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Journal of Chromatography A, 849 (1999) 293–297

JOURNAL OF CHROMATOGRAPHY A

Short communication

Simultaneous analysis of thiols, sulphides and disulphides in wine aroma by headspace solid-phase microextraction–gas chromatography

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Received 8 February 1999; received in revised form 19 April 1999; accepted 20 April 1999

Abstract

This paper deals with the improvement of a headspace solid-phase microextraction (HS-SPME) method, developed in a previous work, in order to analyse, simultaneously, thiols, sulphides and disulphides in wines. This can be achieved by applying Carboxen–polydimethylsiloxane fibres and a cryogenic trap to focus the analytes. Under optimum conditions, the HS-SPME procedure developed shows low limits of detection for the sulphides and disulphides studied (0.05–3 μg/l) and the thiols can also be analysed and detected at very low levels (0.5–1 μg/l) with acceptable recoveries and repeatability.

Keywords: Headspace analysis; Extraction methods; Wine; Food analysis; Sulphur compounds

1. Introduction

Thiols, sulphides and disulphides are important in wine aroma because of their high volatility and low perception levels. Low concentrations of volatile S-compounds give wines a distinctive aroma, but in higher concentrations they have a negative effect [1–3]. So, a quick and easy method for detecting and quantifying S-compounds becomes increasingly important for controlling the quality of wine.

In previous studies, we used the new technique of headspace solid-phase microextraction (HS-SPME) [4–6] to analyse sulphides and disulphides in wines, by using different fibres [7–9]. The results showed that the Carboxen–polydimethylsiloxane fibre (CAR–PDMS) has the strongest affinity for S-compounds, although there were some problems in the direct desorption of thiols and the values of reproducibility and repeatability are not so good than the ones obtained with other fibres [9–12]. The purpose of the present study is to obtain a HS-SPME procedure which allows the simultaneous analysis of all the wine volatile S-compounds we studied by using CAR–PDMS fibres.

2. Experimental

2.1. Chemicals and reagents

The volatile S-compounds we studied were: hy-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00538-5

drogen sulphide (SH_2) [7783-06-4], methanethiol (MeSH) [74-93-1], ethanethiol (EtSH) [75-08-1], dimethyl sulphide (Me_2S) [75-18-3], diethyl sulphide (Et_2S) [352-93-2], methyl-*n*-propyl sulphide (MeSPr) [3877-15-4], methyl thioacetate (MeSAc) [1534-08-3], ethyl thioacetate (EtSAc) [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]. Ethylmethyl sulphide (EtSMe) [624-89-5] and thiophene [110-02-1] were used as internal standards (I.S.). The thiols were obtained from their respective sodium salts: ethanethiol sodium salt [811-51-8], methanethiol sodium salt [5188-07-8] and sodium sulphide hydrate [7783-06-4].

2.2. Preparation of standard solutions

The ethanolic standard solutions of 2000 μg/l of each sulphide and disulphide were prepared as described in a previous work [9]. For thiols, solutions of each thiol salt in alkaline medium have been used but, since the thiol solutions in this medium could easily be oxidised, we followed the procedure developed in a previous study [13] to prepare standard salt thiol solutions and check their real concentration.

2.3. Sample preparation

Since the optimum conditions found for sulphides and disulphides [9] were also good for thiols, we used the same parameter values in this study. So, 25 ml of sample (either natural or synthetic wine both adjusted to 12% of EtOH) was poured into a 50-ml glass vial with 2.92 g of NaCl (2 *M*) and 0.15 g of ethylenediaminetetraacetic acid (EDTA). Each sample was spiked with the internal standards (10 μg/l of EtSMe and 2.5 μg/l thiophene) and the vial was tightly capped with a PTFE-faced silicone septum and shaken. This was done at 4°C to avoid losses of the most volatile compounds. When the samples had to be spiked with thiols, we put a suitable amount of each salt thiol into an Eppendorf microtube under a nitrogen stream. The opened microtube was put into the vial and, when the vial was capped and shaken, the thiol salts came into contact with the wine at pH 3–4, so the thiols were instantly released into the vial without loss [13].

2.4. Headspace and SPME

The liquid-headspace samples were equilibrated for 30 min at 25°C. The CAR–PDMS fibre (Supelco, Bellefonte, PA, USA) was then inserted manually through the vial septum and exposed to the headspace of the sample. After 30 min the fibre was removed from the vial and inserted into the injection port of the gas chromatography (GC) system for thermal desorption at 300°C for 1 min [9].

2.5. Chromatography

Chromatographic analyses were made on a gas chromatograph equipped with a flame photometric detection (FPD) system. Separation was performed, as in a previous work [9], using an SPB-1 Sulfur column (30 m×0.32 mm I.D., 4 μm) with helium as carrier gas with a flow-rate of 1.2 ml/min but, since in this study it was necessary to separate the thiols, the oven temperature program was: 35°C (8 min), 15°C/min, 150°C, 40°C/min, 280°C (5 min). Furthermore, 50 cm of uncoated and deactivated column (0.32 mm I.D.) was placed between the injector and the chromatographic column. Thirty cm of this uncoated column, used as a cryogenic gap, was placed outside the oven. The SPME injection was made in the splitless mode for 1 min at 300°C and cryogenically trapped and focused by chilling 20 cm of the gap column in liquid nitrogen. The gap was then submerged in boiling water to volatilise the focused and condensated analytes and introduce them in the chromatographic column.

