

Short communication

# Determination of pyrimethanil and kresoxim-methyl in green groceries by headspace solid-phase microextraction and gas chromatography–mass spectrometry

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## Abstract

A method for determination of trace amounts of the fungicides pyrimethanil and kresoxim-methyl in green groceries, previous headspace solid-phase microextraction (HSSPME), was developed using gas chromatography–mass spectrometry and selected ion monitoring (GC–MS, SIM). Both fungicides were extracted with a fused-silica fiber coated with 85  $\mu\text{m}$  polyacrylate. The effects of pH, ionic strength, extraction and desorption times as well as the extraction temperature were studied. The linear concentration range of application was 12.5–250  $\text{ng g}^{-1}$  for both compounds, with detection limits of 1.8–2.0  $\text{ng g}^{-1}$  for pyrimethanil and 2.8–3.1  $\text{ng g}^{-1}$  for kresoxim-methyl. SPME/GC–MS analysis yielded good reproducibility (RSD between 7.4 and 15.0%). It was applied to check the eventual existence of pyrimethanil and kresoxim-methyl above the detection limits on grapes, strawberries, tomatoes and ketchup samples. The method validation was completed with spiked matrix samples. It can be applied as a monitoring tool in grapes, strawberries, tomatoes and ketchup samples.

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## 1. Introduction

Pyrimethanil, *N*-(4,6-dimethylpyrimidin-2-yl)aniline (Fig. 1), a novel anilino-pyrimidine fungicide (AgrEvo) is used for the control of grey mould and leaf scab on grape, strawberry, tomato, fruit,

vegetables and ornamentals [1]. Kresoxim-methyl, methyl- $\alpha$ -(methoxyimino)-2-[(2-methylphenoxy)-methyl]phenyl acetate (Fig. 1) a recently developed

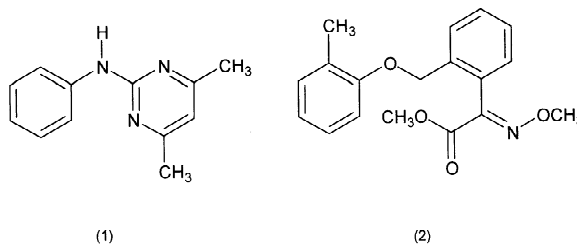


Fig. 1. Chemical structure of (1) pyrimethanil and (2) kresoxim-methyl.

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strobilurin fungicide (BASF) is used for the control of powdery mildew and scab in apples, pears, grapes, tomatoes, strawberries and vegetables.

Few analytical methods have been reported for the determination of these fungicides [2–5].

Since its introduction, solid-phase microextraction (SPME) has gained popularity as a simple, solvent-free, reliable and flexible tool for the sampling of a variety of volatile and semivolatile compounds.

SPME has been extensively used for direct extraction of pesticides from aqueous samples [6–10]. On the other hand, food like fruit and vegetable are mostly in a solid and heterogeneous form, not allowing a direct extraction. However, the SPME fibre can also be suspended in the headspace above the homogenised sample. This option named headspace solid-phase microextraction (HSSPME) eliminates interference problems because the fibre is not in contact with the complex matrices of fruits and vegetables. Several classes of chemical compounds have been extracted from complexes matrixes with HSSPME [11–15]. To date, pyrimethanil and kresoxim-methyl have not been investigated by direct or headspace SPME.

This paper describes the application of HSSPME in combination with GC–MS for the determination of fungicides pyrimethanil and kresoxim-methyl in grapes, strawberries, tomatoes and ketchup.

## 2. Experimental

### 2.1. Materials

A manual fibre holder for SPME was purchased from Supelco (Bellefonte, PA, USA). Three types of fiber, 100  $\mu\text{m}$  polydimethylsiloxane (PDMS), 85  $\mu\text{m}$  polyacrylate (PA), and 75  $\mu\text{m}$  carboxen/polydimethylsiloxane (CAR/PDMS) were obtained from the same manufacturer. The fibres were conditioned as recommended by the manufacturer.

