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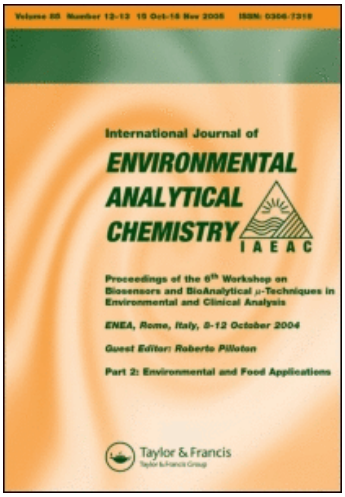

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The present paper reports on an analytical method for the routine analysis of oxadiazon (5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3H)-one) and oxyfluorfen (2-chloro-α,α,α-trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether) residues in thyme (*Thymus vulgaris L.* and *Thymus zygis subsp. gracilis*). Samples were extracted by sonication with a water-acetonitrile mixture and the herbicides were partitioned into dichloromethane. Residue levels in thyme were determined by gas chromatography with electron-capture detection (ECD). Confirmation analysis of herbicides was carried out by GC/MS in the selected ion monitoring (SIM) mode. The identification of compounds was based on retention time and on comparison of the primary and secondary ions. The average recovery by the GC-ECD method obtained for these compounds varied from 89.0 to 108.5% with a relative standard deviation between 1.9 and 6.1%. The detection limit by the GC-ECD for the pesticides studied varied from 4.9 to 13.9 $\mu g\,kg^{-1}$. The proposed method was applied to thyme samples grown in the Region of Murcia (Spain). The results showed that this method provided a simple, rapid and sensitive way to analyse oxadiazon and oxyfluorfen residues in thyme.

Keywords: thyme; herbicides; oxadiazon; oxyfluorfen; gas chromatography-electron capture detection

1. Introduction

Aromatic plants have high essential oils content, are rich in aromatically active components, and have therapeutic properties, which justify their great demand in the pharmaceutical, cosmetic and food industries [1,2]. In particular, aromatic plants that belong to the *Thymus* species have been used traditionally for food seasoning. Their essential oil richness confers many healing effects, in addition to acting as nourishing agents [3–8].

In Spain, thyme has normally been cultivated in the mountainous regions. However, due to problems associated with mountains and to the increasing demand for thyme, our Institute is carrying out studies of cultivation under irrigated conditions of the two species

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of thyme of greater interest: *Thymus vulgaris L.* and *Thymus zygis Subs. gracilis* (Boiss.) R. Morales (red thyme) [9,10].

Weeds are one of the main problems in thyme cultivation, and as a consequence it is necessary to use herbicides for their control. Oxadiazon and oxyfluorfen are two herbicides that can be used for weed control in thyme cultivation. These herbicides are usually applied individually but their alternate application could lead to their residues appearing together in the samples. The maximum residue limits (MRLs) for both analytes in thyme are not established. For agricultural crops, the MRL established by the Spanish legislation is $50\,\mu g\,kg^{-1}$ for both herbicides [11].

Determination of oxadiazon and oxyfluorfen normally is made by gas chromatography (GC) with a nitrogen-phosphorus detector (NPD) [12,13], electron-capture detection (ECD) [14,15], or mass spectrometer detector (MSD) [16–20]. HPLC has also been used [21,22]. Nevertheless, we have not found to date, an analytical method for the individual or simultaneous determination of these compounds in thyme.

Our aim in this work was to develop a simple, fast and sensitive method for the simultaneous determination of the herbicides oxadiazon and oxyfluorfen in thyme. Final determination was made by GC using ECD with confirmation by GC-MSD.

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2. Experimental

2.1 *Materials and standards*

Oxadiazon (purity 99%) and oxyfluorfen (purity 97%) standards were purchased from Dr Ehrenstorfer (Augsburg, Germany) and their logarithmic octanol-water partition coefficients ($\log K_{ow}$) are listed in Table 1. Stock solutions ($1{,}000\,\mu g\,mL^{-1}$) of individual pesticide standards were prepared by dissolving 0.025 g of the pesticide in 25 mL of ethyl acetate/cyclohexane (1/1, v/v). Working solutions were obtained by appropriate dilutions with ethyl acetate/cyclohexane (1/1, v/v).