The column used to confirm the identity of the analytes in real samples was an HP-Innowax column (50 m×0.2 mm I.D., 0.2 μm) [9].

3. Results and discussion

The method was assessed by estimating the repeatability, the reproducibility, the linear range and the limits of detection and quantification. These values were obtained using a synthetic wine which contained other volatile compounds [9] to reproduce

the influence of the wine matrix on the extraction and under the SPME conditions specified in Section 2.

The samples spiked with sulphide and disulphide standard solutions were analysed jointly but, when they were spiked with thiol standard solutions, they were analysed separately because thiols are very reactive [14–16] and easily oxidised into disulphides which gave its corresponding peaks. This made it impossible to quantify the compounds studied accurately. So, further studies were made twice: one for sulphides and disulphides and the other for thiols.

The FPD response is a power function so the S-compounds linear calibration graphs were constructed by plotting the log [S-compound/I.S.] peak area ratios against the log [S-compound/I.S.] concentration ratios. The range of linearity was obtained from four replicates of six calibration standard solutions over concentrations 1–40 μg/l for the thiols, 0.25–80 μg/l for the sulphides and 0.125–40 μg/l for the disulphides. To calculate these calibration graphs, linear least-squares regression was used and, in all cases, the correlation coefficients were good (r^2>0.99). The efficiency of SPME varies when different CAR–PDMS fibres are used, so all the experiments should be performed with a single fibre and, if more than one is used, the calibration graphs must be recalculated.

The limits of detection (LODs) (signal/noise=3) of sulphides and disulphides were similar to the ones obtained without cryogenic trap and the values of thiols ranged between 0.5 and 1 μg/l, values low enough to determine these S-compounds in real samples (Table 1).

To evaluate repeatability, five identical samples of synthetic wine, fortified with 5–10 μg/l of each S-compound, were extracted once with the same CAR–PDMS fibre. For each extraction, the peak area ratios, with their relative standard deviations (RSDs) were calculated. In this study we have seen that, for sulphides and disulphides, these values were not influenced by the cryogenic trap (3–20%). For thiols, the values of RSDs obtained ranged between 5 and 30%. These higher RSDs may be due to the low repeatability of this fibre and also to the chemical instability of these compounds.

To calculate the recovery, the standard addition technique was applied to white and red wines. The analytes were added to wines at three different concentration levels: 1 μg/l, 0.5 μg/l and 0.25 μg/l (first level), 5 μg/l, 2.5 μg/l and 1.25 μg/l (second level) and finally, 30 μg/l, 25 μg/l and 12.5 μg/l (third level) for thiols, sulphides and disulphides, respectively. Samples from each level were extracted six times using two different fibres and the results are shown in Table 2. The recoveries for sulphides and disulphides are close to 100%. These values, as with the RSDs, are similar to those obtained without the cool trap [9]. Thiols also give values close to 100%, but RSD values are higher because they may suffer oxidation in the wine during the sampling time.

Table 1
Limits of detection (LODs) of the method (HS-SPME) by using CAR–PDMS fibre

S-Compound	LOD (μg/l)
SH_2	0.50
MeSH	0.50
EtSH	1.00
Me_2S	4.00
CS_2	0.07
MeSAc	1.00
Et_2S	0.15
MeSPr	0.10
Me_2S_2	0.07
EtSAc	0.50
Et_2S_2	0.05

Fig. 1 shows a typical chromatogram of a sample of wine. Peaks are well shaped and resolved for all the volatile S-compounds studied, although a high SO_2 peak appeared at the beginning of the chromatogram.

This method was applied successfully to determine the volatile S-compound contents of different varietal wines from the experimental vineyard of the Faculty of Oenology in Tarragona. The ranges (μg/l) of the results obtained from triplicate extraction of the samples are shown in Table 3. These results are similar to those reported in the literature [1,8,9,13,17–19].

4. Conclusions

The HS-SPME technique, using the CAR–PDMS fibre, allows the simultaneous determination of

Table 2
Recovery percentages and relative standard deviations (in parentheses) (conditions given in text)

