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Determination of imidacloprid in vegetables by high-performance liquid chromatography with diode-array detection

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Abstract

An HPLC method is described for the determination of imidacloprid residues in vegetables at levels ranging from 0.01 to 0.60 mg/kg. The selection of the extraction and clean-up procedure is discussed. Spectral data obtained with diode-array detection allow the identification of imidacloprid residues. Thermospray mass spectrometric studies were carried out in combination with HPLC. The mean recoveries and standard deviations were 95% and 4.7%, respectively, in the various crops tested. Registration of the analytical results for a control sample in quality control charts demonstrated the performance of the method. Data for incurred residues of imidacloprid in vegetable samples routinely applying the proposed method are also presented.

1. Introduction

Gas chromatography (GC) still appears to be the major analytical technique in monitoring pesticide residues in agricultural foods, owing to its high separation power, the availability of selective detectors and a historical knowledge of laboratories involved in pesticide residue analysis. However, the number of compounds or breakdown products of concern which cannot be analysed directly by GC owing to their poor volatility, polarity and/or thermal instability has grown dramatically in the last few years. Hence the use of high-performance liquid chromatography (HPLC) on alkyl-bonded silica stationary

phases for screening purposes is becoming popular, especially since the introduction of sensitive diode-array detection (DAD) owing to its suitability in selecting the optimum wavelength for maximum sensitivity and confirmation from UV spectral information [1–3]. However, its application in residue analysis is hampered mainly by the necessity for critical sample preparation because of the lack of sensitivity of DAD and the large amounts of UV interferences in vegetable extracts.

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] (Fig. 1) is a recent systematic and contact insecticide, which cannot be determined directly by GC, recently introduced in Europe by Bayer (Leverkusen, Germany) for insect control in a variety of

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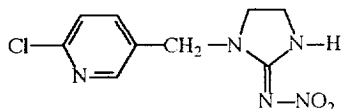


Fig. 1. Structure of imidacloprid.

vegetables. The development, activity, mode of action and effectiveness have been described by Leicht [4] and its physical, chemical and toxicological properties have been summarized in a pesticide manual [5]. Imidacloprid was used for the first time during the agricultural season 1993–94 in Almería, a Spanish province, located on the mediterranean coast, where over 500 000 tonnes of vegetables are produced annually for export. The Spanish tolerances [6] in pepper, tomatoes and cucumber are 0.5, 0.1 and 0.1 mg/kg, respectively. Its use is mainly to control greenhouse whiteflies. No method has been published for the determination of residues of this insecticide in vegetables. Placke and Weber [7] measured residual levels of imidacloprid in different fruits and vegetables by HPLC–UV detection, but their method is too laborious with high solvent consumption. Sample extraction is followed by a multi-step clean-up involving three evaporations plus either partitions or the use of a solid-phase clean-up cartridge.

This work is part of wider studies in our laboratory to develop HPLC–diode-array detection (DAD) multi-residue analytical methods for pesticides currently used on vegetable matrices in mediterranean areas. The aims of our work were as follows: (1) to evaluate the efficiency of well known solvent extraction systems used for multi-residue methods (MRMs), such as the Luke [8,9], Mills [10,11] and Leary [12–14] methods for extraction and clean-up of residues of imidacloprid followed by HPLC–DAD analysis; (2) to develop a simpler, faster and sensitive methodology for determining imidacloprid residues in vegetables by HPLC–DAD, considering aspects such as quantification, recoveries, analysis of control samples (Shewhart charts) and identification problems and also characterization by HPLC–MS; and (3) the study the applicability of the proposed method in routine practice by

analysing 54 real crop samples containing incurred residues of imidacloprid.

2. Experimental

2.1. Instrumentation

Analysis was carried out using a Hewlett-Packard (HP) HPLC system consisting of a Model 1050 pump (Hewlett-Packard, Waldbronn, Germany), a Rheodyne six-port injection valve with a 20- μ l loop, an HP Model 1040 photodiode-array detector and an HP 500 DeskJet printer. Data acquisition and processing were performed with an HP microcomputer using HPLC3D Chemstation(DOS Series) software.

