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Determination of imidacloprid and its metabolite 6-chloronicotinic acid in greenhouse air by application of micellar electrokinetic capillary chromatography with solid-phase extraction

A. Segura Carretero*, C. Cruces-Blanco, S. Pérez Durán, A. Fernández Gutiérrez
Department of Analytical Chemistry, Faculty of Sciences, University of Granada, 18071 Granada, Spain

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Abstract

A method is described for the analysis of the insecticide imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] and its metabolite 6-chloronicotinic acid by micellar electrokinetic chromatography with diode-array detection at 270 and 227 nm, respectively. The best results were obtained using sodium dodecyl sulphate at a concentration of 60 m*M* and a running buffer of NH_4Cl/NH_3 at 15 m*M* (pH 8.5). The selection of instrumental parameters such as voltage at 30 kV with an injection time of 20 s gave the best resolution with an analysis time of less than 6 min. The method yields similar sensitivity for the parent compound and for the metabolite, with detection limits of 0.71 and 1.18 μg/ml for imidacloprid and 6-chloronicotinic acid, respectively. The sampling and analysis of these two pesticides in greenhouse air was carried out using personal samplers connected to XAD-2 cartridges as sampling media, investigating the dissipation of analytes in a 24-h period after their application.

Keywords: Air analysis; Environmental analysis; Imidacloprid; Chloronicotinic acid; Pesticides

1. Introduction

In recent years the presence of pesticides, parent compounds and their major metabolites or conversion products in air has become a serious environmental concern. Therefore, suitable analytical methods are needed to identify and accurately quantify pesticides [1]. For air analysis, the sampling procedure is of even greater importance as a key step in obtaining reliable measurements than for most other environmental media [2].

Methods of preconcentration such as adsorption on solids are becoming more widely employed because of the advantages obtained when selecting the most appropriate sorbent [3,4]. Diffusive air sampling using prepacked adsorbents in sample tubes has been used extensively in environmental applications.

The wide application range, long-term stability, ease of use, low cost and improved selectivity (diode array) mean that UV detection is widely used in residue analysis [5–7]. However, UV detection does not deliver high sensitivity; hence, preconcentration procedures are usually required in trace analysis.

*Corresponding author. Tel.: +34-958-248-593; fax: +34-958-249-510.
E-mail address: ansegura@ugr.es (A. Segura Carretero).

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Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] is a systemic nitroguanidine insecticide that belongs to the neonicotinoid family. Various investigations have been carried out based on the study of the oral acute and chronic toxicity of imidacloprid and its main metabolite, 6-chloronicotinic acid [8,9], where it has been demonstrated that no significant toxicity was observed with 6-chloronicotinic acid in the range of doses tested.

Although there are reports dealing with the determination of imidacloprid residues by various spectroscopic [10] or electrochemical techniques [11], the application of separation techniques to real sample analysis is usually needed. Among these techniques, gas chromatography–mass spectrometry (GC–MS) has been widely applied to their determination in vegetables, urine samples of agricultural workers exposed to the insecticide and industrial water [12,13]. Also, high-performance liquid chromatography (HPLC) with diode-array detection has been employed, but few methods have been published for the simultaneous determination of residues of both imidacloprid and its metabolite 6-chloronicotinic acid [14–16].

The application of capillary electrophoresis (CE) methods to pesticide and environmental samples has been reviewed [17,18]. Isotachophoretic methods were the first capillary electrophoretic applications in the analysis of pyrethroids [19], bipyridilium salts (diquat and paraquat) [20] and *s*-triazines [21]. Capillary zone electrophoresis (CZE) enables the rapid separation of ionic compounds and has been successfully applied to the analysis of herbicides [22,23], organophosphoric acids [24] and sulfonylureas [25]. Several classes of pesticides and their degradation products have been separated using micellar electrokinetic chromatography (MEKC) [26–29], including imidacloprid with differential-pulse polarography detection [30].

The objective of this study was to confirm the potential of CE for the detection and separation of a widely used insecticide and its metabolite in greenhouse air carried out with different solid sorbent procedures as an alternative to the well-established chromatographic techniques with diode-array detection [14,16]. The development and validation of the methodology using standard pesticide vapors is also described.