2.2 *Analytical apparatus*

Analysis of the final extract was performed on an Agilent (Waldbronn, Germany) model HP 6890 gas chromatograph equipped with an electron-capture detector and automatic split-splitless autosampler injector model Agilent 7683. A HP-5MSI fused silica capillary column ($30\,m \times 0.25\,mm$ i.d.) and $0.25\,\mu m$ film thickness, supplied by Agilent Technologies, was employed, with nitrogen as the makeup gas at $25\,mL\,min^{-1}$. Helium was used as the carrier (constant pressure eluting, bromophos 20.08 min). A 1 μL sample

Table 1. Logarithmic octanol-water partition coefficients ($\log K_{ow}$), retention time (RT, min), molecular mass (MW), target (T), qualifier ions (Q_1, Q_2 and Q_3) (m/z) and abundance ratios (%) of qualifier ion/target ion (Q_1/T and Q_2/T)[a] of the studied herbicides.

Herbicide	$\log K_{ow}^{b}$	RT	MW	T	Q_1	Q_2	Q_3	Q_1/T	Q_2/T
Oxadiazon	4.91	24.42	345.2	175	177	258	260	63.1	54.8
Oxyfluorfen	4.47	24.73	361.7	252	302	331	361	41.9	44.2

Notes: [a]Q/T (%) ratios are the results of abundance values of the qualifier ion (Q_1, Q_2) divided by the abundance of the target ion (T) × 100. [b]Taken from reference Tomlin [23].

was injected into the GC using splitless mode. The injector and detector were operated at 250 and 325°C, respectively. The column temperature was maintained at 70°C for 2 min and then programmed at 25°C min^{-1} to 150°C, increased to 200°C at a rate of 3°C min^{-1} followed by a final ramp to 280°C at a rate of 8°C min^{-1}, and held for 10 min.

An Agilent model HP 6890 gas chromatograph equipped with a model 5973N mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 50 to 500 at 3.21 s per scan. The interface temperature was 230°C and the quadrupole temperature 150°C. A solvent delay of 4.5 min was employed. Gas chromatography was performed under the same conditions used in GC/ECD.

Analysis was performed with selected ion monitoring (SIM) mode using primary and secondary ions. The target and qualifier abundances were determined by injection of individual herbicide standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from m/z 50 to 500. Table 1 lists the herbicides along with their retention times, molecular mass, the target and qualifier ions, and their qualifier to target abundance ratios. Herbicides were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier-to-target ratios. Retention times had to be within ±0.1 min of the expected time, and qualifier-to-target ratios had to be within a 10% range for positive confirmation. The concentration of each compound was determined by comparing the peak areas in the sample with those found for mixtures of herbicide standards of known concentration.

For the extraction of samples, a sonic dismembrator 200 W generator equipped with standard titanium probe (Dr Hielscher GmbH. Stahnsdorf, Germany) was used.

An Eppendorf model 5810R centrifuge (Hamburg, Germany) and a Büchi model R-205 rotavapor (Flawil, Switzerland) were used in the centrifugation and evaporation to dryness of samples, respectively.

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2.3 *Sample preparation*

2.3.1 *Thyme samples*

The thyme samples (*Thymus vulgaris* and *Thymus zygis*) were raised in Campo de Cartagena, Murcia (south-east, Spain). Fresh plant material was dried in a forced-air dryer at 35°C for 48 h, until it reached a constant weight. Herbicide-free thymes were used as blank to spike samples for recovery studies.

2.3.2 *Extraction procedure*

Thyme (0.5 g of powder) was weighed in a 100 mL beaker. Samples were extracted with 30 mL of acetonitrile/water (2/1) by sonication (15 min at 0.5 cycles and 60% amplitude). After sonication, 20 mL of dichloromethane were added and then centrifuged for 5 min at 3,000 g. Extract was filtered quantitatively through a glass funnel containing a filter paper DP302, 150 mm diameter (Albet, Barcelona, Spain). The organic phase was concentrated to dryness using rotary vacuum evaporation. The residue was redissolved in 5 mL of ethyl acetate/cyclohexane (1/1, v/v) and an aliquot analysed using GC-ECD under conditions described above.

Four solvents (acetonitrile, dichloromethane, acetone and ethyl acetate) were tested as extractants for all compounds studied.

