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Application of headspace solid-phase microextraction to the determination of sulphur compounds with low volatility in wines

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Abstract

Headspace solid-phase microextraction (HS-SPME) has been used for determining sulphur compounds with low volatility in wines. With this technique, handling of samples is mimimal so undesirable loses and reactions between compounds are prevented. Furthermore, this kind of extraction is fast and does not require any organic solvent. Under optimal conditions, the HS-SPME, using a new fibre coated with Stable Flex divinylbenzene–Carboxen–polydimethylsiloxane, makes possible the quantification of sixteen sulphur compounds with low volatility which may be present in wines. The limits of detection for the analytes studied ranged between 0.05 and 10 μg/l, and the recovery and repeatability found were acceptable. The method developed was successfully applied to determine the concentration of the target analytes in varietal wines from the Catalonian region (Spain) with some aromatic defects such as an odour of rubber, onion, rotten, unpleasant herbaceous, etc. The results show that the contents of the sulphur compounds studied in these wines are higher than in those without defects. This shows a relationship exists between the presence of sulphur compounds and the quality of the wine aroma.

Keywords: Headspace analysis; Wine; Food analysis; Organosulfur compounds

1. Introduction

Volatile sulphur-containing compounds are found in many foods and beverages. They mainly contribute to unpleasant aromas, although some of them have been reported to play a positive role in the overall aroma profile [1–7]. This paradoxical behaviour has to be considered, particularly when dealing with products with a high mark-up like wine.

It is possible to make some distinction between the different kinds of sulphur compounds which can be found in wine on the basis of their functional groups (thiols, sulphides, disulphides, etc.), but their physical and chemical properties also allow other classifications. These compounds are usually classified, based on the boiling point of 3-methylthiopropanol (b.p. 90 °C), as light sulphur compounds (b.p.<90 °C) and heavy sulphur compounds (b.p.>90 °C) [8,9]. This classification becomes very helpful when deciding which analytical technique can be used because, depending on the boiling points of the analytes, some techniques give better results than others.

The light sulphur compounds are responsible for very bad odours in wine (rotten eggs, cabbage,

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rubber, garlic, onions, etc.) because of their high volatility and low perception levels, nevertheless most of them can be eliminated with simple wine aeration. On the other hand, the heavy sulphur compounds can have a detrimental effect on wine quality because they cannot be eliminated and, usually, their aroma is unpleasant even at low concentrations [8]. However, it is also known that very low levels of some of these compounds are typical of some varieties and give these wines a distinctive aroma [9–11]. So, a quick and easy detection and quantification method for the less volatile sulphur-containing compounds becomes increasingly important for the quality control of wine.

The most widely used technique for analysing these compounds is gas chromatography (GC), but a preconcentration step is required because, usually, the sulphur compounds are found at trace levels in wines. The most-used sampling and concentration techniques, performed before chromatographic analysis, are either liquid–liquid extraction [8,12,13] or purge and trap [14].

Headspace solid-phase microextraction (HS-SPME) is a solventless technique for extracting and concentrating analytes from a sample in a single step. It uses a polymer-coated silica fibre to extract the analytes from the headspace of a variety of matrices and transfer them directly into the injector of a GC for thermal desorption and analysis [15–17]. Developed by Pawliszyn and coworkers in the early 1990s, HS-SPME has proved to be a fast and reliable technique in environmental analyses [18–20] and, later, also in a lot of applications, among which are food and beverage analyses [21–29]. In previous studies we used polydimethylsiloxane (PDMS) [30,31], polyacrylate (PAc) [30,31] and Carboxen–polydimethylsiloxane (CAR–PDMS) [32,33] coated fibres to analyse thiols, sulphides and disulphides in wines using the HS-SPME technique. The fibre coated with CAR–PDMS also gave good results in the analysis of the heavy organic sulphur compounds in wine using the direct sampling mode (DI-SPME) [34]. The problem we encountered in this last study was the presence of matrix interferences, so the purpose of the present study is to test the suitability of the headspace sampling mode for analysing the less volatile sulphur compounds in wines.

