Flow injection analysis of the insecticide imidacloprid in water samples with photochemically induced fluorescence detection

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Abstract
A flow injection analysis (FIA) system, combined with photochemical induced fluorescence (PIF) detection is developed for the sensitive and rapid determination of imidacloprid. It is based on the conversion of imidacloprid into the fluorophore 1-(6-chloro-3-pyridyl-methyl)-2-(hydroxyimino)-3,4-didehydroimidazolidene. In an aqueous medium, this compound shows native fluorescence with an excitation maximum at 334 nm and an emission maximum at 377 nm. The linear concentration range of application was 1.0–60.0 ng ml\(^{-1}\) of imidacloprid, with a relative standard deviation of 2.1% (for a level of 10 ng ml\(^{-1}\)) and a detection limit of 0.3 ng ml\(^{-1}\). The method was applied to check whether imidacloprid was present above this limit in waters from Cuenca and Granada (Spain). It was validated applying a recovery test (Student’s \(t\)-test). Recovery levels of the method reached around 100% in all cases. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] (Fig. 1) belongs to a new group of active ingredients, the chloronicotinyl insecticides. It has a new mode of action, low toxicity for warm-blooded animals, good systemic properties and a lasting action.

This insecticide, introduced by Bayer (Leverkusen, Germany), is used for the control of mites present in vegetable crops [1]. Its development, activities, mode of action and effectiveness have been described by Leicht [2] and its physical, chemical and toxicological properties have been summarised in pesticide manuals [3].

Some high-performance liquid chromatography (HPLC) methods for the detection and determination of imidacloprid have been proposed. For instance, Placke and Weber [4] measured residual levels of imidacloprid in different fruits and vegetables by HPLC-UV. Here, sample extraction is followed by multi-step cleanup involving three evaporations followed by either partition or an elution through a clean-up solid-phase cartridge.

Fernández-Alba et al. [5] proposed an HPLC-diode array detection method for the determination of imidacloprid residues extracted from vegetables involving acetone and C\(_{18}\) reverse-phase cartridges. Also, Ruiz de Erenchun et al. [6] have developed an HPLC-pulse reductive amperometric detection method for the determination of imidacloprid in soils.

Since imidacloprid has low volatility, gas chromatography (GC) seems to be ruled out as a determination method. However, gas chromatographic–mass
spectrometric (GC–MS) methods for the determination of imidacloprid in water and soil samples [7] and vegetable samples [8] have been proposed. Here, the pesticide is first transformed into an adequate volatile compound by hydrolysis in a basic medium after which liquid–liquid extraction with chloroform is carried out for the extraction and preconcentration of the hydrolysis product.

Recently, a differential-pulse polarographic method for the determination of imidacloprid in commercial formulations was proposed [9]. Additionally, the use of UV radiation to produce fluorescent photoproducts by direct irradiation of the liquid solutions containing the analyte is a valuable method for the rapid screening of non-fluorescent compounds in clinical, pharmaceutical, biochemistry and environmental analysis [10].

Generally, photochemical kinetics are relatively rapid and in the majority of compounds, the photochemical reaction yields a photoproduct with an enhanced molecular absorption coefficient and/or higher fluorescence quantum yield relative to those of analytes [11]. For these reasons, the photochemical-induced fluorimetric methods might have some advantages such as simplicity, high sensitivity, high selectivity and cleanliness over other derivatisation methods.

In the field of pesticide and environmental analysis, laboratory-constructed instruments and modifications to commercial instruments for measuring photochemically-induced fluorescence in various media have appeared in the last years. Methods for chlorophenols [12], phenylurea herbicides [12], dinitroanilines [13], phenylcarbamates [14], phenylamides [14], pyrethroid insecticides [15], chlorophenoxyacid herbicides [16,17], phenylurea herbicides [18] and sulfonylurea herbicides [19,20] have been recently described.

Photochemical reactions in flow-based methodologies have enabled improvements in the fluorescence detection of selected light-sensitive compounds [21–23]. A multi-commutated flow network is another tool to improve versatility, design and sample volumes to be used [24].

This paper presents an on-line method based on a photochemical reaction and fluorescence intensity measurements for the determination of imidacloprid. The method has been applied to the determination of imidacloprid in natural water samples. The photochemical-fluorescence approach used here allows achieves, in general, better detection limit than existing methods with no matrix interference.

2. Experimental

2.1. Apparatus and software

All spectrofluorimetric measurements were performed using a Perkin-Elmer (Norwalk, CT, USA) LS-50 luminescence spectrometer fitted with accessories described previously [25] and a Hellma (Mülheim/Baden, Germany) 176.052-QS flow cell (20 μl inner volume). The LS-50 spectrometer was interfaced with an IBM (Armonk, NY, USA) PC 300-100DX4 microcomputer supplied with FL Data Manager Software (Perkin-Elmer) for spectral acquisition and the subsequent manipulation of spectra.

