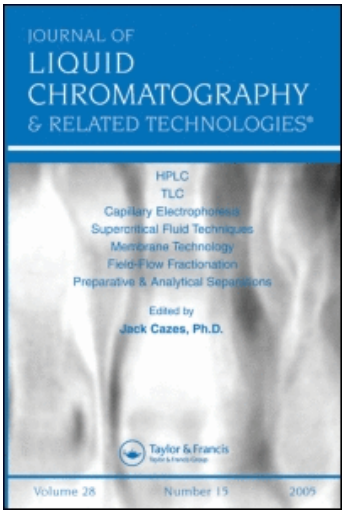

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DETERMINATION OF ACETAMIPRID BY HPLC-FLUORESCENCE WITH POST-COLUMN PHOTODERIVATIZATION AND HPLC-MASS SELECTIVE DETECTION

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ABSTRACT

Two methods are described for determining the highly polar pesticide acetamiprid in vegetables. Residues are extracted with ethyl acetate and coextratives are removed with a graphitised Carbon-based packing ENVI-carb cartridge. Analysis is performed with goods results, by HPLC with post-column photoderivatization, and fluorescence detection and by HPLC-Electrospray ionisation mass spectrometry. The clean-up step is not necessary in this last case. LODs were $6\,\mu g\,L^{-1}$ for HPLC-fluorescence detection using matrix matched standards and $1.5\,\mu g\,L^{-1}$ for HPLC-ES-MS detection. Recovery data were

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obtained by fortifying vegetable matrix at 0.01, 0.1, and 0.5 mg kg^{-1}, with recoveries between 65 and 75% for the HPLC-fluorescence and between 72 and 77% for HPLC-ES-MS.

INTRODUCTION

Acetamiprid is a systemic insecticide for soil and foliar application. It is used to control Hemiptera, especially aphids, Thysanoptera and Lepidoptera on a wide range of crops, especially vegetables, fruits, and tea. This pesticide acts on the central nervous system causing irreversible blocking of postsynaptic nicotinergic acethylcholine receptors.[1] It is being widely used in some countries as Japan, but not in the United States and the European Union Countries.

Nowadays, the United States Environmental Protection Agency (EPA) is examining dietary, ecological, residential, and occupational risks concerning organophosphates (OP), with the purpose of reducing or eliminating the use of some "high-risk" pesticides. Therefore, EPA is registering pesticides,[3,4] taking into account if they are "reduced-risk" pesticides and OP alternatives, among others. Environmental Protection Agency's "Reduced-Risk" Pesticide Committee evaluates pesticide characteristics and makes decisions about whether the registrant fulfils these requirements. The Agency has accelerated the reviews for some of the emerging OP alternatives based upon data from growers. In this way, the Registration Division's FY2002 Work Plan[4] has selected 16 new chemical candidates, seven of which are for reduced-risk chemicals, and three potential alternatives for organophosphate. Among them, only two pesticides (acetamiprid and milbectin) fulfill both requirements.

Environmental Protection Agency expects to complete its decision-making for most of these chemicals within the next 18 months. Therefore, it seems advisable to find new, simple and rapid methods with accuracy and precision enough to reach possible maximum residue levels (MRLs).

The Southern of Spain, mainly the province of Almería, is concerned by a growing agricultural activity, which implies the use of increasing quantities of pesticides. Among them, a high percentage are OP. In this area, trips and white fly are between the most problematical pests and, therefore, highly effective pesticides must be used, such as the OP methamidophos. However, this systemic pesticide, with acaricide and insecticide activity, is a restricted use pesticide, only authorized for cotton, potato, and tomato in USA. The European Union establishes 0.01 mg kg^{-1} as the MRL for methamidophos in vegetables; therefore, the preharvest intervals established for this pesticide by the Spanish Government are too high: 35 days for cotton, broccoli, citric, cabbage of Brussels, hop, corn, and cabbage, whereas in vegetables, methamidophos cannot be used after the beginning of the first flowering. Besides, methamidophos is a metabolite of

another registered pesticide, acephate, and consequently, residues would be from both pesticides. Therefore, as a consequence of this problematical situation, acetamiprid would be an alternative to these OP pesticides, and it seems interesting to find new simple and rapid methods with enough accuracy and precision to reach possible European MRLs.