2. Experimental

2.1. Reagents and solutions

The standards of the different sulphur-containing compounds studied, with a purity above 98%, were supplied by Sigma–Aldrich (Madrid, Spain), Fluka (Madrid, Spain), Lancaster (Bischheim, France) and Interchim (Montluçon, France). These, and their CAS numbers (given in brackets) were as follows: methyl thioacetate (1534-08-3), ethyl thioacetate (625-60-5), dimethyl disulphide (624-92-0), diethyl disulphide (110-81-6), 2,5-dimethylthiophene (638-02-8), 2-methyltetrahydrothiophen-3-one (13679-85-1), 3-(methylthio)propanaldehyde or methional (3268-49-3), methyl 3-(methylthio)propionate (13532-18-8), ethyl 3-(methylthio)propionate (13327-56-5), 3-(methylthio)propyl acetate (16630-55-0), 2-mercapto-ethanol (60-24-2), 2-(methylthio)ethanol (5271-38-5), 3-(methylthio)-1-propanol or methionol (505-10-2), 4-(methylthio)-1-butanol (20582-85-8), 3-(methyl-thio)propionic acid (646-01-5) and benzothiazole (95-16-9). Propyl isothiocyanate (628-30-8) was used as internal standard (I.S.).

An individual standard solution of 2000 mg/l of each sulphur compound was prepared in ethanol and stored in darkness at 6 °C. A global standard solution, containing all the analytes, was prepared with an aliquot of each individual solution and subsequently diluted with ethanol using suitable volumetric flasks. Since some of these compounds (3-(methylthio)propanaldehyde, methyl 3-(methyl-thio)propionate and ethyl 3-(methylthio)propionate) can suffer some oxidation, the individual standard solutions of these compounds, as well as the global one, were prepared and handled under nitrogen atmosphere.

To study the effect of some parameters we used a synthetic wine solution. As in previous studies [31–33] this solution was prepared in such way that it was as similar as possible to a real wine. To reproduce the matrix influence, we injected the solid-phase microextracts of different real wines (white, rosé and red) into the GC–MS and we determined which compounds found in wines were extracted by the different fibres, because these could compete with the sulphur compounds on the SPME process.

So, these interferences were taken into account and the synthetic wine was obtained with a solution (in Milli-Q quality water) of 3.5 g/l of L-(+)-tartaric acid, 120 ml/l of ethanol, 125 mg/l of methanol, 75 mg/l of ethanal, 100 mg/l of ethyl acetate, 5 mg/l of isoamyl acetate, 250 mg/l of isoamyl alcohol, 0.4 mg/l of ethyl hexanoate, 0.5 mg/l of ethyl octanoate, 1.5 mg/l of ethyl decanoate, 7.5 mg/l of diethyl succinate, 75 mg/l of 2-phenylethanol, 5 mg/l of octanoic acid, 3 mg/l of decanoic acid and 275 mg/l of potassium metabisulphite, all of which had a purity of over 98%. They were supplied by Aldrich. Finally, the pH was adjusted to 3.5 with 1 *M* NaOH.

2.2. Headspace and SPME

The SPME holder for manual sampling, the CAR–PDMS and the Stable Flex divinylbenzene–Carboxen–polydimethylsiloxane of 2 cm (DVB–CAR–PDMS) coating fibres were purchased from Supelco (Bellefonte, PA, USA). These fibres were conditioned and cleaned by inserting them into the GC injector at recommended temperatures (0.5 h at 270 °C for the CAR–PDMS and 4 h 280 °C for DVB–CAR–PDMS) and were used immediately to prevent contamination.

The different parameters that influenced the HS-SPME analyses were studied, as is discussed in Section 3, and the optimal conditions obtained were as follows: for each analysis 10 ml of the sample was placed in a 20-ml vial capped with a silicon septum, which contained 3 g of (5 *M* NaCl) and a little magnetic stirrer. The SPME fibre was then inserted manually through the vial septum and exposed to the headspace over the liquid sample at 35 °C for 2 h. Afterwards, the fibre was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system, for thermal desorption, at 270 °C in the splitless mode (1 min).