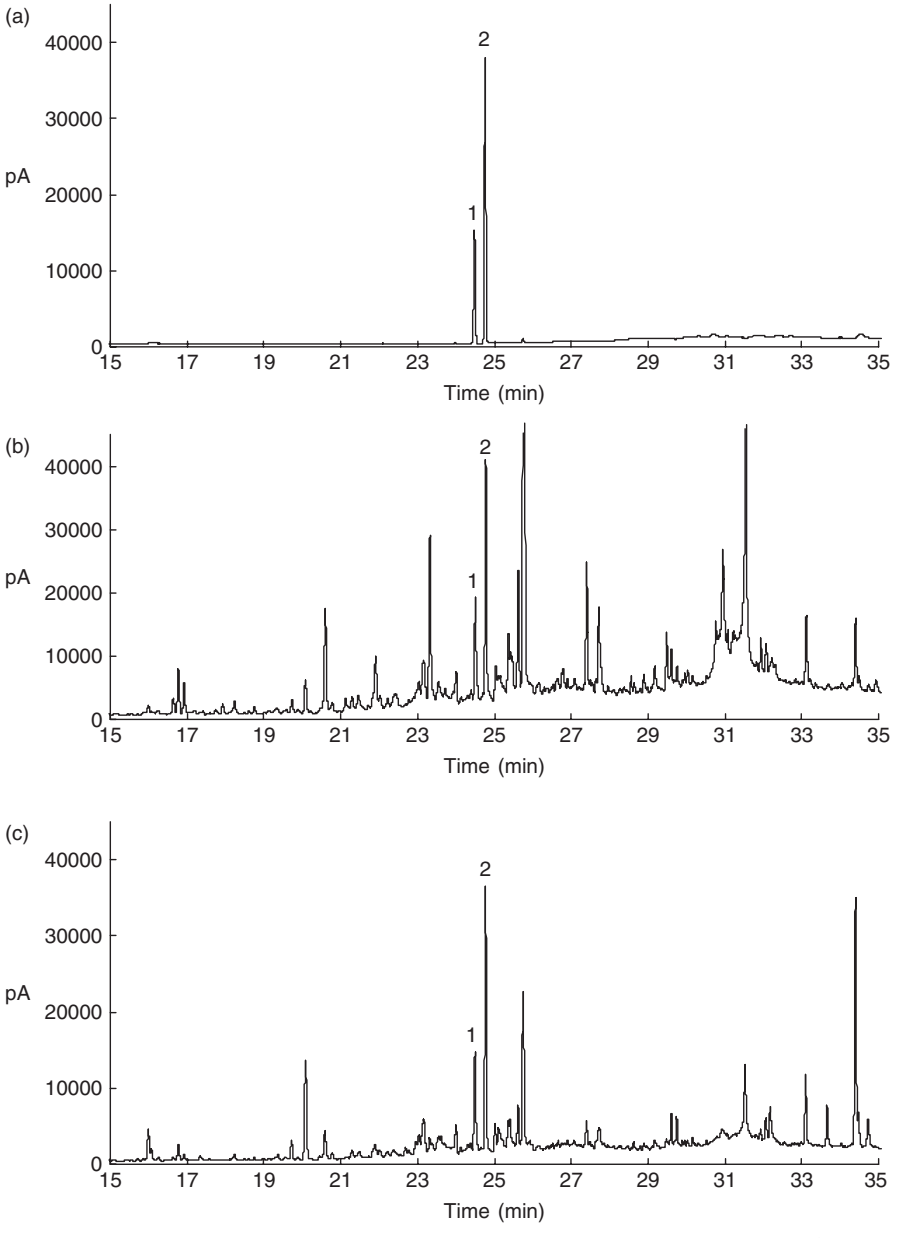

Figure 1. Chromatograms (ECD) obtained for (a) standard solution (2000 μg kg^{-1}). (b) Spiked thyme (*Thymus zygis*) sample (2000 μg kg^{-1}). (c) spiked thyme (*Thymus vulgaris*) sample (2000 μg kg^{-1}). 1 = oxadiazon; 2 = oxyfluorfen.

3. Results and discussion

3.1 *Gas chromatographic determination*

The chromatograms obtained for a standard herbicide mixture and two thyme (*Thymus vulgaris* and *Thymus zygis*) samples spiked with the compounds of the standard solution are shown in Figure 1.

