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Determination of oxadiazon residues by headspace solid-phase microextraction and gas chromatography–mass spectrometry

Alberto Navalón, Avismelsi Prieto[1], Lilia Araujo[2], José Luis Vílchez*

Department of Analytical Chemistry, University of Granada, Avda. Fuentenueva s/n, E-18071 Granada, Spain

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Abstract

A method for the determination of trace amounts of the herbicide oxadiazon was developed using headspace solid-phase microextraction (HS-SPME), gas chromatography–mass spectrometry (GC–MS) and selected ion monitoring. It was applied to determine oxadiazon in ground water, agricultural soil, must, wine and human urine samples. To determine oxadiazon in liquid samples, a response surface methodology generated with a Doehlert design was applied to optimize the HS-SPME conditions using a 100 μm polydimethylsiloxane fibre. For the analysis of soil samples, they were mixed with water and the SPME fibre suspended in the headspace above the slurry. Ground water, human urine and must show linear concentration range of application of 0.5–50 ng ml^{-1} with detection limits ≤0.02 ng ml^{-1}. HS-SPME–GC–MS analysis yielded good reproducibility (RSD values between 6.5 and 13.5%). The method validation was completed with spiked matrix samples. The developed analytical procedure is solvent free, cost effective and fast.

Keywords: Soil; Environmental analysis; Water analysis; Food analysis; Wine; Headspace analysis; Factorial analysis; Oxadiazon; Pesticides

1. Introduction

The use of pesticides yields increased agricultural outputs. However, slow degradation of pesticides in the environment and improper usage by farmers could lead to environmental contamination in water, soil, air, several types of crops and biological samples, e.g. urine and blood [1]. Oxadiazon, 5-*tert.*-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3H)-one, is a selective contact herbicide used in pre- and post-emergence control of bindweed, annual broad-leaved weeds and grasses, in fruit trees, vines, cotton, rice, onions, soy beans, sunflowers, roses and ornamental trees [2].

Ambrosi et al. [3] studied its degradation in various types of soil and in an aquatic model ecosystem where the residue could be easily transferred through the biological chain. They found that oxadiazon degraded slowly in all soils tested. Li and Wong [4] also found that 73–96% of the applied oxadiazon remained after 144 days in three soils duly saturated with water or flooded.

*Corresponding author. Tel.: +34-958-243-326; fax: +34-958-243-328.

E-mail address: jvilchez@ugr.es (J. Luis Vílchez).

[1]Current address: Faculty of Agronomy, University of Zulia, PO Box 6610, Maracaibo, Venezuela.

[2]Current address: Faculty of Engineering, University of Zulia, PO Box 10259, Maracaibo, Venezuela.

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The most time consuming and difficult task in chemical analysis of oxadiazon is the extraction and purification of the analyte from their matrices, especially for a complicated matrix like soil. The most commonly used procedures for the analysis of oxadiazon are liquid–liquid extraction and solid-phase extraction in combination with GC or HPLC. These techniques have been applied to the analysis of oxadiazon in water, soils, grapes, wine and hops samples [5–8].

Solid-phase microextraction (SPME) is an advance in the samples preparation for trace analysis. Because organic compounds are adsorbed directly from an aqueous or gaseous sample onto a fibre coated with an appropriate stationary phase, and then the fibre needle can be directly injected into a GC injection port for analysis. The SPME fibre can also be suspended in the headspace above the aqueous or solid sample. This option, named headspace SPME (HS-SPME), eliminates interference problems because the fibre is not in contact with the sample [9]. Direct sampling has been considered superior to headspace sampling for semivolatile compounds, although the elimination of sample matrix–fibre interferences provided by headspace methods can be invaluable. For this reason, the method developed in this work is for headspace analysis. HS-SPME has been used in the determination of some pesticides in different matrices [10–13].

This paper describes the development and application of HS-SPME in combination with GC–MS for the determination of oxadiazon in water and soil samples, must, wine and human urine. A response surface methodology has been used to optimize the conditions to determine oxadiazon in liquid samples. We demonstrate that the optimization provides good linear ranges and improved detection limits.

2. Experimental

2.1. Materials

A manual fibre holder for SPME was purchased from Supelco (Bellefonte, PA, USA). Polydimethylsiloxane (PDMS) fibres (100 μm) were obtained from the same manufacturer. The fibres were conditioned as recommended by the manufacturer.