Mass spectrometric analysis was carried out using a Hewlett-Packard (Palo Alto, CA, USA) Model 5988A thermospray quadrupole mass spectrometer and a Hewlett-Packard 59970C instrument for acquisition and data processing. In all experiments the filament was on.

2.2. Reagents and materials

HPLC-grade solvents (Merck) were used. Ammonium acetate was purchased from Merck (Darmstadt, Germany). Mobile phases were degassed with helium prior to use. Distilled water was obtained from a Millipore Milli-Q water-purification system. To prepare dihydrogenphosphate–NaOH buffer (2 mol/l), KH_2PO_4 (121 g) was dissolved in deionized, distilled water (500 ml), the pH was adjusted to 7.2 with 1 mol/l NaOH and the solution was diluted to 1.0 l. Sodium chloride was added to the buffer solution to achieve saturation, while stirring constantly.

Cartridges prepacked with Sep-Pak Vac RC C_{18} chemically bonded silica (0.1 g) were used (Waters). All cartridges were conditioned with acetonitrile (5 ml) and acetonitrile–water (25:75) (5 ml).

Imidacloprid pesticide standard was obtained from Bayer (Leverkusen, Germany). A stock standard solution was prepared by dissolving 25

mg of the pesticide in 100 ml of acetonitrile. A working standard solution was prepared by diluting 5 ml of the stock standard solution and making up to 100 ml with acetonitrile. All solvents were filtered through Millipore membrane filters before injection on to the column.

2.3. HPLC separations

HPLC separations were conducted using a LiChrospher C₁₈ (5 μm particle size) column (125 × 4 mm I.D.) (Merck).

Low-level detection requires that the interfering peaks in vegetable samples should be not too large. The shape of the matrix peaks depends on the nature of the sample and also on the organic mobile phase content. For better evaluation of the results, we selected a mobile phase gradient of acetonitrile–water; with the following acetonitrile content: 0–2 min, 25%; 12 min, 90%; and 15 min, 100%. The equilibration time was 5 min, the injection volume 20 μl, the mobile phase flow-rate 1 ml/min and the detection wavelength 270 nm. The void time was obtained by using methanol as the unretained component for all mobile phases. All chromatographic runs were performed in duplicate; the reproducibility of the retention times was ±0.5% or better.

2.4. Comparison study of different solvent extraction systems

Different aliquots of untreated pepper, tomato and cucumber samples (50 g fresh mass) collected from greenhouses in the vicinity of Almería, Spain, were fortified as follows. A 1-ml volume of the imidacloprid working standard solution was added to 50 g of chopped samples in a high-speed blending jar and the sample was homogenized for 2 min. After 30 min the sample was again homogenized for 2 min and immediately extracted according to the following three procedures. Each recovery assay from these fortified samples containing 0.25 mg/kg of imidacloprid was repeated for each commodity and the extraction procedure three times.

Procedure A. Extraction with acetonitrile

A 50-g amount of fortified sample is blended with 200 ml of acetonitrile for 10 min, the mixture is filtered through a 12-cm Buchner filter. The filtered is rinsed with 50 ml of acetonitrile, the filtrate is transferred to a separating funnel (500 ml) and 10 ml of phosphate buffer solution are added. The separating funnel is shaken vigorously for 1 min and the filtrate is allowed to separate into two phases. The acetonitrile phase is filtered through a layer of anhydrous sodium sulphate (ca. 10 g) placed in a funnel plugged with glass-wool. The acetonitrile extract is evaporated in a rotary evaporator using a 40–50°C water-bath. The final volume is adjusted to 5 ml with acetonitrile–water (1:3) and sonicated for 5 min. This final solution is filtered through a membrane filter disc attached to the end of a syringe (10 ml) and is ready for HPLC analysis.

Procedure B. Extraction with acetone

A 50-g amount of fortified sample is blended with 100 ml of acetone for 5 min and the mixture is filtered through a 12-cm Buchner filter. The filter is rinsed with 50 ml of acetone, the filtrate is transferred to a separating funnel (500 ml) and 200 ml of dichloromethane–light petroleum (1:1) are added. The separating funnel is shaken vigorously for 1 min and the filtrate is allowed to separate into two phases. The dichloromethane phase is filtered through a layer of anhydrous sodium sulphate (ca. 10 g) placed in a funnel plugged with glass-wool. The dichloromethane extract is evaporated in a rotary evaporator using a 40–50°C water-bath. The volume is adjusted to 5 ml with acetonitrile–water (1:3) and sonicated for 5 min. This final solution is filtered through a membrane filter disc attached to the end of a syringe (10 ml) and is ready for HPLC analysis.

