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Article

HPLC Determination of Oxadiazon in Commercial Pesticide Formulations

*Guillermo Quintás, Sergio Armenta, Javier Moros, Salvador Garrigues, Agustín Pastor and Miguel de la Guardia**

Department of Analytical Chemistry, University of Valencia, Edifici Jeroni Muñoz, 50th Dr. Moliner 46100 Burjassot, Valencia, Spain

Um procedimento simples, rápido e preciso de cromatografia líquida de alta eficiência (HPLC) foi desenvolvido para a determinação de oxadiazona em formulações concentradas de pesticidas emulsificáveis. 20 µL de amostra, diluída em acetronitrila, foram injetados em uma coluna Kromasil C18 (250 × 4,6 mm, 5 µm), usando acetonitrila:água (80:20) como fase móvel, numa velocidade de fluxo de 1 mL min^{-1}. Oxadiazona foi determinada por medidas de absorbância a 292 nm. Obteve-se, usando-se o procedimento desenvolvido, um limite teórico de detecção de 0,02 µg mL^{-1}, um limite de quantificação de 0,047 µg mL^{-1}, correspondendo a 0,02 e 0,07% m/v na amostra original e desvio padrão relativo de 0,08%, para três análises em replicata de amostras contendo 25% m/v de oxadiazona. A precisão do método foi evidenciada pelas porcentagens de recuperação de 98% a 99% para amostras contendo 0,015 a 0,085 mg de oxadiazona, assim como pela comparação entre os resultados encontrados pelo procedimento recomendado e pela espectrofotometria de infravermelho com transformada de Fourier (FTIR), para amostras reais de produtos formulados.

A simple, fast and precise high performance liquid chromatographic (HPLC) procedure has been developed for the determination of oxadiazon in emulsifiable concentrated pesticide formulations. 20 µL of diluted sample in acetonitrile were injected in a Kromasil C18 (250 × 4.6 mm, 5 µm) column, using acetonitrile:water (80:20) as mobile phase at 1 mL min^{-1} flow rate and oxadiazon determined by absorbance measurement at 292 nm. A theoretical limit of detection of 0.02 µg mL^{-1}, a limit of quantification of 0.047 µg mL^{-1}, corresponding to a 0.02 and 0.07% m/v in the original sample, and a relative standard deviation of 0.08% for three replicate analysis of samples containing 25% m/v. Oxadiazon were achieved using the developed procedure. The accuracy of the whole method was evidenced by recovery percentages from 98% to 99% for samples spiked with 0.015 to 0.085 mg oxadiazon as well as by the good comparability between results found by the recommended procedure and by Fourier Transform Infrared (FTIR) spectrophotometry for actual samples of formulated products.

Keywords: oxadiazon, high performance liquid chromatography, UV-Vis detection, pesticide formulations

Introduction

Oxadiazon, 5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3H)-one, is a selective pre-emergence herbicide used for the control of annual grasses and many broadleaf weeds in turf and landscape ornamentals. The relatively low toxicity by ingestion and by dermal route of this herbicide is one of the reasons for its widespread use.[1]

*e-mail: miguel.delaguardia@uv.es

Formulated products containing oxadiazon are presented as 25% (m/v) emulsifiable concentrates and are widely available in the market.[2]

Methods based on gas chromatography with flame ionization,[3,4] electron capture,[4] mass spectrometry[5] and nitrogen–phosphorous[4] detection, have been proposed for the determination of oxadiazon residues at trace levels after different clean-up and preconcentration steps, being used the aforementioned procedures in various types of matrices such as river and tap waters, foods and mussels.

High pressure liquid chromatography (HPLC) with UV-Vis detection is one of the most common

techniques employed in the analysis and quality control of formulated pesticides, due to the high sensitivity and selectivity achieved.[6] Despite of this fact, there are only two precedents in the literature about the use of HPLC-UV for the determination of oxadiazon. One of these was recently published in English about the determination of oxadiazon in water at trace level[7] and a second one concerning 12% concentrated oxadiazon formulations,[8] published in Chinese, is not available from the Analytical Abstract data base.

The objective of this work has been the development of a simple and fast HPLC-UV procedure which could be applied to the routine quality control analysis of formulated products containing oxadiazon as active ingredient.