A magnetic stirrer/temperature-controlled oil bath (Agimatic-N, Selecta, Spain) was used during the sampling process.

The STATGRAPHICS [14] software package was used for the design and evaluation of the chemometric studies, the statistical analysis of the data and regression analysis.

All reagents were of analytical-reagent grade unless stated otherwise. Water was purified with a Milli-Q plus system (Millipore). Oxadiazon (99.5%) was supplied by Dr. Ehrenstorfer (Leverkusen, Germany). Working solutions were obtained by appropriate dilutions with methanol.

2.2. Instrumentation

A Hewlett-Packard (HP) system consisting of a 5890 GC system fitted with a splitless injector for the HP-1 fused-silica capillary column (30 m×0.25 mm I.D., 0.25-μm film thickness), a 5971 mass spectrometer, a HP-UX A 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 fibre was inserted into this injector using the splitless mode with the split closed for 3 min. The injector temperature was set at 250 °C and the transfer line temperature was 280 °C. The oven temperature was held at 75 °C for 3 min, then heated to 270 °C at a heating rate of 30 °C min^{-1}, followed by holding at 270 °C for 3 min. The carrier gas was helium (purity 99.999%) at a flow-rate of 2 ml min^{-1}. The mass spectrometer detector was tuned by maximum sensitivity autotune. Values of 175 and 258 m/z were selected in the electron impact ionization mode by selected ion monitoring (SIM).

2.3. Procedures for the determination of oxadiazon by HS-SPME

A 7.00-ml volume of water, human urine, must or wine was placed in a 14-ml vial; in the case of wine samples, they were diluted 2:1 with deionized water. For the HS-SPME analysis, the spiked matrix samples were prepared by adding $<0.1\%$ of the appropriate standard in methanol to the aqueous matrix and then homogenized. A 2.52-g amount of NaCl was added and agitated, then 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 700 rpm during the HS-SPME experiments. The PDMS fibre was exposed to the headspace over the liquid for 25 min. After the extraction, the fibre was directly exposed to the hot injector of the GC for 5 min and the chromatogram was registered.

Calibration graphs were thus constructed using solutions of oxadiazon of known concentrations. Air-dried soil samples were spiked with oxadiazon standard in methanol and to ensure that oxadiazon was well distributed, a reasonable volume of acetone was added and carefully agitated. These samples were allowed to stand for 24 h prior to analysis. A 0.75-g amount of soil was added into a 4.7-ml vial, 1.00 ml of water was added and a 1-cm stir bar was placed into the vial, which was sealed. Then the procedure was identical to the case of aqueous samples.

3. Results and discussion

3.1. Experimental design

The extraction temperature, headspace volume and addition of NaCl were included as variables in a factorial design response surface. Other variables affecting adsorption, such as extraction time and fibre type were tested in preliminary studies. Extraction time profiles were studied extracting samples of 10 ng ml^{-1} of oxadiazon. Equilibrium was not obtained even after 90 min. Therefore, a 25-min extraction time was adopted, even though oxadiazon had not reached partition equilibrium at this time point, but the analytical sensitivity was sufficient. An exposure time of 25 min is a reasonable compromise between acceptable time and a good response of oxadiazon. For quantitative analysis it is not necessary for the analyte to have reached equilibrium, but only for sufficient loading onto the fibre and reproducible extraction times [9]. Among the SPME fibres tested, 100 μm PDMS proved to be the most satisfactory phase for this study. Sample pH was not adjusted because there were no differences in response when acidic, neutral or basic pH values were used. The desorption temperature and desorption time were as in a previous paper [15].

For the three factors selected, a Doehlert design was used. This model allows the direct evaluation of the variables considered, and also the first and second order interaction terms. The response surface experiment with the levels of the factors is listed in Table 1. The complete design consisted of 16 experiments including the central point repeats. The

Table 1
Doehlert experiment matrix and response values

Experiment no.	Headspace volume (%)	Extraction temperature (°C)	NaCl (g ml^{-1})	Results (counts)
1	80	70	0.18	364 688
2	40	70	0.18	953 671
3	70	100	0.18	3 708 465
4	50	40	0.18	134 771
5	70	40	0.18	163 176
6	50	100	0.18	4 657 587
7	70	80	0.36	2 104 522
8	50	60	0	72 153
9	70	60	0	228 472
10	60	90	0	1 172 328
11	50	80	0.36	2 932 386
12	60	50	0.36	287 243
13	60	70	0.18	983 137
14	60	70	0.18	1 163 270
15	60	70	0.18	1 022 046
16	60	70	0.18	1 095 714

experiments shown in Table 1 were carried out in a random order with a standard solution containing 10.0 ng ml^{-1} of oxadiazon.

