ELSEVIER

Journal of Chromatography A, 808 (1998) 211–218

JOURNAL OF CHROMATOGRAPHY A

Headspace solid-phase microextraction analysis of volatile sulphides and disulphides in wine aroma

M. Mestres*, O. Busto, J. Guasch

Departament de Química Analítica i Química Orgànica (Unitat d'Enologia, CeRTA), Escola d'Enologia de Tarragona, Universitat Rovira i Virgili, Avda. Ramón y Cajal 70, E-43005 Tarragona, Spain

Received 23 October 1997; received in revised form 29 January 1998; accepted 30 January 1998

Abstract

Sulphur compounds (S-compounds) are important constituents of wine off-flavours. Headspace solid-phase microextraction (HS-SPME) combined with gas chromatography coupled to flame photometric detection (GC–FPD) was used to develop a suitable method to analyse volatile sulphides and disulphides. This is a very simple and fast technique which gives good reproducibility at μg/l levels (relative standard deviations<10%). The analytes were extracted from the headspace of the samples by using either polydimethylsiloxane or polyacrylate coated fused-silica fibers in an SPME unit. Then, the fiber was inserted into the injector of a gas chromatograph and the extracted S-compounds were thermally desorbed. The influence of different parameters, such as ionic strength, stirring, headspace volume, ethanol concentration, time and temperature of extraction, was studied. The extraction of the fibers varies considerably for the different sulphur compounds studied. The most volatile compounds were the least extracted by the coating fibers tested. The standard additions technique, applied to real samples, gave the recoveries >94%. The detection limits range between 3 μg/l and 50 ng/l. The overall process was successfully applied to identify and quantify S-compounds in white and red wines.

Keywords: Wine; Food analysis; Extraction methods; Headspace solid-phase microextraction; Sulphur compounds, volatile

1. Introduction

Volatile sulphur (S) compounds play an important role in the aroma quality of foods and beverages [1–3]. They are commonly found in foods that are in bad condition, giving them unpleasant flavour. From the oenological point of view, the presence of these compounds in wines is usually considered as an off-flavour and means that the conditions under which they were produced were wrong. So, if wine production is to be controlled, the way in which S-compounds are formed needs to be understood and there should be a method for determining their concentration.

Volatile S-compounds in wines, particularly dimethyl sulphide and carbon disulphide, have been identified by some investigators [4–6]. Concentrations are usually low but they can increase with cloudy grape juices [7], the use of sulphur containing pesticides [8], thermal and photochemical reactions (during and after fermentation) [9,10], ageing [11] and many more conditions. Thus, volatile S-com-

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00100-9

pounds have been identified in wine at concentrations which depend on these factors.

Several analytical methods have been described for determining these analytes: thermometric titration [12], spectrophotometric determination using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) [13], application of biosensors [14], gas chromatography (GC) using the purge and trap [15,16], static headspace techniques [13,17] or other methods. However, GC with chemiluminiscence detection (SCD) [17,18] or flame photometric detection (FPD) [13,19] are the most widely used.

To determine volatile S-compounds in foods and beverages, a concentration step is usually required. Therefore, this study proposes the new procedure involving solid-phase microextraction (SPME) [20,21]. This technique has been utilized for the evaluation of environmental samples [22,23] and flavor of different beverages [24,25] and foods [26–28], but not for the analysis of volatile S-compounds in wine.

The SPME unit consists of a length of fused-silica fiber, coated with different phases and bonded to a stainless steel plunger and a holder that looks like a modified syringe. The technique involves immersing this fiber into either the liquid sample or, as in this work, the gas headspace above it. The analytes are absorbed on the fiber and then thermally desorbed inside the GC injection port.

The speed of extraction depends on the distribution constants of the analytes and the phase. Agitation, ionic strength, surface of contact between liquid–gas phases and temperature of absorption are other parameters that influence the extraction. They have all been tested in this study.

