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Analysis of low-volatility organic sulphur compounds in wines by solid-phase microextraction and gas chromatography

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Abstract

A method for analysing low-volatility sulphur compounds using solid-phase microextraction has been developed. The analytes were extracted directly from the liquid sample using fibres coated with different stationary phases. The best extraction efficiency was obtained with Carboxen–polydimethylsiloxane coating. Ionic strength, sample volume, time and temperature of the extraction were optimised and the matrix effect studied. The method enables 15 sulphur compounds in wine to be determined at trace levels with recoveries close to 100% and limits of detection between 0.05 and 5 μg/L. The overall method was successfully applied to the determination of the sulphur compounds studied in several red, white and rosé wines. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Flavour of foods and beverages plays a very important role as a result of their interaction with the sensory organs. As in most food products, wine aroma is influenced by the action of hundreds of different chemicals. Among these substances, the organic sulphur compounds have a considerable influence on the sensorial quality because they usually give unpleasant odours, although some of them may contribute to the desirable aroma of wine [1–4].

Organic sulphur compounds can be classified into two groups: those with a boiling point lower than 90°C (volatile compounds) and those with a boiling point above 90°C (heavy compounds). The volatile compounds contribute significantly to the possible unpleasant aroma of wine due to their low sensory thresholds [1]. The low-volatility sulphur compounds can seriously affect the aroma either because of the concentration at which they are present [5] or because they become precursors of volatile sulphur compounds, which degrade throughout the elaboration and storage of wine. In any case, the sulphur compounds are normally found at trace levels in wines.

The analytical determination of the less-volatile sulphur compounds has usually been carried out by combining chromatographic separation with analyte concentration techniques. The most widely used concentration techniques have been liquid–liquid extraction [4–6] and purge and trap [7]. However, liquid–liquid extraction has two important disadvantages: it requires large volumes of organic solvents and it is time consuming since multiple steps in sample handling are usually needed. This may lead to a low recovery and a not very good repeatability.

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The purge and trap technique avoids these disadvantages but requires specific instrumentation coupled to the gas chromatograph. Solid-phase microextraction (SPME) is a solvent-free extraction technique that enables the extraction and the concentration steps to be performed simultaneously [8–10]. Therefore, SPME appears to be an excellent alternative to the purge and trap technique since it requires minimal sample treatment and simple instrumentation.

In previous studies, polydimethylsiloxane, polyacrylate and Carboxen–polydimethylsiloxane [11–14] fibres have been used to analyse thiols, sulphides and disulphides in the headspace of wine samples. The best extraction results were obtained with fibres whose stationary phase contained carbon. The purpose of the present study was to test and compare the results obtained using different coating fibres for analysing the low-volatility organic sulphur compounds in wine.

2. Experimental

2.1. Chemicals and reagents

The following sulphur compounds were studied (CAS number in brackets): methyl thioacetate [1534-08-3], ethyl thioacetate [625-60-5], dimethyl disulphide [624-92-0], diethyl disulphide [110-81-6], 2,5-dimethyl-thiophene [638-02-8], 2-methyl-tetrahydro-thiophen-3-one [13679-85-1], 3-(methylthio)propanaldehyde [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-mercaptoethanol [60-24-2], 2-(methylthio)ethanol [5271-38-5], 3-(methylthio)-1-propanol [505-10-2], 4-(methylthio)-1-butanol [20582-85-8], 3-(methylthio)propionic acid [646-01-5], benzothiazole [95-16-9]. Propyl isothiocyanate [628-30-8] was used as internal standard (I.S.). The standards, with a purity above 98%, were supplied by Sigma–Aldrich (Madrid, Spain), Fluka (Madrid, Spain), Lancaster (Bischheim, France) and Interchim (Montluçon, France).

Standard solutions of 2000–3000 mg/L of each sulphur compound were prepared in ethanol and stored at 5°C. A global standard solution containing all the analytes in the range 3–1000 mg/L was prepared by mixing an aliquot of each individual solution and diluting with ethanol. Some of these compounds are readily oxidised. Therefore, the individual standard solutions of these compounds, as well as the global one, were prepared and handled under nitrogen atmosphere.