Experimental

Apparatus and reagents

The HPLC system included an HP1050 chromatograph from Hewlett-Packard (Palo Alto, CA, USA) with a variable wavelength UV-Vis detector HP1050 and a Kromasil C18 (250 × 4.6 mm, 5 µm) analytical column from Scharlau (Barcelona, Spain). A Hewlett-Packard 8452-A diode array spectophotometer was employed for the measurement of the UV-Vis spectra.

A Nicolet Magna 750 FTIR spectrometer (Madison, WI, U.S.A.) with a temperature-stabilized DGTS detector, a long-lasting Ever-Glo source and a KBr beamsplitter, was employed for IR measurements, using a Specac microflow cell with ZnSe and CaF_2 windows and 0.11 mm pathlength. The equipment employs the 2.1 version of the OMNIC software developed by Nicolet Corporation for the acquisition and processing of the FTIR absorbance data.

Oxadiazon standard of PESTANAL⊃ grade supplied by Riedel de Haën (Seelze, Germany) and acetonitrile of HPLC grade, supplied by Scharlau (Barcelona, Spain) were employed. Formulated samples were obtained directly from the local market.

Recommended HPLC-UV procedure

25 mg of sample were accurately weighted, inside a 25 mL volumetric flask and diluted to the volume with CH_3CN, being sonicated during 5 minutes in an ultrasound water bath to extract oxadiazon from the matrix and to ensure a complete homogenization. 1 mL of the extract was diluted to 10 mL and filtered through a 0.22 µm nylon filter. 20 µL of this latter solution were directly injected in the HPLC system using a 80:20 acetonitrile:water mobile phase and a 1 mL min^{-1} carrier flow. Oxadiazon was determined in the isocratic mode by absorbance measurements at 292 nm using area values of the chromatogram peaks obtained at a retention time of 10.37 min for samples and interpolating them in an external calibration line established from oxadiazon standard solutions in acetonitrile with concentrations from 3.81 to 38.1 mg L^{-1} measured in the same conditions as the samples.

Alternative FTIR procedure

A previously developed FTIR procedure[9] was used as alternative method for the validation of the HPLC determinations. 50 mg of homogenised sample were diluted with 7 g of $CHCl_3$. This solution was passed through a 0.22 µm nylon filter and then introduced in the FTIR measurement cell by using a peristaltic pump. The spectra were obtained in the stopped-flow mode at 4 cm^{-1} nominal resolution and by accumulating 25 scans *per* spectrum from 4000 to 900 cm^{-1} using a background of the cell filled with the solvent.

Peak area values of the first-order derivative spectra between 1770 and 1774 cm^{-1}, corrected with a single point baseline established at 1950 cm^{-1} after a 5 points smoothing, were employed to quantify oxadiazon. Data found from samples were interpolated in an external calibration line obtained with 5 standard solutions of the pesticide in the concentration range between 0.68 and 2.91 mg g^{-1} and measured in the same conditions than samples.

Results and Discussion

UV spectra of oxadiazon

Inset in Figure 1 shows the oxadiazon UV spectrum in the wavelength region from 190 to 400 nm. As it can be seen, the spectrum presents a maximum peak at 208 nm and a second band at 292 nm with two shoulders at 224 and 242 nm. The molar absortivity of oxadiazon was 42759 and 4206 a.u. mol^{-1} L cm^{-1} for the bands at 208 and 292 nm, respectively.

HPLC parameters

In order to select the most appropriate chromatographic conditions for the HPLC determination of oxadiazon, the effect of flow rate, mobile phase composition and detector wavelength were studied.

The two absorption maxima wavelengths at 208 and 292 nm were employed for detection, and the resolution of the oxadiazon chromatographic peak was evaluated by using different mobile phase compositions.