2. Experimental

2.1. Chemicals and reagents

The S-compounds studied were: dimethyl sulphide (Me_2S) [75-18-3], diethyl sulphide (Et_2S) [352-93-2], methyl-*n*-propyl sulphide (MeSPr) [3877-15-4], methyl thiolacetate (MeThioAc) [1534-08-3], ethyl thiolacetate (EtThioAc) [625-60-5], carbon disulphide (CS_2) [75-15-0], dimethyl disulphide (Me_2S_2) [624-92-0], diethyl disulphide (Et_2S_2) [110-81-6]. Ethyl-methyl sulphide (MeSEt) [624-89-5] and thiophene [110-02-1] were used as internal standards (I.S.s).

Ethyl-methyl and diethyl disulphides were supplied by Aldrich (Beerse, Belgium). The other sulphides and disulphides were supplied by Fluka (Madrid, Spain). They were all chosen with a purity of above 98%.

An individual standard solution of 2000 mg/l of each sulphide and disulphide was prepared in HPLC-grade ethanol (Scharlau, Barcelona, Spain) and stored in darkness at −10°C. Dilutions were made at 4°C.

Solutions for further studies were prepared by dissolving different amounts of standards in a synthetic wine solution. The synthetic wine was obtained by dissolving 3.5 g of L-(+)-tartaric acid (Scharlau) and 120 ml of ethanol in a suitable amount of deionized water to give 1 l of solution. The pH was adjusted to 3.5 with 1 *M* NaOH (Scharlau).

Other auxiliary reagents used in the different studies were Na_2EDTA-2-hydrate and NaCl, both supplied by Scharlau.

2.2. Equipment

2.2.1. SPME

The SPME holder, for manual sampling, and fibers used in this investigation were purchased from Supelco (Bellefonte, PA, USA). Two fibers were tested and compared: one was coated with 100 μm of polydimethylsiloxane (PDMS) and the other with 85 μm of polyacrylate (PA).

The SPME fibers were conditioned by inserting them into the GC injector, before they were used. For the PDMS and PA fibers, the injector was kept at 250°C for 1 h and at 300°C for 2 h, respectively.

2.2.2. Chromatography

A Hewlett-Packard 5890 gas chromatograph equipped with an HP Model 19256A flame photometric detector in sulphur mode was used for GC analysis.

The injection was made in the splitless mode and, because the diameter of the GC injection liner has an important influence on the peak shape [29], an inlet liner of 0.75 mm I.D. was used. For the thermal

desorption of the analytes inside the GC injection port, the temperature was 250°C for the PDMS fiber, and 275°C for the PA fiber.

Chromatographic separations were performed using an HP-Innowax (50 m×0.2 mm I.D., 0.2 μm film thickness) and the oven temperature was programmed as follows: 35°C for 8 min, then ramped at 50°C/min to 220°C and held for 10 min. The carrier gas was helium with a flow-rate of 0.4 ml/min.

The temperature of the detector was 200°C and it was fed with 75 ml/min of hydrogen, 86 ml/min of synthetic air and 57 ml/min of helium as auxiliary gas. The detector signals were sent to an HP Chemstation, where they were collected, integrated and recorded.

2.3. Headspace and SPME

Conditions were optimal when 25 ml of sample (natural or synthetic wine) with addition of 5.8 g of NaCl and 0.15 g EDTA was placed into a 50-ml glass vial [30]. The vial was tightly capped with a PTFE-faced silicone septum and placed in a thermostatted bath under constant stirring. EDTA was added to form complexes with metal ions, so preventing these ions from having a catalytic effect on the oxidation of S-compounds [31]. The effect of NaCl is to increase the efficiency of extraction [21,29].

After the fiber, which is housed in a stainless steel needle, had been conditioned, it was pushed out of the housing and exposed for a fixed time and temperature to the headspace generated in the sample vial. After extraction, the fiber was pulled into the housing and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption.