Working solutions used in the SPME parameter optimisation were obtained by diluting the global standard solution in a synthetic wine solution (3.5 g tartaric acid and 120 mL ethanol diluted with a suitable amount of Milli-Q quality water to give 1 L of solution; pH 3.5).

2.2. SPME fibres

The fibres used (Supelco, Bellefonte, PA, USA) were coated with different stationary phases and various film thicknesses: polydimethylsiloxane (100 μm), polydimethylsiloxane–divinylbenzene (65 μm), polyacrylate (85 μm), Carbowax–divinylbenzene (65 μm), Carboxen–polydimethylsiloxane (75 μm) and Stable Flex divinylbenzene–Carboxen–polydimethylsiloxane (50/30 μm). They were conditioned by inserting them into the GC injector, which was kept at a suitable conditioning temperature for each fibre.

2.3. Direct SPME sampling

As mentioned above, samples were prepared under nitrogen atmosphere. For each SPME analysis, they were placed in a vial with a small stir magnet and were spiked with an appropriate amount of propylisothiocyanate (internal standard) to obtain a concentration of 2.5 μg/L. The vials were sealed with silicone septa and shaken to obtain an homogenous mixture. The SPME needle then pierced the septum and the fibre was extended through the needle to place the stationary phase in contact with the liquid sample. The fibre was withdrawn into the needle after the optimum sampling time. Finally, it was removed from the vial and inserted into the injection port of the gas chromatograph for 1 min. The extracted chemicals were desorbed thermally and transferred directly to the analytical column.

Fibres were cleaned before each microextraction process to prevent contamination. Cleaning was performed by inserting the fibre in the auxiliary

injection port, which was kept at the conditioning temperature of each fibre.

2.4. Chromatography

The analyses were carried out on a Hewlett-Packard 5890 gas chromatograph equipped with an HP Model 19256A flame photometric detection (FPD) system in the sulphur mode. The injection was made in the splitless mode for 1 min using an inlet of 0.75 mm I.D. which improved the GC resolution. 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 a HP Chemstation, where they were collected, integrated and recorded.

The separations were performed using a HP-Innowax column (50 m×0.2 mm I.D., 0.2 μm) with an oven temperature programme of 40°C (5 min), 3°C/min, 130°C, 40°C/min, 220°C (15 min). The carrier gas was helium with a flow-rate of 0.4 mL/min. The column used to check the identity of the analytes in real samples was an SPB-35 column (30 m×0.25 mm I.D., 0.25 μm) with an oven temperature programme of 50°C (10 min), 5°C/min, 280°C. The carrier gas was helium with a flow-rate of 1 mL/min.

To identify other wine volatiles, which were also extracted by the fibre, a Hewlett-Packard 5890 (series II) gas chromatograph equipped with a HP-5972 mass-selective detector was used. The chromatographic conditions were the same as described for FPD. The detector operated in impact electron mode (70 eV) at 280°C. Detection was performed in the scan mode between 30 and 300 u.

3. Results and discussion

3.1. Fibre screening

Six different types of commercially available fibres were tested. Identical samples of synthetic wine spiked with a mixture of all the analytes were extracted with these fibres using either headspace-SPME or direct-SPME mode. In these experiments, magnetic stirring was used to accelerate the transfer of the analytes from the sample to the fibre coating. Furthermore, the samples were saturated with NaCl because the extraction efficiency is improved by adding soluble salts to the sample. The temperature during the extraction was 25°C and the times of extraction were 1 and 3 h, respectively.

The results of this screening showed that, in general, the extraction efficiency was better when the fibre was immersed directly into the liquid sample. Furthermore, the highest chromatographic peaks were obtained with fibres whose stationary phase contained carbon, whatever the period of time applied. Therefore, Carboxen–polydimethylsiloxane (CAR–PDMS) and Stable Flex divinylbenzene–Carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fibres were selected for the method optimisation.