All reagents were of analytical-reagent grade unless stated otherwise. Water was purified with a Milli-Q plus system (Millipore). Pyrimethanil (99.5%) and Kresoxim-methyl (99.5%) were supplied by Riedel-de Haën (Seelze, Germany). Working solutions were obtained by appropriated dilutions with methanol.

Grapes, strawberries, tomatoes and ketchup commercially available were used.

We used a magnetic stirrer/temperature-controlled oil bath (Agimatic-N, Selecta, Spain) during the sampling process. A blender was used to homogenise the fruit samples (Phillips)

The Statgraphics [16] software package was used for the statistical analysis of the data and regression analysis (linear model).

### 2.2. Instrumentation

A Hewlett-Packard system consisting of a 5890 GC system fitted with a splitless injector for the HP-1 fused-silica capillary column (30 m $\times$ 0.25 mm I.D., 0.25  $\mu\text{m}$  film thickness), a 5971 mass spectrometer, a HP-UX Chemsystem computer and the proprietary software was used. A silanized narrow-bore injector liner (0.75 mm I.D.) for the SPME injections was installed and the fiber was inserted into this injector using the splitless mode with the split closed for 3 min. The injector temperature was set at 250  $^{\circ}\text{C}$  and the transfer line temperature was 280  $^{\circ}\text{C}$ . The oven temperature was held at 100  $^{\circ}\text{C}$  for 3 min, then heated to 300  $^{\circ}\text{C}$  at a heating rate of 30  $^{\circ}\text{C min}^{-1}$ . Temperature was held at 300  $^{\circ}\text{C}$  for 1.5 min. The carrier gas was helium (purity 99.999%) at a flow-rate of 2 ml  $\text{min}^{-1}$ . The mass spectrometer detector was tuned by maximum sensitivity autotune. The following  $m/z$  values were acquired in the electron impact ionization mode by single ion monitoring and used for quantification of the fungicides: 116, 131 and 206 for kresoxim-methyl and 198 for pyrimethanil.

### 2.3. Procedures for the determination of pyrimethanil and kresoxim-methyl

Samples were blended and then the spiked matrix samples were prepared by adding the appropriate standard in methanol to the homogenised matrix. Samples were agitated for 60 min to ensure that pyrimethanil and kresoxim-methyl were well distributed. Grapes, tomatoes, ketchup and strawberry homogenates were diluted with buffer Britton-Robinson 0.2 M (dilution 1:2 in weight) and the pH was adjusted to 7.0.

An aliquot of 6.00 g of diluted sample was placed

in a 14 ml vial. Then, 2.16 g of NaCl was added and agitated, the vial was sealed with a headspace aluminium cap with a PTFE-faced septum. The vial was immersed in a temperature-controlled oil bath during the sampling process at 100 °C. The samples were agitated with a magnetic stirring bar at 500 rpm or  $\text{rev min}^{-1}$  during the HSSPME experiments. The polyacrilate fibre was exposed into the headspace for 25 min. After the extraction, the fiber was directly exposed to the hot injector of the gas chromatograph for 5 min and the chromatogram was acquired.

Calibration graphs in green groceries were thus constructed using solutions of pyrimethanil and kresoxim-methyl of known concentrations.

### 3. Results and discussion

#### 3.1. Optimization of conditions for SPME

Preliminary experiments were carried out to optimise the main parameters affecting the SPME of the fungicides investigated. In these studies Milli-Q water samples spiked with the appropriate amount of the standard solution were used.

The more adequate fiber was found by comparing the extraction behaviour on three commercial SPME fibers, PDMS, PA and CAR/PDMS at NaCl concentration over the range 0–0.36  $\text{g ml}^{-1}$ . PA fibre in combination with 0.36  $\text{g ml}^{-1}$  NaCl exhibited the highest extraction performance and was chosen for the rest of experiments.

The effect of pH on the extraction yield was investigated by changing the pH in the range 3.0–10.0. The extraction of kresoxim-methyl is practically independent of pH variation. In the case of pyrimethanil the response is higher when  $\text{pH} > 7.0$ . The relation between the pH and extraction yield is as expected, because pyrimethanil is mainly in its neutral form when the pH is neutral or basic. A value pH of 7.0 was chosen to prevent the possible hydrolysis of fungicides at conditions more basic.