2.3. Chromatography

Chromatographic analyses were made on a Hewlett-Packard (HP) 5890 gas chromatograph with an HP Model 19256A flame photometric detection system (FPD) in the sulphur mode. 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 injector temperature was 270 °C and injection was made in the splitless mode (1 min).

A CP-WAX 57 CB (50 m×0.25 mm I.D., 0.2 μm film thickness) fused-silica capillary column was used as the analytical column, with helium as carrier gas at a flow-rate of 1 ml/min. The oven temperature was programmed as follows: 40 °C (5 min), 3 °C/min, 130 °C, 10 °C/min, 220 °C (10 min).

Compounds were identified by comparing GC retention times using two different chromatographic columns. The column used to confirm the identity of the analytes in real samples was a SPB-35 (30 m×0.25 mm I.D., 0.25 μm film thickness) with an oven temperature programme of 50 °C (10 min), 5 °C/min, 280 °C (5 min). The carrier gas was helium at a flow-rate of 1.2 ml/min.

To determine the identity of other wine compounds which were also extracted by the fibre, as well as the sulphur-containing compounds, a HP 5890 (series II) gas chromatograph equipped with an HP-5972 mass-selective detector was used. Injection was made in the same way as with the FPD system. The detector was operated in the electron impact ionisation mode (70 eV) at 280 °C. Detection was made in the scan mode between 30 and 300 u.

3. Results and discussion

In a previous study [29], we had tested six different types of commercially available fibres. The results showed that the highest chromatographic peaks were obtained with fibres whose stationary phase contained carbon whatever the sampling method used (direct or headspace). Therefore, in this study we have only used CAR–PDMS and DVB–CAR–PDMS fibres, which are coated with porous carbon.

In order to optimize headspace microextraction, several factors influencing these equilibria [35] were considered: magnetic stirring, ionic strength, sample volume, temperature and time of extraction. Experi-

ments were carried out with a red wine (12%, v/v), because we wanted to take into account the matrix effect in the optimization of the different SPME parametres.

The first studies showed that the tendency of the analytes to pass to the headspace and, therefore, to the fibre, could be accelerated by a high ionic strength and with magnetic stirring of the liquid sample, so the optimization of the rest of the parameters was made using stirred samples with 5 *M* NaCl.

The influence of sample volume in SPME was also taken into account. So, we tested 50- and 20-ml vials with 25 and 10 ml of sample, respectively. These are the maximum sample volumes used, because greater volumes would imply the immersion of the fibre into the liquid sample. The results showed that the 20-ml vials gave a higher repeatibility, so we decided to use them.

Since the sulphur compounds studied do not have a high volatility, the time to reach the equilibrium between the three phases was expected to be long. Also, it is well known that the extraction rate is strongly influenced by temperature, so both parameters (time and temperature) were studied simultaneously.

In order to select the optimum values of these variables, five experiments were performed corresponding to a 2^2 factorial design plus an experiment in the center of the domain. The experimental domain ranged from 10 to 60 °C for the temperature and from 1 to 4 h for the time. The different experiences were randomly performed with three different fibres coated with CAR–PDMS to take the fibre variability into account. The same experiment was done with the fibres coated with DVB–CAR–PDMS.

The optimal conditions corresponded to the central point (2 h at 35 °C) for both types of fibres. Although equilibrium was not reached in 2 h, the response obtained was good enough because most of the sulphur compounds studied only improved their response by 10–20% when equilibrium state was reached after 4 h.

Although we had to work in a nonequilibrium situation, this did not cause any problem as several papers [36,37] had demonstrated that SPME quantification before reaching equilibrium was feasible, once the extraction conditions were held constant.

Since the CAR–PDMS fibre gave poorer results on the extraction of methional and benzothiazole, we decided to carry out the study and validate the method using only the DVB–CAR–PDMS fibre.

The method had to be assessed by estimating the linear range, limits of detection, recoveries and standard deviations. In previous studies [32,33], where we used HS-SPME and carbon coated fibres, these values were calculated using a synthetic wine solution that contained the volatile compounds which could interfere with the extraction. Therefore it seemed appropriate to repeat the experiment using the solution specified in Section 2.1, to reproduce the influence of the wine matrix on the HS-SPME.