3. Results and discussion

The parameters which were optimized in order to obtain the best sensitivities were temperature and time of extraction, relation between liquid and gas surface, ionic strength, stirring and the effect of the ethanol concentration. To determine the optimum value of each parameter, five solutions of synthetic wine spiked with a mixture of all the compounds studied (40 μg/l of sulphides and 20 μg/l of disulphides) were analysed. When the spiking was carried out, the sample in the capped vial was homogenized before the extraction with the SPME unit.

Fig. 1 shows the chromatograms of the same sample obtained with PDMS (a) and PA (b) fibers under the optimal conditions described above. EtSMe, Et_2S and MeSPr are more efficiently extracted with the PDMS fiber and the thiophene with the PA fiber. There are no important differences between the two fibers for the other compounds studied.

As been mentioned above, two internal standards have been used. This is because the fibers extract MeSEt and thiophene in different proportions in such a way that the chromatographic area of the thiophene is greater than the MeSEt. So, for the more volatile compounds, which are generally extracted to a lesser extent, MeSEt is used, while for the compounds which give a greater response it is more appropriate to work with thiophene.

To study the influence of the extraction temperature, 30, 45 and 60°C were tested and compared. The analyte peak areas decreased as the temperature increased independently of the SPME fiber coating, so 30°C was considered to be the optimum value. Furthermore, as the temperature rises, new peaks appear which indicate that the sample decomposes at higher temperatures. For practical reasons, we did not decrease the temperature to below 30°C. The extraction time in all of these assays was 15 min.

To determine the optimum sampling time, periods of 5, 15 and 30 min were tested. There are no significant differences between them so we did not increase the time to over 30 min. This behaviour is due to the high volatility of the analytes, which are quickly transferred from the liquid to the gas phase. The extraction time for subsequent analyses was fixed at 15 min because is the suitable time for the routine procedure of preparing the sample and carrying out the extraction and chromatographic analyses.

The equilibration time can be reduced by using a smaller headspace, because the analytes will take less time to diffuse through the headspace to the fiber [29]. This was confirmed with several experiments with a constant extraction time of 15 min. Different volumes (10, 20 and 25 ml) of the same sample were

