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Short communication

Determination of imidacloprid in water and soil samples by gas chromatography–mass spectrometry

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Abstract

A method for determination of imidacloprid in water and soil samples, previous hydrolysis in basic medium, followed by gas chromatography–mass spectrometry and selected ion monitoring. A 250-ml sample water was previously heated in basic medium to give a hydrolysis compound of adequate volatility. The hydrolysis product which was extracted and isolated with chloroform was identified and found to be suitable for gas chromatography analysis. Further, a clean-up is not necessary using the selected ion monitoring mode. $[^2H_{10}]$Anthracene was used as an internal standard. The applicable concentration range was 5–20 $\mu g\,l^{-1}$. Detection limit was 0.16 $\mu g\,l^{-1}$ for water and 1 $\mu g\,kg^{-1}$ for soil samples. Their relative standard deviations established for different concentration levels were between 0.3 and 1%. It was applied to the check whether there was imidacloprid above these limits on waters and soil from Granada (Spain). The method was validated applying the standard addition methodology. Recovery levels of the method reached 100% in all cases.

Keywords: Soil; Water analysis; Environmental analysis; Pesticides; Imidacloprid

1. Introduction

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimida-zolidin-2-ylideneamine] (Fig. 1) is a systemic and contact insecticide introduced by Bayer AG. It is used for the control of mites present in vegetable crops [1]. The development, activities, mode of action and effectiveness have been described by Leicht [2].

High-performance liquid chromatography (HPLC) methods for detection and determination of imidacloprid include the method of Placke and Weber [3] involving HPLC–UV, which is tedious and requires high solvent consumption, and the HPLC-diode array detection method of Fernández-Alba et al. [4], covering the range 0.01–0.60 $mg\,kg^{-1}$.

Imidacloprid has low volatility, so gas chromatography (GC) seems to be ruled out. However, in our gas chromatography–mass spectrometry (GC–MS) method for water and soil samples imidacloprid is transformed into a volatile compound by hydrolysis in a basic medium. Then a liquid–liquid extraction

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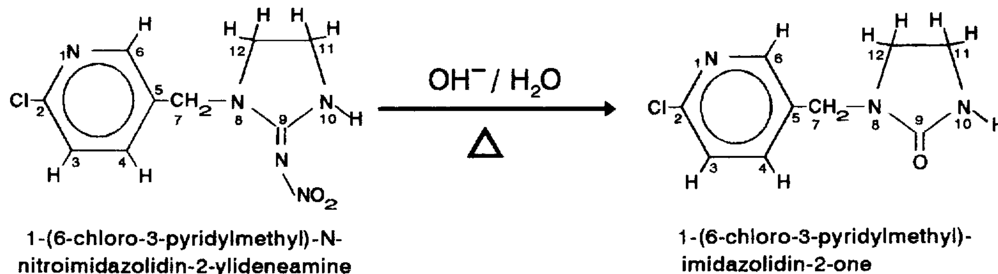

Fig. 1. Hydrolysis of imidacloprid in a basic medium.

with chloroform is made of the hydrolyzed product to ensure adequate extraction and preconcentration.

2. Experimental

2.1. Apparatus and software

A Hewlett-Packard system consisting of a 5890 GC system fitted with a 7673 autosampler, a splitless injector for the HP-5MS fused-silica capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) and a 5971 mass spectrometer, a HP-UX Chemsystem computer and the proprietary software was used. Carrier gas was helium (purity 99.999%).

The Lack-of-fit test from Statgraphics software [5] was applied to check the linearity of the calibration graphs, according to the Analytical Methods Committee [6].

2.2. Reagents

Imidacloprid (purity>99%) was supplied by Bayer (Leverkusen, Germany). All other reagents, except the internal standard [$^2H_{10}$]anthracene (Cromlab, Barcelona, Spain), were of analytical-reagent grade (Merck, Darmstadt, Germany).

2.2.1. Imidacloprid stock solution

A solution of 400 μg ml^{-1}, was prepared with the product and deionized water. It was stable for at least two weeks if stored in the dark at 4°C. Working solutions were obtained by appropriated dilutions.