Sulphur compound	White wine			Red wine		
	1st level	2nd level	3rd level	1st level	2nd level	3rd level
SH_2	103 (25)	97 (15)	97 (25)	96 (29)	98 (22)	104 (21)
MeSH	94 (32)	97 (17)	99 (31)	99 (18)	96 (26)	106 (27)
EtSH	– –	104 (28)	105 (19)	– –	105 (28)	103 (24)
MeSMe	– –	– –	98 (15)	– –	– –	100 (12)
CS_2	112 (16)	100 (11)	106 (22)	99 (16)	100 (5)	98 (19)
MeSAc	– –	104 (7)	99 (15)	– –	109 (15)	112 (9)
EtSEt	98 (19)	100 (9)	97 (10)	99 (18)	103 (7)	95 (14)
MeSPr	97 (18)	99 (7)	99 (17)	100 (4)	100 (2)	95 (12)
MeSSMe	98 (21)	101 (11)	105 (19)	93 (16)	105 (8)	98 (16)
EtSAc	97 (15)	106 (18)	102 (20)	110 (13)	105 (15)	102 (17)
EtSSEt	93 (19)	91 (14)	106 (21)	101 (9)	99 (10)	97 (14)

thiols, sulphides and disulphides in wines at μg/l–ng/l levels. We have demonstrated how a cryogenic trap can solve the problems caused by poor desorption of the most volatile S-compounds. When the sample is heated to be volatilised, this cool trap does not affect the determination of sulphides and disulphides. Results for repeatability, recovery and limits of detection are acceptable for all the S-compounds studied. When the method was applied to real samples, results are within the same range as those reported using current headspace techniques which are slower and more difficult to use.

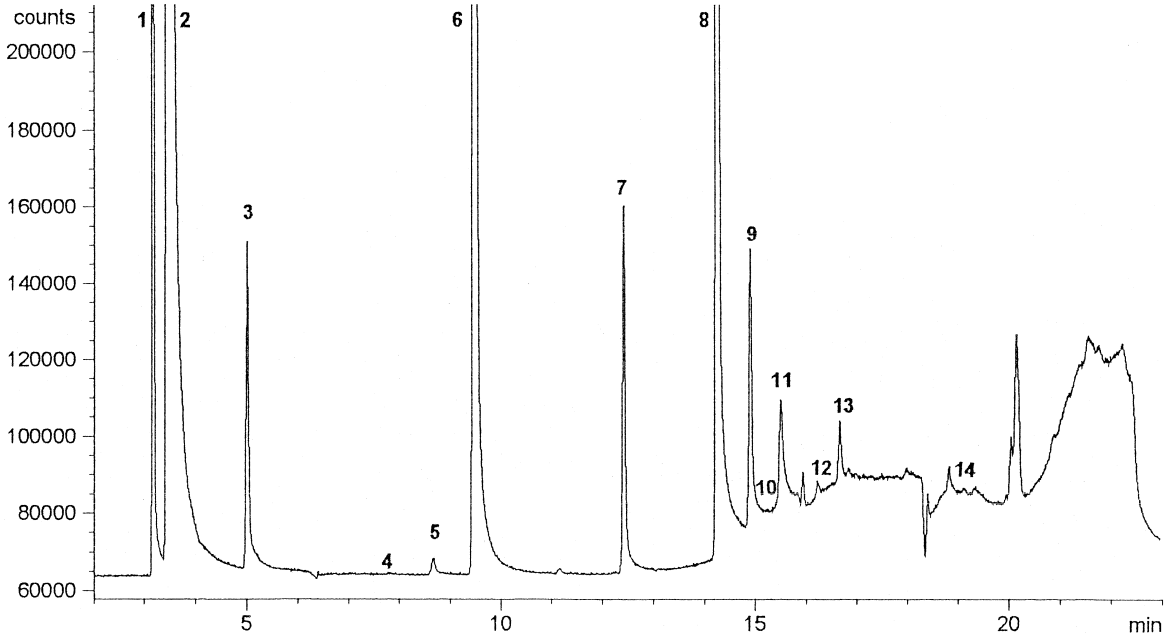

Fig. 1. Chromatographic response of a real sample of wine analysed using the proposed procedure. 1=Hydrogen sulphide, 2=sulphur dioxide, 3=methanethiol, 4=ethanethiol, 5=dimethyl sulphide, 6=carbon disulphide, 7=ethylmethyl sulphide (I.S.), 8=tiophene (I.S.), 9=methyl thioacetate, 10=diethyl sulphide, 11=methylpropyl sulphide, 12=dimethyl disulphide, 13=ethyl thioacetate, 14=diethyl disulphide. Compounds 4 and 10 do not appear in this sample but their retention times are indicated.

Table 3
Sulphur compound contents range (μg/l) in varietal wines

S-Compound	White wine (n=8)	Rosé wine (n=2)	Red wine (n=7)
SH_2	1.5–21.5	5.3–34.6	nq[b]–8.5
MeSH	1.5–16.3	1.1–10.8	nq–9.3
EtSH	nd[a]–3.5	nd–3.2	nd–2.8
Me_2S	nd–20.2	nd–10.2	3.2–21.2
CS_2	0.3–7.8	0.2–5.9	0.1–4.5
MeSAc	nq–53.8	nd–15.8	nq–24.8
Et_2S	nd–nd	nd–nq	nd–5.3
MeSPr	nd–2.7	nd–1.7	nd–1.7
Me_2S_2	nd–0.6	nd–0.2	nd–0.4
EtSAc	nq–3.2	nd–nq	nq–4.2
Et_2S_2	nd–nq	nd–nq	nd–nq

[a] nd=Not detected.
[b] nq=Not quantified (nq=3.3×nd).

Acknowledgements

The authors wish to thank CICYT (project ALI97-0765) for the financial support given.

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