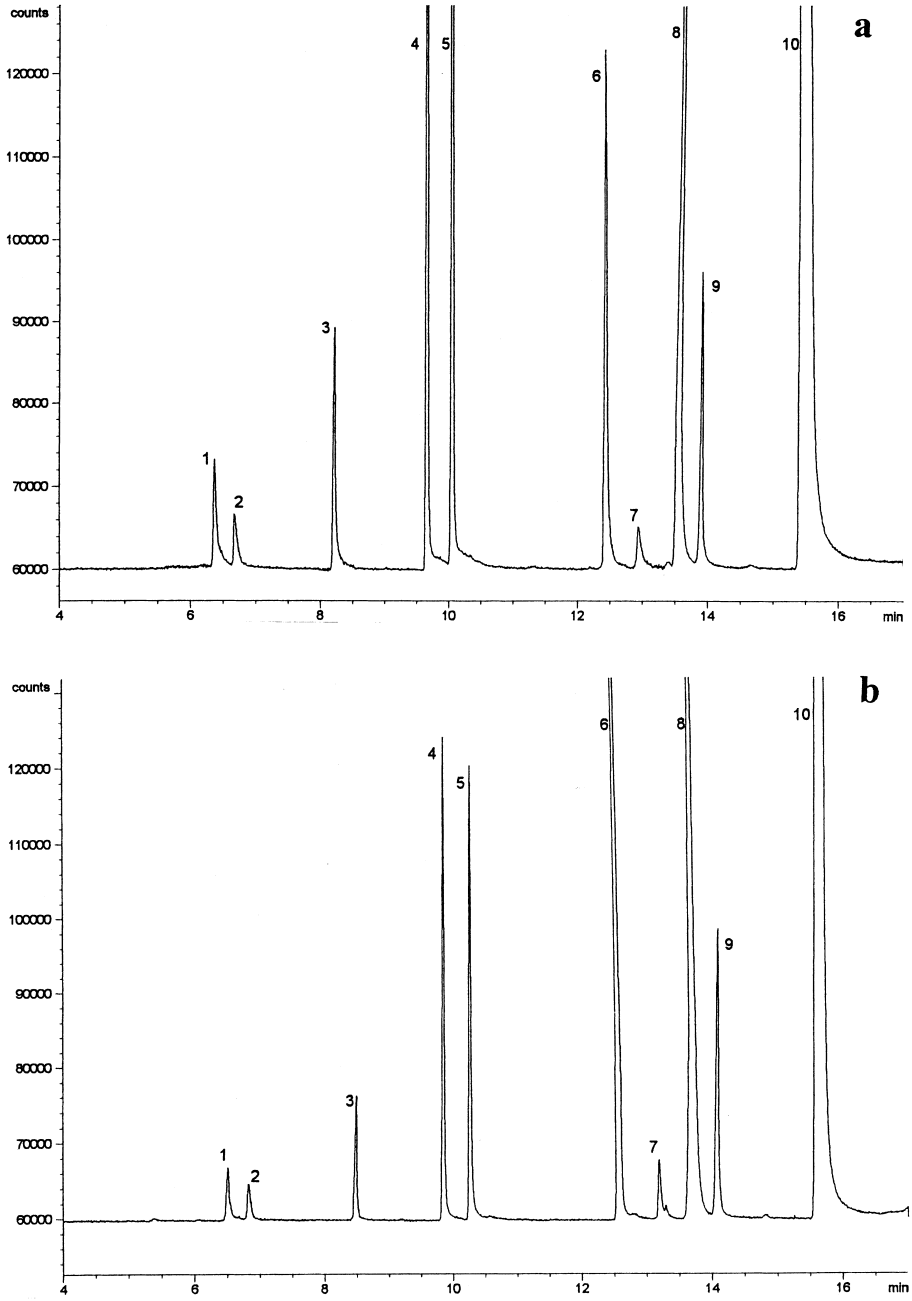

Fig. 1. Chromatographic response of a standard solution of S-compounds in synthetic wine after microextraction with PDMS fiber (a) and with PA fiber (b). 1=Carbon disulphide, 2=dimethyl sulphide, 3=methyl-ethyl sulphide (I.S.), 4=diethyl sulphide, 5=methyl-propyl sulphide, 6=thiophene (I.S.), 7=methyl thioacetate, 8=dimethyl disulphide, 9=ethyl thioacetate, 10=diethyl disulphide.

placed into a 50-ml sampling vial to obtain different volumes of headspace. Absorption increases while the headspace volume decreases. This may be due to the fact that the analytes diffuse quickly to the coating when the headspace volume is smaller, since the concentration in the three phases (liquid, gas and

coating) only depends on the partition coefficients. The volume of 25 ml of sample was the maximum value considered in order to guarantee that the fiber did not touch the liquid.

The influence of the headspace volume was also tested by working at a constant ratio of liquid–gas phases (1:1) with 20- and 50-ml vials. Because of the high diffusion rate mentioned above, the absorption obtained with 20-ml vials was higher than with 50-ml vials, but repeatability was lower. Thus, the 50-ml vials were selected.

The effect of the ionic strength was also studied by adding NaCl to the samples. It seems that the nature of the matrix can be modified by adding a salt to affect the liquid–gas partition coefficients of the analytes. As observed by other authors [21,29], the analyte peak areas increased considerably when NaCl was added to the sample and, therefore, samples were saturated with NaCl in all the SPME experiences.

The stirring effect was also taken into account. The results of the experiments show that magnetic stirring did not affect the extraction, due to the fact that the compounds studied have high volatility and they can be easily transferred into the gas phase. However, stirring was necessary to dissolve the NaCl and to homogenize the samples. Also a higher repeatability was observed.

