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Journal of Chromatography A, 869 (2000) 497–504

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of imidacloprid and its metabolite 6-chloronicotinic acid in greenhouse air by high-performance liquid chromatography with diode-array detection

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Abstract

A method is described for analysing and sampling imidacloprid and its metabolite 6-chloronicotinic acid in greenhouse air by high-performance liquid chromatography (HPLC) with diode-array detection (DAD). The trapping efficiency of two solid sorbents, Amberlite XAD-2 and Amberlite XAD-4 and the use of different desorption procedures have been tested. To validate the methodology, standard atmospheres containing known concentrations of these pesticides and with different relative humidities were generated. No breakthrough was observed in the range of concentrations studied. Dissipation of analytes was investigated in a 24 h period after application by using personal samplers in a field experiment.

Keywords: Air analysis; Imidacloprid; 6-Chloronicotinic acid; Pesticides

1. Introduction

Imidacloprid [1-6(chloro-3-pyridylmethyl)-*N*-nitio-imidazolidin-2-ylideneamine] belongs to a new group of active ingredients, the chlornicotinyl insecticides. It has a new mode of action, low toxicity to warm-blooded animals, good systemic properties and a lasting action [1]. The development, activity, mode of action and effectiveness have been described by Leicht [2] and its physical, chemical and toxicological properties have been summarised in the pesticide manual [3]. The compound was introduced in Europe by Bayer (Leverkusen, Germany). The parent and its main metabolite (6-chloronicotinic acid) are polar compounds with high solubility in water.

Residues of imidacloprid are analysed commonly by HPLC with UV detection [4], although pulse reductive amperometric detection has been also used [5]. However, some papers have been found using GLC based techniques [6] but derivatization is required. Other methods, such as a photochemical-fluorimetric method [7] or a differential pulse polarographic method [8] have been proposed. Several works have been published for the determination of imidacloprid residues in different matrices, such as vegetables [9,10], waters [6,7,11], and soils [6,12,13]. No methods have been published for the determination of residues of this insecticide and its metabolite in air.

Nowadays the potential risk of exposure to pesticide residues in working environments is high. On one hand, pesticide use has increased dramatically in and around businesses and homes through applica-

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)00879-1

tions by professional laws care and pest control firms and by homeowners. Many of these applications are performed routinely on a weekly or monthly to using insecticides. Depending upon the type of work performed, individuals can spend long hours in an indoor environment where they come in contact with pesticide residues from both dermal and inhalation exposures. In addition, greenhouse operations involve heavy use of pesticides to control pests, and the potential for worker exposure is high. Safety measures for farm workers (mainly small-holders) are very poor until now. Many of them do not strictly follow the manufacturer's directions in using the formulations.

On the other hand, regulations concerning acceptable levels of insecticides in air in working environments have been in place for years because of the potentially high residue levels that might be encountered during a working day [14], as average eight hours.

The EPA Air program is poised for growth and change. Implementation of the clean Air Act amendments will require new methods and regulations [15]. Information on current contaminants and their pervasiveness will be required to assess the state of air quality. Routine monitoring will require the development of rugged methods suitable for a great variety of situations.

In order to evaluate the exposure to pesticides, accurate, reliable and sensitive analytical methods for monitoring organic trace constituents in the atmosphere are necessary. 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 [16].

Methods of preconcentration as adsorption on solids is becoming more widely employed because of its advantages in the selection of the most appropriate sorbent or a given group of pollutants [17,18]. Diffusive air sampling using prepacked adsorbents in sample tubes has been used extensively in environmental applications. It has been extended successfully to other environmental application for air and water sampling in Europe.

As part of a project for monitoring pollutants in air, we are interested in the development of a method for the determination of imidacloprid and its metabolite 6-chloronicotinic acid in air samples by HPLC–DAD. This technique is particularly suitable in this case due to their strong absorbance between 200 and 270 nm.

This paper reports the results of studies carried out with different solid sorbents and using several extraction procedures in order to establish the optimum conditions for sampling and analysing imidacloprid and 6-chloronicotinic acid in greenhouse air. The development and validation of the methodology using standard pesticide vapours was also described.

The procedure was applied to the determination of the mentioned pesticides in air, after an experimental application carried out in a greenhouse with a high volume application system in the Nijar zone (Almería, Spain).

