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Journal of Chromatography A, 773 (1997) 261–269

JOURNAL OF CHROMATOGRAPHY A

Chromatographic analysis of volatile sulphur compounds in wines using the static headspace technique with flame photometric detection

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Received 3 December 1996; revised 14 February 1997; accepted 18 February 1997

Abstract

This study describes the development of a method for determining eleven sulphur compounds in wine, which takes into account that thiols are easily oxidizable. The equilibria of the analytes between air and aqueous ethanol were studied and optimised using static headspace gas chromatography in order to obtain the best sensitivities. The influences of parameters such as temperature, time, ionic strength, headspace volume and the volume of headspace injected were determined. A cryogenic trap was used to concentrate the headspace analytes and they were chromatographically analysed using GC temperature programming on a poly(ethylene glycol) capillary column with FPD detection at 394 nm. The power relationship was observed between the chromatographic response and a concentration of sulphur compounds in the range 2–150 $\mu g\ l^{-1}$ in the sample. Recoveries were determined by the standard addition technique and were higher than 90% for sulphides and disulphides and close to 80% for thiols. The overall method was succesfully used to determine the sulphur compounds in white and red wines.

Keywords: Wine; Headspace analysis; Sulfur compounds, volatile

1. Introduction

For many years it has been known that sulphur compounds (S-compounds) are present in a variety of foods, including cheese [1], fish [1], poultry [1], meat [2] and mushrooms [3], as well as in wine [4,5], whisky [6,7], beer [8,9] and some other alcoholic beverages [10].

These compounds can be classified according to their molecular mass. Compounds which contain sulphur and which have a low molecular mass, such as thiols (R-SH), sulphides (R-S-R) and disulphides (R-S-S-R), have a considerable influence on the aroma of foods and beverages, even at trace amounts. This is because of their high volatility and low sensory threshold. This last property is an aspect that should be taken into account for thiols, because they are very oxidizable and quickly convert into disulphide forms in the presence of oxygen. As a result, making standard solutions and working with samples which contain thiol is cumbersome and requires special care.

In the case of wine, some of these compounds, such as dimethyl sulphide, may have a beneficial effect [11,12], but generally the influence of those compounds is considered to be negative and they

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PII S0021-9673(97)00188-X

contribute to strong, objectionable flavours. Sulphurous off-flavours, which cause some of the major defects in the quality of wine aroma, [13], lead to unpleasant tastes and odours which can be described as rubber, onion, garlic, cabbage, and so on [9,14,15].

Different S-compounds have been identified in wine [5,11,16]. Their concentration depends on the conditions of wine production, because they have different origins: natural causes [17], the use of some S-containing phytosanitary products [13,18], thermal treatments (Maillard and Strecker reactions) [2,14], production during alcoholic fermentation [19,20] and photochemical reactions [15].

Amperometric, colorimetric, fluorimetric, gas chromatographic, potentiometric and titrimetric techniques have all been used to determine these compounds [8,21,22]. However from the point of view of sensitivity, specificity and reliability, the usual method is gas chromatography with sulphur-specific detection: flame photometric detection [4,5] or chemiluminescence detection [23,24].

Because of the low boiling point of some of the S-compounds studied, liquid–liquid or solid-phase extraction could not be used as the concentration technique. So, the most widely used techniques are headspace, either static [5,23] or dynamic [25], and purge and trap [26,27].

For the procedure proposed here, the static headspace technique with cryogenic trap was tested to improve the recoveries for the determination of volatile S-compounds. The method was then used to determine these compounds in red and white wines.

2. Experimental

2.1. Chemicals and reagents

The S-compounds studied were: hydrogen sulphide [7783-06-4], methanethiol [74-93-1], ethanethiol [75-08-1], dimethyl sulphide [75-18-3], diethyl sulphide [352-93-2], methyl-*n*-propyl sulphide [3877-15-4], methyl thiolacetate (S-methyl acetate) [1534-08-3], ethyl thiolacetate (S-ethyl acetate) [625-60-5], carbon disulphide [75-15-0], dimethyl disulphide [624-92-0] and diethyl disulphide [110-81-6]. Ethyl-methyl sulphide [624-89-5] and thiophene [110-02-1] were chosen as internal standards (istd), since both have suitable retention times which are not the same as those of the other analytes and they do not occur naturally in wines [4,10]. We used two istds because the ethyl-methyl sulphide may be overlapped by the SO_2 peak if the latter is very large, while if we inject a great deal of sample, the thiophene peak is distorted by the effect that the ethanol has on the baseline.