Acetamiprid has been analysed by Gas Chromatography (GC),[5,6] High Performance Liquid Chromatography (HPLC)[7] and by Enzime Linked Inmunosorbent Assay (ELISA) based on monoclonal antibody,[8] but the high water solubility of this pesticide ($4200\,mg\,L^{-1}$)[1] makes more adequate its determination by HPLC than by GC.

The aim of this study was to develop extraction and clean up procedures for the highly water soluble pesticide, acetamiprid, from vegetable matrices and to describe and compare the performance of two HPLC methods, using fluorescence after photolysis and MS detection, respectively.

EXPERIMENTAL

Chemicals and Solvents

Acetamiprid ((E)-*N*-[(6-chloro 3-pyridil)methyl]-*N′*-cyano-*N*-methylacetamidine (pestanal quality) was obtained form Dr. Ehrenstorfer (Augsburg, Germany).

Analytical reagent grade solvents, methanol (MeOH), acetonitrile (AcN), dichloromethane, ethyl acetate, petroleum ether, and anhydrous sodium sulfate for pesticide residue analysis were obtained from Scharlaw (Barcelona, Spain). Solid phase extraction (SPE) cartridges of 1000 mg of graphitised carbon black (GCB) (Supelco, Bellefonte, PA, USA) were used to clean up vegetable samples.

Mobile phases were filtered through a 0.45 μm cellulose acetate (water) or politetrafluoroethylene (PTFE) (AcN) and degassed with helium prior to and during use. All standards and samples were filtered through Millipore membrane PTFE filters (0.45 μm particle size) before injection into the chromatographic column.

Distilled water from a Milli-Q water purification system Millipore (Bedford, MA, USA) was used.

HPLC-Fluorescence Analysis

The HPLC system was a Waters, composed of a Model 600E multisolvent delivery system, a Rheodyne 7725i manual injector valve with a 400 μL sample loop, a temperature control system, and a Model 474 scanning fluorescence

detector. Millenium 32 (Chromatography Manager, Waters) software was used for acquisition and treatment of data. Separations were performed with a Waters spherical silica-based stationary phase 150 × 3.9 mm (4 μm particle size) column.

The photochemical reaction was carried out in a post-column photochemical reactor (Softron, Gynkotek HPLC, Germering, Germany), fitted with a knitted open tube reactor coil (5 m × 1.6 mm O.D. × 0.3 mm I.D.).

Volumes of 400 μL were injected at a mobile phase flow rate of 0.5 mL min^{-1}, isocratically with AcN : water 80 : 20 (v/v).

HPLC-ES-MS Analysis

The system consisted of a Hewlett Packard (HP, Palo Alto, CA, USA) series 1100 with a HP Chemstation for recording chromatograms and quantitative measurements. A Grandient Model G1312A pump from HP was used for delivering the mobile phase and a HP autosampler Model G1313A was used for injecting aliquots into a 20 μL sample loop. A HP model G1946A LC-API-MS system with quadrupole mass spectrometer and a HP model G2710AA instrument for data acquisition and processing were also used. The LC-MS interface was used in ES positive mode and the capillary and fragmentor voltage were 3 kV and 100 V, respectively. The source was maintained at 350°C. Nitrogen drying gas flow-rate and nitrogen nebulizer gas pressure were 10 L min^{-1} and 50 psi, respectively.

Scanned spectra were acquired over the range m/z 50–600 with a scan time of 1.8 s $cycle^{-1}$. Selective ion monitoring (SIM) was performed, using for quantification of the ion an (m/z) value of 223. The dwell time for each channel was 0.3 s.