Procedure C. Extraction with ethyl acetate

A 50-g amount of fortified sample is blended with 200 ml of ethyl acetate for 5 min. The supernatant liquid is filtered under suction through a filter-paper and a layer of 20 g of anhydrous sodium sulfate. The filter is rinsed with 50 ml of ethyl acetate and the combined

extracts are evaporated on a vacuum rotary evaporator using a 40–50°C water-bath. The residue is dissolved in 5 ml of acetonitrile–water (1:3) and sonicated for 5 min. This final solution is filtered through a membrane filter disc attached to the end of a syringe (10 ml) and is ready for HPLC analysis.

2.5. Clean-up

A representative portion of sample, sampled according to the EC Directive 79/700 [15], collected in the vicinity of Almería, as prepared following procedure B described above and is subjected to a clean-up step on a 0.1-g C₁₈ disposable cartridge (Fig. 2). A 20- μ l volume of this cleaned-up extract is injected into the HPLC–DAD system.

2.6. Identification of imidacloprid

The identification of imidacloprid was based on the retention time and UV spectrum by constructing a spectral chromatographic library using the HPLC3D Chemstation (DOS Series) software to compare spectra generated from an unknown sample with the spectrum acquired under the same HPLC mobile phase conditions of a 2.5 mg/l standard solution of imidacloprid and stored in the library on disk under the following conditions: spectral range, 200–500 nm; number of points, 5; and smooth, 10.

The HPLC–thermospray mass spectrum and the detection limit of imidacloprid were studied in the isocratic mode [methanol–water (50:50) with the addition of 0.05 M ammonium acetate]. The thermospray temperatures were 100°C (stem) and 200°C (tip). The ion source temperature was set at 240°C.

2.7. Recovery studies

The proposed sample preparation method was validated by carrying out a recovery study on fresh pepper, tomato and cucumber samples, which were not treated with the pesticide studied, and fortified following the procedure described above with 1 and 0.2 ml of the working

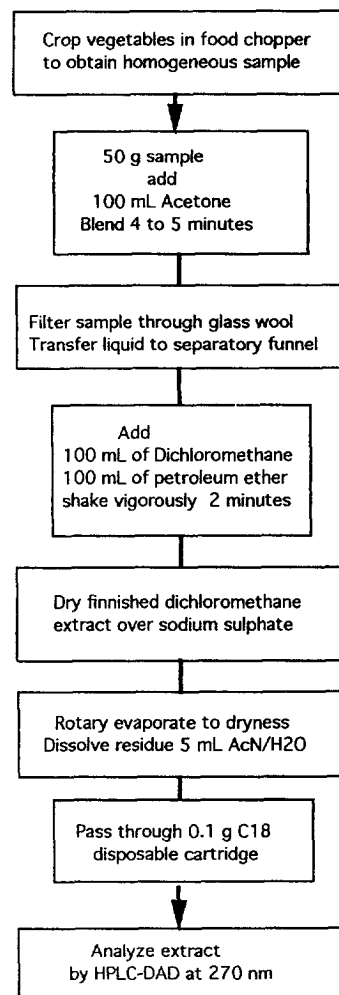


Fig. 2. Flow diagram of the method.

standard mixture solution. These values represent 0.25–0.05 mg/kg of imidacloprid in the sample. The recovery assays were replicated five times.

