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Resolution of imidacloprid pesticide and its metabolite 6-chloronicotinic acid using cross-sections of spectrochromatograms obtained by high-performance liquid chromatography with diode-array detection

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Abstract

The analysis by HPLC–DAD of imidacloprid and its main metabolite 6-chloronicotinic acid (two very polar compounds) has been carried out by using a graphical computer-aided technique. It is based on performing cross-sections through the three-dimensional data matrix (A, λ, t) to obtain selective analytical information for each of the analytes. The procedure allows the simultaneous determination of these pesticides in mixtures and has been successfully applied to their determination in groundwater at $\mu g\, l^{-1}$ levels, after extraction with dichloromethane. © 1998 Elsevier Science B.V.

Keywords: Pesticides; Imidacloprid; 6-Chloronicotinic acid

1. Introduction

In recent years the presence of pesticides, parent compounds and major metabolites or conversion products in surface and ground waters has become a serious environmental concern. Therefore, analytical methods are needed which are suitable for identifying and accurately quantifying pesticides and their major conversion products in aquatic systems and for adaptations to regional situations.

In comparison with GC-based techniques, reversed-phase liquid chromatography (RPLC) is a more suitable technique for the determination of polar analytes in water [1–4], since derivatisation is usually superfluous and in the analysis of aqueous samples the mobile phase system is fully compatible resulting in a high potential for automation. The wide application range, long-term stability, ease of use, low cost and improved selectivity (diode array) means that UV detection is widely used in residue analysis [5–7]. However, UV detection does not deliver high sensitivity. Hence, in trace analysis it usually requires concentration procedures.

Imidacloprid, 1-(6-chloronicotinyl)-2-nitroimino-imidazolidine, is a recent systemic and contact insecticide with high activity especially against sucking pests [8]. In the research to develop this insecticide, it became clear that the 6-chloro-3-pyridylmethyl group was an essential structural element for insecticidal activity, and it has been called imidacloprid and its related insecticide class

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chloronicotinoids after the common name of the 3-pyridylmethyl group [9].

Electrophysiological studies of the chloronicotinoids [10] and their inhibition of α-bungarotoxin binding indicated that they had the same mode of action as natural insecticides [9].

Imidacloprid, recently introduced in Europe by Bayer (Leverkusen, Germany) and its main metabolite are very polar compounds with high solubility in water. The development, activity, mode of action and effectiveness have been described by Leicht [11] and its physical, chemical and toxicological properties have been summarized in a pesticide manual [12]. Although some papers have been found which deal with the determination of imidacloprid residues in vegetables [13] no methods have been published for the determination of residues of this insecticide and its metabolite in water.

Imidacloprid and 6-chloronicotinic acid cannot be determined directly by GC and they are analysed by HPLC [9,13]. HPLC coupled to UV detection is particularly suitable in this case due to their strong absorbance between 230 and 270 nm.

RPLC can be used to determine polar organic compounds. However, when working with highly polar analytes, problems are encountered. Retention is small even on highly hydrophobic C_{18}-bonded silica phases and, as a result only a limited separation capacity is available to separate the analyte(s) from early eluting interferences.

The objective of this work was the determination of the pesticide imidacloprid and its metabolite 6-chloronicotinic acid simultaneously by using a methodological approach developed by us and successfully applied in the resolution of pesticide mixtures by HPLC–DAD [14–16].

The procedure was applied to the determination of these pesticides in groundwater at $\mu g\, l^{-1}$ levels after liquid–liquid extraction (LLE) with dichloromethane.

2. Experimental

2.1. Chemicals and solvents

HPLC grade solvents were used. The pesticide standards (pestanal quality), were obtained from Riedel-de Haën (Seelze, Germany). Solid standards were dissolved in acetonitrile and stored at 4°C in the dark, where they were stable for several months. Working solutions were prepared daily by appropriate dilution with acetonitrile. Mobile phases were degassed with helium prior to use. Distilled water was obtained from a Millipore (Bedford, MA, USA) Milli-Q water purification system. All solvents and samples were filtered through Millipore membrane filters before injection into the column.

