

Headspace Solid-Phase Microextraction Analysis of Methyl Isothiocyanate in Wine

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Methyl isothiocyanate (MITC) is used as soil fumigant for several vegetable diseases and as antifermentative agent in wine. A new headspace solid phase microextraction (HS-SPME) method for analysis of MITC in wine is here described. This method is fast, inexpensive and does not require any solvent. Moreover, it shows a low minimum detectable limit and a wide linearity range. The quantification of MITC is performed by the standard additions method and does not require the use of internal standard, as in other methods.

Keywords: *Wine analysis; antifermentative agents; methyl isothiocyanate; HS-SPME*

INTRODUCTION

Methyl isothiocyanate (MITC) is used as soil fumigant for nematodes, fungi, and other diseases in vegetables, fruits, etc. This compound is illegally employed as antifermentative substance in wine. The addition of antifermentative agents in wines is controlled by EC and non-EC regulations. In particular, the Italian legal system does not allow the use of MITC in wines and requires the control of all wines to be exported (Gaetano and Matta, 1992). The methods used for the analysis of MITC are based on solvent extraction and concentration procedures (Carbone, 1987; Gaetano and Matta, 1992; Ottad et al., 1978; Saito et al., 1994). In this study we have applied an innovative, fast and inexpensive method based on headspace solid phase microextraction (HS-SPME). This technique was developed by Pawliszyn and co-workers and it is commercially available since 1993 (Arthur and Pawliszyn, 1990; Arthur et al., 1992). In some cases it offers many advantages because it does not require any solvent, nor any sample pretreatment; moreover it is also fast and inexpensive. However, there are still some reproducibility and repeatability problems to overcome for some types of compounds (Pelusio et al., 1995). In order to obtain good recovery by SPME, the compound must be lipophilic and highly volatile.

The method suggested here has a short analysis time and does not need sample pretreatment, which makes it particularly suitable for routine analysis in public health and state laboratories.

MATERIAL AND METHODS

Wines. Wines were sampled by the Inspectorate of Italian Agriculture Department. White, red, and rosé wines were obtained from different northern Italian countries.

General HS-SPME Procedure. The SPME device included a fused silica fiber, partially cross-linked with Carbowax-divinylbenzene (65 μ), which was purchased from Supelco Co. (Bellefonte, PA).

For headspace sampling, 5 mL of wine was placed in a 10 mL vial capped with a silicon septa, giving a headspace volume equal to 5 mL. 1.25 g of sodium chloride (25% w/v) was then

added to the sample. During extraction, the fused silica fiber was not immersed in the liquid phase; the fiber was exposed to the vapor phase generated by the liquid sample while it was subjected to continuous stirring. The extraction time is the result of a compromise between the time required to perform the analysis and the reproducibility of the analysis. As shown in Table 2, the detector response was already quite high after 5 min, but higher reproducibility was obtained after 30 min, which was thus chosen as sample extraction time.

Official Extraction Method. We used the Italian official method (Gaetano and Matta, 1992), in order to compare it with the method of HS-SPME. 100 μ L of 4-ethylpyridine (as internal standard) was added to 100 mL of wine. The wine pH was taken to 7 with sodium hydroxide solution. The sample was then extracted three times with 15 mL of *n*-pentane. Anhydrous sodium sulfate was added to the solvent, which was then concentrated to 0.3 mL by using a rotary evaporator at 40 °C; no vacuum was applied in order to avoid MITC loss.

Instrumental Analysis. HS-SPME analysis was performed with an MFC 500 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with two detectors: FID and NPD. Other methods for determining MITC use either the NPD or FPD detector (Carbone, 1987; Gaetano and Matta, 1992; Ottad et al., 1978; Saito et al., 1994).

The FID and NPD temperatures were 230 °C and 240 °C, respectively. A split/splitless injector was used in the splitless injection mode; the injector temperature was set at 230 °C. A 30 m, 0.25 mm i.d., 0.25 μ m film thickness Stabilwax column (Restex Corporation, Bellefonte, PA) was used. The GC oven temperature was programmed as follows: 40 °C held for 2 min, increased to 60 °C at a rate of 20 °C/min, 60 °C held for 1 min, increased to 75 °C at a rate of 1 °C/min, 75 °C held for 2 min, increased at the end to 220 °C at a rate of 8 °C/min and at this temperature held for 5 min.

Helium was used as carrier gas at a flow rate of 1 mL/min. For thermal desorption, the SPME fiber was kept in the injector for 2 min.

RESULTS AND DISCUSSION

Wine samples were firstly analyzed by HS-SPME using the FID detector. This type of detector was chosen because it is widely used for routine analysis. Figure 1 shows the GC traces of three different wines. It is important to point out that these wines belong to different types and do not have the same alcohol degree and geographic origin. Table 1 lists the main characteristics of these wines. As observed from Figure 1, the wine GC traces were very similar among them, in particular in the eluting zone of the MITC peak.