HPLC separation was performed on a Superspher RP-18, LiCroCART 125 × 4.6 mm (4 μm particle size). The mobile phase gradient was initially 1 min isocratic with ACN : Water 90 : 10 (v/v), 14 min linear gradient to 100% AcN, maintaining this mobile phase composition for 1 min and, finally, 4 min linear gradient to the initial conditions, ACN : Water 90 : 10 (v/v). Volumes of 20 μL were injected at a mobile phase flow rate of 1 mL min^{-1}.

Preparation of Standards and Spiked Samples

Standard solutions of acetamiprid (200 mg L^{-1}) were prepared by exactly weighing and dissolving the solid compound in ACN. This standard solution was stable for at least three months. Dilutions were freshly prepared for the working solutions. All solutions were protected against light with aluminium foil and stored in a refrigerator at 4°C.

For recovery studies, 50 g samples of finely chopped vegetable were spiked with 0.5, 5.0, and 25.0 μg of acetamiprid (0.01, 0.10, and 0.50 mg kg^{-1},

equivalent to 0.1, 1.0, and 5.0 μg mL^{-1} in the final extract) and with 0.1 and 0.7 μg of acetamiprid (2 and 7 μg kg^{-1}, equivalent to 0.02 and 0.07 μg mL^{-1}), for working in HPLC-fluorescence and HPLC-MS, respectively. The spiked samples were allowed to stand for a few minutes before extraction, to allow the spiked solution to penetrate the test material.

A model PT 2100 Polytron (Kinematica, Luzern, Switzerland) and a Model BV-401C blender (Fagor, Guipuzcoa, Spain) were used for blending the samples. A model VV2000 LIF rotary vacuum evaporator (Heidolpf) thermostated by water circulation with an N-010 KN-18 vacuum pump (Telstar) was used to evaporate the extracts.

Procedure for Determining Acetamiprid in Vegetables

A 50 g vegetable sample was homogenized with 100 mL of ethyl acetate for 2 min with a Polytron. Then, 80 g of sodium sulfate anhydrous were added and the mixture was homogenized again for 1 min. The extract was filtered thorough a 12 cm Buchner funnel and washed with two successive 30 mL portions of ethyl acetate. The rinsings were added to the extraction fraction and evaporated to dryness in a rotating vacuum evaporator with a water bath at $60 \pm 1°C$. The residue obtained from the extract was redissolved in 5 mL MeOH. Twenty microliters of this solution was analyzed by HPLC-ES-MS, as described above.

An ENVI-Carb GCB cartridge was preconditioned with 4 mL of ACN, followed by 4 mL of MeOH and then, 1 mL of sample extract was brought into the SPE column. The pesticide was eluted with 4 mL of AcN, concentrated nearly to dryness, in a rotating vacuum evaporator with a water bath at $60 \pm 1°C$ and the remaining solvent was allowed to evaporate under a slight N_2 stream. This final residue was redissolved in 1 mL of AcN : water 50 : 50 (v/v) and then filtered through a 0.45 μm PTFE filter. Four hundred microliters of this solution were analyzed by HPLC-fluorescence.

RESULTS AND DISCUSSION

Analysis by HPLC-Fluorescence

According to Aaron,[9] the main advantages of photochemically-induced fluorimetry (PIF) are: (i) the use of photons for analyte conversion does not require a mixing system, and therefore, the analyte must not be diluted; (ii) since most photochemical reactions take place via free radicals, the reactions rates are generally fast, resulting in short conversion times; (iii) the use of room temperature; (iv) the technique requires low cost equipment and it is suitable to

various experimental conditions, such as stationary liquid solutions[10–13] or dynamic systems, including flow injection analysis[14,15] and HPLC with photo post column photoderivatization.[16–20] This last option is especially useful, because it combines advantages described above with the performance of separation techniques.

Initial experiments have shown that in an aqueous medium, acetamiprid does not show fluorescent properties, but when aqueous solutions are irradiated with UV radiation, a fluorescent photoproduct is obtained with an excitation maximum at 255 nm and an emission maximum at 385 nm (Fig. 1).