In a previous paper [34], where we studied the same analytes but with direct SPME, we found a matrix effect related to the colouring material of wine which changed the distribution constants [38].

Taking these observations into account, we decided to test two different matrices to check their suitability. Thus, we constructed the calibration graphs using, as matrices, the synthetic wine (SW) described in Section 2.1, and, also, a representative real wine.

To obtain a real wine as representative as possible so that the calibration graphs were applicable to the majority of real samples, we prepared a ‘mixed’ wine (MW) for each type (white, rosé and red). MW were obtained by mixing five different wines coming from different regions and with different proof grading and aromatic properties. None of them showed organoleptic defects.

Calibration solutions, in the range specified in Table 1, were prepared by suitable dilution of the global solution in the synthetic wine and in the real MW of each type. It is well known that the FPD response is a power function so, to obtain the S-compounds linear calibration graphs, these were constructed by plotting the log [S-compound/I.S.] peak area ratios against the log [S-compound/I.S.] concentration ratios. To calculate these calibration graphs, linear least-squares regression was used and, in all cases, the determination coefficients were good ($r^2>0.99$). The efficiency of SPME varies when different DVB–CAR–PDMS fibres are used, so the

Table 1
Concentration ranges of calibration graphs and limits of detection (LODs) of the HS-SPME method

Sulphur compound	Range calibration graphs (μg/l)	LOD (μg/l)			
		SW	RW	WW	RsW
2-Mercaptoethanol	10–200	6	5	5	5
Methyl thioacetate	5–100	4	2	2	2
Dimethyldisulphide	0.5–15	0.25	0.25	0.25	0.25
Ethyl thioacetate	5–100	1.5	1	1	1
2,5-Dimethylthiophene	0.5–20	0.05	0.05	0.05	0.05
Diethyldisulphide	0.2–5	0.04	0.04	0.04	0.04
Methional	5–100	12.5	2.5	2.5	2.5
Methyl 3-(methylthio)propionate	5–100	2	1.5	2	2
2-Methyltetrahyrothiophen-3-one	10–150	5	5	5	5
(Methylthio)ethanol	10–200	8	5	8	8
Ethyl 3-(methylthio)propionate	5–100	1	0.5	1	1
3-Methylthiopropyl acetate	2–50	0.75	0.75	1	1
Methionol	50–2000	10	10	15	15
(Methylthio)butanol	10–200	10	6	7	7
Benzothiazole	2–50	1	0.5	0.75	0.75
3-(Methylthio)propionic acid	20–200	25	8	10	10

SW, synthetic wine; RW, red wine; WW, white wine; RsW, rosé wine.

experiments were performed with different fibres and the results were averaged.

The results showed that while some of the sulphur compounds studied gave a different calibration graph depending on the matrix, other compounds gave a similar response whatever matrix was used (Fig. 1).

In order to compare the calibration graphs obtained with the different matrices, we added known amounts of analytes to different wines and, after extraction, we compared the real concentration added and the concentration values obtained by interpolation in the different calibration graphs. In this way, we calculated the recovery of the method. This parameter was defined as the percentage ratio between concentration of analyte found and concentration of analyte added. If the values are close to 100% it means that the method works because the behaviour of the sample can be predicted by the calibration graphs.

To calculate the different recovery values we used three red, three white and three rosé wines which were different to those used to make the mixed wines. These wines were spiked with concentrations of each analyte at three different levels (low, middle and high concentration of the calibration graphs), and then they were extracted under conditions described before. Each wine was analysed twice and the recoveries calculated. Table 2 shows the average recoveries with their relative standard deviations (RSDs) for each kind of wine, at the three levels, using the two calibration graphs.

To compare the recovery values calculated using the calibration graphs obtained with SW matrix or MW matrix, we applied a statistical *t*-test. The results showed that, in general, the values were comparable. However, as can be seen in Table 2, SW gave recoveries and RSDs higher than those obtained with MW. In fact, while with SW the values of recovery and RSD were around 115 and 20%, respectively, when MW was used, these values were around 100 and 15%.