3.2. SPME parameter optimisation

The parameters optimised were ionic strength, sample volume, extraction time and temperature of the sample during extraction. The interference of the matrix was also taken into account. All the experiments were performed with constant magnetic stirring because this has a positive influence on the mass transfer to the different phases [8–10].

The addition of salt to the samples (salting out effect) can modify the extraction efficiency, because the partition coefficients are partially determined by the matrix–analyte interactions [8–10]. Therefore, to check the ionic strength effect, different amounts of sodium chloride (0–5 *M*) were added to identical samples. For CAR–PDMS fibres the best results were obtained without salt, perhaps due to the formation of a thin layer of salt around the fibre. However, when the DVB–CAR–PDMS fibre was used, the extraction efficiency improved as the NaCl concentration increased, thus samples were saturated with NaCl (5 *M*).

Another important parameter to check in SPME is the sample volume. It is reported [15] that, as the sample volume increases, the extent of SPME initially increases rapidly and then remains relatively constant at larger volumes. Therefore, in the present study, volumes of 20 and 50 mL were tested. The results were the same when times of 4 and 6 h were used for the 20 and 50 mL samples, respectively.

Thus, in order to reduce the analytical time, 20 mL samples were analysed.

The SPME is strongly influenced by temperature because the partition coefficients are temperature dependent and the extraction of the analytes by the fibre coating is an exothermic process [16]. Furthermore, temperature and time of extraction are closely connected, so both parameters were studied simultaneously. Periods of time of 2, 3, 4, 6 and 10 h were tested at 10 and 25°C. Higher temperatures were not used to prevent analytes from degrading and to help the extraction. The best results were obtained at 10°C, whatever the time of extraction and the fibre used. We also observed that the efficiency of extraction improves up to 4 h for all the analytes studied. Thus, 4 h and 10°C were selected as the optimum conditions.

3.3. Matrix effect

The distribution constants between the liquid phase and the coating are strongly dependent on the matrix [8–10]. Since ethanol is one of the major constituents of wines, it may compete with the sulphur compounds in the extraction. When dealing with the headspace of the samples there is an inversely proportional dependence between the concentration of ethanol and the extraction efficiency [12–14,17,18]. However, no differences were observed between samples with different alcohol contents (9–15%, v/v) when dealing with direct liquid sampling and whatever fibre was assayed.

In wine samples, besides ethanol, there are hundreds of compounds that can interfere in the SPME [19–23]. To check the matrix interference, several samples of synthetic and real wines, spiked with the same amount of sulphur compounds, were extracted with both fibres, and the resulting chromatographic peak areas were compared. Some kind of competition between the interfering substances and analytes was observed, because the peak chromatographic areas of the sulphur compounds became worse for real than for synthetic wines. Furthermore, when dealing with real samples the DVB–CAR–PDMS fibres gave the highest chromatographic peak areas, but only when new, because they lost efficiency after about six analyses. Therefore, the CAR–PDMS fibres, which produce repetitive chromatographic peak areas, were selected to analyse the sulphur compounds studied in wines.

As in previous studies [13,14], in order to reproduce the influence of the matrix, we tried to obtain a matrix which was as similar as possible to a real wine. For this reason, we injected the solid-phase microextracts of four different samples of red, rosé and white wines into the GC–MS system and we determined which organic compounds commonly found in wines, besides the analytes studied, were extracted by the fibre. These were ethyl acetate, 3-methylbutyl acetate, hexyl acetate, 2-phenylethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, 2,3-butanediol, *meso*-butanediol, 1-hexanol, 2-phenyl ethanol, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl lactate, hexanoic acid, octanoic acid and decanoic acid. Therefore, in the calibration step, we worked with the synthetic wine described in Section 2.1, but we added the organic compounds cited above in similar concentrations to those found in wines [24].

To check if the matrix of the above synthetic wine was similar enough to real wines, both kinds of wines were extracted and the results compared. Chromatographic responses were similar for all the analytes studied in rosé, white and synthetic wines, whereas the response of the red wines was unexpectedly higher. This different behaviour can be observed in Fig. 1, where the chromatograms obtained with a red wine and a white wine, both spiked with the same amount of sulphur compounds, are shown. Therefore, it seems that some characteristic substances present in red wines (perhaps polyphenolic compounds) interfere in the SPME of the compounds studied. Hence, the standard addition technique was used to validate the method.