All continuous measurements were carried out at the maximum excitation and emission wavelengths, previously established. With the aim of choosing a mobile phase to give maximum responses, minimal broadening on the chromatograms, and a reduced time of analysis, different MeOH : water mixtures were evaluated as mobile phases. The best results were obtained using AcN : water 80 : 20 (v/v).

In addition, the effect of the flow-rate through the column and the photoreactor (residence time) and the loop volume were studied. It was found that, analytical response increases as the flow rate decreases between 1.2 and 0.2 mL min^{-1} [Fig. 2(a)]. On the other hand, the analytical signal became constant when the volume of injected sample increases over 500 μL [Fig. 2(b)]. In

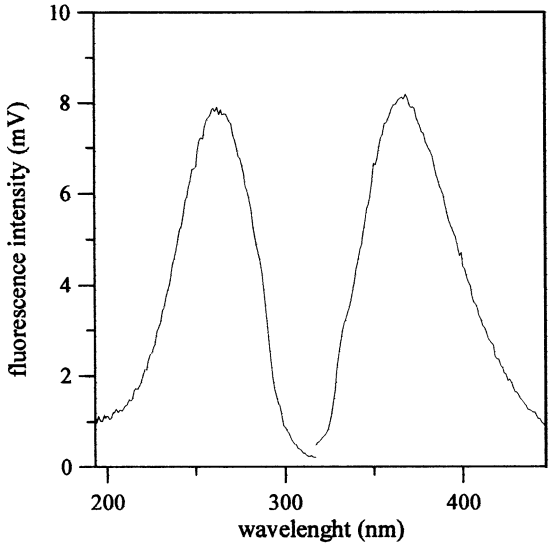

Figure 1. Excitation (a) and emission (b) spectra for 0.1 μg mL^{-1} of acetamiprid in aqueous solution after UV irradiation during 10 min.

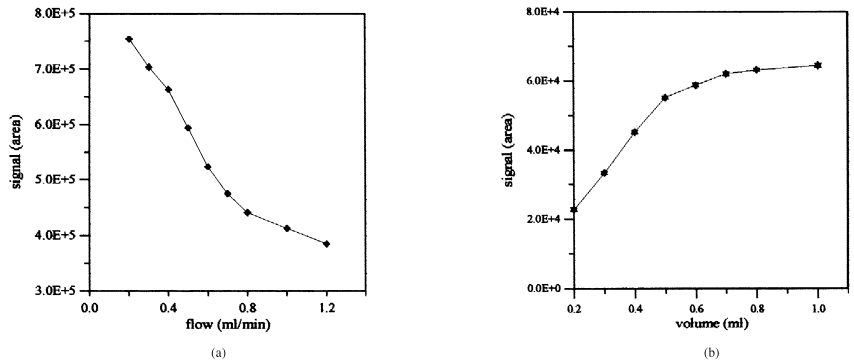

Figure 2. (a) Influence of the flow of the mobile phase and (b) of the injection volume in the analytical signal.

this way, a flow rate of $0.5\,mL\,min^{-1}$ was chosen as a compromise between the residence time in the reactor and the band broadening on the chromatogram, and a 400 μL volume loop was fixed for obtaining more favorable conditions related to the sensitivity of the method.

The effect of pH on the fluorescence intensity was also studied by using different buffer solutions ($C_T = 0.01–0.1\,M$). A change in the pH between 3 and 8 does not modify the analytical signal. Therefore, the mobile phase was not buffered. Figure 3(a) shows the chromatogram corresponding to $0.5\,\mu g\,mL^{-1}$ of acetamiprid in the selected conditions.