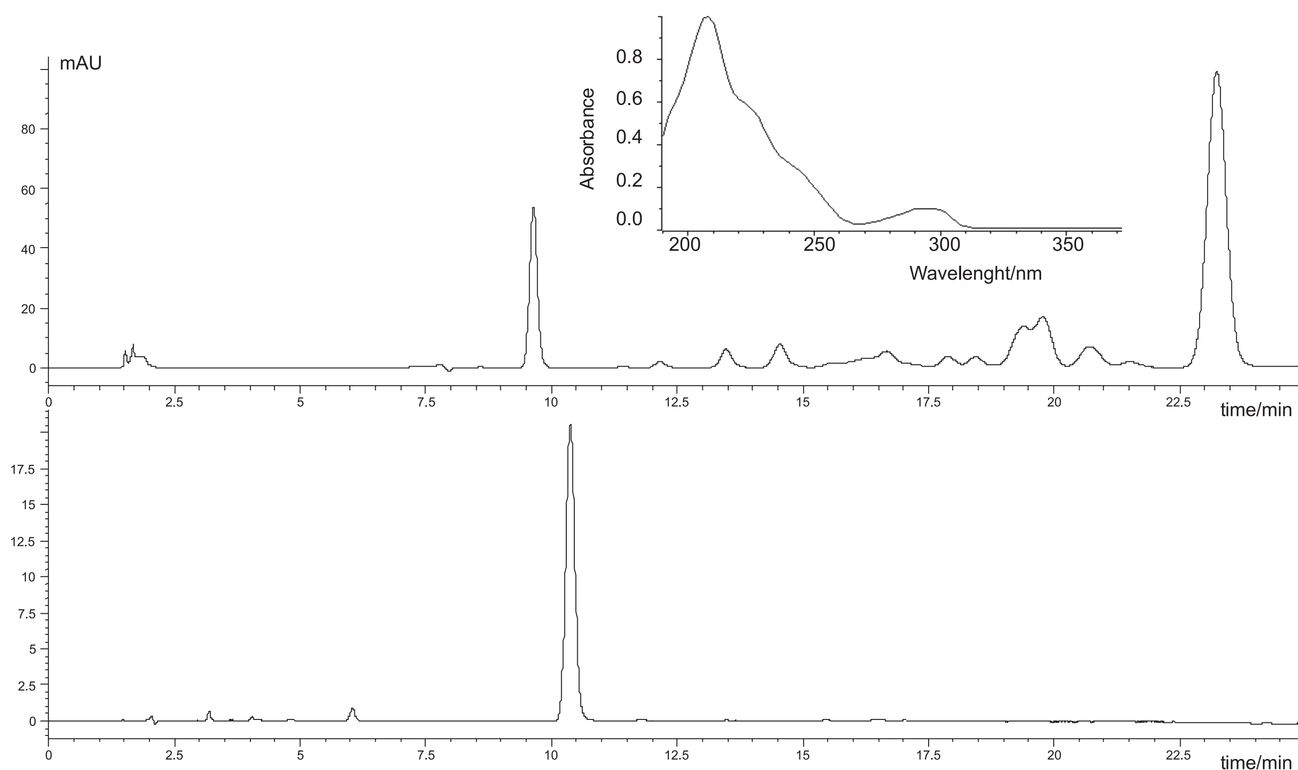

Figure 1. Chromatograms obtained for sample extracts using different conditions. Top: Detection wavelength, 208 nm; mobile phase composition, $CH_3CN:H_2O$ (70:30) (v/v). Bottom: Detection wavelength, 292 nm; mobile phase composition, $CH_3CN:H_2O$ (80:20) (v/v). Inset: UV spectrum of a 5 mg L^{-1} oxadiazon solution in CH_3CN.

The effect of using increasing flow rates of the mobile phase from 0.5 to 2 mL min^{-1} provided a decrease of the retention time of oxadiazon from 41.8 to 10.4 min. On the other hand, it was observed an increase of the pressure from 55 bar to 230 bar. So, in order to ensure a compromise between the time of analysis and an appropriate pressure in the column, a flow rate of 1.00 mL min^{-1}, which provides a retention time of 19.8 min and a pressure of 108 bar, was chosen.

Chromatograms depicted in Figure 1 show that the use of 292 nm as detection wavelength permits to increase the percentage of acetonitrile in the mobile phase, thus reducing both, the total analysis time and the pressure of the system, which varies from 131 bar for 60% (v/v) CH_3CN to 62 bar for 100% CH_3CN on using a flow of 1 mL min^{-1}.

The use of an 80% (v/v) acetonitrile provides a retention time for oxadiazon of 10.37 min, as compared with the 23 min found on using 70% (v/v).

Analytical features of the developed procedure

The regression line between peak area data and concentration of oxadiazon in µg mL^{-1} provided a slope of 8.977, ten times smaller than that found on using 208 nm and 70% (v/v) CH_3CN but in both cases with a reduced value of the intercept and an excellent linear regression coefficient (see Table 1).