The limits of detection (LODs) for each analyte were obtained by performing HS-SPME of synthetic wine and of each mixed wine, spiked with the required amount of each S-compound to produce a signal-to-noise ratio of 3. Table 1 shows the LOD values obtained under the described conditions. It can be observed that these values are slightly better than those obtained using direct sampling [34], except for four compounds: methional, 2-methyltet-

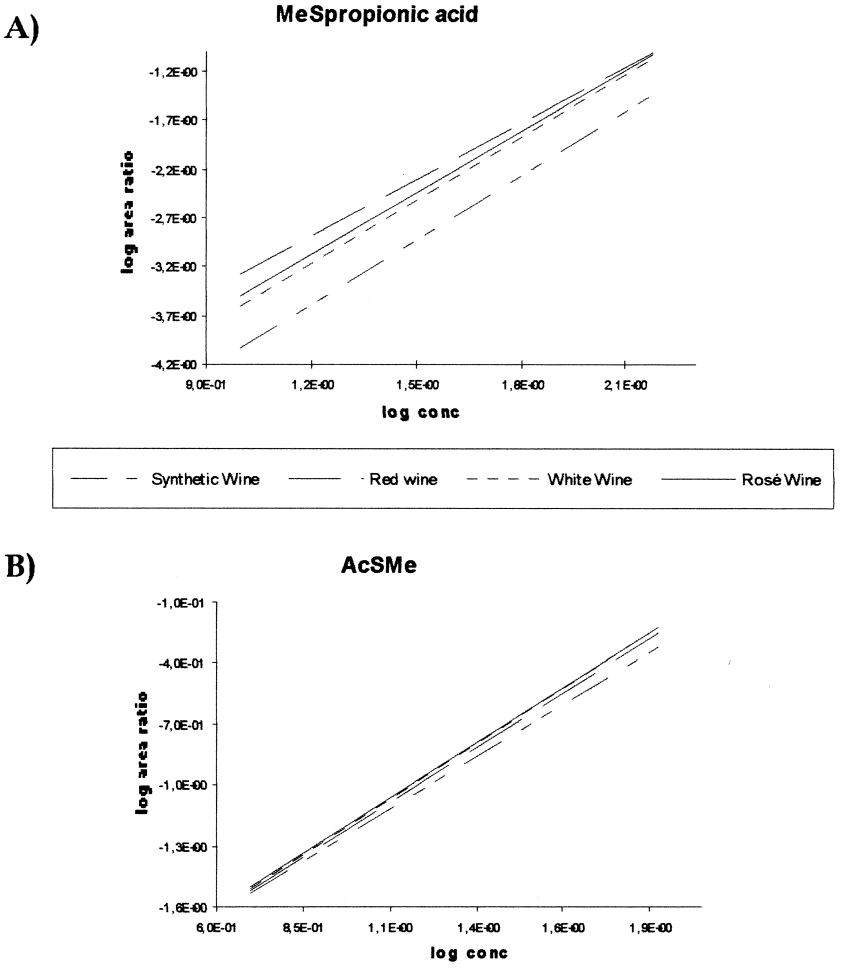

Fig. 1. Examples of calibration graphs obtained from solid-phase microextraction of sulphur compounds in different matrices. (A) with matrix effect (B) without matrix effect.

rahydrothiophen-3-one, methylthiobutanol and 3-methylthiopropionic acid.

The proposed HS-SPME procedure was applied successfully to determine the sulphur compound contents of different varietal wines from Catalonia (Spain) (using the calibration graphs made with MW). The wines analyzed showed aromatic defects like odour of rubber, onion, plastic, garlic, disagreeable herbaceous, rotten, etc. This type of off-flavor is usually related to sulphur compounds, so the analysis of these wines was undertaken in order to determine a possible relationship between these aromas and high contents of sulphur compounds.

The results (Table 3) showed that wines with this type of aroma defect had higher contents of the compounds studied than those without defects. Fig. 2 illustrates this behaviour with two chromatograms: one of a wine without aromatic defects and the second of a wine with defects. The second one contains much more sulphur compounds.