The sulphides and disulphides were supplied by Fluka (Madrid, Spain), except ethyl-methyl and diethyl disulphides, which were supplied by Aldrich (Beerse, Belgium).

The thiols were obtained from their respective sodium salts: sodium ethanethiolate [811-51-8] and sodium methanethiolate [5188-07-8], which were supplied by Fluka, and sodium sulphide hydrate [7783-06-4], which was supplied by Merck (Darmstadt, Germany).

Other auxiliary reagents used in the preparation of the standards were: NaOH (Scharlau, Barcelona, Spain) to help dilute the thiols; KH_2PO_4 and Na_2HPO_4 (Scharlau) to prepare the buffer needed in the reaction between the thiols and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Aldrich) and L-(+)tartaric acid (Scharlau) to prepare the synthetic wine used in the calibration experiments. Finally, ethylenediaminetetraacetic acid disodium salt 2-hydrate and sodium chloride were also supplied by Scharlau.

2.2. Preparation of sulphide and disulphide standard solutions

For these compounds, which are liquid at room temperature, an individual standard solution of 2000 mg/l of each one was prepared in HPLC-grade ethanol (Scharlau). Since the boiling point of some of them is not very high, the dilutions were made at 4°C and stored in darkness at −18°C. A global standard solution containing all the analytes was prepared with an aliquot of each individual solution and subsequently diluted with ethanol in a volumetric flask. The diluted solutions used in the different studies were prepared by diluting this standard solution with ethanol.

2.3. Preparation of thiol standard solutions

Preparing the standard solutions of methanethiol, ethanethiol and hydrogen sulphide was difficult because of their low boiling points (−60°C to 35°C) and oxidizability. It would have been possible to work with gaseous standards, but because our matrix was wine and we were interested in determining the quantity of S-compounds it contained, it seemed more appropriate to work with liquid solutions of the S-compounds. On the other hand, thiol salts can easily be oxidised to form disulphides in an alkaline medium and it is difficult to know the real concentration of the thiol standard solutions. In order to prevent these problems, some researchers have used the strong affinity of mercuric salts to thiols to obtain stable standard solutions. A known amount of 4-(hydroxymercuri)benzoic acid is used to fix the thiol gas samples, since it forms stoichiometric stable complexes with thiol groups in alkaline medium. The excess thiol is eliminated with nitrogen steam, and the S-compounds are then liberated by adding glutathione [5]. In this study salt thiols were used instead of gas thiols, but their excess was very difficult to eliminate. For this reason, the new procedure described below for preparing and validating standard thiol solutions was established with thiol sodium salts.

As these thiols are oxidiseable in alkaline medium, the concentration of thiol in each standard was spectrophotometrically determined with Ellmans reagent. The spectrophotometric calibration curve was constructed in the following way:

(1) A stock solution of about 500 mg l^{-1} of thiol was prepared in a 0.1 *M* alkaline medium. The exact concentration of this solution was determined by adding an excess of 0.1 *M* acidified iodine and titrating the excess of iodine with a standard arsenite solution, with starch as the indicator. (2) A bank of dilutions with concentrations between 0.5 and 5 mg l^{-1} was therefore prepared from the titrated stock solution. To avoid oxidation, dilutions were made at a low temperature (4°C) and under a N_2 steam. (3) An excess of DTNB solution (360 mg l^{-1}) was added to each dilution and the yellow colouring, the intensity of which was proportional to the number of SH^- groups, was measured at 412 nm [28]. This reaction requires the pH to be 7 and this was obtained with a buffer solution of KH_2PO_4–Na_2HPO_4. (4) The spectrophotometric calibration line was constructed by regressing the concentrations vs. the measured absorbances.

With the above method, the concentration of the standards solutions can be accurately determined after successive dilutions, even if oxidation occurs.

Synthetic wine with a pH of 3.5 was used to obtain the calibration curves. The standard solutions could not be added directly to wine without incurring losses due to the high volatility of RSH compounds with an acid pH. So, the following method was used:

(1) A suitable amount of an alkaline thiol standard solution was put into an Eppendorf microtube under a N_2 steam. (2) The microtube was opened under the N_2 steam and then put into a 20 ml glass vial containing synthetic wine at pH 3.5. (3) Then it was tightly capped and shaken to allow the salts to come into contact with the wine. So the thiols were instantly released into the capped vial without losses. The headspace of this solution was used to construct the calibration curves.

