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Solid-phase microextraction and gas chromatography–mass spectrometry for the rapid screening of triazole residues in wine and strawberries

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Abstract

A solid-phase microextraction gas chromatography–mass spectrometry method has been developed for the determination of triazole residues, such as triadimefon, propiconazole, myclobutanil and penconazole. The method has been successfully applied to the analysis of strawberries and wine samples. The procedure is solvent-free, simple and highly sensitive. Within-day and day-to-day RSDs ranged between 2–11% and 7–28%, respectively. Detection limits estimated at a signal-to-noise ratio of 3 ranged between 30 (propiconazole) and 100 ng/kg (triadimefon). Since the detection limits achieved by this method are well below the maximum residue levels for wine (or grapes) and strawberries recommended by the European legislation, it can be conveniently used as a low-cost rapid screening method for the contamination of the considered samples.

Keywords: Wine; Fruits; Food analysis; Triazoles; Pesticides

1. Introduction

Triazoles are a class of systemic fungicides widely employed to control powdery mildews, rusts and other fungal pests on cereals, fruits, vegetables, turf, shrubs and trees. These compounds are classified as acutely toxic because of their potential to cause adverse chronic effects at low to moderate oral dose levels. Triazoles have been associated with changes in the liver functionality, decreased kidney weights, altered urinary bladder structure and acute effects on the central nervous system in treated laboratory animals [1]. Due to their persistence, they can easily contaminate to a variable extent agricultural products and derivates such as fruit juices and wines. Thus, because of possible health effects, there is an increasing need to monitor these fungicides in food commodities. In fact, the Italian government fixed the limits of detection of these compounds in wine and strawberries between 100 and 500 μg/kg.

The determination of pesticides in foodstuffs is usually accomplished by chromatographic techniques and involves many preliminary steps like sampling, extraction, clean up or interference removal. Existing liquid chromatography (LC) and gas chromatography (GC) methods for the determination of triazoles have been mainly based on complex sample

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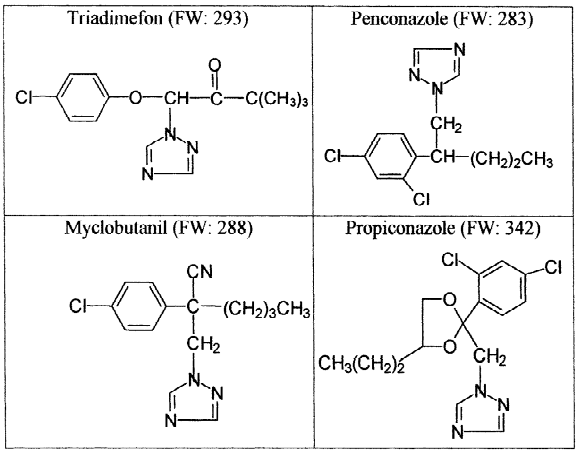

Fig. 1. Chemical structures of the selected triazoles. FW = formula mass.

preparation and/or extensive use of toxic organic solvents [2–7].

Solid-phase microextraction (SPME) is an extracting method [8] providing a good alternative to traditional extraction techniques for chromatographic analysis. In fact, it can be easily automated [9] and allows, in a single step, preconcentration and a rapid direct extraction, without the use of organic solvents. SPME methods have been developed for the determination of a large number of pesticides in different environmental matrices [10]. The technique has been also widely applied for sample preparation in pesticide residue analysis in food; the relevant existing literature has been recently reviewed [11].

In the present paper, a method has been developed for the rapid screening of triazoles residues in wine and strawberries. In this study, SPME–GC–mass spectrometry (MS) conditions have been optimised for the target compounds, triadimefon, propiconazole, myclobutanil and penconazole (Fig. 1). The developed procedure has been then successfully applied to the analysis of food samples such as wine and strawberries, in order to assess their possible contamination.