3.4. Validations of the method

The standard addition method was used to obtain calibration graphs as representative as possible and to avoid the influence of the matrix. Therefore, four different wines of each type (red, rosé and white) were mixed to obtain a standard wine of each class. The four varieties used to obtain each red, rosé and white standard wine came from different regions and had different proof grading.

Calibration graphs were obtained from 20 mL of

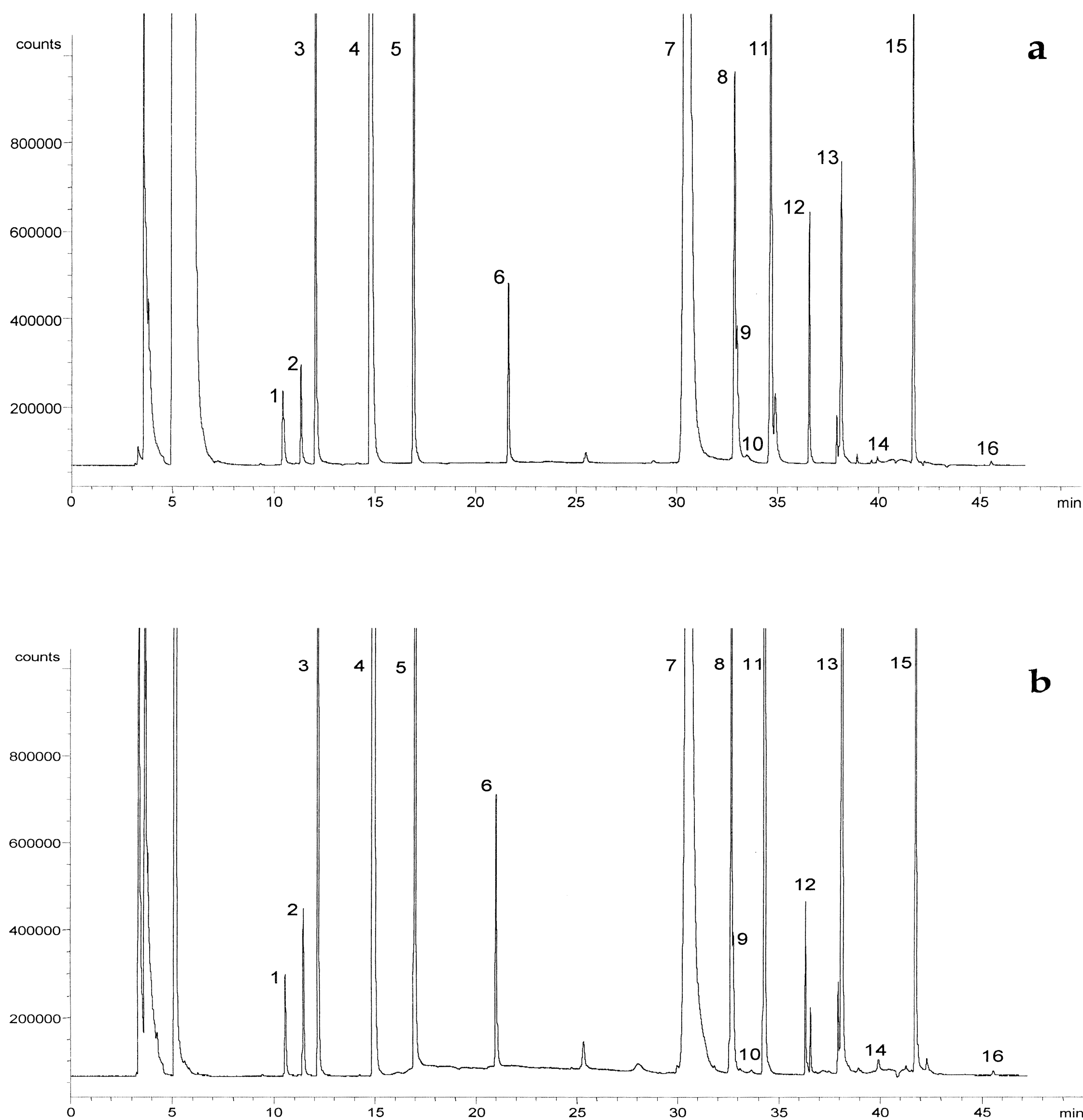

Fig. 1. Chromatographic response of a white wine (a) and red wine (b) both fortified with the same amount of sulphur compounds. 1: Methyl thioacetate (56.8 μg/L), 2: dimethyl disulphide (5.4 μg/L), 3: ethyl thioacetate (53.2 μg/L), 4: 2,5-dimethylthiophene (35.8 μg/L), 5: diethyl disulphide (3.3 μg/L), 6: propyl isothiocyanate (I.S.), 7: methional (28.3 μg/L), 8: methyl 3-(methylthio)propionate (50.6 μg/L), 9: 2-methyltetrahydrothiophen-3-one (100.4 μg/L), 10: (methylthio)ethanol (100 μg/L), 11: ethyl 3-(methylthio)propionate (51.7 μg/L), 12: 3-methylthiopropyl acetate (20.3 μg/L), 13: methionol (956.8 μg/L), 14: (methylthio)butanol (102.4 μg/L), 15: benzothiazole (21.4 μg/L), 16: 3-(methylthio)propionic acid (110.5 μg/L).

the standard wine (white, rosé or red) spiked with six different concentrations of sulphur compounds. Table 1 shows these concentration ranges which are in accordance with the usual contents of each compound in wines [5,6,25–28]. 2-Mercaptoethanol was not quantified because its peak overlapped with other peaks at the beginning of the chromatogram. Three replicates were analysed at each concentration. To minimise fibre variability, which we observed in the Carboxen fibres [13,14], the samples were ran-

Table 1
Concentration ranges of calibration graphs and limits of detection (LOD) of the method for white, rosé and red wines

Sulfur compound	Range (μg/L)	LOD (μg/L)
Methyl thioacetate	5–125	2–3
Dimethyl disulphide	0.5–15	0.2–0.4
Ethyl thioacetate	5–110	2–3
2,5-Dimethyl-thiophene	0.5–20	0.1–0.2