2.4. Preparation of synthetic wine solution

The chromatographic calibration curves were obtained by dissolving different amounts of standards in a synthetic wine solution. The synthetic wine was obtained by dissolving 3.5 g of tartaric acid and 120 ml of ethanol in a suitable amount of deionized water to give 1 l of solution. The pH was then adjusted to 3.5 with 1 *M* NaOH.

2.5. Equipment

Chromatographic experiments were performed using a Hewlett-Packard 5890 gas chromatograph with an HP Model 19256A flame photometric detector at λ=394 nm. The detector was at 200°C and fed with 86 ml/min of synthetic air, 75 ml/min of hydrogen and 57 ml/min of helium as auxiliary gas. The carrier gas was helium with a flow-rate of 0.4 ml/min. Separation was performed using an HP-INNOWAX (50 m×0.2 mm I.D. and 0.2 μm film thickness). The first 30 cm of the capillary column were placed outside the oven. Chromatographic data were collected and recorded on an HP Chemstation version A.04.01.

The headspace was injected at 220°C via splitless mode, with a Hamilton 1005 gastight (5 ml) syringe, and cryogenically trapped by chilling the outermost 25 cm of the capillary column in a liquid nitrogen trap. A 2-ml volume of headspace was injected in 1 min. The liquid nitrogen dewar flask was then removed. The chromatographic separation started at an oven temperature of 30°C for 8 min which was then finally increased at 50°C/min to 220°C.

Spectrophotometric measurements were performed using a Hitachi U-2000 spectrophotometer with the wavelength set at 412 nm.

2.6. Headspace technique

The headspace was generated with 20-ml glass vials in which 10 ml of a synthetic wine, 2.32 g of NaCl and 0.05 g of EDTA were added to different amounts of the S-compounds. The vials were then tightly capped with teflon-faced silicone septa and shaken. Equilibrium was reached in 2 h at 60°C. A volume of 3–4 ml of headspace was taken into the syringe before being adjusted to 2 ml and injected under the conditions described in Section 2.5.

3. Results and discussion

Volatile S-compounds were chromatographically analysed using the headspace technique. Therefore, different parameters were studied in order to obtain the best sensitivities. The parameters optimised were: temperature, time, ionic strength, volume of headspace and volume of headspace injected. EDTA (50 mg/10 ml wine) was added to form complexes with the metals, so avoiding their catalytic effect in the oxidation of thiols [29].

The first parameter examined was the temperature of the sample vials. If experiments were done at room temperature, headspace equilibrium of sulphides and disulphides was reached in 10–12 h, but in the same time the thiol concentration in the headspace decreased, presumably due to oxidation (Fig. 1). However, if the temperature was 60°C (Fig. 2), the equilibrium of all S-compounds was reached in 2 h. Higher temperatures tend to further favour the oxidation of thiols. So, the optimal time and temperature to reach the headspace equilibrium was 2 h at 60°C.

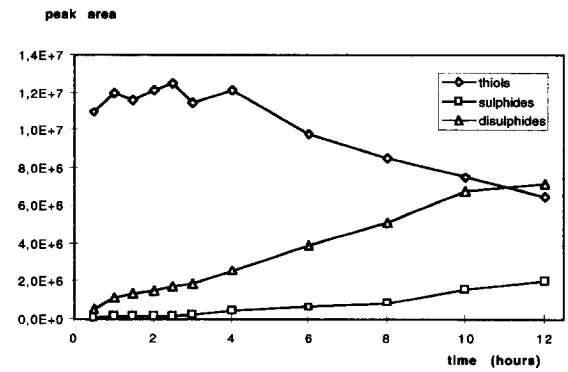

Fig. 1. Effect of time on the equilibrium in the headspace samples at room temperature.

Another variable studied was the ionic strength. Different concentrations of sodium chloride were tested and compared (Fig. 3). As the concentration increased up to ionic strength 4 *M*, the chromatographic responses of the non polar compounds (sulphides and disulphides) were also seen to increase. Once this value was exceeded, there was no further increase in the responses. Because of their polarity, the thiols were not affected by the changes in ionic strength.