3. Results and discussion

3.1. Selection of extraction procedure

A different chromatographic profile was obtained for each type of vegetable, with the pepper extract resulting in the most complex chromatogram. Fig. 3 shows typical chromato-

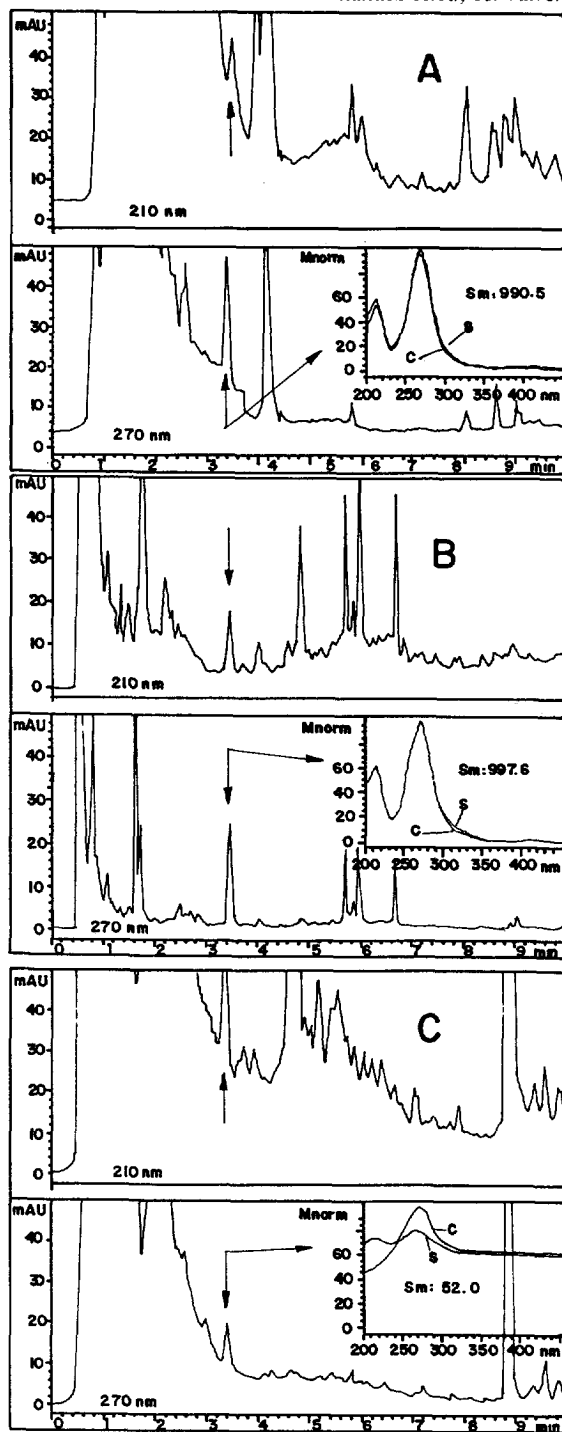


Fig. 3. HPLC–DAD at 210 and 270 nm following application of procedures A, B and C (see text) to pepper samples spiked at 0.25 mg/kg (injection volume 20 μ l). Comparison of standard spectra (s) and unknown peak (c) spectrum for imidacloprid.

grams at 210 and 270 nm of the extracts obtained with procedures A, B and C for pepper samples fortified with imidacloprid at 0.25 mg/kg and the spectral matches obtained at 3.4 min, the retention time of imidacloprid. The spectral matches for method C show low values owing to the high background level at this time. Recoveries for imidacloprid with the three procedures are given in Table 1. It can be seen that good recoveries are obtained with methods A and B and lower values with method C, probably as consequence of the low solvating power of ethyl acetate [16]. Procedure A shows unsatisfactory chromatograms at 210 nm because of the large number of co-eluting peaks, specially in the first 6 min of the chromatogram where imidacloprid elutes. In comparison, the acetone procedure is cleaner, can be used with commodities with a high sugar content because it does not form a two-phase system with water in the presence of sugar [9] and the solvent evaporation step is faster. We therefore selected this procedure for the determination of residues of imidacloprid in vegetables.

In order to achieve a cleaner extract, free of pigments and polar plant co-extractives, and to preserve the chromatographic system, a single clean-up step was incorporated by passing the final plant extract (acetonitrile–water) through a C_{18} reversed-phase cartridge (0.1 g). Most of the plant pigments are removed and the eluate is pale yellow, light green or colourless, depending on the vegetable sample. In Fig. 4 chromatograms are displayed at 210 and 270 nm, corresponding to the extracts obtained from pepper

Table 1
Recovery of imidacloprid from fortified pepper samples using procedures A, B and C

Crop	Spike level (mg/kg)	Recovery (%) ^a		
		A	B	C
Pepper	0.25	123	101	52
Tomato	0.25	114	98	69
Cucumber	0.25	102	105	47

^a Recovery data are mean values of three determinations.