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 at the concentration level of 200 $\mu g\ ml^{-1}$ and stored at 4°C in the dark, where they were stable for several months. Working solutions of the pesticides with concentrations ranging from 0.25 to 5.0 $\mu g\ ml^{-1}$ were prepared daily by appropriate dilution in the mobile phase. Mobile phase was 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 a 0.45 μm Millipore membrane filters before injection into the column.

A phosphate buffer (0.01 *M*, pH 3.0) was prepared from anhydrous disodium hydrogen 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. Acetonitrile solution at apparent pH=2 was obtained by addition of chloride acid solution.

The sorbents used were Amberlites XAD-2 and XAD-4 (Supelco, Bellefonte, PA, USA).

2.2. Apparatus

A Waters (Milford, Massachusetts, 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 200–290 nm.

HPLC separations were carried out using a Hypersyl Shandon Green Environ-C_{18}-column (15×0.46 cm ID; 5 μm particle size).

A Konik Model Cromatix KNK-2000 gas chromatograph and a silanized hollow glass column (2 m length and 5 mm ID) were used to generate the standard atmosphere.

A rotary vacuum evaporator (Buchi 461) with thermostatic water-bath and vacuum pump was used.

2.3. HPLC operating conditions

The mobile phase was acetonitrile/0.01 *M* phosphate buffer (pH 3.0) (25/75 (v/v)). 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. Photometric detection was performed at 227 nm for 6-chloronicotinic acid and 270 nm for imidacloprid. The solvents were filtered daily through a 0.45 μm cellulose acetate (for water) or politetrafluoroethylene (for acetonitrile) membrane filter (Millipore) before use and degassed with helium during and before use.

2.4. Clean-up procedure of sorbents

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 under a nitrogen current and stored in a clean glass container in the dark. After, the sorbents were packed under nitrogen current in cartridges containing 500 mg of each sorbent and kept in darkness in a precleaned, capped vessel at room temperature.

2.5. Desorption procedure

The sorbents were spiked with 6 μg of each pesticide using a micropipette and dried with nitrogen current for 10 min. A sonication was used by treating the sorbents with three sequential portions of 20 ml each of acetonitrile or acetonitrile at pH=2 for 15 min each. Consequently, the sorbent was washed with 15 ml of acetonitrile or acetonitrile at pH=2. A second method, by Soxhlet, was used siphoning at 20 cycles min^{-1} for 4 h using 100 ml of acetonitrile as extractant. In both cases, the final extracts were preconcentrated in rotary vacuum evaporator and concentrated afterwards to dryness with a nitrogen stream to avoid loss in the evaporation step. The dry extracts were redissolved in 2 ml of the mobile phase and 20 μl of this solution was injected into the HPLC system. Another method in which the sorbents were packed in cartridges containing 500 mg of each one, spiked with the pesticides and extracted with 3 ml of acetonitrile followed of 3 ml of 0.01 *M* phosphate buffer (pH=3.0) aqueous solution was tested. 20 μl volumes of the 6 ml overall extract were injected into the system.

2.6. Method validation

A system to generate standard pesticide vapours similar to that described in Ref. [19] was used in order to validate the ability of the sorbent to trap both analytes from air. With this purpose standard pesticide vapours were obtained by injecting 28 μg of imidacloprid and 21 μg of its metabolite in the device under the following conditions: injector, oven and detector temperature: 225°C; carrier gas: dry air at 2 l min^{-1} for 30 min. Recovery rates and precision of the methodology including the sampling step, was calculated analysing ten replicates carried out during two weeks.

Breakthrough was studied as is described in Ref. [19], considering a high concentration being sampled during short time (typical ''ceiling concentration'' case), and considering low concentration being sampled during eight hours (typical time weighted average concentration during a normal working day); if a 5% of the amount analysed in the first cartridge is found in the second, breakthrough has occurred [20].

The influence of the humidity was also tested sampling standard pesticide vapours generated using

either dry air or saturated air (100% relative humidity).

Limits of detection (LOD) and limits of quantification (LOQ), considering the whole methodology, were obtained measuring the signal of ten extracts from uncontaminated XAD-2 at the retention time of each analyte and their sensitivity in the range of low concentrations, between 250 and 400 μg l^{-1} [21].