2. Experimental

2.1. Chemicals

Triadimefon, propiconazole, myclobutanil and penconazole certified standards were purchased by Labor Dr Ehrenstorfer-Schafers (Augsburg, Germany). A triazoles mix (1 μg/μl) was prepared, by dissolving the selected triazoles in ethanol, and stored in the dark at 4 °C. More diluted solutions were prepared just before use. Sodium chloride 99.5% was obtained from Merck (Darmstadt, Germany). Other chemicals were analytical grade reagents.

2.2. Apparatus

GC–MS analysis was performed with a Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with a HP 5890 GC split/splitless injector and interfaced, by a GC transfer line, to a VG Trio-2000 quadrupole mass spectrometer (VG Biotech, Altrincham, UK). The carrier gas was helium; contaminants were removed by using a drying tube, a Supelpure-HC trap and an OMI-1 Indicating Purifier (all Supelco) in series.

The GC chromatographic column consisted of a Supelco fused-silica SPB-5 capillary column (30 m×0.20 mm I.D. with 0.25 μm film thickness) connected to the split/splitless injector.

2.3. Chromatographic and detection conditions

Initial experiments to optimise the gas-chromatographic and MS detection conditions were carried out by direct injection of 1 μl of the standard mix in ethanol. The optimised oven temperature program was 50 °C (0.5 min) to 150 °C at 25 °C/min, then 150 °C to 270 °C at 3 °C/min (final temperature held for 1 min). A column head pressure of 15 p.s.i. and an injector temperature of 250 °C were used (1 p.s.i. = 6894.76 Pa). The GC transfer line was maintained at 280 °C. The mass spectrometer was operated in the electron impact positive ion (EI^+) mode with a source temperature of 200 °C. The electron energy was 70 eV and the filament current 200 μA.

Mass spectra were acquired in the mass range from m/z 50 to 450, using a scan time of 0.9 s and an inter-scan time of 0.1 s. Detection of analytes was also accomplished in selected ion monitoring (SIM) mode, using the following fragment ions and time windows: m/z 128, 210, 293, (triadimefon), from 23 to 25 min; 145, 173, 259 (propiconazole), from 25 to 28 min; 179, 206, 288 (myclobutanil), from 28 to

32.5 min; 159, 161, 248 (penconazole), from 32.5 to 37 min. The dwell time and the mass span were 0.2 s and 0.4 u, respectively, for each fragment.

2.4. Solid-phase microextraction

A silica fibre coated with 85 μm thick polyacrylate (PA) film and a manual SPME device (Supelco) were employed as described elsewhere [8]. One fibre was used throughout all the experimental work. Standard solutions were prepared by spiking 5 ml of triply distilled water into 7 ml clear vials (Supelco). Then, the vials were sealed with hole caps and PTFE-faced silicone septa (Supelco). The extraction was carried out at 50 °C for 45 min under magnetic stirring in order to improve mass transfer from the aqueous sample into the fibre coating. Thermal desorption (5 min desorption time) was performed directly into the GC injection port maintained at 250 °C.

2.5. Food samples

2.5.1. Strawberries

Samples were purchased from a local store. Then, 50 g were homogenized and centrifuged for 30 s at 10 000 rev/min; then, a 25 g aliquot of the homogenate was transferred into a 50 ml PTFE tube, mixed with 40 ml of tridistilled water and centrifuged again at 5000 rev/min for 20 min. The aqueous phase was then recovered and brought to 100 ml with an aqueous solution containing 0.2 g/ml of NaCl. Finally, 5 ml of the resulting solution were transferred in a 7 ml clear vial and subjected to SPME. The remaining solution was stored at 4 °C in the dark.

2.5.2. Wine

Wine samples were purchased from a local store. Samples were filtered through a 0.45 μm Millex-HV type filter and diluted 1:2 with an aqueous solution containing 0.2 g/ml of NaCl. Finally, 5 ml of the resulting solution were transferred in a 7 ml clear vial and subjected to SPME.

Quantitation was performed by the standard addition method. Three standard additions performed covering one concentration decade (duplicate measurements on each addition).