Diethyl disulphide	0.1–5	0.05
Methional	2.5–100	1–2
Methyl 3-(methylthio)propionate	5–100	2–3
2-Methyltetrahyrothiophen-3-one	5–200	2–4
(Methylthio)ethanol	30–200	25
Ethyl 3-(methylthio)propionate	5–100	2–3
3-Methylthiopropyl acetate	2–50	1–1.5
Methionol	50–2000	5–10
(Methylthio)butanol	5–200	4–5
Benzothiazole	2.5–50	1–1.5
3-(Methylthio)propionic acid	7.5–200	5–7

domly extracted with three different CAR–PDMS fibres for 4 h at 10°C and then chromatographed. Since FPD was used and its response to the amount of sulphur is a power function, in order to obtain a linear response the log(sulphur compound/I.S.) peak area ratios were plotted against the log(sulphur compound/I.S.) concentration ratios. For all the sulphur compounds studied the regression coefficient was good ($r^2 > 0.99$).

The limits of detection (LODs) were obtained from the amount of analyte required to give a signal-to-noise ratio of 3 when injecting the microextract of white, rosé and red standard wines. Table 1 shows the results obtained, which ranged between 0.05 and 5 μg/L for all the compounds studied except for (methylthio)ethanol, which has a LOD around 25 μg/L. These values are low enough to determine these sulphur compounds in real samples. Since the response varies as the type of wine changes, the LOD ranges are included for the wines analysed.

The recovery of the method was determined by the standard addition technique applied to four red, four rosé and four white wines, different from those used to obtain the wine standards. The analytes were added to each wine at three different concentrations, at the lower, middle and higher concentration of the calibration range specified in Table 1. Three samples of each level were extracted using different CAR–PDMS fibres. Table 2 shows the average recoveries with their relative standard deviations (RSDs), which were obtained for all the samples analysed at each level. It can be seen that the recoveries were about 90–100% and their RSDs were lower than 20%. Only 3-methylthiopropyl acetate shows recoveries less than 90%.