Extraction and SPE Clean-Up

Fifty-gram samples were spiked with $0.1\,mg\,kg^{-1}$ of acetamiprid and extracted using several polar solvents (ethyl acetate, acetone, hexane, MeOH, petroleum ether, AcN, dichloromethane, and mixtures of them). Then, a SPE clean-up was tested using different sorbents (aminopropyl, florisil, silica, diol, alumine, and ENVI-carb) and eluents (MeOH, AcN, ethyl acetate, and dichloromethane). The best results were obtained when extraction was carried out with ethyl acetate using ENVI-carb GCB and AcN and MeOH in the precondition and elution steps. Figure 3(b) shows the chromatogram corresponding to a blank extract of cucumber obtained after a clean up step, as described above. It can be seen that, no interference peaks appear at the retention time of acetamiprid.

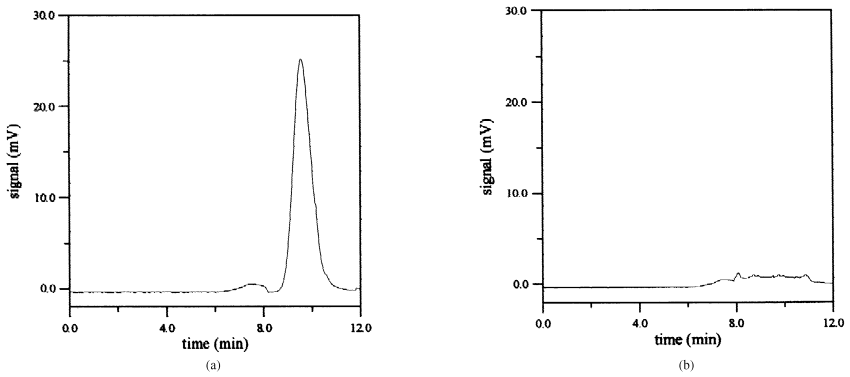

Figure 3. (a) Chromatogram corresponding to $0.5\,\mu g\,mL^{-1}$ of acetamiprid and (b) chromatogram corresponding to a blank extract of cucumber.

Analytical Parameters

Among the parameters used for validation, detection and quantification capabilities represent fundamental performance characteristics of measurement processes. Firstly, parameters for validation were estimated in pure solvent (Table 1). Detection and quantification limits (LOD and LOQ, respectively) were calculated statistically on the value of the blank at the retention time of the analyte (six injections), according to the IUPAC criterion[21] and as the injected amount that results in peaks with a height at least 3, and 10 times as high as noise level, respectively. In addition, LOQ was calculated as the amount that gives a previously defined precision (RSD = 5% in our case)[22] by injecting six samples, each time with decreasing amounts of the analyte.

Linear range was established between 0.01 and $5.00\,\mu g\,mL^{-1}$, with the lower limit, LOQ, calculated according to the EURACHEM Guidance[22] and the upper limit, the concentration for which the signal deviates from the linearity in

Table 1. Validation Parameters Obtained Using Standards Prepared in Solvent

LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	Linear Range ($\mu g\,L^{-1}$)	Slope	Intercept	*P*-value
4[a]	15[a]	20–5,000	1,715,896	68,914	0.87
5[b]	16[b]				
	20[c]				

[a]According to IUPAC criterion.
[b]According to S/N ratio criterion.
[c]According to EURACHEM Guidance.

3–5%[23] (Table 1). Calibration graphs were obtained by triplicate and Analysis of Variance (ANOVA) was used to detect lack of fit in the regression. The results obtained (P-value = 0.0087; $\alpha = 0.05$), shows that a linear relationship exists between signal and concentration.

To asses the efficiency of the proposed extraction and clean-up methods, recovery and precision studies were performed at three concentration levels (0.01, 0.1, and 0.5 mg kg^{-1}, corresponding to 0.1, 1.0, and 5.0 μg mL^{-1} injected), with recoveries too high for the lowest one, and RSD (%) lower than 4% in all cases (Table 2).