2.7. Field experiment

A 1 ha greenhouse cropped with tomato (2.5 m height) was sprayed with Confidor 20 LS using a high volume sprayer operating at 30 atmospheres, at a flow-rate of 3 l min^{-1}. The concentration of spray tank was 150 mg l^{-1} and it was applied using a gun with three nozzles producing a fog that remained in the air. Theoretical concentration value of the pesticide in the greenhouse air was 0.5 mg m^{-3}.

Air samples were taken using XAD-2 cartridges connected to three personal samplers placed at 1.65 m high into the greenhouse and working at a flow-rate of 2 l min^{-1}. The operator during the application held another personal sampler. After sampling, the cartridges were stored into glass capped tubes, out of light at 4°C until analysis. The sampling times ranged between 60 min during the application to 8 h the day after. During the application the sprayer 7 samples were collected in each sampling location and analysed in order to study the dissipation process in the air.

3. Results and discussion

Imidacloprid and 6-chloronicotinic acid pesticides are polar compounds with high molar absorptivity in the UV–Vis region. 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, HPLC with diode-array detection is one of the primary detectors used in their determination. In addition, the advent of DAD increases the utility of absorbance detectors due to the spectral confirmation capability required to eliminate false positives.

In Fig. 1A, the HPLC chromatogram corresponding to a mixture of both imidacloprid and 6-chloronicotinic acid pesticides, using the mobile phase previously indicated, is presented. A satisfactory chromatographic resolution of the analytes was achieved.

The efficiency of the clean up of sorbents can be observed in Fig. 1B, where a HPLC chromatogram corresponding to an extract from a cleaned Amberlite XAD-2 is shown.

3.1. Performance of the chromatographic method

Retention times are summarised in Table 1. The linearity of the detector response was determined injecting 20 μl of standard solutions of the pesticides with concentrations ranging from 0.25 to 5.0 μg ml^{-1}. Calibration graphs and the different parameters associated with them were studied using both areas and heights. Good linearity was found in the concentration range studied, with correlation coefficients in both cases above 0.997. The precision ($n=3$) of quantitative measurement of pesticides was studied at two concentration levels for each pesticide, 4.7 μg ml^{-1} and 0.5 μg ml^{-1} for imidacloprid and 3.5 μg ml^{-1} and 0.5 μg ml^{-1} for 6-chloronicotinic acid finding RSD (%) values lower than 10%.

3.2. Desorption procedure

Sorbents were spiked with both pesticides and dried with a nitrogen current for studying the reliability of the desorption procedure using both Soxhlet extractor and ultrasonic bath. The results obtained are summarised in Table 2.

Good recoveries (90.3–100.2%) for the imidacloprid pesticide were obtained in all the conditions tested, i.e. with both extraction methods, both types of sorbents and extraction solvents. However, poor recoveries ranging from 25.2 to 59.0% were always obtained for the 6-chloronicotinic acid.

To improve these results, it was decided to modify the extraction procedure. For that, the sorbents were packed in cartridges, spiked, at two different concentration levels of 6 μg and 18 μg respectively of each pesticide, and extracted with different volumes of acetonitrile followed by 0.01 *M* phosphate buffer (pH=3.0) solution or with mixtures of these solvents in different percentages. The best results for both