Finally, the method was applied to determine the low-volatility organic sulphur compounds studied in different commercial red, white and rosé wines. Table 3 shows the concentrations found. As can be

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

Sulfur compound	Recovery (%)		
	Low level	Middle level	High level
Methyl thioacetate	102.0 (13.4)	99.4 (10.9)	97.7 (10.1)
Dimethyl disulphide	97.6 (12.0)	97.5 (13.5)	94.5 (13.5)
Ethyl thioacetate	93.4 (12.6)	92.0 (12.7)	91.8 (12.0)
2,5-Dimethyl-thiophene	97.7 (7.3)	98.1 (8.7)	95.3 (7.9)
Diethyl disulphide	94.9 (10.3)	91.8 (7.2)	90.1 (16.5)
Methional	98.7 (11.8)	97.0 (10.4)	90.0 (12.3)
Methyl 3-(methylthio)propionate	98.6 (14.2)	89.8 (15.5)	94.3 (10.9)
2-Methyltetrahyrothiophen-3-one	91.7 (20.6)	86.3 (20.5)	94.7 (11.4)
Ethyl 3-(methylthio)propionate	94.0 (16.9)	92.6 (17.9)	93.5 (18.9)
3-Methylthiopropyl acetate	82.0 (10.0)	81.5 (8.6)	85.7 (9.4)
Methionol	101.0 (12.6)	99.5 (12.4)	95.0 (12.9)
(Methylthio)butanol	95.7 (13.3)	92.3 (10.4)	90.7 (14.8)
Benzothiazole	102.1 (12.6)	99.0 (8.1)	97.5 (9.4)
3-(Methylthio)propionic acid	97.8 (9.2)	95.8 (7.6)	99.7 (10.5)

Table 3
Range of sulfur compound contents in commercial wines. nd, not detected; nq, not quantified (below the lowest point of the calibration graph)

Sulfur compound	Content (μg/L)		
	White wine	Rosé wine	Red wine
Methyl thioacetate	5–10	6–15	5–15
Dimethyl disulphide	nq–1	nd–1.5	nq–2
Ethyl thioacetate	5–8	nq–5	5–10
2,5-Dimethyl-thiophene	nd–nq	nd–nq	nd–nd
Diethyl disulphide	nd–0.1	nd–0.1	nd–0.2
Methional	5–25	nq–15	4–35
Methyl 3-(methylthio)propionate	nd–8	nd–nq	nd–5
2-Methyltetrahyrothiophen-3-one	nq–10	nd–5	nq–15
(Methylthio)ethanol	nd–35	nd–nq	nd–30
Ethyl 3-(methylthio)propionate	nq–8	nd–10	5–10
3-Methylthiopropyl acetate	nd–4	nd–nq	nd–5
Methionol	250–1570	195–1750	220–1850
(Methylthio)butanol	nd–8	nd–10	nd–7
Benzothiazole	nq–10	nd–6	nq–10
3-(Methylthio)propionic acid	nd–8	nd–10	nd–8

seen the majority of the analytes studied are not found or found at low levels, which is quite normal since all the wines had been stored in optimum conditions of darkness and temperature. Only methyl thioacetate and methionol were found in all wines, the latter being the one which was found at high concentrations, but still in the normal range. These results are similar to those obtained by other authors [5,6,25–28].

4. Conclusions

Direct solid-phase microextraction has been successfully applied to the analysis of low-volatility organic sulphur compounds in wines. This solvent-free technique uses a very simple instrumentation and avoids multiple steps in the sampling stage, which are important advantages over more classical methodologies. The standard addition technique was used for quantitative analysis since matrix interference was observed and a synthetic matrix, similar enough to a real wine, could not be found. Using this technique, the matrix effect is taken into account and the method developed appears to be satisfactory for determining a large number of low-volatility organic sulphur compounds in wines.

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