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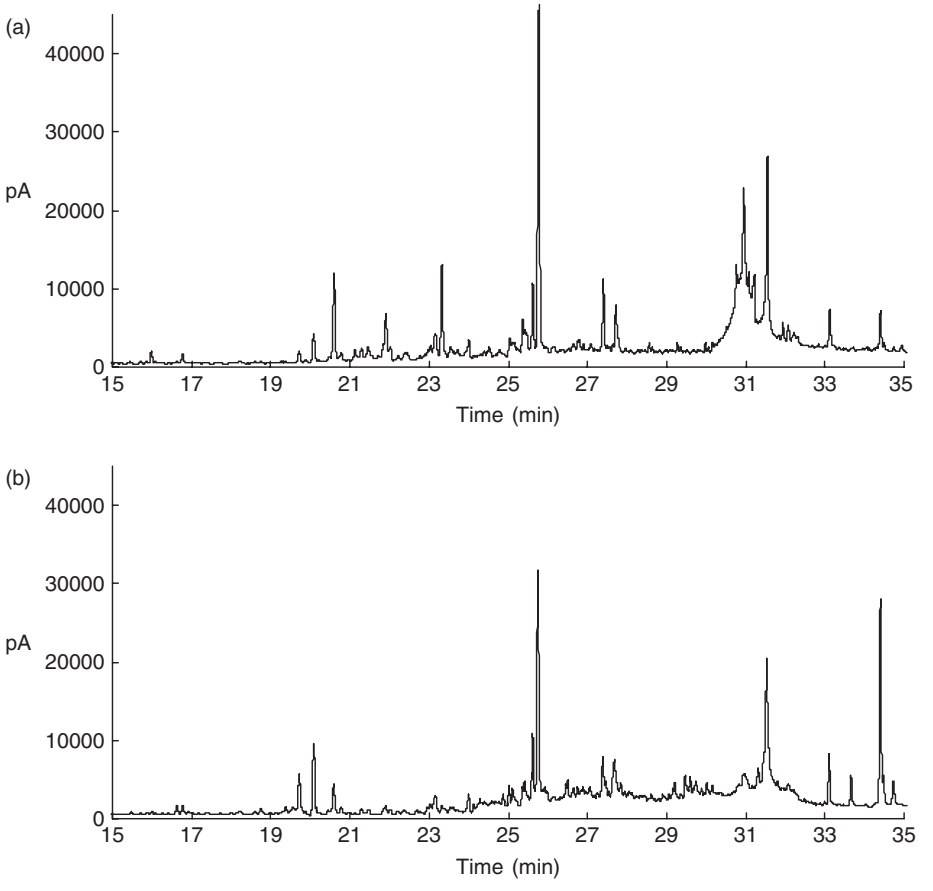

Figure 2. Chromatograms (ECD) obtained for (a) a control thyme (*Thymus vulgaris*) sample. (b) a control thyme (*Thymus zygis*) sample.

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The best results were obtained using acetonitrile and dichloromethane with recoveries between 89 and 108%, meanwhile recoveries for acetone and ethyl acetate were under 75%. Accordingly, acetonitrile and dichloromethane were selected as extractants. Oxadiazon and oxyfluorfen were satisfactorily separated with high sensitivity and selectivity (Figure 2). Chromatograms showed no co-eluted compounds at the retention time of analytes, so cleanup of thyme samples was not required.

3.2 *Method validation*

3.2.1 *Linearity and detection limit*

Standard solutions, with concentrations of 20 to 1000 $\mu g\,L^{-1}$ were injected in CG/ECD and CG-MSD to calculate the linearity of detector response and the detection limits of the studied compounds. The ECD and MSD response for oxadiazon and oxyfluorfen was linear in the concentration assayed with determination coefficients 0.9998 and 0.9999, respectively. Table 2 summarizes the limits of detection (LOD; obtained at

Table 2. Limits of detection (LOD, $\mu g\,kg^{-1}$), limits of quantification (LOQ, $\mu g\,kg^{-1}$), standard deviations for repeatability (RSD_r, $n = 10$) and standard deviations reproducibility (RSD_R, $n = 7$) of the studied herbicides by GC-ECD and GC-MSD in thyme (*Thymus vulgaris L.* and *Thymus zygis subsp. gracilis*).