Dichloromethane was of residue analysis quality (Riedel-de Haën). A 0.01 *M* phosphate buffer (pH 3.0) was prepared from anhydrous di-sodiumhydrogen phosphate (Merck, Darmstadt, Germany) and ortho-phosphoric acid (Panreac, Barcelona, Spain) in Millipore Milli-Q purified water (HPLC-grade water); chemicals were of analytical-reagent grade or better.

2.2. Apparatus

A Waters (Milford, MA, USA) Model 990 liquid chromatographic system was used, equipped with a Model 600E constant-flow pump, a Rheodyne six-port injection valve with a 20-μl sample loop and a Model 990 photodiode-array detector. The spectral resolution used was 1.4 nm per diode in the range 200–350 nm.

HPLC separations were carried out using a Microspher Chrompack 100×4.6 mm (3-μm particle size) C_{18} column.

A rotary vacuum evaporator (Buchi 461) with thermostatic waterbath and vacuum pump was used.

2.3. Software

An IBM 486 DX personal computer was used for acquisition and treatment of the data. The Waters 991 software controlling the instrument generates a three-dimensional file (A, λ, t) in binary format. The analysis and manipulation of the data was carried out with SURFER V. 5.02 and GRAPHER V. 1.27 [17].

2.4. HPLC operating conditions

The operating conditions were: flow-rate: 1 $ml\, min^{-1}$; chart speed: 0.5 $cm\, min^{-1}$; detector sensitivity: 0.02 a.u.f.s.; column at room temperature.

The mobile phase was acetonitrile–0.01 *M* phosphate buffer (pH 3.0) (25:75, v/v). The solvents were filtered daily through a 0.45-μm cellulose acetate (water) or polytetrafluoroethylene (acetonitrile) membrane filter before use, and degassed with helium during and before use.

3. Results and discussion

Imidacloprid and 6-chloronicotinic acid display overlapping absorption spectra with absorption maxima located at 200, 230 and 270 nm for 6-chloronicotinic acid and at 210 and 270 nm for imidacloprid. 6-Chloronicotinic acid and imidacloprid are easily eluted by aqueous methanol or aqueous acetonitrile with 15% of organic modifier. Aqueous methanol gives retention times higher than aqueous acetonitrile but peaks were broader than with acetonitrile and R_s values were lower. The peak corresponding to 6-chloronicotinic acid approached the solvent peak as the percentage acetonitrile increased and for 25% of acetonitrile they overlapped, as shown in Fig. 1.

In addition, under these conditions the retention time of imidacloprid showed poor repeatability, with R.S.D. values higher than 10%.

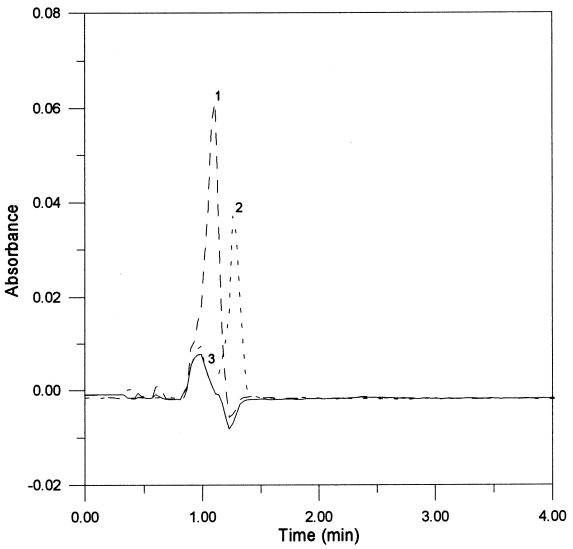

Fig. 1. LC chromatograms of 8 μg ml^{-1} of 6-chloronicotinic acid (peak 1) 8 μg ml^{-1} of imidacloprid (peak 2) and solvent (peak 3) at 220 nm. Mobile phase: ACN–water (25:75, v/v).