2.2.2. Internal standard solution

3 μg ml^{-1} of [$^2H_{10}$]anthracene in *n*-hexane (starting solution 100 μg ml^{-1}) stored also at 4°C.

2.3. Sample treatment

Water samples were filtered through a cellulose acetate filter (Millipore HAWP 04700, pore size 0.45 μm), collected in a glass bottle previously cleaned with HCl and stored at 4°C. The usual precautions were taken to avoid contamination [7].

2.4. Basic procedure for determination of imidacloprid in water and soil samples

2.4.1. Water samples

Mix 0.4 g of NaOH with 250 ml of water sample containing between 5 and 20 μg l^{-1} of imidacloprid and stir well. Heat the mixture in a water bath at 85°C for 15 min, cool to 20°C and neutralize with HCl 1:1. Adjust the final volume to 250 ml with deionized water and transfer to a separatory funnel. Add 25 ml of chloroform and shake the mixture for 3 min, collecting then the organic phase. The extraction is repeated once again.

The extracts are mixed, dehydrated with anhydrous sodium sulfate, filtered and concentrated to a few millilitres in a rotatory vacuum evaporator and transferred and evaporated to 1 ml in a micro-snyder column. 200 μl of the concentrate from the column were spiked with 4 μl of [$^2H_{10}$]anthracene internal standard solution described above (see Section 2.2.2) before injecting and registering each chromatogram in the GC–MS system.

Calibration graphs were thus constructed using solutions of imidacloprid of known concentrations.

2.4.2. Soil samples

A mixture of 50 g of soil sample and 100 ml of deionized water is treated in an ultrasonic bath for 15 min. Then the mixture is filtered through Whatman No. 1 paper, the procedure repeated again, and both filtrates are introduced into a 250-ml calibrated flask, the volume being completed with deionized water. Then the procedure is identical to the case of water (see Section 2.4.1).

2.5. GC–MS analysis

A 2-μl aliquot of the extract was injected using the splitless mode with the split closed for 2 min. The GC–MS parameters are shown in Table 1. We chose m/z 211 (base peak) as target ion as well as m/z 126 and m/z 99 as qualifiers in selected ion monitoring (SIM) analysis for hydrolized imidacloprid and m/z 188 for [$^2H_{10}$]anthracene, respectively. The concentrations of the pesticide were calculated by the internal standard method.

2.6. Identification of the imidacloprid hydrolyzed product

2.6.1. Elemental analysis

Found: C 51.19%, H 4.73%, N 19.66%. Calc. for $C_9H_{10}N_3Cl$: C 51.06%, H 4.73%, N 19.86%.

Table 1
GC–MS conditions

Gas chromatograph		Mass spectrometer	
Total flow	100 ml min^{-1}	Interface temperature 280°C	
Septum purge	3 ml min^{-1}	Electron multiplier voltage between 1750 and 2100 V	
Head column pressure	105 kPa	Scan mode	SIM mode
Purge-off time	2 min	m/z Range 45–300	Selected ion m/z 211, 126, 99
Injector temperature	200°C		
Injected volume	2 μl		
Oven program	76°C (1 min), 30°C/min, 270°C (3 min)		

2.6.2. IR spectrum (KBr discs)

The disappearance of the stretching band of the imidacloprid C=N group (1571 cm^{-1}) and the appearance of a new band at 1689 cm^{-1}), is tentatively ascribed to δC=O in the IR spectrum of the hydrolysis product.

2.6.3. ^{1}H NMR spectrum of hydrolysis product

δ: 3.3(m, 2H), 3.45(m, 2H), 4.38(s, 2H), 7.3(d, J=8 Hz, 1H), 7.63(dd, J_1=8 Hz, J_2=2 Hz, 1H), 8.32(d, J=2 Hz, 1H).

2.6.4. ^{13}C NMR spectrum of hydrolysis product

δ: 38.12(C_7), 44.47(C_{11} and C_{12}), 124.63(C_3), 131.87(C_5), 139.06(C_4), 149.14(C_6), 150.9(C_2), 163.32(C_9).

