formed by the action of the reagent on solvent impurities or even on the solvent itself (case of PEG)), or because some chromatographic distortion most likely induced by the accumulation of the less-volatile solvents at the head of the chromatographic column. Better results were obtained by using water/acetone solutions of the reagent, although in this case the amount of reagent transferred is limited by the solubility of PFBBr in these media, as shown in Fig. 2. The best blanks were obtained by the exposure of the fibre to a solution containing 200 mg L^{-1} of the reagent in an aqueous solution containing 10% (v/v) of acetone. The mass of reagent transferred in this case is 3.0 ± 0.2 nmol, a figure well below the mass fixed by other authors [28]. However, this tiny amount of reagent will be enough to get signals from some analytes, and not large enough to cause blank problems,

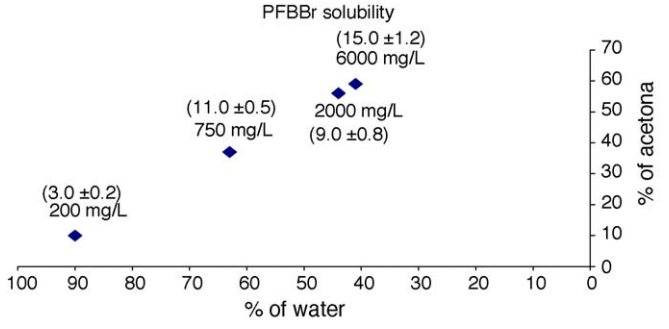

Fig. 2. Solubility of PFBBr in different water/acetone solutions and amount (in nmol) of reagent fixed after the exposure of the SPME fibre to such solutions (5 min at 55 °C).

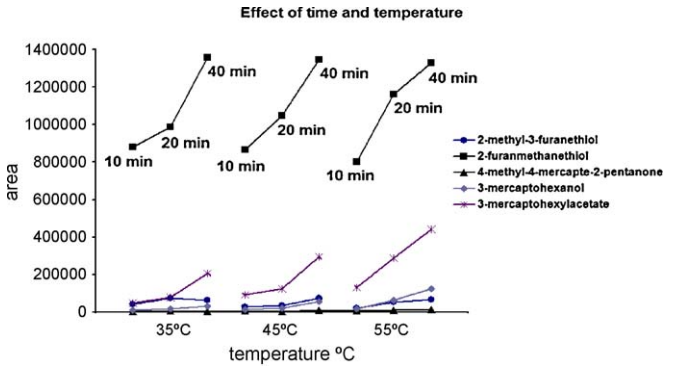

Fig. 3. Effect of time and temperature in the extraction-derivatisation of analytes contained in a synthetic wine.

a fast degradation of the fibre, the chromatographic column or the mass selective detector.

3.2.2. SPME optimisation

The effect of the extraction time and temperature on the signal of analytes is seen in Fig. 3. As shown in this figure, the longer the time and the higher the temperature, the higher the signal which clearly indicates that the equilibrium cannot be reached. This result is expected from the particular thermodynamics of the system: The chemical equilibrium is displaced towards the fibre, since the derivatives are strongly retained in the fibre and not in the vapour or in the liquid phases, because of their low volatility and high hydrophobicity. Therefore, the analyte will be, slowly but continuously, transferred to the fibre, where the reaction takes place. The kinetics of the system is the result of two slow processes: the transference of analytes from the liquid phase to the fibre, and the chemical reaction. Both processes are speeded up by temperature and, therefore, it is not easy to say from data in Fig. 3, which one is the critical step.

The addition of salt and the alcoholic content of the sample were also investigated. None of these parameters seemed to exert a major influence on the signal obtained, except the addition of salt on the signal of 3-mercaptohexanol (data not shown). As noted in a previous work [30] alcohols are much more affected by the addition of salt. These results suggest that, except in that case, the critical parameter in the signal is most likely not the transference of analytes to the fibre but the speed of the reaction.