The next parameter to be examined was the volume of headspace. The different liquid–gas ratios essayed were 5:15, 10:10 and 15:5 in 20-ml vials. The results showed that a volume of solution of 5 ml is too small (as shown by the chromatographic responses) and that a volume of 15 ml was difficult

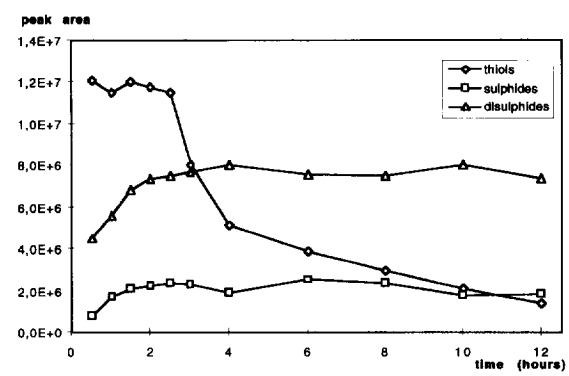

Fig. 2. Effect of time on the equilibrium in the headspace samples at 60°C.

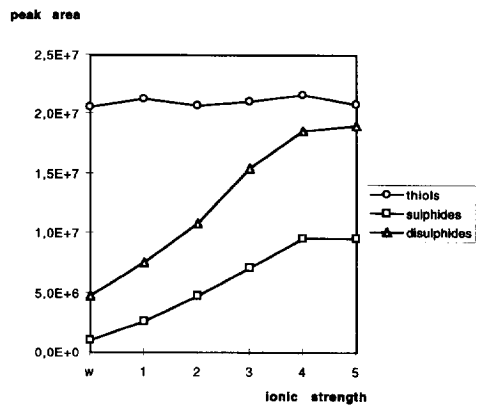

Fig. 3. Effect of ionic strength on the headspace chromatographic response; w is the ionic strength of wine.

to extract because it was 75% of the total volume of gas in the vial. Thus, the ratio 10 ml of solution:10 ml of gas was fixed as the best.

Finally, the volume of headspace injected was also tested. It is obvious that the more gas is injected, the greater the response obtained. A volume of 2 ml was considered sufficient to obtain suitable responses at suitable injection times (1 min).

In the chromatographic analysis, the different S-compounds were identified by comparing their retention times with standard solutions. Fig. 4 shows the chromatogram which resulted from injecting a standard solution (20–40 $\mu g\ l^{-1}$) of studied sulphur compounds under the conditions described above. Good resolution was obtained among the above-mentioned peaks.

The FPD response is of the type: response=kC^b (where $1<b<2$ depending on the analyte) [30,31]. This behaviour is attributed to the detectors' specific response to each compound. In order to verify the FPD response at 394 nm at the working concentration of each analyte, standard solutions were