UV irradiation was carried out with an Oriel (Barcelona, Spain) Model 6035 “pencil style” mercury (argon) lamp connected to an AC power supply Model 6048. The lamp (51 mm × 10 mm Ø) operates at a 4 W and the main spectral lines are 253.7, 302.2,
312.6, and 365.0 nm. A continuous photochemical reactor, designed and constructed by the authors, was used to irradiate the sample while it circulated through a long PTFE tube (0.8 mm i.d. × 125 cm) coiled around the lamp. The reactor is placed into a PVC cylinder internally fitted with a flexible aluminium mirror.

A Gilson (Villiers, France) Minipuls 3 peristaltic pump and a Crison (Barcelona, Spain) 501 digital pH-meter with a combined glass-saturated calomel electrode were also used. A schematic diagram of the set-up for the method with on-line photochemical reaction and fluorescence measurement is shown in Fig. 2.

Statgraphics Plus [26], Alamin [27] and Grams/286 [28] software packages were used for the statistical treatment of the data, regression analysis (linear model) and integration of peak areas.

2.2. Reagents

All the experiments were performed with analytical-reagent grade chemicals. Water was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA).

A stock standard solution of imidacloprid (400 μg ml\(^{-1}\)) was prepared by dissolving the product (Bayer) (purity > 99%) in purified water. The solution was stable for at least 2 weeks if stored in the dark at 4°C. Working solutions were obtained by appropriate dilution with water. Britton–Robinson buffer solutions of required pH were prepared in the usual way, i.e. by adding to a solution 0.04 M in orthophosphoric acid (Merck, Darmstadt, Germany), 0.04 M in acetic acid (Merck) and 0.04 M in boric acid (Merck) the appropriate amount of 0.2 M sodium hydroxide (Merck) solution.

2.3. Treatment of water samples

Water samples were filtered through a Millipore HAWP 04700 cellulose acetate filter (0.45 μm pore size) and collected in dark glass bottles previously cleaned with 2 M hydrochloric acid and washed with deionised water. The samples were stored in the dark at 4°C until the analysis was performed with the minimum possible delay [29].

2.4. Analytical procedure

The sample solution (1100 μl) containing between 1 and 60 ng ml\(^{-1}\) of imidacloprid diluted with carrier was inserted into the carrier stream (0.04 M Britton–Robinson buffer solution, pH 11.8) at a flow
rate of 1.25 ml min\(^{-1}\). After circulating through the continuous photochemical reactor (0.628 cm\(^3\)), the relative fluorescence intensity (at 15.0 ± 0.5°C) of the sample was recorded operating at an emission wavelength of 377 nm and an excitation wavelength of 334 nm. A blank solution of deionised water was injected into the manifold in the same way as the sample. The calibration graph was constructed with imidacloprid solutions of known concentrations using as analytical parameters both the maximum fluorescence intensity and the peak area.

3. Results and discussion

3.1. Photochemically-induced fluorescence properties

Initial experiments have shown that in an aqueous medium imidacloprid does not have fluorescent properties, but when aqueous solutions are irradiated with UV radiation, a fluorescent signal is obtained. A previous imidacloprid photodegradation study [30] enabled later isolation and identification of the fluorescent transformation product [31], i.e. 1-(6-chloro-3-pyridylmethyl)-2-(hydroxyimino)-3,4-didehydroimidalozolidene (Fig. 1). In aqueous solutions this compound shows native fluorescence with an excitation maximum at 334 nm and an emission maximum at 377 nm.

3.2. Effect of experimental variables

To establish the optimum conditions for the production of fluorescence, a series of experiments were conducted. The studies were performed by modifying each variable in turn while keeping the others constant, i.e. the univariate method. Variables influencing the system can be divided into two groups: chemical variables and flow system variables.

3.2.1. Physicochemical variables

The relative fluorescence intensity of the photodegraded compound reaches its maximum in the pH range 11.5–12.2. The selected buffer was Britton–Robinson of pH 11.8. Changes in the buffer concentration from 0.01 to 0.04 M increase the fluorescence signal doubly. A 0.04 M concentration of buffer was selected to obtain an adequate buffering capacity and sensitivity and was used as a carrier solution.

The fluorescence is independent of the ionic strength, adjusted with NaCl or NaClO\(_4\), up to 1 M. In the temperature interval ranging from 5 to 18°C, the relative fluorescence intensity is practically constant, decreasing with the rise of temperature between 20 and 70°C. This effect is completely reversible. All other measurements reported here were taken at 15.0 ± 0.5°C.