3. Results and discussion

3.1. GC–MS

Preliminary experiments were performed by direct injection of triazoles for GC–MS conditions optimisation; the temperature programme developed was capable of a good separation of the investigated analytes, including the two geometric stereoisomers of propiconazole.

Fig. 2 shows the EI^+ mass spectra of the selected triazoles. As apparent, molecular ions were observed, even though of low intensities, especially in the case of penconazole. The choice of the ions for SIM acquisition was made in order to obtain the best S/N ratios. For instance, as far as triadimefon is concerned, the m/z ion 210 was selected instead of the more intense m/z ion 208, since the latter was very abundant in the background noise.

3.2. SPME conditions

The extraction time profiles were established by plotting the area counts versus the extraction time. Three different extraction temperatures, room temperature (ca. 20 °C), 50 and 80 °C, were explored. Fig. 3, reports the results obtained at 50 °C. As apparent, the equilibrium was almost reached after about 120 min for all the analytes; this behaviour was observed at all the investigated temperatures. Higher absolute responses were observed with increasing temperatures; however, when working at 80 °C data repeatability deteriorated heavily. Thus, 50 °C was chosen as optimal working temperature. As far as the equilibrium time is concerned, satisfactory and reproducible extraction yields were also obtained in non-equilibrium conditions. In fact, the amount of the analyte extracted into the fibre is proportional to the initial concentration in the sample matrix, provided the mass transport conditions and the sampling time are strictly controlled; hence, SPME quantitation is feasible [12] even before equilibrium is reached. In the present case, an extraction time of 45 min revealed a good compromise between sample throughput and peak response and was then chosen for further experiments.

Generally speaking, salt addition often improves the SPME recovery, especially in the case of polar (hydrophilic) compounds. Indeed, the addition of

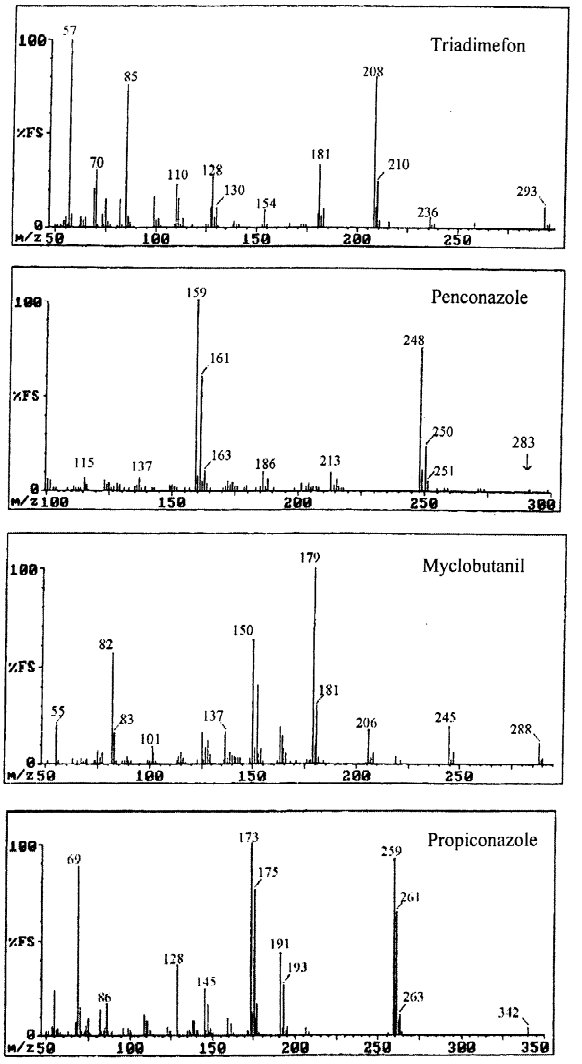

Fig. 2. Electron impact mass spectra of the selected triazoles. Source temperature: 200 °C; electron energy: 70 eV; filament current 200 μA. Spectra were acquired in the mass range from *m*/*z* 50 to 450 (scan time: 0.9 s; inter-scan time: 0.1 s).

sodium chloride (0.2 g/ml) to the aqueous sample produced a 2- to 3-fold increase in the extraction yields of triazoles. Since sodium chloride concentration higher than 0.2 g/ml produced only marginal effects on the extraction efficiency, this was chosen as the optimal value.