	GC-ECD						GC-MSD					
			RSD_r		RSD_R				RSD_r		RSD_R	
Herbicide	LOD	LOQ	Peak area	RT	Peak area	RT	LOD	LOQ	Peak area	RT	Peak area	RT
Thymus vulgaris												
Oxadiazon	13.9	46.4	2.5	0.02	6.3	0.03	125.3	417.7	3.9	0.03	7.6	0.03
Oxyfluorfen	6.5	21.7	1.8	0.01	3.8	0.02	93.9	313.1	3.7	0.02	7.8	0.03
Thymus zygis												
Oxadiazon	12.3	41.0	3.3	0.02	7.5	0.02	113.4	378.1	4.3	0.03	9.1	0.02
Oxyfluorfen	4.9	16.5	2.7	0.02	4.9	0.02	85.3	284.4	4.4	0.03	8.6	0.02

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a signal-to-signal ratio 3) and the limits of quantification (LOQ, obtained at a signal-to-signal ratio 10) obtained for the individual herbicides in thyme (*Thymus vulgaris* and *Thymus zygis*) by GC-ECD and GC-MSD. In the case of the GC-ECD the LOD and LOQ were a little lower than the ones obtained by GC-MSD (in the SIM mode). Similar LOD and LOQ were obtained for different thyme species.

3.2.2 *Matrix effect*

A comparison between calibration standards prepared in pure solvent and spiked matrix standards (200 to 10,000 $\mu g\,kg^{-1}$) was performed. The responses of herbicides analysed from standard solutions in the solvent were similar to those obtained from standards in thyme. Consequently, the quantification of recovery studies were conducted using calibration standards prepared in pure solvents.

3.2.3 *Repeatability and reproducibility*

The repeatability and reproducibility of our chromatographic method was determined by performing the analysis of samples spiked at 1,000 $\mu g\,kg^{-1}$ of herbicide. Table 2 lists the standard deviations for repeatability (RSD_r, $n = 10$) and reproducibility (RSD_R, $n = 7$). The RSD_r and RSD_R values obtained for peak areas by GC-ECD ranged from 1.8 to 3.3 and 3.8 to 7.5, respectively. The RSD_r and RSD_R values obtained for retention times by GC-ECD ranged from 0.01 to 0.02 and 0.02 to 0.03, respectively (see Table 2).

3.2.4 *Recovery*

Thyme samples were fortified with 500, 1000 and 2000 $\mu g\,kg^{-1}$ of herbicide. After evaporation of the spiking solvent, the samples were allowed to equilibrate for 2 h. before extraction and analysed following the procedures described above. Two thyme species (*Thymus vulgaris* and *Thymus zygis*) are studied to validate the method. The recoveries

Table 3. Recovery of herbicides from spiked thyme samples.[a]

Herbicide	Fortification level, $\mu g\,kg^{-1}$	Mean recovery $\pm$ RSD_s^b (%)[a]	
		Thymus vulgaris	Thymus zygis
Oxadiazon	500	104.4 ± 4.6	108.5 ± 3.9
	1000	100.6 ± 4.4	91.3 ± 2.8
	2000	89.1 ± 1.9	98.2 ± 4.7
Oxyfluorfen	500	94.7 ± 4.9	89.8 ± 5.2
	1000	91.3 ± 3.2	93.4 ± 2.3
	2000	89.0 ± 3.6	94.9 ± 6.1

Notes: [a]$n = 5$; [b]RSD_S = relative standard deviation.

Table 4. Herbicide residues found in thyme samples.

Thyme	Specie	Oxadiazon[a] ($\mu g\,kg^{-1}$)	Oxyfluorfen[a] ($\mu g\,kg^{-1}$)
A	*Thymus zygis*	134 ± 11	
B	*Thymus zygis*		111 ± 9
C	*Thymus vulgaris*	157 ± 7	
D	*Thymus vulgaris*		93 ± 5

Note: [a]Mean of four determinations $\pm$ RSD.

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obtained for all herbicides ranged from 89.0 to 104.4% for *Thymus vulgaris*, and 89.8 to 108.5% for *Thymus zygis* (Table 3). The relative standard deviation (RSD_S) was $<6.1\%$ in the most unfavourable case.