2.6.5. Mass spectrum of hydrolysis product

The more relevant peaks are m/z (rel. int.): 211, 213 (100%)[M^+]; 126, 128 (73.6%)[$C_6H_5ClN^+$]; 99 (58%)[$C_4H_6N_2O$]; 85 (22%)[$C_3H_6N_2O$]

3. Results and discussion

Fig. 1 relates imidacloprid with its hydrolyzed product in a basic medium. Fortunately, its suitable volatility and thermal stability allows us to expect its usefulness in GC.

It was thus thanks to the hydrolysed volatile product obtained, isolated and identified by the methods described above, that we were able to identify and quantify imidacloprid by GC–MS. As the hydrolysis rate in a basic medium is temperature-dependent and very low at room temperature we need only 15 min at 85°C to treat our samples, as we have found experimentally. The chromatographic area peaks remain constant for at least 24 h.

The chromatographic signal increased strongly when the NaOH concentration increased, remaining constant if sodium hydroxide was used in excess. We chose 0.4 g of NaOH as an adequate amount for the range explored, i.e., no more 200 μg l^{-1}.

We used liquid–liquid extraction selecting a 10:1 ratio with chloroform, the more adequate of 6 different solvents tried.

Ionic strength adjusted with NaCl or $NaClO_4$ did

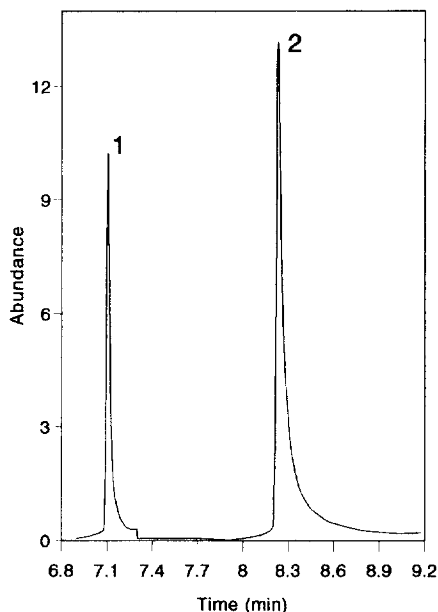

Fig. 2. Typical chromatogram obtained in SIM mode. 200 $\mu g\ l^{-1}$ of imidacloprid, treated as it is indicated in the analytical procedure: 1, [$^2H_{10}$]anthracene; 2, hydrolyzed imidacloprid.

not affect extraction efficiency, and salty waters might be monitored.

Fig. 2 shows a chromatogram, t_R under 9 min.

The mass spectrum in the scan mode is shown in Fig. 3. Notice that the molecular ion appears at 211 *m/z*.

3.1. Analytical parameters

The calibration graph for the samples treated according to the procedure described above (see Section 3, monitored using SIM mode, is linear for the concentration range 5–20 $\mu g\ l^{-1}$. To check the linearity of the calibration graph, the Lack-of-fit test [5] was applied for two replicates and three injections of each standard. Table 2 shows the results for the intercepts (*a*), slopes (*b*), correlation coefficients (R^2) and probability levels (*P*) of Lack-of-fit test. The data yield a good linearity within the range 5–20 $\mu g\ l^{-1}$.

In contrast with other analytical techniques, there is no agreement about how to get the detection limit