The last parameter studied was the ethanol concentration. Ethanol is one of the major constituents of wines, so it can cause some of the partition coefficients of the S-compounds to vary. Some authors [32,33] have found that an increase in the ethanol concentration decreases the extraction efficiency. To check this effect, four extractions were analysed for each of five synthetic wine samples with different alcohol contents (0, 8, 10, 12, 15%, v/v) and fortified with the same amounts of volatile S-compounds (40 μg/l of sulphides and 20 μg/l of disulphides). Although the absolute areas decrease, the S-compound area/I.S. area ratio remains constant and the relative standard deviations (R.S.D.s) are less than 10%. For quantitative analysis the I.S. may be used, so the ethanol concentration does not affect the analytical data.

It was confirmed that the analytes were completely desorbed between one analysis and another. A 3-min period was sufficient to desorb all the extracted analytes in the GC injector port. This was confirmed by desorbing the same fiber for a second time after the initial desorption.

The precision and linearity of the method were then examined. Data obtained from three replicates of headspace-microextraction of the synthetic wine spiked with eight different concentrations of analyte standards were used to establish calibration graphs for each of them. Both I.S.s were added at a level of 40 μg/l.

It is well known that the FPD response is of the type: response$=kC^b$ (where $1<b<2$ depending on the analyte). So, in order to transform this exponential response into a linear response, the calibration graphs of the S-compounds were constructed by plotting the log (S-compound/I.S.) peak area ratios against the log (S-compound/I.S.) concentration ratios. The ULC (univariate linear calibration) computer programme [34] was used to calculate the slope (a) and the intercept (b) with their corresponding standard deviations (s_a and s_b) and the correlation coefficient (r^2) by linear least-squares regression. In all cases, a good correlation coefficient was obtained. The range of linearity, as well as all the other parameters described are shown in Table 1.

The detection limit for each analyte was obtained by applying the HS-SPME method described to a synthetic wine spiked with a known amount of S-compound and decreasing this amount until it gave a $S/N=3$. For each of the compounds studied this value was different, but very similar for both fibers. The results are shown in Table 1.

The recovery of the SPME technique was determined by a standard addition technique with red and white wines, and was tested for both fibers. The analytes were added to real wines at three levels: the first level was 5 μg/l for disulphides and 10 μg/l for sulphides, the second was 25 μg/l and 50 μg/l, and the third was 50 μg /l and 100 μg/l. For each level, three extractions were performed. The recovery average of all extractions was between 94 and 117% and gave R.S.D.s below 9% (Table 2). The values show that both the fibers tested gave good and similar results.

When this method is applied to wines, the SO_2 peak may interfere with the determination of Et_2S and MeSPr because their retention times are very similar. This was observed with both fibers, but to a

Table 1
Linearity and limits of detection of the method (HS-SPME)

	Polydimethylsiloxane					Polyacrylate				
S-Compound	Range (μg/l)	a (s_a)	b (s_b)	r^2	LOD (μg/l)	Range (μg/l)	a (s_a)	b (s_b)	r^2	LOD (μg/l)
CS_2	1–80[a]	1.131 (0.053)	−1.100 (0.060)	0.994	0.40	1–80[a]	2.018 (0.107)	−2.003 (0.141)	0.995	0.50
MeSMe	5–150[a]	1.442 (0.079)	−2.400 (0.145)	0.995	2.00	5–150[a]	1.938 (0.044)	−2.971 (0.081)	0.999	3.00
EtSEt	0.5–150[b]	1.637 (0.049)	−1.999 (0.078)	0.997	0.25	1–150[b]	1.745 (0.104)	−2.729 (0.016)	0.991	0.50
MeSPr	0.5–150[b]	1.595 (0.054)	−1.902 (0.084)	0.997	0.25	1–150[b]	1.745 (0.094)	−2.717 (0.148)	0.992	0.50
MeThioAc	3–150[a]	0.967 (0.024)	−1.960 (0.041)	0.998	1.50	5–150[b]	1.559 (0.084)	−2.508 (0.153)	0.995	2.00
MeSSMe	0.5–80[b]	1.511 (0.048)	−1.461 (0.064)	0.997	0.20	0.5–80[b]	1.748 (0.127)	−1.835 (0.163)	0.996	0.20
EtThioAc	3–150[a]	1.268 (0.037)	−1.554 (0.058)	0.997	1.25	3–150[a]	1.601 (0.071)	−1.742 (0.120)	0.996	1.25
EtSSEt	0.25–80[b]	1.425 (0.093)	0.107 (0.094)	0.998	0.05	0.5–80[b]	1.865 (0.077)	−0.928 (0.102)	0.995	0.10