To improve these results, acetonitrile–water mobile phases, in which the pH of the aqueous portion was modified by using phosphate buffer solution (pH 3), were assayed. The results in Table 1 show that the retention times increased but the peaks were broader and showed overlap. Acceptable results were obtained with 15–25% of aqueous acetonitrile, but this latter percentage gave narrower peaks, which favoured resolution and detection. However, the resolution between the analyte peaks had a value much lower than unity.

In accordance with these results a mobile phase of acetonitrile–0.01 *M* phosphate buffer (pH 3.0) (25:75, v/v) was chosen. Fig. 2 shows LC chromatograms of 6-chloronicotinic acid and imidacloprid with the mobile phase selected at 220 nm, where overlapping among the peaks of 6-chloronicotinic acid and imidacloprid can be observed, but separation from solvent peak has been attained.

In multicomponent mixtures, where the analytes are not resolved in the column but where spectral overlap is minimal, the analytes can be determined simultaneously by monitoring each component at a wavelength which is free of interference.

However, imidacloprid and its metabolite coeluted and their absorption spectra overlapped; in this situation, it is possible to apply chemometric techniques, such as PLS or PCR, in order to extract useful analytical information from the overlapped region [18–20]. Moreover, to make use of the information enclosed in the spectrochromatographic data obtained in HPLC–DAD, it is possible to carry out sections, based on linear trajectories, with variable gradient through the three-dimensional matrix

Table 1
Influence of percentage acetonitrile in 0.01 *M* H_3PO_4–HNa_2PO_4 (pH 3), on retention of 6-chloronicotinic acid and imidacloprid

Acetonitrile (%)	t_R		R_s
	6-Chloronicotinic acid	Imidacloprid	
15	3.02	3.13	0.07
25	2.79	2.98	0.15
35	2.34	2.41	0.09
45	1.87	1.87	0
55	1.60	1.60	0

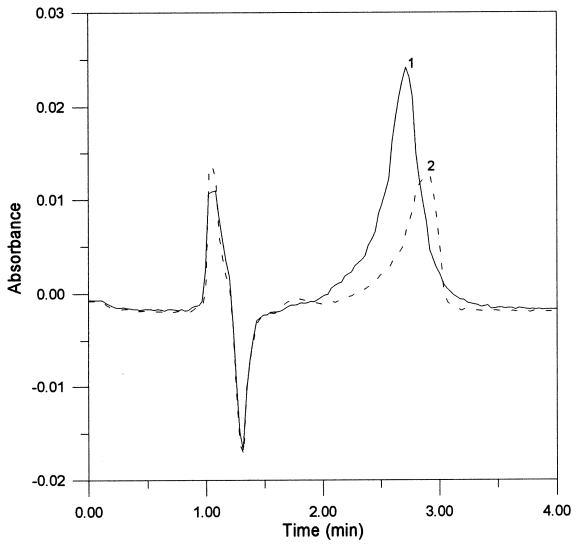

Fig. 2. LC chromatograms of 8 μg ml^{-1} of 6-chloronicotinic acid (peak 1), 8 μg ml^{-1} of imidacloprid (peak 2) at 220 nm. Mobile phase: ACN–0.01 *M* phosphate buffer (pH 3.0), (25:75, v/v).

data. These sections produce a new profile in which the sensibility and/or selectivity of the determination are maximized.

3.1. Cross-sections through the three-dimensional data matrix

Fig. 3 shows the spectrochromatogram corresponding to 8 μg ml^{-1} of 6-chloronicotinic acid and 8 μg ml^{-1} of imidacloprid, presented as a contour plot.