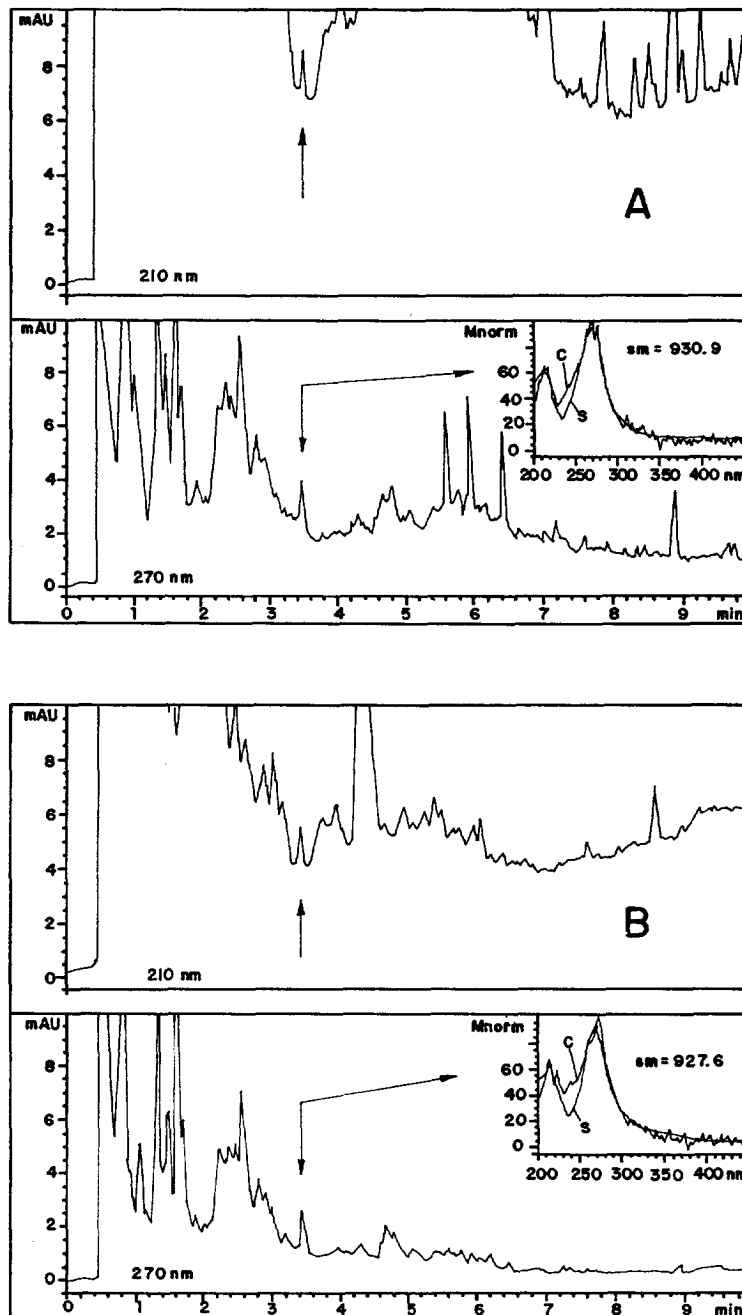


Fig. 4. HPLC–DAD chromatograms of pepper samples spiked at 0.01 mg/kg obtained by operation conditions (A) without clean up, (B) cleaned up by 0.1 g of C18 bonded silica cartridges. Comparison of standard spectra (s) and unknown peak (c) spectrum for imidacloprid.

samples fortified with 0.01 mg/kg of imidacloprid by application of procedure B, (A) without clean-up and (B) with C_{18} clean-up. A decrease in background level can be seen. Chromatographic run times of 15 min for pepper, tomato and cucumber extracts left the column clean for the next analysis.