The theoretical limits of detection (LOD) and quantification (LOQ) of HPLC determination of oxadiazon were calculated for the two wavelengths employed providing values of 0.02 and 0.07 µg mL^{-1}, for 292 nm and 0.015 and 0.029 µg mL^{-1} for 208 nm. These values were determined from the standard deviation of 10 measurements of the peak area at a concentration level of 3.81 mg L^{-1} oxadiazon divided by the analytical sensitivity and multiplying by 3 or 10 for LOD and LOQ, respectively.

In order to determine the repeatability of the method, the relative standard deviation (RSD) was evaluated for six independent measurements of a sample, being found results from 0.08 to 0.17% as a function of the wavelength employed.

To test the accuracy of the proposed analytical method, recovery experiments were carried out by spiking a commercially available formulation with different quantities of oxadiazon and recovery percentages from 98 ± 1% to 99 ± 2% were found (see Table 2).

A typical standard addition line, obtained for a formulated pesticide, provided an equation of $A = (2.089 \pm 0.002) + (8.90 \pm 0.03)C_{ad}$ (µg mL^{-1}) with a regression coefficient R^2 = 0.9999 which provides a slope which

Table 1. Analytical features of the HPLC procedure for oxadiazon determination

	o = 292 nm	o = 208 nm
Regression line	A=(-0.3 ± 0.1) + (8.977 ± 0.005) C (μg mL^{-1})	A=(0 ± 1) + (90.79 ± 0.08) C (μg mL^{-1})
Regression coeficient (R^2)	0.99999	0.99999
RSD %	0.08	0.17
Theoretical LOD / (μg mL^{-1})	0.02	0.01
Theoretical LOQ / (μg mL^{-1})	0.07	0.03
Tested working range / (μg mL^{-1})	3.81-38.1	
A_s	1.0	1.0
Mobile phase (v/v)	CH_3CN:H_2O (80:20)	CH_3CN:H_2O (70:30)
Retention time / min	10.37	23.29

LOD: limit of detection. LOQ: limit of quantification. A_s: asymmetry factor calculated as the ratio (at 10% of the peak height) of the distance between the peak apex and the backside of the chromatographic curve to the distance between the peak apex and the front side of the chromatographic curve.

Table 2. Obtained recoveries (n=3) in the analysis of spiked formulated samples

mg added	mg found	% Recovery
15	14.85 ± 0.04	99 ± 2
57	55.86 ± 0.06	98 ± 1
85	83.3 ± 0.06	98 ± 1

is statistically comparable to that found by external calibration, at a probability level of 99%, being obtained an experimental value of the Student's t of 2.53 which is lower than 2.583, the tabulated value, thus indicating that the HPLC determination of oxadiazon is free from matrix interferences.

Analysis of real samples

Three commercially available pesticide formulation products were analysed using the aforementioned procedure and an alternative method based on FTIR measurements.[9] Results found by both procedures are in good agreement (see Table 3) thus evidencing that HPLC determination of oxadiazon provides a comparable accuracy to that obtained by FTIR.

Table 3. Determination of oxadiazon in actual samples by both, HPLC and the FTIR alternative procedure

Sample	Oxadiazon concentration[a] / (%, m/v)	
	UV-HPLC procedure	FTIR procedure
1	24.9 ± 0.2	25.0 ± 0.4
2	25.0 ± 0.2	24.8 ± 0.3
3	24.9 ± 0.3	25.0 ± 0.2

[a]Values are the mean of three independent analysis ± the standard deviation.

Conclusions

The proposed HPLC method for the oxadiazon determination in formulated commercial pesticides is fast, due to the use of a simple sample pre-treatment and the 10.37 min retention time obtained in the selected conditions.

This method involves the use of reduced amount of solvents, 25 mL CH_3CN to prepare each sample solution, being the sampling throughput of 5 samples per hour. The developed HPLC procedure is faster than previous methodologies based on the use of gas chromatography and does not require the use of chlorinated solvents as it is the case for FTIR determinations. Hence it can be easily and conveniently adopted for routine quality control analysis.

On comparing with the single precedent on the determination of oxadiazon in formulations by HPLC[8] the RSD (0.28%), linear correlation (0.999) and average recovery (100.3%), are of the same order than those obtained by us (See Table 1). However, the determination at 292 nm instead of 220 nm improves the analytical selectivity of the procedure.

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