The effect of temperature was monitored by extracting samples of 10  $\text{ng ml}^{-1}$  of pyrimethanil and kresoxim-methyl in the range of 25–100 °C. A clear increase in the amount of the analytes extracted was observed with increasing temperature up to 100 °C. Therefore, extractions were carried out at

$100 \pm 2$  °C. It must be pointed out that under the experimental conditions used in this work (100 °C and 25 min extraction), hydrolysis of many pesticides will take place. These extraction conditions can then be used only with stable pesticides such as pyrimethanil and kresoxim-methyl.

Extraction time profiles were studied extracting samples of 12.5  $\text{ng ml}^{-1}$  of pyrimethanil and kresoxim-methyl and monitoring the GC area counts as a function of exposure time. Equilibrium was not attained even after 200 min (see Fig. 2). In this case to be viable the analytical method and to keep a reasonable extraction time, operation under nonequilibrium conditions is necessary. Therefore, 25 min extraction time is a reasonable compromise between an acceptable time and adequate response of pyrimethanil and kresoxim-methyl.

To study the carryover effect, blanks were run after extractions of 50  $\text{ng ml}^{-1}$  of pyrimethanil and kresoxim-methyl. No signals were obtained when a 5 min desorption time was chosen, which ensured a complete desorption of pyrimethanil and kresoxim-methyl.

#### 3.2. Application and validation of the proposed method

##### Calibration graphs for Milli-Q water samples

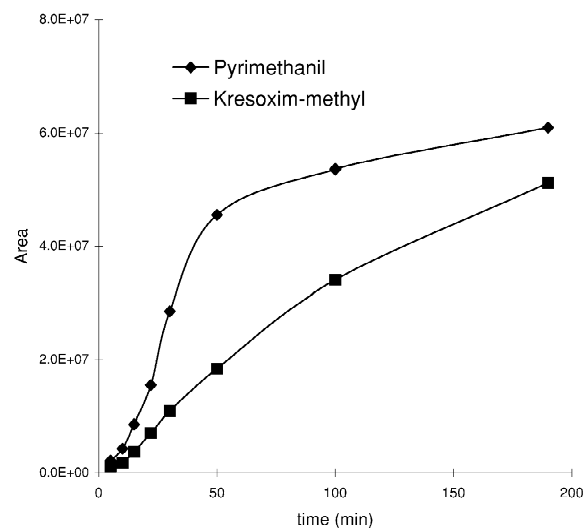


Fig. 2. Absorption time profile of 12.5  $\text{ng ml}^{-1}$  of pyrimethanil and kresoxim-methyl in Milli-Q water with polyacrilate fiber.

treated according to the procedure described previously, monitored using SIM mode, are linear for the concentration range 2.0–64.3 ng ml<sup>-1</sup> ( $r=0.9987$  and 0.9982 for pyrimethanil and kresoxim-methyl, respectively). The *lack-of-fit* test [17] was used to check the linearity of the calibration graph. The test is performed by comparing the variability of the current model residuals to the variability between observations at replicate values of the independent variable  $x$ . Since the  $P$ -values obtained ( $P=0.14$  and 0.33) are greater than 0.10, the linear model appears to be adequate for the observed data. Two replicates were used for each of six prepared standards to obtain the calibration graph. The equations for the calibration graphs were  $y = 688327x - 604732$  for pyrimethanil and  $y = 227424x + 2496$  for kresoxim-methyl.

The optimum headspace SPME sampling conditions for Milli-Q water were applied to the grape, strawberry, tomato and ketchup matrices. Table 1 shows regression equations for pyrimethanil and kresoxim-methyl extracted from matrices studied. The calibration graphs, monitored using SIM mode, are linear in the ranges shown in the table. The *lack-of-fit* test was used to check the linearity of the calibration graph. Two replicates were used for each of five prepared standards to obtain each calibration graph.