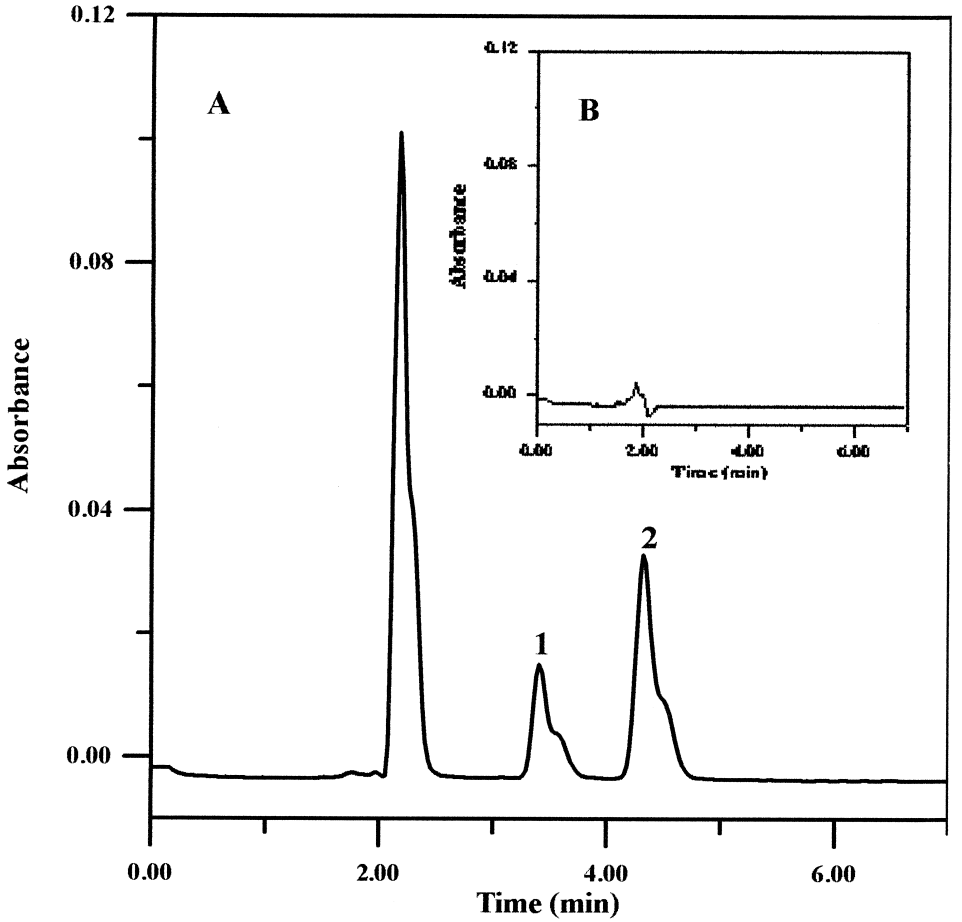

Fig. 1. Chromatogram at 270 nm of: (A) standard mixture of 1 μg ml^{-1} of (1) 6-chloronicotinic acid and (2) imidacloprid and (B) extract from Amberlite XAD-2 after clean up.

Table 1
Retention time, calibration data ($n=7$) and sensitivity for each pesticide

Pesticide	t_R (min)	Equation	r	Sensitivity	
				LOD (μg l^{-1})	LOQ (μg l^{-1})
6-Chloronicotinic acid	3.4	$y=0.0126x+0.0009$[a]	0.9981	80	250
		$y=0.0026x+0.0003$[b]	0.9979		
Imidacloprid	4.3	$y=0.0101x+0.0005$[a]	0.9978	80	250
		$y=0.0025+0.0001$[b]	0.9976		

[a] Using peak height peak.
[b] Using peak area.

Table 2
Recovery percentages (RSD%, $n=3$) of the re-extraction procedure by sonication and Soxhlet

Pesticide	Sonication				Soxhlet	
	XAD-2		XAD-4		XAD-2	XAD-4
	*	**	*	**	*	*
6-Chloronicotinic acid	25.2 (6.6)	46.0 (23.3)	45.2 (25.4)	56.1 (29.1)	59.0 (18.0)	59.0 (15.0)
Imidacloprid	99.7 (4.0)	94.2 (4.5)	90.3 (8.7)	93.4 (6.3)	91.4 (11.0)	100.2 (5.0)

Extraction with acetonitrile (*) or acetonitrile at pH 2 (**).

analytes, with average recoveries ranging 88.5–97.5% for both pesticides and precision values ($n=4$) lower than 8.9%, were obtained with the XAD-2 sorbent and using 3 ml of acetonitrile followed by 3 ml of 0.01 *M* phosphate buffer (pH=3.0) aqueous solution to extract the analytes (Table 3). From these results it is evident that XAD-2 was the best sorbent.

3.3. Method validation

The optima conditions for generating pesticide standard vapours were achieved setting the injector, oven and detector (which acts as an interface) at 225°C and passing through the wide column 60 l of air during 30 min. Recovery rates of the whole process varied respectively from 72.8 to 73.9% for imidacloprid and from 70.0 to 74.9% to the metabolite, with precision values better than 8.6% for both pesticides, being independent of the sampling flow-rates tested (1 and 2 l min^{-1}).