2. Materials and methods

2.1. Reagents

The pesticide standards (Pestanal quality) were obtained from Riedel-de Haën (Seelze, Germany). Stock solutions of the pesticides were prepared in acetonitrile at a concentration of 500 μg/ml and stored at 4 °C in the dark, and were stable for several months. Standard solutions containing 2–8 μg/ml were prepared daily by appropriate dilution of the stock solution with separation buffer. Distilled water was obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). All solvents and samples were filtered through a 0.45 μm Millipore membrane before injection. A NH_4Cl/NH_3 buffer (15 m*M*, pH 8.5) was prepared from NH_4Cl (Panreac, Barcelona, Spain) and sodium dodecyl sulphate (SDS) (60 m*M*) (Riedel-de Haën) in Millipore Milli-Q purified water. The sorbents used were Amberlites XAD-2 and XAD-4 (Supelco, Bellefonte, PA, USA).

2.2. Capillary electrophoresis conditions

Separation of the insecticide and the metabolite was performed using micellar electrokinetic capillary chromatography with a P/ACE MDQ system from Beckman. Separations were carried out with a fused-silica capillary of 75 cm (50 cm to the detector)×75 μm I.D. at a constant temperature of 25 °C. The applied voltage was 30 kV, with an injection pressure of 20 p.s.i. for 20 s with detection conducted with UV diode-array detection at 227 nm (6-chloronicotinic acid) and at 270 nm (imidacloprid) in less than 6 min (1 p.s.i.=6894.76 Pa). The electrolyte buffer was 15 m*M* NH_4Cl/NH_3 (pH 8.5) in the presence of 60 m*M* SDS. At the beginning of each session, the capillary was treated with 0.1 *M* sodium hydroxide for 1 min, then rinsed with ultrapure water for another minute and, finally, with the carrier electrolyte for 10 min.

2.3. Clean-up and desorption procedures

Amberlites XAD-2 and XAD-4 were cleaned using 100 ml of acetone for 16 h in a soxhlet extractor operating at 20 min/cycle. The cartridges were dried and stored in a clean glass container in the dark. The sorbents were then packed in cartridges

containing 1 g of sorbent and kept in the dark in a precleaned, capped vessel at room temperature. For the desorption procedure, the best results were obtained when the sorbent was packed in cartridges containing 1 g, spiked with the pesticides and extracted with 6 ml of water.

2.4. Method validation

A system to generate standard pesticide vapors similar to that described in Ref. [31] was used in order to validate the ability of the sorbents to trap both analytes from air. Hence, standard pesticide vapors were obtained by injecting 28 μg of imidacloprid and 21 μg of its metabolite into the device under the following conditions: injector, oven and detector temperature, 225 °C; carrier gas, dry air at 2 l/min for 30 min. Recovery rates and precision of the methodology, including the sampling step, were calculated by analysing 10 replicates.

2.5. Field treatment

A 400 m^2 greenhouse (2 m height) cropped with tomatoes was sprayed with Condifor 20 LS using a high-volume sprayer operating at 30 atm at a flow-rate of 3 l/min for a sampling time of 25 min (1 atm=101 325 Pa). The concentration of the spray tank was 150 mg/l and it was applied using a gun with three nozzles producing a fog that remained in the air. Twenty-four hours later, air samples were taken using four Amberlite XAD-2 cartridges connected to samplers at a height of 1.65 m in the greenhouse and working at a flow-rate of 2 l/min. The four cartridges were located at increasing distances from the spray tank (5, 10, 15 and 20 m) in order to study the dissipation process in air. After sampling, the cartridges were stored in capped glass tubes in the dark at −20 °C until analysis.

3. Results and discussion

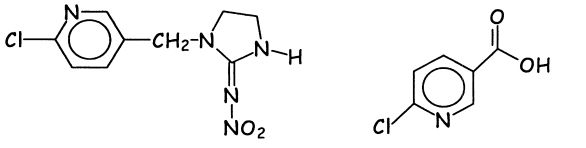
The MEKC methodology allows the separation of neutral pesticides in the presence of charged micelles (i.e. SDS) and an electroosmotic flow. The relative distribution of the pesticides between the mobile aqueous and charged micellar phase is a function of the hydrophobicity of the pesticides and determines the selectivity of the separation.

Imidacloprid and 6-chloronicotinic acid are polar compounds with a high molar absorptivity in the UV–Vis region (see Fig. 1). They display absorption spectra with absorption maxima located at 210 and 270 nm for imidacloprid and 200, 227 and 270 nm for 6-chloronicotinic acid. For this reason, CE with diode-array detection (DAD) is one of the detection methods used in their determination. In addition, the advent of DAD increased the utility of absorbance detectors due to the spectral confirmation capability required to eliminate false positives.