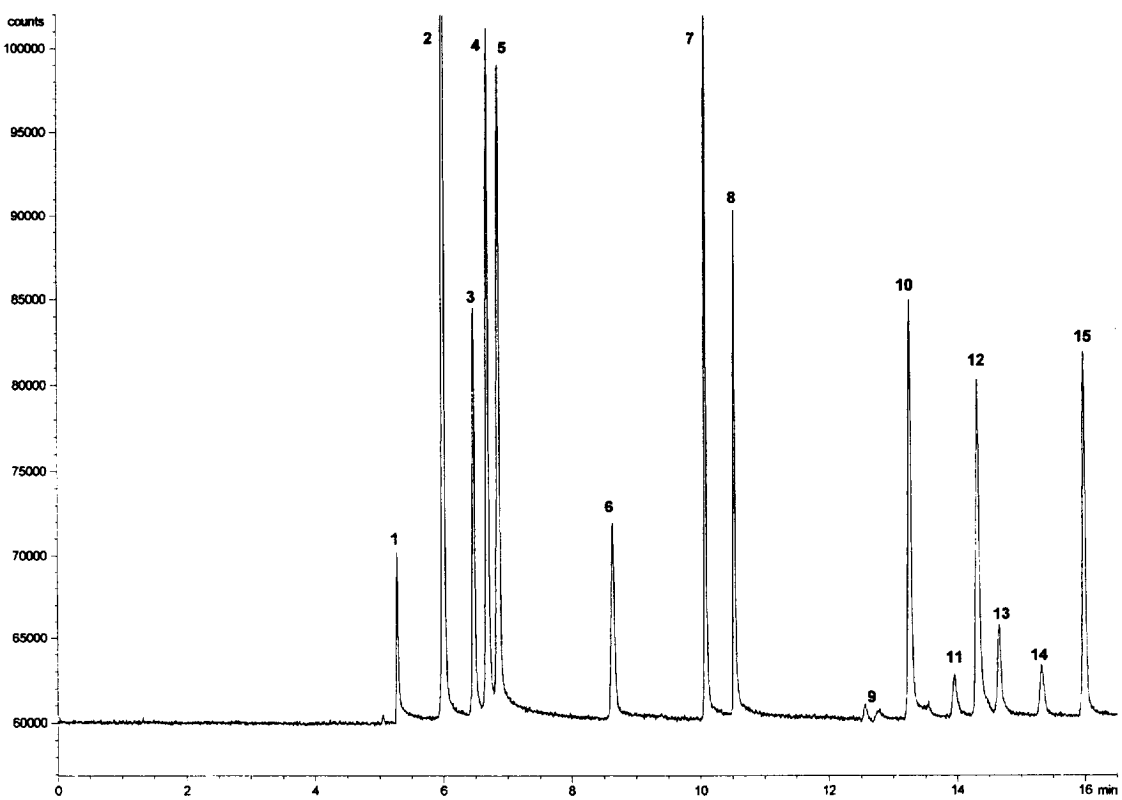

Fig. 4. Optimum chromatographic separation of a standard solution of S-compounds. 1: Hydrogen sulphide, 2: methanethiol, 3: carbon disulphide, 4: ethanethiol, 5: dimethyl sulphide, 6: methyl-ethyl sulphide (istd), 7: diethyl sulphide, 8: methyl-propyl sulphide, 9: ethanol, 10: tiophene (istd), 11: methyl thioacetate, 12: dimethyl disulphide, 13: ethyl thioacetate, 14: ethyl-methyl disulphide, 15: diethyl disulphide.

Table 1
Percentage recoveries (R and R.S.D. in %)

Name	R[a]	R.S.D.[a]	R[b]	R.S.D.[b]
Hydrogen sulphide	81	13.9	79	12.1
Methanethiol	75	14.4	76	15.2
Carbon disulphide	92	6.8	91	6.7
Ethanethiol	78	10.5	81	11.3
Dimethyl sulphide	103	6.1	105	7.1
Diethyl sulphide	100	4.6	99	4.5
Methyl propyl sulphide	–	–	–	–
Methyl thioacetate	101	5.2	98	4.6
Dimethyl sulphide	99	6.7	100	4.9
Ethyl thioacetate	98	5.5	99	5.0
Diethyl disulphide	103	5.1	102	3.8

Results obtained from quintuple analysis of a white[a] and a red[b] wine. Conditions given in Section 2.

diluted in synthetic wine and their headspace injected into the chromatograph to obtain calibration graphs.

Calibration graphs of the S-compounds were constructed by plotting the S-compound to istd peak-area ratios against the S-compound to istd concentration ratios. The calibration curves obtained were power functions of the type described above (response=kC^b), and the values of b were found to be between 1.1 and 1.8, depending on the S-compound. The power regression was calculated by the least squares method, with good correlation coefficients (r>0.991). The range studied was between 1.25 and 80 μg l^{-1} for thiols and disulphides, and between 2.5 and 150 μg l^{-1} for the other compounds. For thiols and disulphides, the highest standard concentrations gave outlier values in relation to the istd response, so they were eliminated.

The detection limits were obtained adding standard solutions to a wine (S-compound free) and determining the minimum amount of each S-compound required to give a S/N=3. They were found to be between 0.6 and 2 μg l^{-1}.

The recovery of the method was determined by a standard addition technique. A vial with 10 ml of