3.2. UV studies of imidacloprid. Identification

The UV spectrum of the imidacloprid standard is obtained in the mobile phase to be used for the chromatographic analysis mentioned above. This absorption spectrum shows two maxima, at 214 and 270 nm, with apparent molar absorptivities of $1.8 \cdot 10^3$ and $3.1 \cdot 10^3$ l/mol·cm, respectively. We selected 270 nm as the detection wavelength for analysis in order to avoid interferences and improve the detection limit. The HPLC–DAD identification studies were based on the retention time and UV spectrum. As the value of the spectral match for a vegetable extract fortified at a concentration of 0.1 mg/l (this represents a residue level of 0.01 mg/kg in a vegetable sample, 10% of the lowest maximum

residue limit (MRL) of imidacloprid in vegetables) is around 920, we consider this value of the spectral match to be the limit of positive identification. This value represents 2 ng absolute of pesticide injected.

3.3. Mass spectrometric study

Apart from demonstrating the applicability of thermospray (TSP) for the identification of imidacloprid in vegetable matrices, the dependence of the ion abundances in the imidacloprid TSP mass spectra on the gas-phase and vaporizer temperatures was also investigated in the positive ionization mode (PI). An increase in the gas-phase temperature led to enhanced fragmentation, as shown in Fig. 5 and as described previously [17]. The base peak is at m/z 256, corresponding to $[M - H]^+$. A fragmentation pattern at m/z 211 corresponding to $[M + 2H - NO_2]^+$ was observed with an ion source temperature of at least 220°C. Variation of the vaporizer temperature had only a slight influence on the fragmentation. Cluster ions $[M + NH_4]^+$ (m/z 273), $[M + H + MeOH]^+$ (m/z 288) and

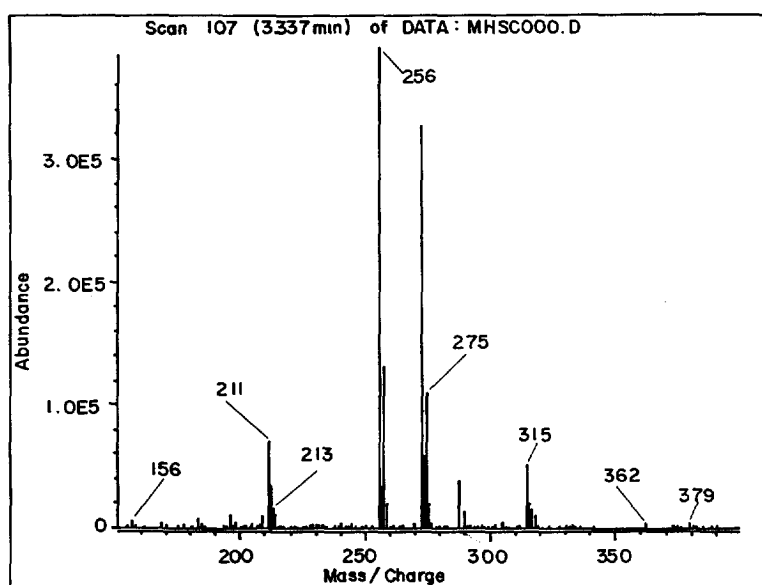


Fig. 5. Flow injection PI-mode TSP mass spectrum of imidacloprid (500 ng). Carrier stream methanol–water (50:50) + 0.05 M ammonium acetate; flow-rate, 1 ml/min. Stem, tip and source temperatures, 100, 200 and 240°C, respectively.

$[M + H + CH_3CONH_2]^+$ (m/z 315), whose relative abundances decreased when the gas-phase temperature increased, were also observed with a water–methanol mobile phase containing ammonium acetate (0.05 M) as an additive. Acetamide is an impurity present in the commercial ammonium acetate salt from Merck and an acetamide adduct appears because imidacloprid has a proton affinity near or below that of acetamide [18]. Induced fragmentation and cluster ions can be very useful for an unambiguous identification of imidacloprid in real samples. The detection limit using an RP C₁₈ column and the PI mode was calculated to be ca. 20 ng in the full-scan mode and 500 pg in the selected-ion monitoring mode using two diagnostic ions (m/z 211 and 256).