In any case, the conditions used in the working procedure were selected more attending to practical considerations (blank and quality of the chromatogram) than to the size of the signal. The selected temperature was 55 °C, and the extraction time 10 min since serious chromatographic distortion and interferences were observed when real wine samples were extracted longer times.

In order to improve the speed of the reaction, we studied the effect of adding an amine (triethylamine, tripropylamine or tributylamine) to the reaction media. Triethylamine is the most commonly used alkali for carrying out the reaction in organic media [24,26,31]. However, it is too volatile (b.p. 88 °C) to be retained in the fibre and only a slight improvement in the signal was obtained. Things worked much better with tributylamine whose boiling point (216 °C) is high enough to ensure it is well retained in the fibre. The easiest way of transferring this alkali to the fibre was by exposing the fibre directly to the vapours of the pure compound at 55 °C, the temperature selected for the extraction and the loading of the reagent. The signal increased by factors 2–5, depending on the analyte.

3.3. Signal stability: metal-complex and oxidation-related problems

An experiment in which 14 vials, containing the same sample spiked with two surrogates, were put in the autosampler tray and were sequentially analyzed (see Section 2) revealed that the signal obtained for the last sample was 20–50% smaller than that obtained in the first analysis. Most of the decrease was attributed to the oxidation of these compounds, and different strategies were explored to minimise such problem. The addition of the antioxidant BHA (5 mg L^{-1}) to the samples did not bring about any improvement in the signal. This may be due to the poor protective effect of normal antioxidants for thiols as noticed by Hoffman et al. [11] or to the fact that oxidation could be take place mainly on the vapour phase, where there is no antioxidant The addition of EDTA (5 g L^{-1}) to the samples showed a positive but very weak effect. Such effect could be due to a competitive effect on complexing metals or to the elimination of their catalytic properties. Which had a definitive effect was a careful elimination of oxygen before sealing the vials, as explained in the proposed procedure, and storing them at 4 °C up to the analysis. No evidence of significant losses of analytes caused by the bubbling of the vial with nitrogen was noticed.

3.4. Application to wine and validation of a procedure

The developed procedure was applied to wine. A typical GC–NCI–MS chromatogram obtained from a wine spiked with 100 ng L^{-1} of 1-hexanethiol and benzylthiol; 40 ng L^{-1} of 2-methyl-3-furan-thiol, and 2-furanmethanethiol and 200 ng L^{-1} of 3-mercaptohexyl acetate is shown in Fig. 4. Hexanethiol and benzylmercaptane were selected as surrogates, since both of them eluted in well separated areas of the chromatogram. However, their use as internal standards did not bring about any improvement, since their behaviour was not equivalent to that of analytes. As shown in the figure, the method failed at determining 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexanol at low levels due to the reasons stated in Section 3.1. The estimated detection limit of the former was 50 ng L^{-1}, while this compound is most often below 20 ng L^{-1} [8,10,11,13,32–37]. The case of 3-mercaptohexanol was still worse, since its LOD was roughly estimated as 300 ng L^{-1}. In the case of 2-methyl-3-furanthiol, the problem found was that the signal was independent of the concentration, i.e., apparently, the dynamic range of the method for this compound is extremely narrow. This could be the consequence of a lack of stability of the derivative which would undergo further transformations in the presence of an excess of reactive.

As for 2-furanmethanethiol and 3-mercaptohexyl acetate, precision (Table 3) and linearity (Table 4) can be considered satisfactory for their determination in wine. In both cases the