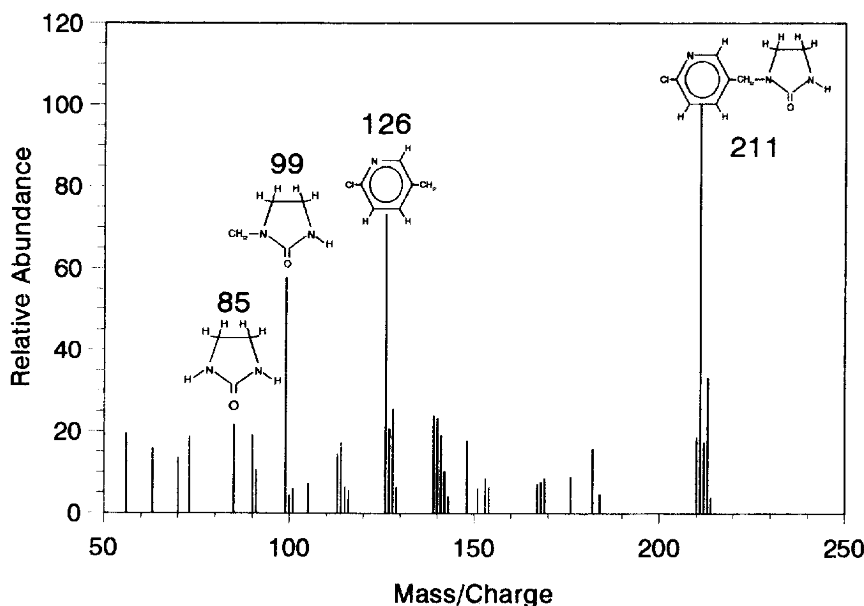

Fig. 3. Mass spectrum of 1-(6-chloro-3-pyridylmethyl)-imidazolidin-2-one (hydrolysis product of imidacloprid).

Table 2
Analytical parameters

Intercept (a)	−0.0294
Slope (b)	0.2244
Correlation coefficient	0.9999
Lack-of-fit test (P-value)	0.23
Linear Dynamic Range ($\mu g\,l^{-1}$)	5–20
Linearity [1−R.S.D.(b)] (%)	99.72
DL ($\mu g\,l^{-1}$)	0.16
QL ($\mu g\,l^{-1}$)	0.6
Precision (R.S.D.) (%)	[10%(0.6 $\mu g\,l^{-1}$)–0.28%(20 $\mu g\,l^{-1}$)] see Fig. 4

(DL) [8] and quantification limit (QL) [9] from the blank standard deviation in gas chromatography, and, frequently, IUPAC recommendations are not strictly used. We believe that our method for calculating DL and QL in pesticides in water [10] is more in line with the IUPAC recommendations. It relies on studying the blank standard deviation in an interval of time corresponding to the peak width in its base, extrapolated to zero concentration.

DL and QL were therefore calculated from calibrated data, set as stated in the above reference, and the results obtained are also summarized in Table 2.

The precision of the method (R.S.D.) was established as in Ref. [11] to the results obtained from the calibration graph (Fig. 4). Notice that the R.S.D. decreases when the concentration of imidacloprid increases.

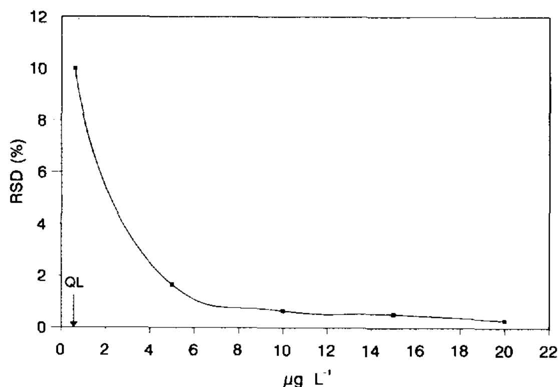

Fig. 4. Relative standard deviation vs. concentration of imidacloprid.

3.2. Validation and applications of the method

We tried to find imidacloprid in ground water and soil samples from Santa Maria farm, near Granada and in tap water from Granada city itself. We did not find imidacloprid above our DL.

Validation of the proposed method for water samples was carried out by using the standard addition methodology [12], whereby three experiments are required to obtain the data set necessary to obtain the proposed statistical protocol.

The same analytical procedure must be applied in each experiment to the 250-ml sample: (a) Standard calibration (SC) as described above; (b) Standard addition calibration (AC) obtained by standard additions of imidacloprid to sampled waters (0, 5, 10 and 15 $\mu g\,l^{-1}$); (c) Youden calibration (YC): a calibration curve was made with the Youden method [13]. Increasing amounts of sample volume (50, 100, 150, 200 and 250 ml, respectively) are checked three times for each of the above stated concentrations. By applying linear regression analysis, the slope, the intercept and the R.S.D. for each curve a, b, c, for each sample can be estimated and thus the whole range of spiking concentrations can be estimated. The parameters obtained from checking these three are shown in Table 3. Student t-test shows the similarity of the representative values of slope of SC and AC and it can be concluded that the method is accurate. On the other hand, the non-significative value of the intercept in the YC reveals the absence of matrix effect.