The extraction temperature was varied from 40 to 100 °C, the NaCl concentration from 0 to 0.36 g ml^{-1} and the headspace volume from 40 to 80%. The results obtained are shown in Table 1. The application of analysis of variance (ANOVA) on the results indicated that the experimental variables have an important influence on the result. The coefficient of multiple regression for the calculated model was 0.993. This indicates that the model as fitted explains 99.3% of the variability in the peak signal area. The lack-of-fit test is designed to determine whether the selected model is adequate to describe the observed data, or whether a more complicated model should be used. Since the *P* value for lack-of-fit test obtained is >0.05, the model appears to be adequate for the observed data at the 95.0% confidence level.

Fig. 1A shows the response surface developed by the model considering percentage headspace and NaCl concentration. The response is maximum between 40 and 55% headspace when the concentration of NaCl is at highest level. The effect of interaction is shown in Fig. 1B, where it is evident that the effect of NaCl concentration is relevant only when the temperature is at the higher level. Fig. 1C shows that the response is maximum when the percentage headspace is at its lowest level and the temperature is at 100 °C. These results give a clear demonstration of the usefulness of the multivariate approach. Since the three interacting variables are already at the limits of their possible values, the optimization can be considered as concluded. The optimal conditions tended to 0.36 g ml^{-1} of NaCl, 100 °C and 50% headspace volume.

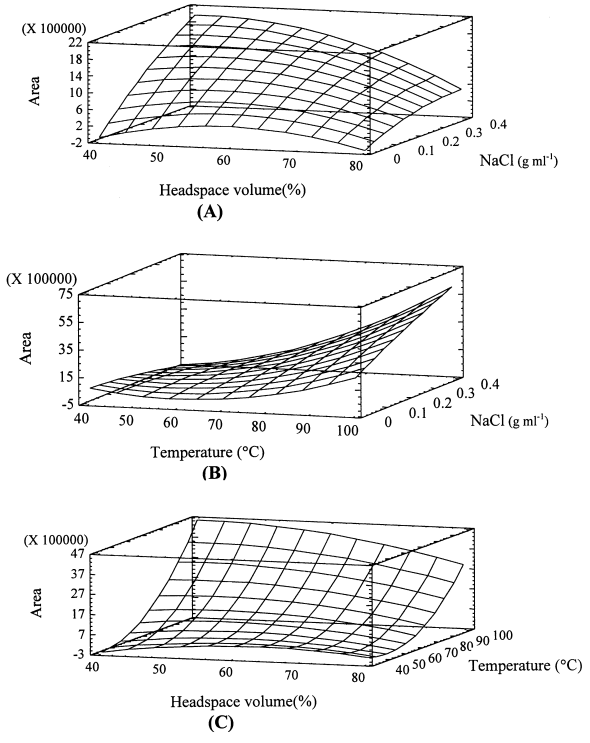

Fig. 1. Response surface for the Doehlert design. (A) NaCl concentration vs. percentage headspace. (B) Temperature vs. NaCl concentration. (C) Percentage headspace vs. temperature.

Under the experimental conditions used in this work (100 °C and 25 min of extraction), hydrolysis of many pesticides will take place. These extraction conditions can therefore be used only with stable pesticides such as oxadiazon.

3.2. Optimization of conditions for soil samples

The extraction of oxadiazon from soil samples was realized using the 100 μm PDMS fibre, 100 °C and 25 min of extraction. The quantity of water added to 0.75 g of soil was investigated next. The experiments were carried out with the addition of water between 0 and 2 ml. Although water vapor in the headspace during the extraction is expected to reduce the sensitivity of SPME slightly [16], a favourable effect of water addition to the soil samples was observed. The results exhibit a maximum response when 1 ml of water is added to the system, with an enhancement of 14 times in area response respect to the dry sample. The results clearly demonstrate that the addition of water to the soil samples is necessary to release oxadiazon into the gas phase. The rest of the experiments were carried out adding 1 ml of water to 0.75 g of soil.