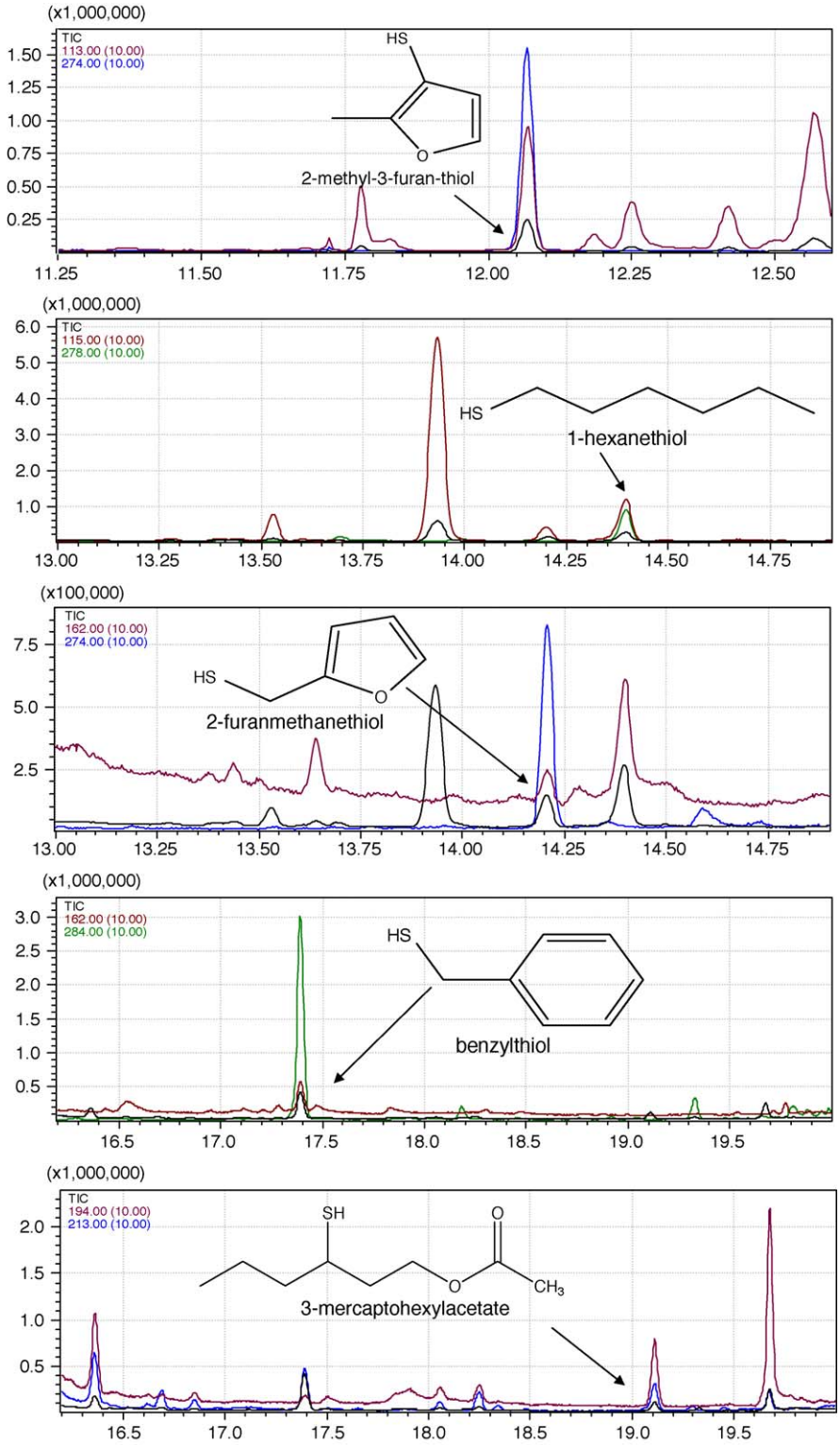

Fig. 4. GC–NCI–MS chromatogram obtained in the analysis of a wine containing 100 ng L^{-1} of 1-hexanethiol and benzylthiol; 40 ng L^{-1} of 2-methyl-3-furan-thiol and 2-furanmethanethiol; and 200 ng L^{-1} of 3-mercaptohexyl acetate.