As expected on the chemical structures of the investigated triazoles, pH had no effect on the response.

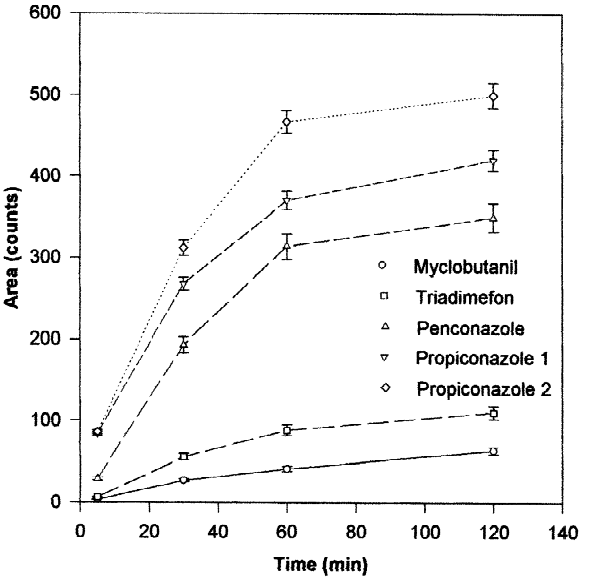

Fig. 3. Area counts (a.u.) vs. extraction time obtained at 50 °C. GC conditions as specified under experimental conditions. MS operated in scan mode.

Desorption time and temperature were optimized in order to prevent ''carry-over'' of the analytes; 5 min at 250 °C were found to be the ideal conditions.

Fig. 4 shows a comparison between two GC–MS-SIM chromatograms relevant to (a) the direct injection of a standard solution of triazoles at a concentration level of 5 μg/kg; and (b) the SPME of the same solution, respectively. Based on the *S*/*N* of the two chromatograms, the described SPME procedure provided an enrichment factor of about 10^2.

3.3. Linear range, detection limits and precision

The dynamic range of the developed SPME–GC–MS procedure (SIM mode) resulted linear for all the analytes over at least two concentration decades, with correlation coefficients better than 0.999 and intercepts not significantly different from zero according to a *t*-test at 95% confidence level.

Detection limits, estimated at a signal-to-noise ratio of 3, ranged between 30 ng/kg (propiconazole) and 100 ng/kg (triadimefon).

Replicate measurements were performed daily, for 5 days on standard solutions of triazoles at a

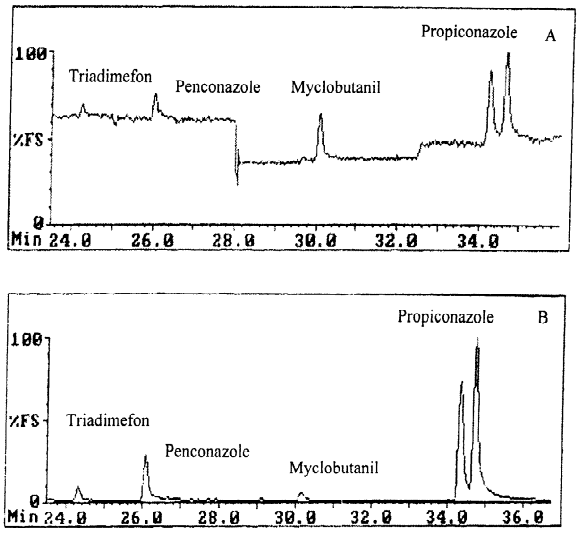

Fig. 4. GC–MS (SIM) chromatograms relevant to (A) the direct injection of a standard solution of triazoles at a concentration level of 5 μg/kg and (B) the SPME of the same solution, respectively. Changes in baseline levels observed in (A) are due to instrumental artefacts originating from SIM acquisition in defined time windows (see Experimental section).

concentration level of 5 μg/kg in order to estimate the within-day ($n=5$) and day-to-day ($n=3$) precision. The relevant results are shown in Table 1.