With the aim of checking the matrix effect, calibration lines obtained using solvent-based standards and matrix-matched standards were compared. The results show an enhancement effect on the analytical signal, as can be seen in Fig. 4. The comparison of the slopes of both straight lines, applying the t-test,[23] shows that significant differences exist between them (P-value = 0.0024; $\alpha = 0.05$) and 20 degrees of freedom. Once proven that matrix effect exists, analytical Figures of merit were calculated using matrix-matched standards. The LODs, LOQs, and the lower limit of the linear range obtained in this way, are slightly higher than the ones obtained using solvent based standards (Table 3). The results of the lack of fit test, also summarized in Table 3, shows, that the relationship between signal and concentration is linear (P-value = 0.64; $\alpha = 0.05$).

Recoveries obtained for spiked samples at three concentration levels (0.01, 0.1, and 0.5 mg kg^{-1}, corresponding to 0.1, 1.0, and 5.0 μg mL^{-1} injected) are summarized in Table 4. It can be seen that, the high recoveries are corrected and the found values are between the ranges accepted in residue analysis, with RSD (%) lower than 7.5% in all cases.

Analysis by HPLC-MS

Mass spectral information was acquired at a concentration of 0.1 μg mL^{-1} with the mobile phase gradient described in experimental section. The mass spectrum of acetamiprid was recorded using ES in positive and negative modes.

Table 2. Recovery Percentages Obtained for Spiked Cucumber Samples Using Standards Prepared in Solvent for the Quantification

Level (mg kg^{-1})	Recovery (%)
0.01	348.3 (1.5)
0.1	105.1 (3.8)
0.5	97.0 (1.6)

RSD (%) in parenthesis; ($n = 6$).

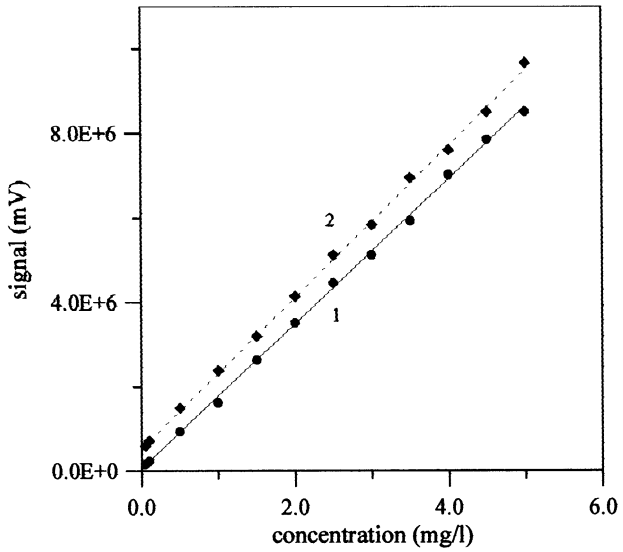

Figure 4. Calibration lines obtained using (1) solvent-based standards and (2) matrix-matched standards, using HPLC-fluorescence.

The best results, taking into account sensitivity and structural information, were obtained in the positive ion mode. Figure 5(a) shows the LC-ES-MS spectrum for acetamiprid under these experimental conditions. The ion used for quantification was m/z 223 and confirmation was carried out using both ions (m/z 223 and m/z 126). A positive result is confirmed if (a) the ion intensity ratios are within approximately 20% of those obtained on the same day from the reference standard and (b) the traces obtained for the ion-chromatograms each have peaks (minimum three data points, signal to noise ratio 3 : 1 or greater, no interfering

Table 3. Validation Parameters Obtained Using Matrix-Matched Standards

LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	Linear Range ($\mu g\,mL^{-1}$)	Slope	Intercept	*P*-value
6[a]	19[a]	0.05–5.00	1,796,901	525,692	0.64
15[b]	40[b]				
	50[c]				

[a]According to IUPAC criterion.
[b]According to S/N ratio criterion.
[c]According to EURACHEM guidance.