Absolute response was lower with green groceries samples than with Milli-Q water. This might be due to the presence of suspended matter as well as dissolved compounds that may interfere with diffusion

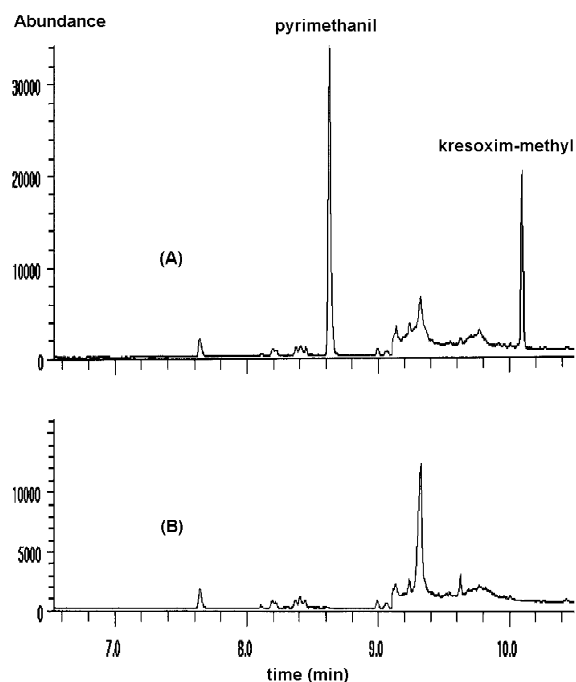


Fig. 3. (A) Chromatogram of spiked ketchup sample at 12.5 ng g<sup>-1</sup> of pyrimethanil ( $m/z$  198) and 25.0 ng g<sup>-1</sup> of kresoxim-methyl ( $m/z$  116–131 and 206). (B) Chromatogram of blank ketchup sample.

and volatilization of analytes decreasing the performance extraction by HSSPME. To improve the extraction yield, samples were diluted with buffer solution.

Quantification of pyrimethanil and kresoxim-

Table 1

Analytical parameters for pyrimethanil and kresoxim-methyl in green groceries samples

Analyte	Sample	Regression parameters <sup>a</sup>			LOD (ng g <sup>-1</sup> )	LOQ (ng g <sup>-1</sup> )	Reproducibility (SDR, %), $n=8$ at 6.25 and 50.0 ng g <sup>-1</sup>
		$a$	$b$	$r$			
Pyrimethanil	Grape	85657	42909	0.9939	1.9	5.7	9.1–7.4
	Strawberry	9122	50862	0.9950	2.0	6.1	9.8–7.8
	Tomato	-202980	94667	0.9965	1.8	5.5	8.8–8.1
	Ketchup	-31511	44099	0.9962	2.0	6.0	9.6–7.6
Kresoxim-methyl	Grape	51957	7582	0.9936	3.1	9.4	15.0–8.8
	Strawberry	77767	10741	0.9963	3.0	9.0	14.4–9.8
	Tomato	-63341	16406	0.9958	2.8	8.5	13.9–8.3
	Ketchup	40996	8902	0.9969	2.9	8.7	13.6–10.0

<sup>a</sup> Referred to range linear 12.5–250 ng g<sup>-1</sup>.

methyl concentrations in green groceries was carried out by the standard addition method because matrix effects were observed. Another option would be the addition of an isotopically labelled analyte as internal standard to the sample [18]. Unfortunately deuterated pyrimethanil and kresoxim-methyl were not available and the last method could not be used.

The precision was measured by performing eight independent determinations. HSSPME/GC–MS analysis yielded good reproducibility (RSD between 7.4 and 15.0%).

The method detection limit and quantification limit were estimated on the basis of results for the 6.25 ng g<sup>-1</sup> concentrations of pyrimethanil and kresoxim-methyl. Limits of detection (LOD) and limits of quantification (LOQ) were calculated with the formula  $LOD=3.33\times SD$  and  $LOQ=10\times SD$ , where

SD is the standard deviation of replicate analysis. The detection limits found were between 1.8 and 3.1 ng g<sup>-1</sup> for pyrimethanil and kresoxim-methyl (see Table 1).