4. Conclusions

On the basis of this study, it is concluded that the determination of the sulphur compounds with low

Table 2
Average recoveries and RSDs (in brackets), calculated from calibration graphs made with synthetic wine (SW), and mixed real wines, white wine (WW), rosé wine (RsW) and red wine (RW); conditions given in the text

Sulphur compound	Recovery (%)											
	Calibration with SW						Calibration with MW					
	White		Rosé		Red		White		Rosé		Red	
2-Mercaptoethanol	116.2	(28.2)	105.7	(11.9)	96.8	(22.6)	111.5	(21.4)	105.0	(9.9)	102.4	(12.8)
Methyl thioacetate	116.6	(18.6)	102.7	(18.4)	102.1	(18.9)	100.2	(15.7)	97.6	(9.5)	97.9	(15.3)
Dimethyldisulphide	99.7	(19.5)	99.5	(21.1)	94.9	(18.6)	101.4	(12.9)	95.4	(14.8)	99.3	(15.0)
Ethyl thioacetate	109.8	(20.2)	101.0	(13.7)	92.7	(19.7)	93.8	(16.6)	96.8	(13.5)	96.4	(15.8)
2,5-Dimethylthiophene	104.7	(20.4)	101.9	(16.4)	92.8	(23.2)	103.2	(18.6)	99.9	(10.4)	98.8	(19.5)
Diethyl disulphide	107.7	(23.7)	110.0	(11.3)	110.8	(18.1)	97.3	(16.8)	102.0	(13.7)	104.9	(16.7)
Methional	122.5	(14.6)	162.5	(20.2)	162.4	(18.9)	100.4	(12.0)	99.1	(9.4)	100.7	(11.5)
Methyl-3-(methylthio)propionate	104.5	(17.9)	104.3	(20.9)	107.9	(31.4)	102.5	(10.3)	99.0	(14.5)	97.3	(18.3)
2-methyl-4H-thiophen-3-one	118.9	(16.9)	116.8	(19.1)	119.9	(21.7)	104.8	(14.5)	100.0	(13.7)	97.3	(17.3)
(Methylthio)ethanol	111.5	(11.4)	106.7	(15.8)	121.1	(21.4)	100.6	(9.3)	99.0	(7.8)	101.6	(15.3)
Ethyl 3-(methylthio)propionate	109.5	(10.5)	106.7	(16.8)	98.5	(16.0)	101.8	(12.4)	105.7	(13.4)	97.1	(12.0)
3-Methylthiopropyl acetate	130.1	(21.1)	129.4	(23.5)	105.6	(22.6)	104.4	(15.4)	101.1	(16.2)	100.3	(18.0)
Methionol	[a]		[a]		[a]		104.3	(24.6)	99.2	(21.2)	99.2	(20.5)
(Methylthio)butanol	115.6	(24.9)	124.6	(22.5)	139.6	(23.3)	101.6	(11.2)	95.9	(11.4)	102.0	(9.8)
Benzothiazole	110.2	(20.5)	113.4	(23.0)	113.3	(28.0)	106.9	(18.4)	100.7	(14.4)	96.8	(16.4)
3-(Methylthio)propionic acid	122.7	(24.1)	144.1	(21.4)	130.5	(18.0)	105.0	(14.7)	101.5	(11.1)	105.0	(11.2)
Total averages	113.3	(19.5)	115.3	(18.4)	112.8	(21.5)	102.5	(15.3)	99.8	(12.8)	99.8	(15.3)

[a] Area ratio methionol:I.S. too high to calculate the recovery values.

Table 3
Sulphur compound contents range in wines

Sulphur compound	Content (μg/l)	
	Wines without off-flavors	Wines with off-flavors
2-Mercaptoethanol	Nd–45	50–150
Methyl thioacetate	Nd–10	Nd–25
Dimethyl disulphide	Nd–0.7	Nq–2.3
Ethyl thioacetate	Nd–5.3	Nq–15
2,5-Dimethylthiophene	Nq–0.5	Nq–0.7
Diethyl disulphide	Nd–0.2	Nq–0.5
Methional	Nd–15	3–88
Methyl-3-(methylthio)propionate	Nd	Nd–23
2-Methyl-tetrahyrothiophen-3-one	Nq–15	Nq–65
(Methylthio)ethanol	Nq–20	Nq–60
Ethyl-3-(methylthio)propionate	Nd–7	Nq–23
3-Methylthiopropyl acetate	Nq–5	Nq–17
Methionol	135–1800	250–3050
(Methylthio)butanol	Nd–12	Nd–190
Benzothiazole	Nd–3	Nd–16
3-(Methylthio)propionic acid	Nd–23	Nq–175