One trajectory has been selected through this plot to define the corresponding cross-section in order to get bidimensional projections with the best analytical selectivity and sensibility. The cross-section is defined by two linear paths, whose initial and final coordinates (λ, t) are (200, 120)–(250, 200) for the first path and (250, 200)–(295, 170) for the second path (Fig. 3).

With the aim of demonstrating that the trajectory taken is free from interferences, the selected cross-section was applied to individual standards of 6-chloronicotinic acid and imidacloprid. The suitable bidimensional projections on the wavelength domain

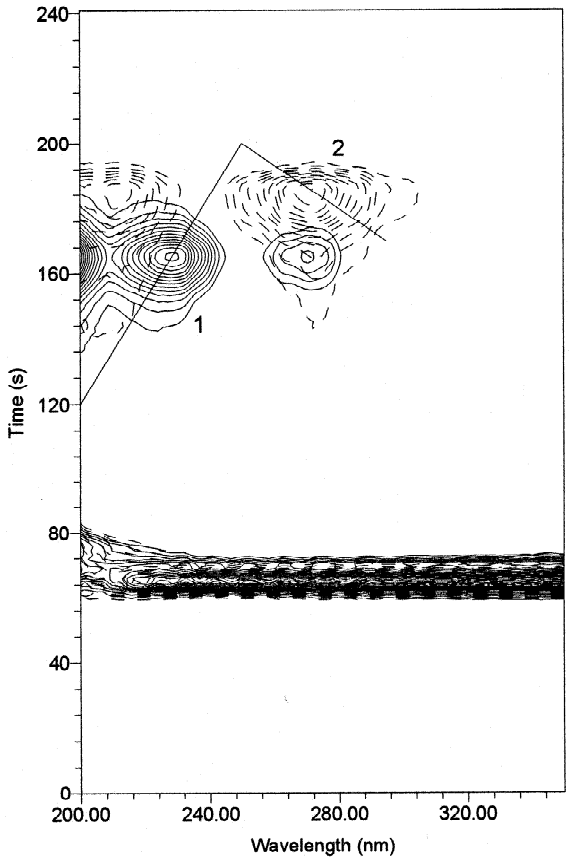

Fig. 3. Contour plot of (1) 6-chloronicotinic acid and (2) imidacloprid at concentrations of 8 μg ml^{-1}, where the trajectory selected is plotted.

are superposed and represented in Fig. 4. It is clear that the combined use of chromatographical and spectral information allows us to carry out the quantitation of each analyte, as the signal from one analyte is free from interferences from the other analyte. From an inspection of Figs. 2 and 4 it is evident that this methodology gives a sensitivity which is better than the application of multivariate calibration methods at a compromise wavelength such as 220 nm, at least for imidacloprid. The above proves the advantage of this approach over the classic techniques of cutting through the three-dimensional matrix at constant wavelength, obtaining chromatograms, or at constant time, obtaining absorption spectra.

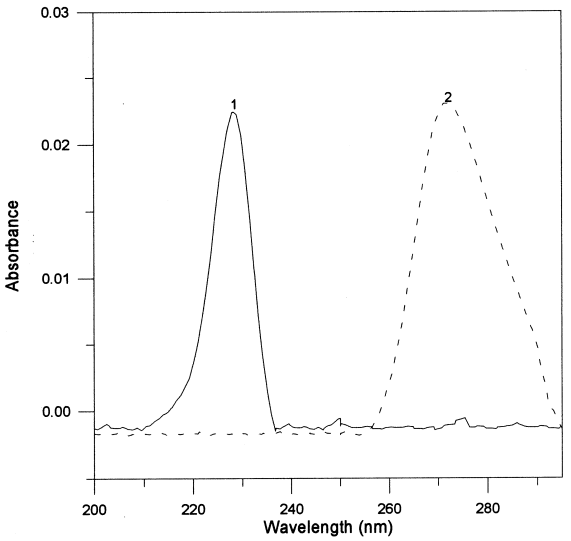

Fig. 4. Two-dimensional projections of the cross-sections produced from the three-dimensional data by plotting absorbance versus wavelength: (1) 6-chloronicotinic acid (8 μg ml^{-1}) and (2) imidacloprid (8 μg ml^{-1}).