[a] S-compound/MeSEt peak ratio
[b] S-compound/thiophene peak ratio.

greater extent with the PA fiber because it gave a higher response for SO_2 than the PDMS fiber. Moreover, the SO_2 peak distorts the baseline and the subsequent peaks may be smaller or distorted. In order to eliminate this interference, ethanal, pyruvic acid or 2-ketoglutaric acid must be added to the wines to bind the free SO_2 [35]. It was observed that 400 mg/l of ethanal is enough to eliminate the interference of SO_2, and the S-compound peaks studied did not decrease, and no new peaks appeared.

The method was applied to different whites and red wines. In these samples (n=10) the ranges (μg/l) of the S-compounds found were: CS_2 (0.8–12.6), (ND–40.5), (ND–1.1), MeThioAc (ND–9.4), Me_2S_2 (ND–3.2), EtThioAc (ND–4.0), Et_2S_2 (ND–3.5). ND means not detected. These results, which are comparable to the ones of other authors [15,36,37], were obtained with duplicates of each sample, and gave standard deviations between 0.5–7% for all the samples. Fig. 2 shows the chromatogram of a white wine analysed with the PDMS (a) and PA (b) fibers under the conditions discussed above. It can be observed that the peak of SO_2 does not interfere with the peaks of the analytes studied.

4. Conclusions

HS-SPME, by using the PDMS and PA fibers, is suitable for determining the S-compounds studied in wines. It is a very simple and fast technique and shows very good reproducibility. It increases the sensitivity of the direct injection of static headspace

Table 2
Percentage recoveries and relative standard deviations (in %)

	Polydimethylsiloxane		Polyacrylate	
S-Compound	White wine	Red wine	White wine	Red wine
CS_2	98.5 (5.8)	96.2 (5.7)	97.5 (5.9)	98.0 (5.6)
MeSMe	98.9 (5.5)	97.3 (5.2)	96.9 (6.4)	97.1 (7.5)
EtSEt	104.4 (5.3)	100.7 (6.9)	100.3 (6.3)	99.4 (5.3)
MeSPr	103.3 (6.6)	101.2 (6.8)	101.2 (5.7)	98.8 (5.5)
MeThioAc	98.6 (6.8)	101.3 (6.4)	98.4 (6.3)	99.6 (6.8)
MeSSMe	105.3 (6.8)	100.6 (6.5)	103.3 (6.2)	94.0 (7.0)
EtThioAc	104.1 (7.1)	101.5 (7.0)	100.7 (7.3)	106.0 (6.2)
EtSSEt	117.1 (7.7)	111.8 (8.0)	106.8 (8.6)	110.4 (7.8)

Conditions given in Section 2.