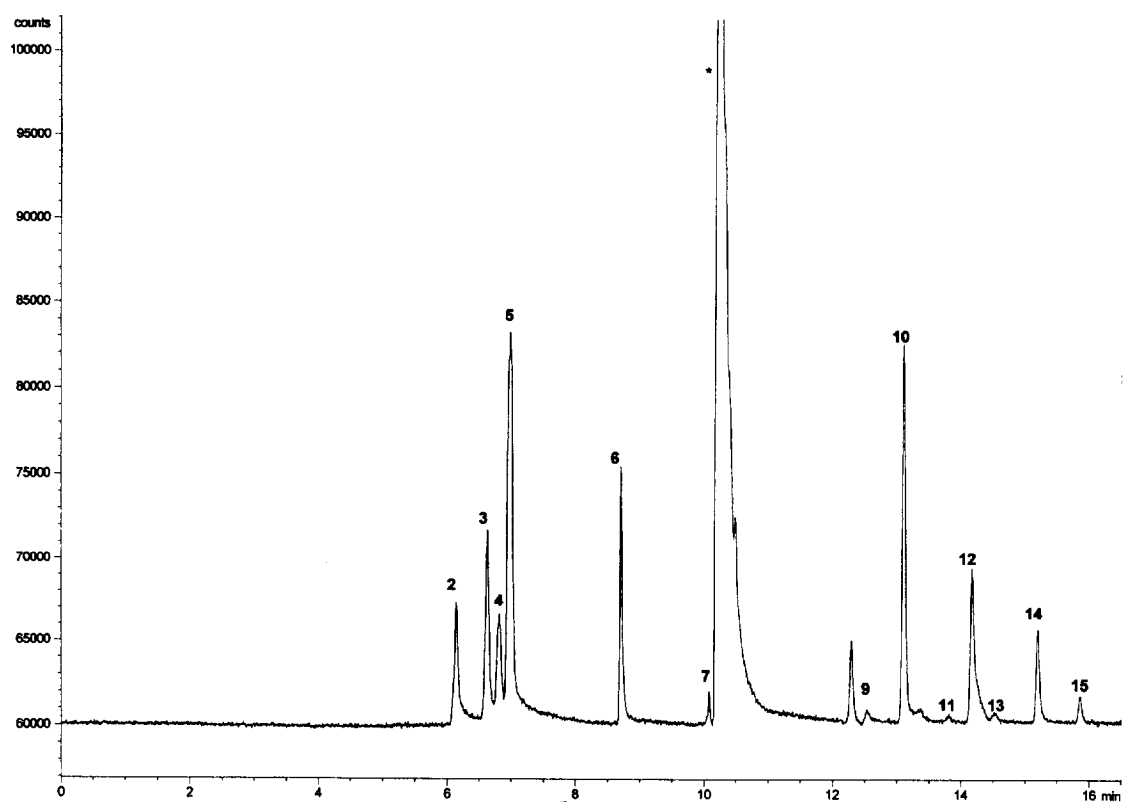

Fig. 5. Chromatogram of a white wine using the proposed procedure. Identification numbers can be seen in Fig. 1. Peak * belongs to SO_2.

wine (S-compound free) was spiked with a known amount of S-compound standard solution (30 μg l^{-1} of each analyte) and kept in the dark at 60°C for 2 h. The results obtained from the corresponding headspace chromatograms are given in Table 1. The recoveries were higher than 90% for most of the S-compounds while for the thiols they were lower (close to 80%). Furthermore, the R.S.D. obtained is acceptable (less than 15% for thiols and less than 7% for the others) with the method described. The thiols may behave in this way because they react with some compounds which are present in wine.

The recovery of methyl-propyl sulphide was not calculated because when this procedure was applied to wines, and particularly to white wines, the SO_2 peak was very large and overlapped with the methyl-propyl sulphide peak.

The method was used to analyze several red and white wines from the Tarragona region. They had all been stored in optimum conditions of darkness and temperature. Figs. 5 and 6 show chromatograms obtained for a white wine and a red wine, respectively. Table 2 shows the concentrations found. These results are comparable to the ones in Refs. [5,16,26]. As can be seen, carbon disulphide, methyl sulphide and dimethyl disulphide were found in all the samples analysed. The first two of these compounds had concentrations that are high enough to be quantified, but this was not so for dimethyl disulphide in some samples. The other S-compounds studied were found in different concentrations in some of the samples analyzed, but in several cases they could not be detected.