3.1. Influence of variables affecting the separation of analytes by MEKC

The instrumental optimization was centred on the selection of different voltages to carry out the separation. Voltages between 20 and 30 kV in 2 kV steps were studied, obtaining a considerable reduction in the retention time of the two analytes from 14 min when employing 20 kV to around 6 min when working at 30 kV, while a good resolution between peaks was achieved. Therefore, a 30 kV voltage was selected for the remaining experimental work. The injection time was also examined between 5 and 30 s and the best results were obtained with 20 s, obtaining good resolution with shorter analysis times.

After the selection of instrumental variables, various buffers (NH_4Cl/NH_3, borate/HCl, glycine/NaOH) were investigated in an attempt to obtain the best resolution between peaks. The best results were obtained with ammonium chloride/ammonia and the concentration was varied from 5 to 30 m*M* and pH values from 8 to 10. For all studies, a 15 m*M* NH_4Cl/NH_3 buffer solution at pH 8.5 under a



Imidacloprid 6-Chloronicotinic acid

Fig. 1. Chemical structure of imidacloprid and its metabolite 6-chloronicotinic acid.

voltage of 30 kV were used to produce a high electroosmotic flow for rapid separations.

To carry out the analysis of these two compounds, SDS concentrations between 40 and 120 m*M* were examined. When 40 m*M* SDS was applied the two target analytes did not resolve sufficiently and a single peak was observed. At concentrations greater than 80 m*M*, migration times greatly increased with no significant improvement in peak resolution. Therefore, the best results for resolution and analysis time were obtained with a 60 m*M* concentration of SDS present in the electrophoretic media (see Fig. 2).

Under these experimental and instrumental conditions, the electropherogram corresponding to a mixture of both imidacloprid and 6-chloronicotinic acid pesticides extracted by Amberlite XAD-2 is presented. Satisfactory electrophoretic resolution of the analytes was achieved (see Fig. 3).

3.2. Performance of the electrophoretic method

Peak quantification by MEKC was carried out using measurements of peak areas and the linearity of the detector response was determined for standard solutions of the pesticides with concentrations ranging from 2 to 10 μg/ml. Calibration and sensitivity data for each pesticide are summarized in Table 1. Good linearity was found in the concentration range studied, with correlation coefficients for imidacloprid and 6-chloronicotinic acid of 0.995 and 0.991, respectively. The precision ($n=3$) for the quantitative measurement of the pesticides was studied at a concentration of 6 μg/ml, with mean values of RSD (%) of 2.67 and 4.79% for imidacloprid and 6-chloronicotinic acid, respectively. The limits of detection (LODs) and limits of quantification (LOQs) for both pesticides are also summarized in Table 1 and were calculated using the method of Cuadros et al. [32].

3.3. Desorption procedure and method validation

Sorbents were spiked with both pesticides at two concentrations, 20 and 40 μg, and dried with a nitrogen current in order to study the reliability of the desorption procedure using 6 ml of distilled water. The best results for both analytes, with average recoveries ranging between 85 and 92% for both pesticides and precision values ($n=5$) less than