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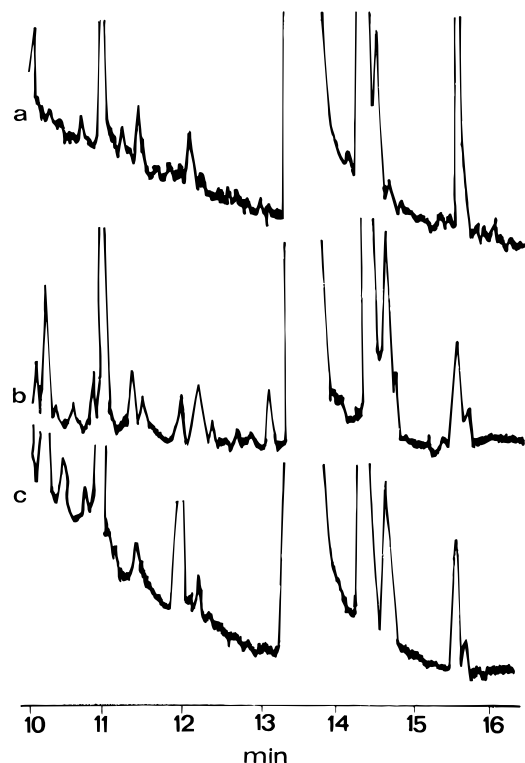


Figure 1. FID chromatograms of a three different wines. These chromatograms were obtained with the HS-SPME method. For characteristics of the wines refer to Table 1. Chromatogram a, white wine; chromatogram b, red wine; chromatogram c, rosé wine.

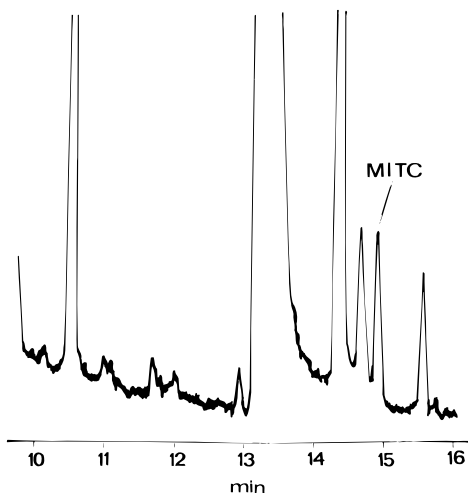


Figure 2. FID chromatogram of a wine sample with addition of 50 ppb MITC. This chromatogram was obtained with the HS-SPME method.

Table 1. Characteristics of Wine Samples

wine	type	geographic origin	alcohol degree (%)	sugar (%)
Trebbiano	white	Romagna	10.42	3.5
San Giovese	red	Romagna	12.00	2.5
Pinot Rosa	rosé	Veneto	10.50	3.0

MITC was identified by spiking the wine sample with an MITC solution in acetone (Figure 2).

The MITC peak coeluted with other unidentified compound, and this did not allow accurate quantitation of MITC. The FID was therefore used to confirm the MITC retention time.

The high selectivity of NPD detector led, however, to the complete elimination of the matrix effect (Figure 3).

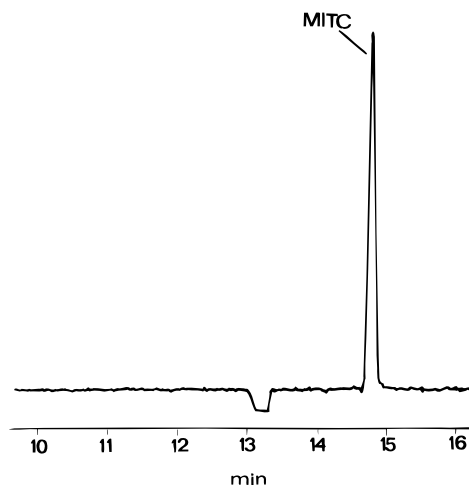


Figure 3. NPD chromatogram of a wine sample with addition of 50 ppb MITC. This chromatogram was obtained with the HS-SPME method.

Table 2. Time Extraction of a Wine to Which Was Added 50 ppb MITC^a

time extraction (min)	MITC peak area (detector counts)	RSD % (three measurements)
5	36 250	9.0
10	39 564	10.4
30	46 950	6.3

^a Red wine was used, as was an NPD detector.

Table 3. Standard Addition of MITC with NPD Detector and HS-SPME Method

addition of MITC (ppb (v/v))	MITC peak area (detector counts)	RSD % (three measurements)
1.04	1048	18.9
5.25	5442	6.3
10.43	10 715	4.0
20.86	26 484	7.1
41.72	43 832	6.6
52.15	48 969	6.5
73.01	67 095	6.2
105	98 220	5.4
208.6	202 593	5.8

Table 4. Accuracy and Precision Results for HS-SPME Method with NPD Detector

MITC concentration added to wine (ppb (v/v))	experimental MITC concentration (ppb (v/v))	RSD % (five measurements)
52	52.5	6.4
104	104.8	4.8
208	208.9	5.0

A faster temperature program could have been used in this case, but the program was not modified in order to verify the MITC retention time observed with the FID.

The NPD detector gave a minimum detectable limit of 1 ppb. The MITC concentration was measured with the standard additions method. A linear detector response was obtained in the 1–200 ppb range (Table 3). The method accuracy data are given in Table 4. The extraction efficiency of the silica fiber decreased after 50 absorption and desorption cycles; it would be advisable to often check the calibration curve. Official method accuracy data are shown in Table 5. Although many methods use the NPD, including the official method, they are not able to obtain minimum detectable limits lower than 10 ppb (Carbone, 1987; Gaetano and Matta, 1992; Ottad et al., 1978).

From these results it can be concluded that the HS-SPME method has a series of advantages that the other

Table 5. Accuracy and Precision Results for Official Method with NPD Detector

MITC concentration added at wine (ppb (v/v))	experimental MITC concentration (ppb (v/v))	RSD % (five measurements)
75	67.5	10.1
200	180	8.9
400	360	8.6

methods do not present: low minimum detectable limit, wide linearity range, short analysis time, and low costs. Furthermore, sample pretreatment is eliminated and solvents are not used.

Future work will be focused on the use of HS-SPME for identification and measurement of complex mixtures of several antifermentative agents. These mixtures are, in fact, illegally used in the wine retail stores, and their compositions are very different. The development of analytical methods for identification of these compounds could be very helpful to eliminate fraud in the enology field.

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