3.2.5 *Sample analyses*

The validity of the method used was checked by performing an analysis of samples collected from an experiment of thyme cultivation, which allowed the determination and identification of herbicides present in the samples. Plants were treated with commercial formulations of oxadiazon and oxyfluorfen. Treatments were carried out at the doses recommended by the manufactures. The thymes were sampled and analysed following the extraction methods described above. Table 4 shows the herbicide levels. The residue concentrations were between 134 and $157\,\mu g\,kg^{-1}$ for oxadiazon and between 93 and $111\,\mu g\,kg^{-1}$ for oxyfluorfen. The residues values for both pesticides were close to the LOQs for the developed method.

4. Conclusions

In the present work a method for analysing oxadiazon and oxyfluorfen in thyme samples was developed. Compared to other methods reported in the literature, the proposed method is more rapid, simple and sensitive. It also avoids the cleanup of samples and offers a good recovery. In addition to this, it uses small volumes of solvents, reducing the risk to human health and the environment.

References

[1] J.F. Morton, *Major Medicinal Plants* (C.C. Thomas Springfield. IL, XIX, 1977), p. 277.
[2] J.A. Duke, *Handbook of Medicinal Herbs* (CRC Press, Boca Raton, Fl, 1985).
[3] S. Karaman, M. Digrak, V. Ravid, and A. Iclim, J. Ethnopharmacol. **76**, 183 (2001).
[4] K.A. Youdim, D.S. Deans, and H.J. Finlayson, J. Essent. Oil Res. **14**, 210 (2002).
[5] A. Singh, R.K. Singh, A.K. Bhunia, and N. Singh, Food Sci. Tec. **36**, 787 (2003).
[6] S. Dragland, H. Senoo, K. Wake, K. Holte, and R. Blomhoff, J. Nutr. **133**, 1286 (2003).
[7] D. Kalemba and A. Kunicka, Curr. Med. Chem. **10**, 813 (2003).
[8] I. Rasooli and S.A. Mirmostafa, J. Agric. Food Chem. **51**, 2200 (2003).
[9] J.A. Sotomayor, R.M. Martínez, A.J. García, and M.J. Jordán, J. Agric. Food Chem. **52**, 5418 (2004).
[10] J.A. Sotomayor, J.M. Berná, M.J. Alcaráz, A.J. García-Moya, and E. Correal, Actas de Horticultura SECH **31**, 2084 (2001).
[11] MAPA Ministerio de Agricultura, Agua y Pesca. Límites Máximos de Productos Fitosanitarios (2007). <http://www.mapa.es/agricultura/pags/fitos/registro/lmrs/conlmr.asp>
[12] C. Sánchez-Brunete, R.A. Pérez, E. Miguel, and J.L. Tadeo, J. Chromatogr. A **823**, 17 (1998).
[13] G.G. Ying and B.D. Williams, Pest. Manag. Sci. **56**, 277 (2000).
[14] A.C. Das, A. Debnath, and D. Mukherjee, Chemosphere **53**, 217 (2003).
[15] D. Duseja and W. Martindale, Commun. Soil Sci. Plant. Anal. **33**, 2693 (2002).
[16] S. Morales-Muñoz, J.L. Luque-García, M.J. Ramos, A. Fernández-Alba and M.D.L. de Castro, Anal. Chim. Acta **552**, 50 (2005).
[17] A. Navalón, A. Prieto, L. Araújo, and J.L. Vílchez, J. Chromatogr. A **946**, 239 (2002).
[18] A. Tanabe, H. Mitobe, K. Kawata, and M. Sakai, J. Chromatogr. A **754**, 159 (1996).
[19] M.A. Aramendía, V. Borau, F. Lafont, A. Marinas, J.M. Marinas, J.M. Moreno, and F.J. Urbano, Food Chem. **105**, 855 (2007).
[20] L. Li, H. Zhang, C. Pan, Z. Zhou, S. Jiang, and F. Liu, J. Sep. Sci. **30**, 2097 (2007).
[21] M.J. Martinez, K. Farsaoui, and R. de Prado, Commun. Agric. Appl. Biol. Sci. **69**, 77 (2004).
[22] A. Laganà, G. Fago, L. Fasciani, A. Marino, and M. Mosso, Anal. Chim. Acta **414**, 79 (2000).
[23] C. D. S. Tomlin, *The Pesticide Manual*, 14th ed. (British Crop Protection Council, Hampshire, England, 2006).

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