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Table 4. Recovery Percentages and RSD (%) Obtained for Spiked Cucumber Samples Using Matrix-Matched Standards for the Quantification

Level (mg kg^{-1})	Recovery (%)
0.01	67.4 (7.1)
0.1	69.4 (5.1)
0.5	74.3 (1.9)

RSD (%) in parenthesis; ($n = 6$).

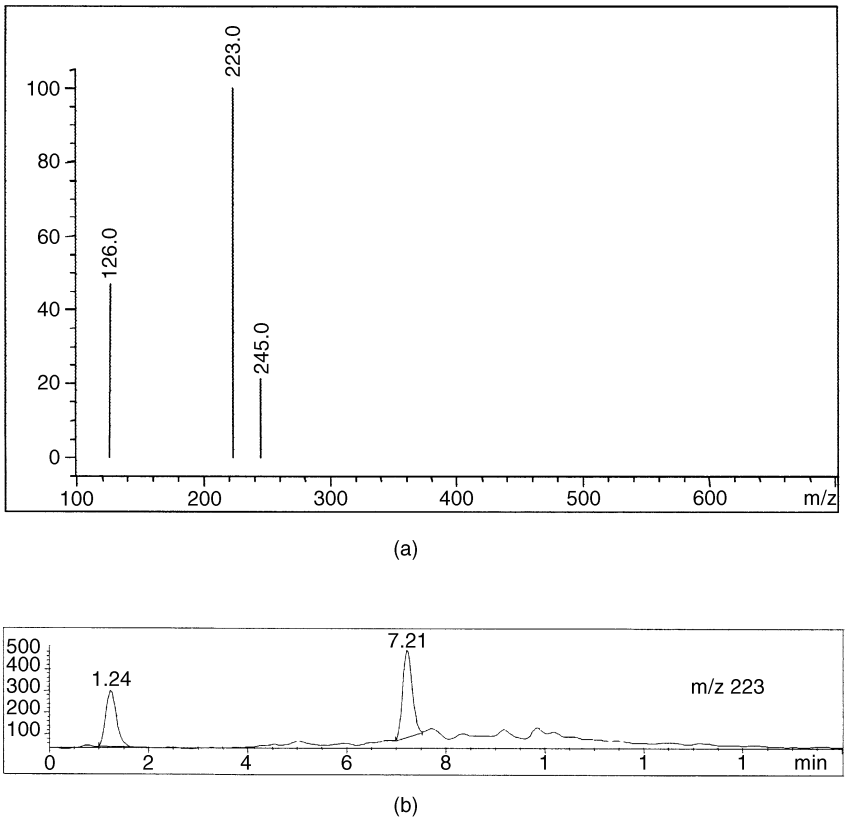

Figure 5. (a) LC-ES-MS spectrum for acetamiprid under experimental conditions and (b) chromatogram for acetamiprid in vegetable matrix using LC-ES-MS.

peaks) with the correct retention time and shape. Quantification of the pesticide was performed when S/N was greater than 10. Acetamiprid was sought at 7.08 ± 0.1 min window.

The linear range was established between 5 and $100\,\mu g\,L^{-1}$ (intercept: 1245.39, slope: 1,847,200) with correlation higher than 0.99.

LOD and LOQ were calculated as the injected amount that results in peaks with a S/N ratio greater than 3 and 10, respectively, obtaining 1.5 and $5\,\mu g\,L^{-1}$, respectively. These results are lower than the ones obtained by the previous HPLC-fluorescence photolysis method, using standards prepared in cucumber blank extract (Table 3).

The absence of matrix effect was checked by comparing the peak area in spiked cucumber blank extract samples with those obtained from standards prepared in solvent. Figure 5(b) shows the LC-ES-MS chromatogram obtained for $50\,\mu g\,L^{-1}$ of acetamiprid, prepared in blank extract matrix .

To determine recoveries, cucumber samples were spiked at 2 and $7\,\mu g\,kg^{-1}$ (corresponding to 0.02 and $0.07\,\mu g\,mL^{-1}$ injected), extracted, and analyzed as described in the experimental section. The mean recovery percentages were 72.1 and 77.0, respectively, both of them being in the range expected for residue analysis and with standard deviations associated lower than 4% ($n = 6$).