3.4. Food samples

The developed method has been then applied to the triazoles determination in food samples, treated as described in the Experimental section.

Fig. 4A shows the SPME–GC–MS-SIM chromatogram relevant to the analysis of a centrifuged strawberries sample. As clearly shown, all the target analytes, with the exception of propiconazole, were detectable in the sample and appeared completely separated from interfering peaks.

Table 1
Within-day ($n=5$) and day-to-day ($n=3$) precision obtained for standard triazoles at 5 μg/kg

Compound	RSD (%)	
	Within-day precision	Day-to-day precision
Triadimefon	5	20
Penconazole	11	17
Myclobutanil	11	28
Propiconazole 1	3	7
Propiconazole 2	2	8

Table 2
Triazoles concentrations estimated in the considered food samples

Compound	Concentration (μg/kg)		
	Strawberries	Wine 1	Wine 2
Triadimefon	11.2	–	–
Penconazole	5.6	1.1	–
Myclobutanil	17.6	–	–
Propiconazole 1	–	1.0	1.7
Propiconazole 2	–	0.8	0.4

Fig. 4B shows the SPME–GC–MS-SIM chromatogram relevant to the analysis of ''wine 1'' sample. As apparent, the peculiar signals of the stereoisomers of propiconazole and the peak of penconazole were observed.

Table 2 resumes the concentrations of triazoles in the food samples estimated by the standard addition method. This approach eliminates any matrix effect and any sample-to-sample variability.

4. Conclusions

In the present paper an SPME–GC–MS method for the determination of selected triazoles has been developed and applied to the analysis of strawberries and wine samples (Fig. 5). The procedure is solvent-free, simple and highly sensitive. It is worth of note that detection limits achieved by the described method are well below the maximum residue limits (MRLs) fixed for wine (or grapes) and strawberries by the European legislation [13,14] (see e.g. Directives 90/642/CE and 86/362/CE). Examples of MRLs are: 200 and 100 μg/kg myclobutanyl in strawberries and wine, respectively; 100 μg/kg penconazole in strawberries; 500 μg/kg propiconazole in grapes (50 μg/kg in most fruits). Consequently, the described procedure can be conveniently used as a rapid screening method for contamination assessment; only those samples giving a ''positive'' response can be analysed by an official method, for assessing their compliance with legal limits.

The developed procedure could be also potentially

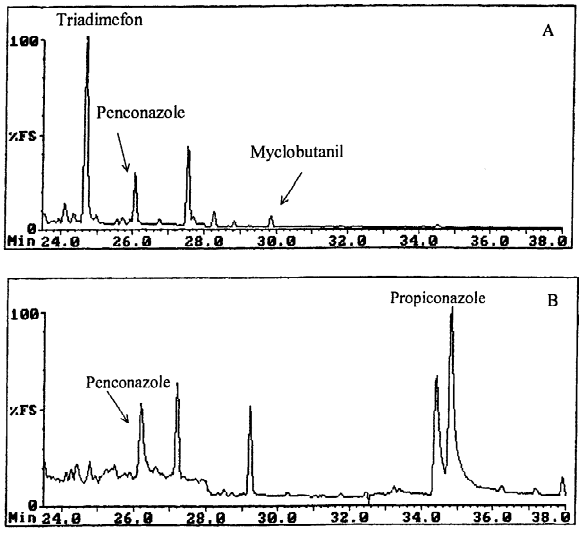

Fig. 5. SPME–GC–MS (SIM) chromatograms obtained on (A) strawberries and (B) wine samples. For sample pre-treatment refer to the Experimental section.

applied to other food commodities. Work in this direction is currently in progress.

Acknowledgements

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