Fig. 3 shows typical chromatograms for HSSPME/GC–MS of a ketchup blank and a ketchup spiked with 12.5 ng g<sup>-1</sup> of pyrimethanil and 25.0 ng g<sup>-1</sup> of kresoxim-methyl.

Validation for spiked green groceries samples was tested using a one-sample test (Student's *t*-test) [19]. We tried to find pyrimethanil and kresoxim-methyl in grapes, strawberries, tomatoes and ketchup acquired in a supermarket. We did not find pyrimethanil and kresoxim-methyl above our detection limit.

Samples were fortified with different levels of pyrimethanil and kresoxim-methyl. Quantification of analytes was completed by the standard additions

Table 2

Results of assays to check the accuracy of the proposed method for pyrimethanil and kresoxim-methyl in spiked grapes, strawberry, tomato and ketchup samples

Analyte	Sample	Spiked (ng g <sup>-1</sup> )	Found <sup>a</sup> (ng g <sup>-1</sup> )	<i>t</i>	<i>P</i> <sup>b</sup>	% Recovery <sup>c</sup>
Pyrimethanil	Grape	12.5	12.8±1.3	0.52	0.62	102.4
		25.0	26.8±3.1	1.40	0.22	107.2
		50.0	47.4±5.0	1.28	0.25	94.8
	Strawberry	12.5	11.5±1.6	1.46	0.20	92.0
		25.0	26.5±2.4	1.57	0.18	106.0
		50.0	52.5±3.1	1.94	0.11	105.0
	Tomato	12.5	11.3±1.1	2.6	0.05	90.4
		25.0	22.6±2.9	2.01	0.10	90.4
		50.0	46.9±5.5	1.35	0.23	93.8
	Ketchup	12.5	11.4±1.3	2.11	0.09	91.2
		25.0	26.6±3.0	0.54	0.61	106.4
		50.0	52.3±5.5	1.02	0.35	104.6
Kresoxim-methyl	Grape	25.0	23.3±2.3	1.79	0.13	93.2
		50.0	52.4±4.7	1.22	0.27	104.8
		75.0	72.6±3.4	1.73	0.14	96.8
	Strawberry	25.0	22.9±2.2	2.31	0.07	91.6
		50.0	48.3±3.9	1.10	0.34	96.6
		75.0	69.5±8.7	1.56	0.18	92.7
	Tomato	25.0	22.9±3.2	1.65	0.16	91.6
		50.0	47.9±3.9	1.31	0.25	95.8
		75.0	77.2±5.0	1.09	0.32	102.9
	Ketchup	25.0	24.4±3.5	0.40	0.70	97.6
		50.0	48.5±3.2	1.15	0.30	97.0
		75.0	71.9±5.2	1.45	0.20	95.9

<sup>a</sup> Average value±standard deviation of six determinations.

<sup>b</sup> *P*-value of the one-sample comparison test.

<sup>c</sup> “% Recovery” refers to the pyrimethanil and kresoxim-methyl concentrations determined rather than the actual percent of analytes extracted by the SPME analysis.

method. The *P*-values calculated, in all cases, are greater than 0.05 and therefore the null hypothesis might be accepted (Table 2).

The HSSPME method proposed is more sensitive than liquid extraction–gas chromatographic methods described for the analysis of residues of pyrimethanil and kresoxim-methyl in grapes [2,3]. The authors reported limits of quantification of 50 ng g<sup>-1</sup> of the fungicides.

#### 4. Conclusions

A simple and practical GC–MS method in combination with HSSPME for the determination of the fungicides pyrimethanil and kresoxim-methyl in grapes, strawberries, tomatoes and ketchup samples is presented. Sensitive responses were obtained using a 85 µm polyacrylate fibre, 0.36 g/ml NaCl, 25 min extraction time, pH 7.0 and 100 °C in combination. Non-equilibrium conditions were adopted in order to reduce the extraction time. Matrix effects can be avoided with the use of the standard addition method for quantitation. In view of its simplicity and sensitivity, the present method is applicable for the quantification of residues of pyrimethanil and kresoxim-methyl in grapes, strawberries, tomatoes and ketchup samples.

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