linear ranges are not very wide, but cover the natural range of occurrence of these compounds in wine. The limits of detection and quantification in wine, shown in Table 3, are below the sensory detection thresholds. The existence of matrix effects was checked by comparing statistically the slopes of calibration curves obtained in three different wines (see Table 4). A single look at the slopes reveals that the sensitivities obtained in the case of the synthetic wine are smaller than those obtained in the analysis of normal wines. These results may support the existence of problems of stability of derivatives in the presence

Table 3
Method repetitivity (expressed as RSD (%)) at two different levels of concentration and method detection limits

	Repetitivity as RSD (%) for verdejo wine		LOD (ng L^{-1})	LOQ (ng L^{-1})
	Low level[a]	High level[b]		
2-Furanmethanethiol (FM)	20.8	15.6	≈0.3	≈1
3-Mercaptohexylacetate (MHA)	15.2	10.1	≈3	≈10

[a] FM 2 ng L^{-1}; MHA 10 ng L^{-1}.
[b] FM 20 ng L^{-1}; MHA 200 ng L^{-1}.

Table 4
Method linearity and diagnose of the existence of matrix effects

		Slope ± s	R^2	Studied range (ng L^{-1})	Linear range (ng L^{-1})
2-Furanmethanethiol	Synthetic wine	492 ± 25	0.9926	0.4–40	0.4 ≥ 40
	Verdejo wine	711 ± 51	0.9798	0.4–40	Up to C_0 + 30
	Red wine	720 ± 40	0.9951	0.4–40	Up to C_0 + 30
3-Mercaptohexylacetate	Synthetic wine	96 ± 6.5	0.9961	2–200	2 ≥ 200
	Verdejo wine	122 ± 13	0.9715	2–200	Up to C_0 + 60
	Red wine	132 ± 14	0.9959	2–200	Up to C_0 + 200

Table 5
Concentration levels of 2-methyl-3-furanthiol and 3-mercaptohexyl acetate in five different wines and results of the recovery experiment

Sample	FM			MHA		
	Determined	Spike	Recovered	Determined	Spike	Recovered
Young red[a]	3.7 ± 0.6	10	9.2 ± 1.0	16.0 ± 2.1	30	26.9 ± 3.8
Verdejo[a]	4.2 ± 0.7	10	8.9 ± 1.4	49.5 ± 5.0	30	33.5 ± 6.1
Chardonnay[b]	11.0 ± 1.5	10	10.4 ± 1.9	6.7 ± 1.3	30	30.4 ± 3.7
Aged rioja[c]	18.1 ± 1.9	10	11.7 ± 2.2	n.d.	30	28.4 ± 4.0
Macabeo[a]	n.d.	10	9.0 ± 1.3	19.4 ± 1.9	30	30.7 ± 4.2

All determinations were carried out in triplicate. Data are in ng L^{-1}.
[a] Young wines without any contact with wood.
[b] Fermented in oak barrel.
[c] Aged in oak barrel.

of an excess of free reagent (higher in the analysis of synthetic samples). Fortunately, the slopes obtained in the case of very different wines do not differ significantly, which suggests that a single calibration in wine can be developed and used for different wines. This was confirmed by a recovery experiment carried out on five different wines, as it can be seen in Table 5. Peak areas were interpolated in the calibration graphs built on the young red wine. As shown in the table, the observed differences between the amounts added and those determined were not significant. Data in the table also suggest that the levels of 2-furanmethanethiol are highest in barrel fermented or barrel aged wines, while the levels of 3-mercaptohexyl acetate are highest in young wines, particularly in verdejo, which would support the sensory relevance of this compound in this type of wine, as it has been recently suggested [6]. The simplicity of the proposed procedure contrasts to the complication of the few previous proposals. Although it is clear that more basic studies aiming the understanding of the reaction mechanisms and kinetics in the fibre are needed, it has been demonstrated that the automated on-fibre derivatisation with PFBBr and subsequent GC–NCI–MS analysis, is a valid and promising alternative for the analysis of thiols and, probably, of other nucleophylic analytes.

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