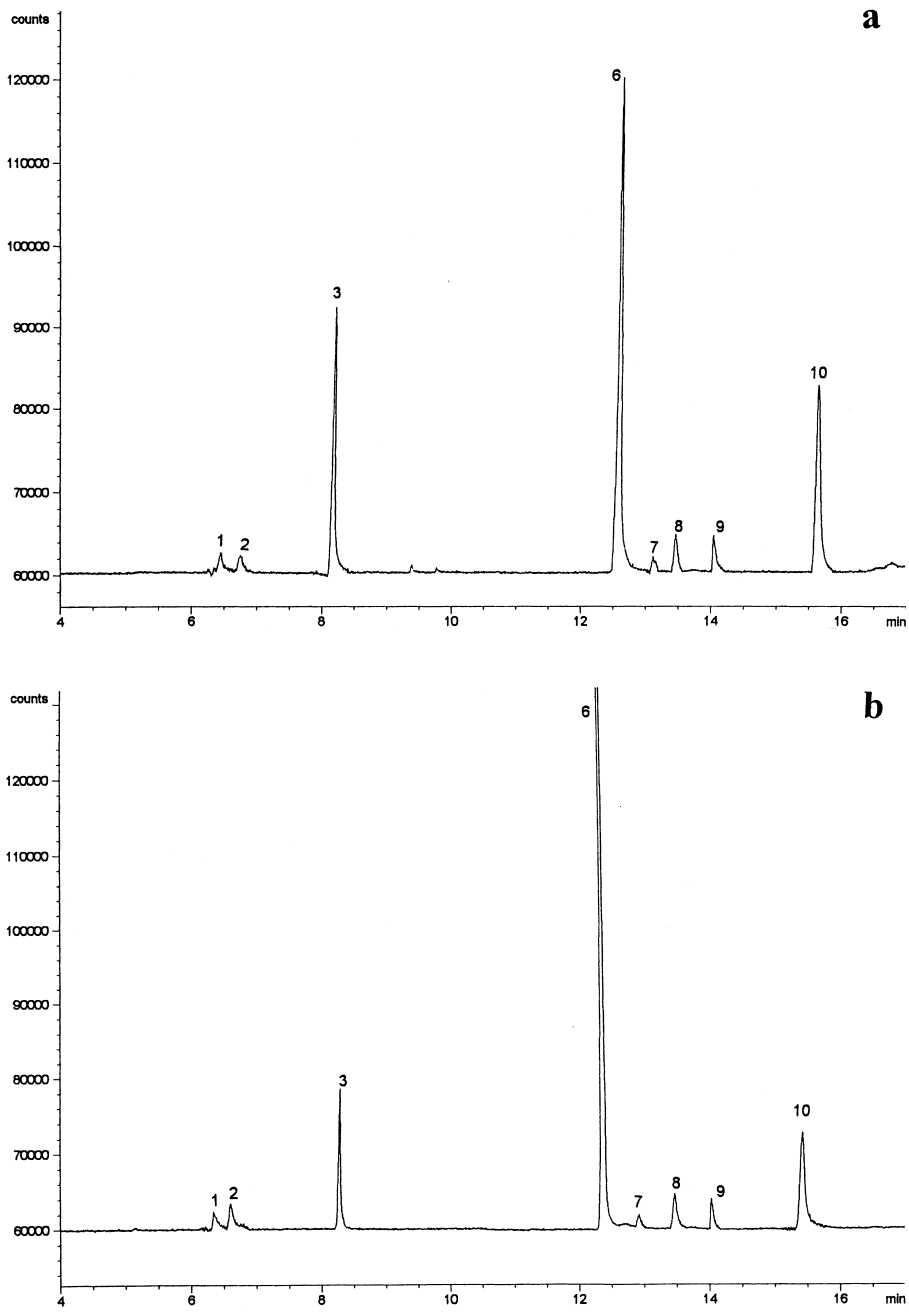

Fig. 2. Chromatogram of a sample of white wine analysed with the proposed procedure by using PDMS fiber (a) and PA fiber (b). Identification numbers can be seen in Fig. 1.

and it provides good recovery for these compounds which may be present in red and white wines.

Acknowledgements

The authors wish to thank CICYT (projects ALI94-0505 and ALI97-0765) for the financial support given.