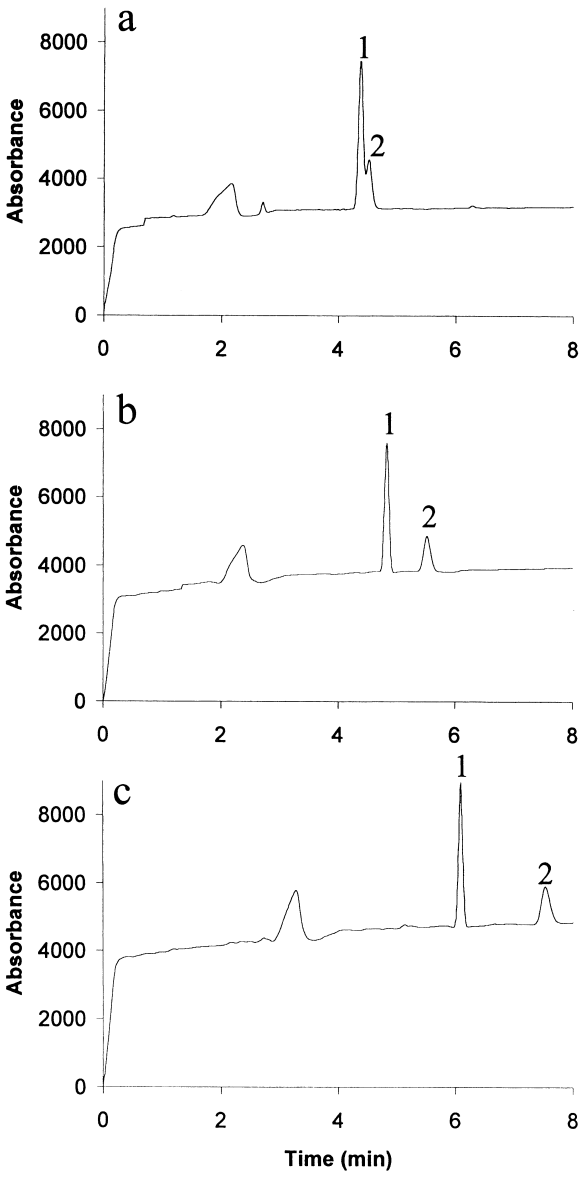


Fig. 2. Electropherograms at 227 nm of a standard mixture of 6 μg/ml of (1) 6-chloronicotinic acid and (2) imidacloprid at different concentrations of SDS [(a) 50 m*M*, (b) 60 m*M* and (c) 70 m*M*]. Fused-silica capillary of 75 cm (50 cm to the detector)× 75 μm I.D., voltage 30 kV, injection pressure 20 p.s.i. for 20 s and electrolyte buffer NH_4Cl/NH_3 15 m*M* (pH 8.5).

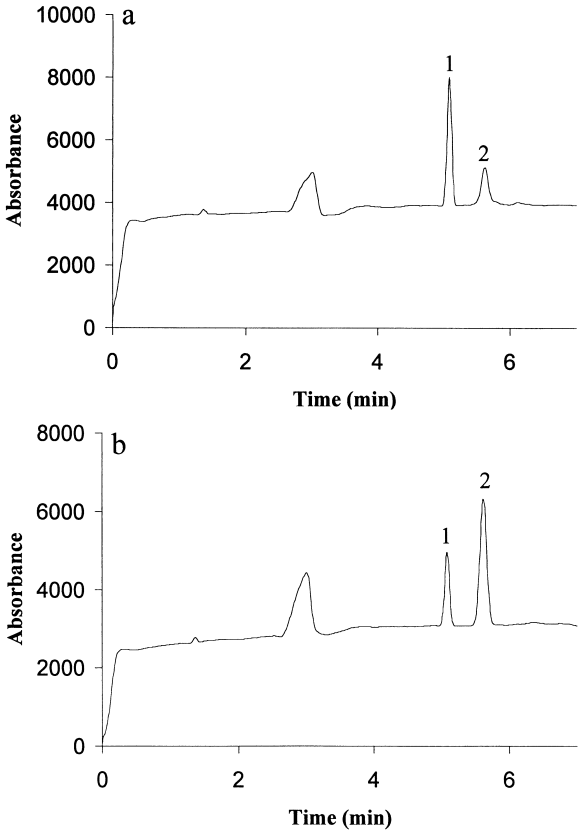


Fig. 3. Electropherograms at 227 (a) and 270 nm (b) of a standard mixture of 6 μg/ml of (1) 6-chloronicotinic acid and (2) imidacloprid. Fused-silica capillary of 75 cm (50 cm to the detector)×75 μm I.D., voltage 30 kV, injection pressure 20 p.s.i. for 20 s, electrolyte buffer NH_4Cl/NH_3 15 m*M* (pH 8.5) and 60 m*M* SDS.

Table 1
Analytical parameters of the proposed method

	Imidacloprid	6-Chloro-nicotinic acid
Linear range (μg/ml)	0.71–10	1.18–10
Sensitivity (μg/ml)	0.25	0.41
Detection limit (μg/ml)	0.71	1.18
Quantification limit (μg/ml)	2.3	3.9
RSD (%)		
2 μg/ml	8.84	9.85
4 μg/ml	4.42	6.53
6 μg/ml	2.67	4.79
8 μg/ml	2.35	3.84

9.2%, were obtained with XAD-2. From these results, XAD-2 was chosen as sorbent.