The validation of our method for soil samples was tested by using a recovery. test (Student t-test)

Table 3
Numerical values of parameters SC, AC and YC

Parameter	SC	Addition C.		Youden C.	
		Tap water	Ground water	Tap water	Ground water
n	30	12	12	15	15
a	−0.03	1.06	1.05	−0.04	−0.02
b	0.2244	0.2226	0.2245	4.47	4.46
S_{yx}	0.0241	0.0477	0.0619	0.0430	0.0481
S_p		0.0321	0.0379		
$t(b)$		0.9474	0.0518		
		p=35%	p=96%		
b_p		0.2240	0.2244		
a'	−0.0259	1.0541			
	−0.0296		1.0547		
YB				0.013	0.005

n: number of measurements; a, intercept; b, slope; S_{yx}, regression standard deviation; S_p, pooled standard deviation of AC and AC; $t(b)$, statistic for slope; b_p, pooled slope of AC and SC; a', corrected intercept; YB, Youden Blank.

Table 4
Results of recovery assays to check the accuracy of proposed method

Soil sample amount	Spiked soil (μg kg^{-1})	Found (μg kg^{-1})	Recovery R (%)	R.S.D. (%)
12.5 g	5	4.80	96.00	2.6
	5	5.07	101.30	
	5	5.13	102.60	
	10	9.70	97.00	
	10	9.80	98.00	
	10	9.80	98.00	
25 g	5	4.98	99.60	2.9
	5	4.75	95.00	
	5	5.09	101.70	
	10	9.96	99.60	
	10	10.20	102.00	
	10	9.60	96.00	
50 g	5	4.85	97.00	2.8
	5	5.03	100.50	
	5	4.75	95.00	
	10	10.20	102.00	
	10	9.63	96.30	
	10	9.60	96.00	

[14,15]. Series of different amounts of soil samples were fortified with different levels of imidacloprid. The P-value calculated, 0.38, is greater than 0.05 and so the null hypothesis might be accepted, i.e., the recovery is close to 100% (Table 4). DL was 1 μg kg^{-1}.

4. Conclusions

A simple and practical GC–MS method for the determination of the pesticide Imidacloprid in water and soil samples (5–20 μg l^{-1}) is presented. It was applied to natural waters and soil samples from Granada (Spain) with good recovery rates.

References

[1] C. de Liñan, Vademecum de Productos Fitosanitarios y Nutricionales, Ed. Embajadores, Madrid, 1995.
[2] W. Leicht, Plfanzenschutz Nachr. Bayer, 4 N° 3 (1993) 17.
[3] F.J. Placke and E. Weber, Plfanzenschutz Nachr. Bayer, 46, No. 64, (1993) 109.
[4] A.R. Fernández-Alba, A. Valverde, A. Agüera, M. Contreras and S. Chiron, J. Chromatogr. A, 725 (1996) 93.
[5] Statgraphics version 6.0, Manugistics Inc. and Statistical Graphics Corporation, USA, (1992).
[6] Analytical Methods Committee, Analyst, 119 (1994) 2363.
[7] APHA-AWWA-WPCF, Métodos Normalizados para el Análisis de Aguas Potables y Residuales, Ed. Díaz de Santos S.A., Madrid, 1992.
[8] IUPAC, Nomenclature, Symbols, Units and Their Usage in Spectrochemical Analysis, Pure Appl. Chem., 45 (1976) 105.
[9] Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry, Anal. Chem., 52 (1980) 2242.
[10] A. González-Casado, L. Cuadros-Rodríguez, E.A. Hernandez and J.L. Vilchez, J. Chromatogr. A, 726 (1996) 133.
[11] L. Cuadros-Rodríguez, A.M. García-Campaña, C. Jiménez-Linares and M. Román-Ceba, Anal. Lett., 26 (1993) 1243.
[12] L. Cuadros-Rodríguez, A.M. García-Campaña, F. Alés-Barrero, C. Jiménez-Linares and M. Román-Ceba, J. Assoc. Off. Anal. Chem., 78 (1995) 471.
[13] M.J. Cardone, J. Assoc. Off. Anal. Chem., 66 (1983) 1283.
[14] K. Doerffel, Fresenius J. Anal. Chem., 348 (1994) 183.
[15] Analytical Methods Committee, Analyst, 120 (1995) 29.