3.2. Calibration graphs

Calibration graphs were obtained from peak heights of two-dimensional projections for samples of mixtures of the two compounds, containing different concentrations of 6-chloronicotinic acid and imidacloprid. Good linearity was obtained for these compounds in the 0.5–8.0 μg ml^{-1} range (r^2>0.99). In order to study the repeatability of the method, a series of six solutions were prepared, containing 3.0 μg ml^{-1} of 6-chloronicotinic acid and imidacloprid, with results of 4% for the both R.S.D. values, which shows the good repeatability of the method.

3.3. Resolution of synthetic binary mixtures

To validate the method, mixtures of 6-chloronicotinic acid and imidacloprid, in the concentration range 0.5–8.0 μg ml^{-1} for each pesticide, were prepared, and chromatograms were recorded according to the described procedure. Satisfactory results were obtained, with recoveries ranging from 90 to 109% for 6-chloronicotinic acid and from 98 to 116% for imidacloprid. The results indicate that the complete resolution of the mixture has been accomplished by the proposed approach, showing the high resolving power of the technique.

3.4. Preconcentration of pesticides in water by liquid–liquid extraction

The proposed method was applied in the determination of pesticides in environmental water samples. A trace enrichment step is necessary to obtain detection limits as low as μg l^{-1} levels. To evaluate the potential of trace enrichment of the pesticides, samples of ultra-pure water, spiked with 3.5 μg l^{-1} of pesticides, were analysed.

A 500-ml water sample (pH 3) was extracted with three separate 100-ml portions of dichloromethane by using a 1000-ml separatory funnel and vigorous shaking for 2 min. The extracts were combined and passed through a column of anhydrous sodium sulfate and then, the total extract concentrated to dryness in a rotary vacuum evaporator. For HPLC analysis the residue was dissolved in 1 ml of acetonitrile, and finally, 20 μl were injected. When the pH of water sample was 2 the results were similar but, at pH 5, 6-chloronicotinic acid was not recovered.

Good linearity was obtained for all substances in the ranges studied (3.5–11.0 μg l^{-1}). The regression coefficient are higher than 0.989 in all cases (n=6). The detection limits [21], calculated statistically, are 0.25 and 0.30 μg l^{-1} for 6-chloronicotinic acid and imidacloprid, respectively. The mean recoveries of the pesticides were 90 and 114% for 6-chloronicotinic acid and imidacloprid, respectively. The repeatability in terms of peak height at various concentrations was studied using the conditions described above. The data obtained for 4 μg l^{-1} indicate that the R.S.D. values ranged from 7% (imidacloprid) to 9% (6-chloronicotinic acid).

Using groundwaters spiked at a level of 3.5 μg l^{-1}, the recoveries were 85 and 115% for 6-chloronicotinic acid and imidacloprid respectively, and the R.S.D. values were 9 and 10%, respectively. A noncontaminated groundwater sample was also analysed. The proposed method was applied to the determination of pesticide levels in groundwaters from Almería (Spain) and the chromatograms obtained showed no peaks of the studied pesticides.

4. Conclusions

The technique of using cross-sections of spectrochromatograms can resolve overlapping chromatographic peaks of analytes with good repeatability and sensitivity. The approach is particularly useful in the separation of several solutes with similar retention times.

The method has been applied to the determination of imidacloprid and 6-chloronicotinic acid in water samples at $\mu g\, l^{-1}$ levels with good results. However, the study has been performed at spiking levels somewhat higher than commonly encountered in real samples. In conclusion, the combination of advanced computational capability with the DAD technology applied in HPLC offers a powerful approach for the resolution of highly overlapping peaks when resolution of the mixture is not possible by modifying the mobile phase, it not being necessary to change the column. This fact is advantageous with respect to the conventional chromatographic methods.

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