Optimal conditions for generating standard pesticide vapors were achieved by setting the injector oven and detector (which acts as an interface) at 225 °C and passing 60 l of air through a wide column for 30 min. Recovery rates for the whole process varied from 70.1 to 74.9% for imidacloprid and from 70.9 to 73.7% for the metabolite, with precision values better than 8.6% for both pesticides.

3.4. Application to real air samples

The applicability of the proposed method was demonstrated by the analysis of the insecticide imidacloprid and its main metabolite in greenhouse air from an important agricultural zone in southern Spain. To carry out the analysis, a greenhouse cropped with tomatoes over 400 m^2 and 2 m height (800 m^3) was sprayed with a tank containing a concentration of 150 mg/l at a flow-rate of 3 l/min for 25 min, which gives a theoretical concentration of pesticides in the greenhouse air of 14.06 mg/m^3.

Twenty-four hours later, four cartridges were located at increasing distances from the tank (5, 10, 15 and 20 m) and, after sampling, they were stored in glass capped tubes. The desorption procedure was carried out with 6 ml of water and taken to analysis by the procedure described. Of the four cartridges analyzed, only one (the cartridge located 5 m from the tank) gave concentrations over the limit of detection of the present method. The resulting concentrations were 0.51 μg/ml for imidacloprid and 2.89 μg/ml for 6-chloronicotinic acid, when the peak area values were introduced into the calibration graph previously established. These values were validated using standard addition.

As the flow-rate of the samplers was established at 2 l/min for 25 min, the total volume extracted was 50 l, which gives a concentration of imidacloprid and 6-chloronicotinic acid in air of 0.061 and 0.347 mg/m^3, respectively (see Fig. 4). These final concentrations of the pesticides in the greenhouse air after 24 h of application indicate that a dissipation process takes place in the air due to the considerable reduction in concentration from the theoretical value of 14.06 mg/m^3 to the final concentrations obtained with the present methodology.

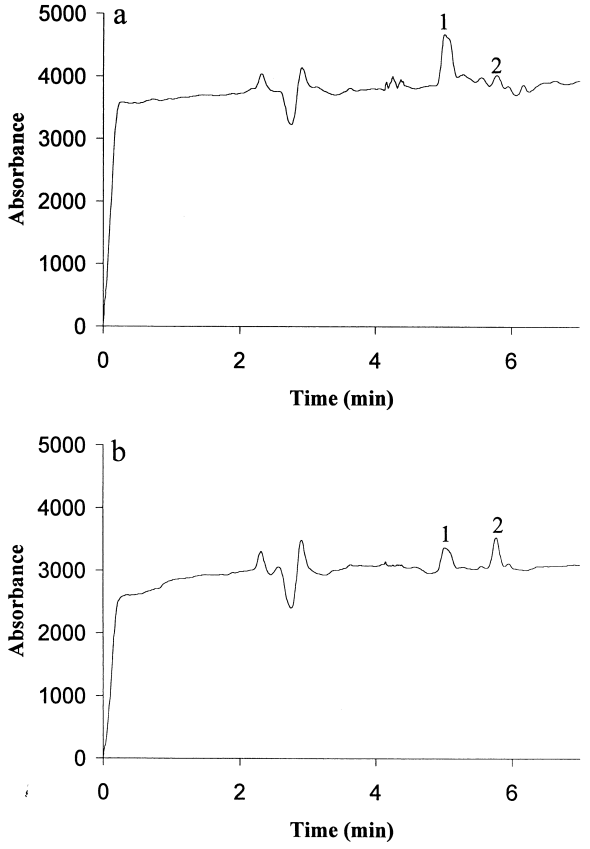


Fig. 4. Electropherograms at 227 (a) and 270 nm (b) of the extract of the XAD-2 cartridge used to sample real air samples 24 h after spraying with Condifor 20 LS. (1) 6-Chloronicotinic acid and (2) imidacloprid. Electrophoretic conditions as in Fig. 3.

4. Conclusions

A method has been developed to sample and analyze imidacloprid and its main metabolite 6-chloronicotinic acid in greenhouse air using a MEKC–DAD methodology. The results indicate that this methodology produces similar results with respect to repeatability, sensitivity and retention times as other HPLC methods [14] and that it can be considered as an alternative, particularly considering the low solvent consumption.

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