The role of ionic strength was investigated using sodium chloride. NaCl amounts (0.18 and 0.36 g) were added to vials containing 0.75 g of soil and 1 ml of water. The results show a negative effect in the response after the addition of salt. The area response for the sample without NaCl added was 1.8 and 6 times that for the samples with 0.18 and 0.36 g NaCl

added, respectively. Possibly, the enhancement of ionic strength decreases the competition between oxadiazon and the water for active sites of the soil. This would decrease the release of oxadiazon into the aqueous and gas phases. The rest of the experiments were made without using salt.

3.3. Analytical parameters

The calibration graph for deionised water treated according to the procedure described previously, monitored using the SIM mode, is linear for the concentration range 0.5–50 ng ml^{-1} ($r=0.9974$). The lack-of-fit test [17] was used to check the linearity of the calibration graph. Since the P value obtained ($P=0.72$) is >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 equation for the calibration graph was $y=351\,550x-128\,565$.

The detection limit was calculated by comparing the signal-to-noise ratio (S/N) of the lowest detectable concentration to a $S/N=3$. The detection limit found was 0.01 ng ml^{-1}. A S/N of 10 was applied for the calculation of the quantification limit. The quantification limit found was 0.04 ng ml^{-1}.

The precision was measured for oxadiazon concentrations of 1, 10 and 25 ng ml^{-1} by performing ten independent determinations. The relative standard deviations (RSDs) were 10.3, 7.9 and 6.3%, respectively.

3.4. Application and validation of the proposed method

The optimum HS-SPME sampling conditions for deionised water were applied to the ground water, human urine, must and wine matrixes. Table 3 shows regression equations for oxadiazon extracted from liquid matrixes studied. The calibration graphs, monitored using the 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. The absolute response was lower with human urine, must and wine samples than with pure matrix. The calibration response slopes for must, human urine and wine were 19, 24 and 85%, respectively lower than in deionised water. This might be due to the presence of additional organic and inorganic compounds in must, urine and wine, which may affect the extraction process. The concentration of ethanol present in the wine samples (15%, v/v) increases the oxadiazon solubility in the matrix. This fact has a direct influence on the partition coefficient, k, which diminishes, the partition equilibrium is displaced to the wine sample and the recovery of oxadiazon decreases. To improve the extraction yield, wine samples were diluted 2:1 with water. In the case of ground water, Student's t-test shows the similarity of the value of slope respect to deionised water ($P>0.05$). External calibration was used in the evaluation of oxadiazon in ground water and standard addition calibration was used in the rest of matrices. With standard addition calibration, matrix effects are nullified.

The detection limits found were between 0.01 and 0.12 ng ml^{-1} for oxadiazon. The precision was measured by performing eight independent determinations. HS-SPME–GC–MS analysis yielded good reproducibility (RSD between 6.5 and 13.5%).

The extraction efficiencies were calculated by comparing the peak areas obtained from the extracts of the spiked samples with those obtained by direct GC injection of non-extracted oxadiazon dissolved in methanol. By the nature of the SPME methodology, based on partition equilibrium between phases, recovery of oxadiazon from the samples was far from 100%. The analytical parameters are summarized in Table 2.

The calibration curve for oxadiazon in agricultural soil samples exhibited an r factor of 0.9976 in the concentration range of 0.01–1.00 μg g^{-1}. The calibration graph was $y=1.99\cdot10^{7}x-76\,567$. The detection and quantification limits found were 1 and 4 ng g^{-1}, respectively. A relatively satisfactory precision of the HS-SPME analysis of oxadiazon in soil was obtained. Seven consecutive extractions of soil, spiked with methanolic solution of oxadiazon were carried out at 100 °C. The RSD obtained was 12.1% for 0.01 μg g^{-1} and 8.7% for 0.04 μg g^{-1}. The efficiency of extraction of oxadiazon in soil samples by HS-SPME was 7.0%.

Fig. 2 shows typical chromatograms for HS-

Table 2
Analytical parameters for oxadiazon in liquid matrices

	Ground water	Urine	Must	Wine
Intercept (*a*)	−126 311	−94 661	227 272	−67 511
Slope (*b*)	338 886	267 764	283 569	51 343
Correlation coefficient	0.9972	0.9966	0.9965	0.9952
Lack-of-fit test (*P* value)	0.32	0.94	0.74	0.94
Linear dynamic range (ng ml^{-1})	0.10–50.00	0.10–50.00	0.10–50.00	0.5–150.0
Detection limit (ng ml^{-1})	0.01	0.02	0.02	0.1
Quantification limit (ng ml^{-1})	0.04	0.07	0.07	0.4
Extraction efficiency (%)	12.2	9.5	9.9	3.7
Reproducibility (RSD, %), $n=8$				
1.0 ng ml^{-1}	10.9	12.2	11.8	13.5 (5.5 ng ml^{-1})
10.0 ng ml^{-1}	7.8	8.5	9.3	11.1 (11 ng ml^{-1})
25.0 ng ml^{-1}	6.5	8.1	7.9	10.4 (33 ng ml^{-1})

SPME–GC–MS of soil blank and soil spiked with 0.02 μg g^{-1} of oxadiazon.