CONCLUSIONS

Two LC methods, using either fluorescence after photolysis or MS detection, have been developed and validated for the analysis of acetamiprid in cucumber samples. Both methods offer good linearity and sensitivity for the determination of the target pesticide at trace levels.

In the LC-fluorescence method, the matrix effect is overcome by using matrix matched standards. However, this effect does not appear in LC-MS, and therefore, this method is more rapid and gives better accuracy.

The LC-fluorescence method needs less expensive equipment and its LOD and LOQ are comparable with those obtained by LC-MS.

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REFERENCES

1. *Metabolic Pathways of Agrochemicals*; Roberts, T., Hutson, D., Eds.; The Royal Society of Chemistry: Cambridge, UK, 1999.
2. http://www.epa.gov/opppmsd1/PR_Notices/pr97-2.htmL.
3. http://www.epa.gov/opppmsd1/PR_Notices/pr98-7.htmL.
4. http://www.epa.gov/pesticides/chemreg.htm.
5. Tokieda, M.; Ozawa, M.; Gomyo, T. J. Pest. Sci. **1999**, *24*, 181.
6. Mateu, M.; Moreno, M.; Arrebola, F.J.; Martínez Vidal, J.L. Pest. Sci. *submitted.*
7. Tokieda, M.; Tanaka, T.; Ozawa, M.; Gomyo, T. J. Pest. Sci. **1998**, *23*, 296.
8. Wanatabe, S.; Ito, S.; Kamata, Y.; Omoda, N.; Yamazaki, T.; Munakata, H.; Kaneko, T.; Yuasa, Y. Anal. Chim. Acta **2001**, *427*, 211.
9. Coly, A.; Aaron, J.J. Talanta **1999**, *46*, 815.
10. Coly, A.; Aaron, J.J. Anal. Chim. Acta **1998**, *360*, 129.
11. Coly, A.; Aaron, J.J. Talanta **1999**, *49*, 107.
12. Maafi, M.; Mahedero, M.C.; Aaron, J.J. Talanta **1997**, *44*, 2193.
13. Gil García, M.D.; Martínez Galera, M.; López López, T.; Martínez Vidal, J.L.; Mahedero, M.C.; Salinas, F. Talanta **2001**, *53*, 915.
14. Coly, A.; Aaron, J.J. Analusis **1996**, *24*, 107.
15. García, L.F.; Aaron, J.J. Mikrochim. Acta **1997**, *126*, 289.
16. Miles, C.J.; Moye, H.A. Chromatographia **1987**, *24*, 628.
17. Miles, C.J.; Moye, H.A. Anal. Chem. **1988**, *60*, 220.
18. Patel, B.M.; Moye, H.A.; Weinberger, R. Talanta **1991**, *38*, 913.
19. Martínez Galera, M.; López López, T.; Gil García, M.D.; Martínez Vidal, J.L.; Parrilla Vázquez, P. J. Chromatogr. A **2001**, *918*, 79.
20. Lopez Lopez, T.; Gil Garcia, M.D.; Martínez Vidal, J.L; Martínez Galera, M. Anal. Chim. Acta **2001**, *447*, 101.
21. Curie, L.A.; Pure Appl. Chem. **1995**, *67*, 1699.
22. EURACHEM Guidance Document No. 1. WELAC Guidance Document No. WGD 2. *Accreditation for the Chemical Laboratories: Guidance on the Interpretation of the EN 45000 Series of Standards and ISO/IEC Guide 25*; 1993. Available from the EURACHEM Secretariat, PO Box 45, Teddington, Middlesex, TW11 ONH, UK.
23. Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De long, S.; Lewi, P.J.; Smeyers-Verbeke, J. *Handbook of Chemometrics and Qualimetrics: Part 2*; Elsevier Science: Amsterdam, The Netherlands, 1997.

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