4. Conclusions

The method developed appears to be suitable for determining a relatively large number of usual volatile S-compounds in wines. The interference of

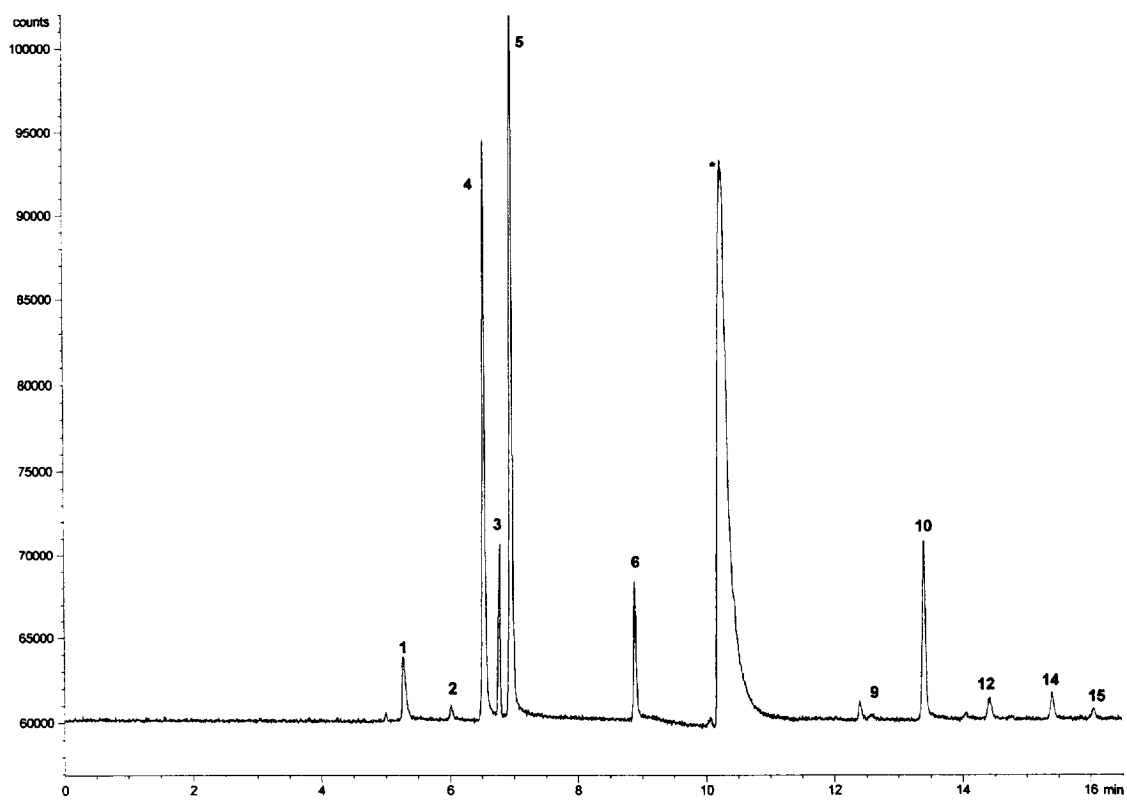

Fig. 6. Chromatogram of a red wine using the proposed procedure. Identification numbers can be seen in Fig. 1. Peak * belongs to SO_2.

Table 2
Sulphur compound content (mg l^{-1}) in wines from the Tarragona region

Name	Red wines (n=8)		White wines (n=8)	
	Range (mg l^{-1})	Median	Range (mg l^{-1})	Median
Hydrogen sulphide	3.0–nd	nd	2.2–nd	nd
Methanethiol	nq–nd	nd	7.1–nd	2.1
Carbon disulphide	17.8–0.9	10.2	2.3–0.4	2.1
Ethanethiol	11.1–nd	3.3	3.5–nd	nq
Dimethyl sulphide	209.0–20.3	109	60.8–9.1	39.9
Diethyl sulphide	5.4–nd	nd	7.8–nd	nd
Methyl thioacetate	16.8–nd	nq	8.4–nd	nq
Dimethyl disulphide	3.0–nq	2.0	3.3–2.2	2.5
Ethyl thioacetate	5.1–nd	nd	6.2–nd	nd
Ethyl methyl disulphide	nd–nd	nd	nq–nd	nd
Diethyl disulphide	3.2–nd	nd	nq–nd	nd

Results from the triplicate injection of the samples. nd means not detected and nq means not quantified (nq=3.3×nd).

other compounds in a matrix as complex as the wine was eliminated by using a specific detector and S-compound low concentrations could be detected by concentrating the headspace with a cryogenic trap.

Furthermore this new procedure for preparation and validating standard thiol solutions enables accurate concentration standards of these volatile and oxidizable compounds to be used.

The method also seems be appropriate for oenology laboratory work because of the simplicity of the instrumentation used.

Acknowledgments

The authors wish to thank CICYT (project ALI 94-0505) for the financial support given.

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