Validation for spiked ground water, urine, must, wine and soil samples was carried out by using a one-sample test (Student's *t*-test) [18]. Samples were fortified with different levels of oxadiazon and analyzed by the proposed method. External calibration was used in the quantification of oxadiazon in ground water and standard addition calibration was used in the rest of matrixes. Table 3 shows the results obtained. The *P* values calculated in all cases were >0.05 and the null hypothesis might be accepted.

Validation of the HS-SPME method for soil samples was also made by comparison with an

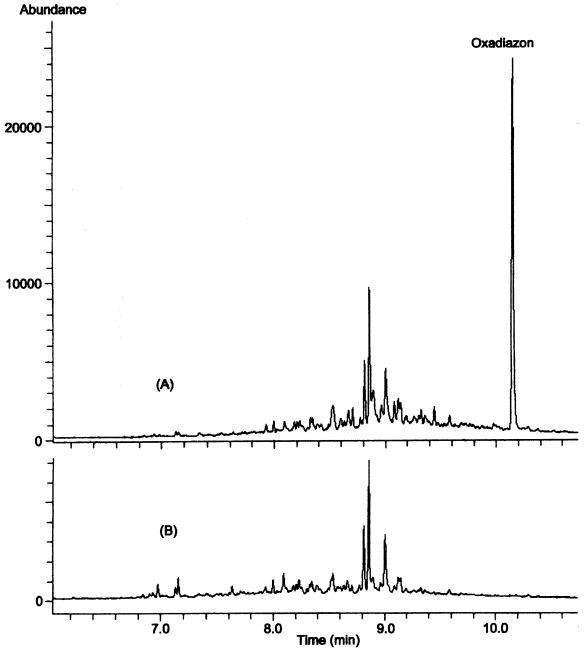

Fig. 2. (A) Chromatogram of spiked soil sample at 0.02 μg g^{-1} of oxadiazon. (B) Chromatogram of blank soil sample.

Table 3
Results of assays to check the accuracy of the proposed method for oxadiazon in spiked ground water, urine, must and wine samples (concentration in ng ml^{-1}) and spiked soil samples (concentration in μg g^{-1})

Sample	Spiked	Found[a]	*t*	*P*[b]
Ground water	0.20	0.21±0.01	1.30	0.25
	1.00	1.07±0.12	1.60	0.15
	5.00	5.16±0.42	1.11	0.30
Urine	0.20	0.18±0.02	1.63	0.16
	1.00	1.08±0.11	2.12	0.07
	5.00	4.84±0.38	1.17	0.28
Must	0.20	0.22±0.02	2.54	0.05
	1.00	0.96±0.12	0.84	0.43
	5.00	4.71±0.41	2.01	0.08
Wine	1.18	1.35±0.21	2.07	
	5.36	5.76±0.55	2.04	0.08
	10.72	11.54±1.56	1.49	0.18
Soil	0.01	0.01±0.002	1.99	0.09
	0.04	0.04±0.004	2.34	0.05
	0.12	0.13±0.014	2.23	0.06

[a] Average value±standard deviation of six determinations.
[b] *P* value of the one-sample comparison test.

ultrasonic extraction method reported by Sánchez-Brunete et al. [19] as a reference method. Spiked soil samples with concentrations of 0.10 and 0.25 μg g^{-1} of oxadiazon were analyzed by both methods. Similar results were obtained by both methods. The statistical comparison of these results by means of a Student's *t*-test showed no significant difference (*P* value of 5%).

4. Conclusions

A simple and practical GC–MS method in combination with HS-SPME for the determination of the herbicide oxadiazon in ground water, urine, must, wine and agricultural soil is presented. Maximum responses were obtained using a 100-μm PDMS fibre, a 25-min extraction time and a temperature of 100 °C. In view of its simplicity and sensitivity, the present method is recommended for the quantification of oxadiazon in the matrices studied in environmental and toxicological studies.

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