Nd, not detected; Nq, not quantified (nq=3.3×nd).

volatility in wines using the technique of HS-SPME, is conditioned by the matrix effect, as it was in the direct mode. This problem can be solved either using a synthetic wine with added aromas or using a representative real matrix to construct the calibration graphs. The validation of the method was satisfactory and the method could be successfully applied to real samples. Recovery values and limits of detection are acceptable for all the compounds studied. From a practical point of view, the use of the headspace sampling mode of SPME considerably minimizes the deterioration of the SPME fibres and the chromatographic column that occurs when the direct sampling mode is used.

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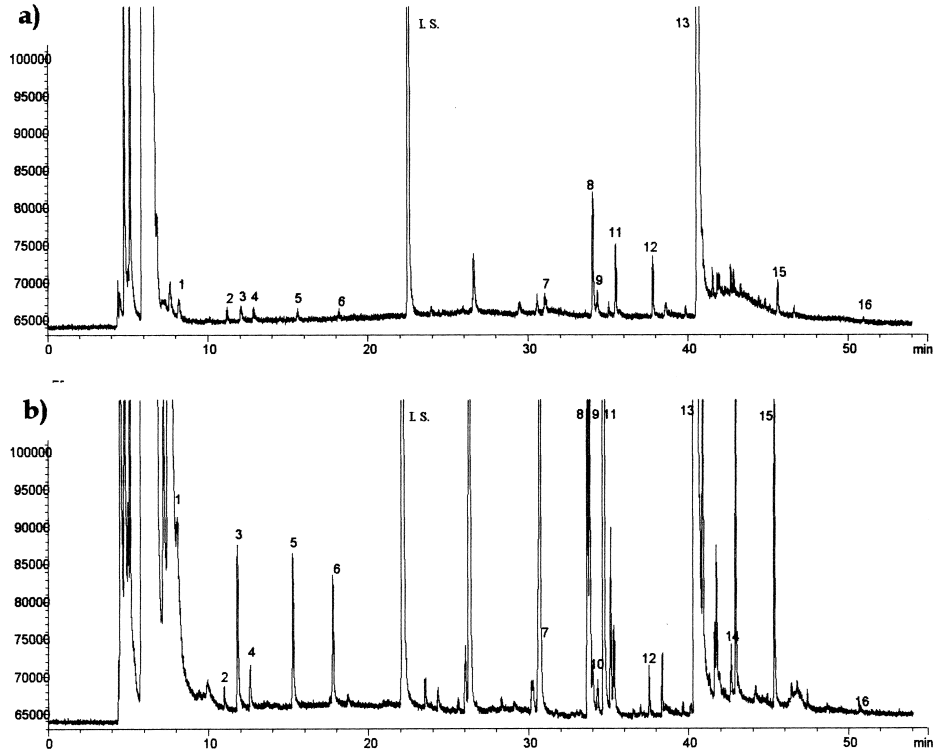

Fig. 2. Chromatographic response of a wine without (a) and with (b) aromatic defects, which are analysed with the proposed procedure using HS-SPME technique with Stable Flex fiber. 1=Mercaptoethanol; 2=methyl thioacetate; 3=dimethyl disulphide; 4=ethyl thioacetate; 5=2,5-dimethylthiophene; 6=diethyl disulphide; I.S.=internal standard (propyl isothiocyanate); 7=methional; 8=methyl methylthiopropionate; 9=2-methyl-4H-thiophen-3-one; 10=methylthioethanol; 11=ethyl methylthiopropionate; 12=methylthiopropyl acetate; 13=methionol; 14=methylthiobutanol; 15=benzothiazole; 16=methylthiopropionic acid.

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