No breakthrough was observed when 300 μg m^{-3} where sampled during 30 min (expected ceiling air concentration) neither when 1.5 μg m^{-3} where sampled during 8 h (expected time weighted average concentration during a working day).

Recovery rates and precision obtained at 100% relative air humidity were similar to these obtained with dry air and previously presented. The methodology yielded similar sensitivity for the parent compound or for the metabolite, being LOD and LOQ for both analytes 80 μg l^{-1} and 250 μg l^{-1} respectively.

3.4. Dissipation process in air

Fig. 2 shows a chromatogram corresponding to an air sample extract at the concentration level of 25 μg m^{-3}. During the application the concentration of imidacloprid decreased 1 h later until values lower than the LOD. The % RSD between sampling locations was <20%. No imidacloprid or metabolite was found the first hour after the application.

4. Conclusions

A method has been developed to sample and analyse imidacloprid an 6-chloronicotinic acid in greenhouse air using personal samplers connected to XAD-2 cartridges as sampling media, acetonitrile and buffer to extract the analytes from the sorbent and HPLC–DAD analysis. Analytical parameters of

Table 3
Recovery percentages (RSD%, $n=4$) of the improved extraction procedure

Pesticide	XAD-2				XAD-4	
	*[a]	**[a]	**[b]	*[a]	**[a]	**[b]
6-Chloronicotinic acid	79.3 (9.6)	93.9 (8.0)	97.5 (6.8)	73.7 (18.2)	72.6 (24.5)	74.5 (21.5)
Imidacloprid	87.2 (9.1)	88.5 (8.9)	90.1 (8.0)	74.7 (12.4)	75.4 (16.8)	76.5 (14.3)

Extraction with 2 ml acetonitrile+2 ml buffer (*) or 3 ml acetonitrile+3 ml buffer (**).
[a] Spiking level 1 mg l^{-1}.
[b] Spiking level 3 mg l^{-1}.

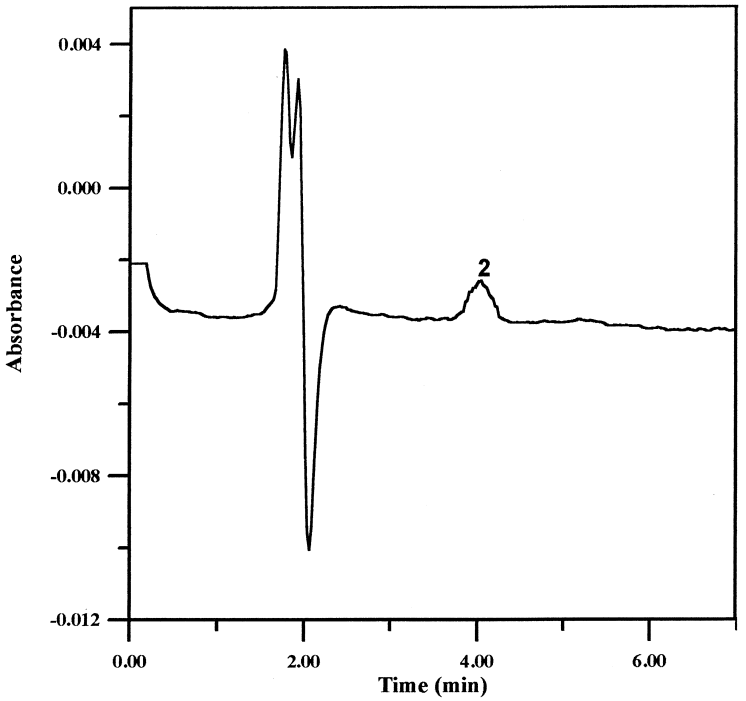

Fig. 2. Chromatogram at 270 nm of a real air samples containing 25 μg m^{-3} of imidacloprid (2).

methodology have been validated using standard pesticide vapours. Finally an application in a greenhouse showed that the concentration of imidacloprid in the air is very low and descends to below the detection limit 1 h after the application. On the other hand, neither residues of the 6-chloronicotinic acid were found.

Acknowledgements

This study has been financially supported by the European Union Standard Measurement and Testing Programme, contract number SMT4-CT96-2048 and by the CICYT Project AMB97-1194-CE.

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