3.4. Linearity, reproducibility and recovery

The linear dynamic range of the UV response was checked for pepper, tomato and cucumber extracts fortified with imidacloprid and appeared to be 2.0–120.0 ng (0.01–0.60 mg/kg) absolute amount injected and the correlation coefficients were all satisfactory ($R^2 > 0.991$) using the peak height at 270 nm. Non-fortified samples were also analysed in order to ensure that no peak was obtained at the retention time of the analyte. An important point is that the calibration equations are virtually identical when constructed from each type of vegetable studied. A concentration of 0.01 mg/kg can be considered as the detection limit of the method as lower concentrations do not always allow a proper definition of the spectrum, or the spectral match obtained is lower than 920. This level is low enough for monitoring imidacloprid in vegetables.

The retention time was 3.49 min and the reproducibility of the retention time was tested by the analysis of five fortified pepper samples. The within-day relative standard deviation (R.S.D.) 0.2%.

The procedure was validated by carrying out a recovery study on pepper, tomato and cucumber samples, fortified at two levels, 0.25 and 0.05 mg/kg. The recovery of imidacloprid was complete and independent of the commodity and

fortification level. The average recoveries for five replicates were 88–94% for addition of 0.25 mg/kg and 96–99% for addition of 0.05 mg/kg. The overall average recovery for 30 determinations was 95% with an R.S.D. of 4.7%.

3.5. Control sample

As a quality assurance measure, a control sample was repeatedly analysed over a period of 2 months. Green pepper was chosen as the matrix, and 500 g of blended pepper were fortified with imidacloprid at 0.25 mg/kg following the procedure described above. Seven aliquots of this fortified sample were packed separately in polyethylene bags and kept frozen at -18°C until analysis. The analysis of the samples was performed every nine days during a period of 2 months. The analytical data for the fortified control sample were registered on a Shewhart control chart and all of data points fell inside the control limits ($\pm 3s$). These results confirm the overall reliability of the optimized method in routine analysis.

3.6. Application of the method to real samples

Fifty-four vegetable samples (peppers, tomatoes and cucumbers) with incurred residues, from three different greenhouse plantations, treated with Confidor 20LS [Bayer; 20% (w/v) of imidacloprid] at different doses and collected during the week after treatment, were selected to evaluate the effectiveness of the proposed HPLC method. Residues of imidacloprid were found in all samples in the range 0.01–0.3 mg/kg. The results of the analysis are shown in Fig. 6.

4. Conclusions

Of the three liquid–liquid extraction systems assessed for extraction of residues of imidacloprid in vegetables, the acetone–dichloromethane method was selected owing to the good recovery values and small number of co-extractives obtained. This method combined with HPLC

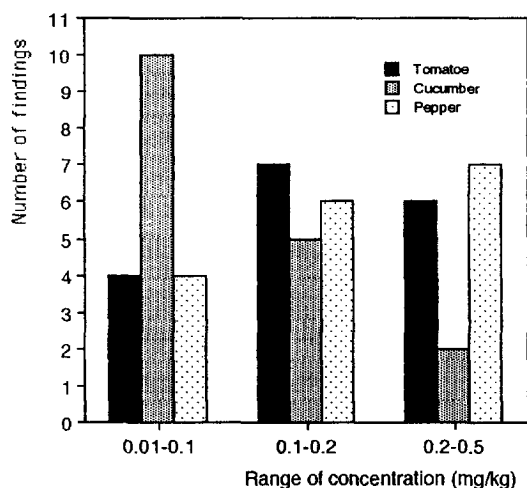


Fig. 6. Residue findings of imidacloprid within intervals of concentration 0.01–0.10, 0.10–0.20 and 0.20–0.30 mg/kg obtained in the analysis of 54 vegetable (tomatoes, cucumbers and peppers) samples with incurred residues.

diode-array UV detection, using a wavelength of 270 nm in the quantification step, permits the identification and determination of imidacloprid residues in vegetables at levels ≥ 0.01 mg/kg. The applicability of the method was demonstrated by recovery studies, quality control charts and data for real samples. Further, the HPLC–thermospray MS technique has been demonstrated to be adequate for confirming the presence of imidacloprid in vegetable samples.

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