References

[1] C.J. Mussinan and M.E. Keelan (Editors), Sulfur Compounds in Foods (ACS Symposium Series, No. 564), American Chemical Society, Washington, DC, 1993, ch. 1, p. 1.
[2] L. Nykänen and H. Suomalainen, in Aroma of Beer, Wine and Distilled Alcoholic Beverages, Reidel, Dordrecht, 1983, ch. 16, p. 233.
[3] D. Rauhut, H. Kürbel, H.H. Dittrich, Vitic. Enol. Sci. 48 (1993) 214.
[4] S.J. de Mora, S.J. Knowles, R. Eschenbruch, W.J. Torrey, Vitis 26 (1987) 79.
[5] D.J. Spedding, R. Eschenbruch, A. Purdie, Vitis 19 (1980) 241.
[6] D.J. Spedding, P. Raut, Vitis 21 (1982) 240.
[7] V. Lavigne, J.N. Boidron, D. Dubourdieu, J. Int. Sci. Vigne 26 (1992) 75.
[8] D. Rauhut, in P. Ribereau-Gayon and A. Lonvaud (Editors), Actualités Œnologiques 89, Dunod, Paris, 1990, p. 482.
[9] A. Maujean, M. Nedjma, J.P. Vidal, R. Cantagrel and L. Lurton, in R. Cantagrel (Editor), Elaboration et Connaissance des Spiritueux, BNIC, Cognac, 1993, p. 110.
[10] A. Maujean, N. Seguin, Sci. Aliment. 3 (1983) 589.
[11] P. Chatonnet, D. Dubourdieu, J.N. Boidron, Wine Ind. J. February (1991) 73.
[12] J.W. Stahl, J. Jordan, Anal. Chem. 59 (1987) 1222.
[13] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 773 (1997) 261.
[14] S.B. Adeloju, S.J. Shaw, G.G. Wallace, Chem. Australia 62 (1995) 26.
[15] Y. Le Fur, M. Feuillat, P.X. Etievant, Rev. Fr. Oenol. 128 (1991) 7.
[16] A.W. Dercksen, I. Meijering, B. Axcell, J. Am. Soc. Brew. Chem. 50 (1992) 93.
[17] M. Nedjma, A. Maujean, J. Chromatogr. A 704 (1995) 495.
[18] M.S. Burmeister, C.J. Drummond, E.A. Pfisterer and D.W. Hysert, Amercian Society of Brewing Chemists, 1992, p. 53.
[19] A. Anocibar Beloqui, P. Guedes de Pinho, A. Bertrand, Am. J. Enol. 46 (1995) 84.
[20] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[21] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[22] K.D. Buchholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.
[23] Z. Zhang, J. Pawliszyn, J. High Resolut. Chromatogr. 16 (1993) 689.
[24] L.-K. Ng, M. Hupé, J. Harnois, D. Moccia, J. Sci. Food Agric. 70 (1996) 380.
[25] A. Steffen, J. Pawliszyn, J. Agric. Food Chem. 44 (1996) 2187.
[26] J. Song, B.D. Gardner, J.F. Holland, R.M. Beaudry, J. Agric. Food Chem. 45 (1997) 1801.
[27] R. Artacho, M.F. Olea, M.D. Ruiz, Food Chem. 53 (1995) 91.
[28] B. Denis Page, G. Lacroix, J. Chromatogr. 648 (1993) 199.
[29] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[30] M. Mestres, O. Busto and J. Guasch, in First Symposium Proceedings of the In Vino Analytica Scientia, Bordeaux, 12–14 June 1997, p. 325.
[31] M.D. Walker, J. Sci. Food Agric. 67 (1995) 25.
[32] L. Urruty, M. Montury, J. Agric. Food Chem. 644 (1996) 3871.
[33] C. Fisher, U. Fischer, J. Agric. Food Chem. 45 (1997) 1995.
[34] R. Boqué, F.X. Rius, J. Chem. Educ. 71 (1994) 230.
[35] D. Rauhut, H. Kürbel, K. MacNamara and M. Grossmann, in First Symposium Proceedings of the In Vino Analytica Scientia, Bordeaux, 12–14 June 1997, p. 169.
[36] V. Lavigne, J.N. Boidron, D. Dubourdieu, J. Int. Sci. Vigne Vin 27 (1993) 1.
[37] S.K. Park, R.B. Boulton, E. Bartra, A. Noble, Am. J. Enol. Vitic. 45 (1994) 341.