3.2.2. FIA variables

The effect of reactor length, carrier flow rate, length of tubing after photochemical reaction and

![Fig. 3. Influence of flow rate at the reactor lengths of (A) 125 cm; (B) 150 cm; (C) 75 cm.](image-url)
sample loop volume on the intensity and width of the FIA peaks were all studied. Changes in the flow rate between 1 and 4 ml min$^{-1}$ modify the fluorescence intensity. Several reactor lengths of 75, 125 and 150 cm were tried at different carrier flow rates. Fig. 3 indicates in all cases a decrease in the fluorescence signal when the flow rate increases because of a decrease in the time of exposure of the analyte to the UV radiation. The maximum fluorescence signal was obtained for a 125 cm photochemical reactor length, probably due to a balance between photoconversion and dispersion of analyte in the FIA system. In order to select the optimum flow rate we took into account the best intensity of the narrower peak width and the highest flow rate getting 1.25 ml min$^{-1}$. Under these conditions residence time for imidacloprid in the photochemical reactor was 30 s. A negligible influence of the imidacloprid initial concentration on the kinetic of photodegradation was observed as well.

An increase in the sample loop volume between 200 and 2400 l produces a non linear increase in the analytical signal, due to the better performance of photodegradation for smaller sample volumes. We selected an 1100 l sample loop volume to get a good signal without excessive peak widening. The stability of the fluorescent photoproduct was constant for at least 2 h when away from diffuse daylight and light from the instrument xenon lamp.

### 3.3. Analytical parameters

The calibration function for the samples treated according to the procedure described above is linear in the concentration range 3.0–100.0 ng ml$^{-1}$ of imidacloprid (using the peak height as an analytical parameter) and in the concentration range 1.0–60.0 ng ml$^{-1}$ of imidacloprid (when using the area peak as an analytical parameter).

The lack-of-fit test [32] was used to check the linearity of the calibration graphs. Three replicates were used for each of five standards prepared to obtain the calibration graphs.

The standard deviation of the background fluorescence measured for the blank, which is necessary for the estimation of the IUPAC detection limit ($K = 3$) [33] and quantification limit [34] was taken as the average of 10 determinations and noted as RSD units.

The repeatability of the proposed method was determined at three imidacloprid concentration levels of 10, 20 and 40.0 ng ml$^{-1}$ by performing 10 independent determinations for each one. The relative standard deviations (RSD) were 2.1, 1.7 and 1.5%, respectively, using the peak area as the analytical signal, and using the peak height were 1.6, 1.2 and 1.1% for imidacloprid concentrations of 10, 40 and of 80.0 ng ml$^{-1}$, respectively. The precision (RSD) of the fluorescence

### Table 1

Analytical figures of merit

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>Height</th>
<th>Area</th>
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<tbody>
<tr>
<td>Intercept ($a$)</td>
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<tr>
<td>Slope ($b$)</td>
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<td>298.41</td>
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<tr>
<td>Correlation coefficient</td>
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<td>Lack-of-fit test (P-value)</td>
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<td>Linear dynamic range (ng ml$^{-1}$)</td>
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<td>1.0–60.0</td>
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<td>Linearity [1 – RSD($b$)] (%)</td>
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<td>99.4</td>
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<tr>
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<td>Quantification limit (ng ml$^{-1}$)</td>
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### Table 2

Methods for the determination of imidacloprid* 

<table>
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<th>Technique</th>
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<th>Linear dynamic range (ng ml$^{-1}$)</th>
<th>RSD (%)</th>
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<td>100–600</td>
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<td>5–20</td>
<td>10–0.28</td>
<td>[7]</td>
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<td></td>
<td>Soils</td>
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<td>5–20</td>
<td>10–0.28</td>
<td>[7]</td>
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<td></td>
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<td>2.5</td>
<td>1–20</td>
<td>5.6–0.3</td>
<td>[8]</td>
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<td>DPP</td>
<td>Commercial formulations</td>
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<td>10–200</td>
<td>3.2–1.5</td>
<td>[9]</td>
</tr>
<tr>
<td>FI-PIF</td>
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<td>1–60</td>
<td>2.1–1.5</td>
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</tr>
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</table>

The proposed method was compared with others that are among the most sensitive reported to date in the literature for the determination of imidacloprid. Table 2 show that the photochemical-fluorescence approach used here allow achieves, in general, better detection limit with a very simple methodology.

The proposed method was applied to the determination of imidacloprid in mineral water from the Solán de Cabras spa (Cuenca), ground water from the Santa María farm, near Granada, and spring water from Güejar Sierra (Granada). The imidacloprid content of these water samples was smaller than the above-stated detection limit (0.3 ng ml\(^{-1}\)). The analysed water samples were checked to ensure there were no interferences nor matrix effect through the extraction of water samples of 250 ml, in triplicate, with 25 ml of dichloromethane, evaporation of the extracts, dissolution in water and application of the proposed procedure.

It was also made certain that no significant difference existed between the calibration slopes and the standard addition method for the waters above cited. For this reason, the validation of the proposed method for water samples was tested using a recovery test (Student’s \(t\)-test) \[35,36\]. Water samples were fortified with different levels of imidacloprid. Since the \(P\)-values calculated, 63% for mineral water, 96% for ground water and 68% for spring water, are greater than 5.0%, the null hypothesis appears to be valid, i.e. recovery is close to 100% (Table 3).

### 4. Conclusions

A simple and practical flow injection photochemically induced spectrofluorimetric (FIA-PIF) method for the determination of the pesticide imidacloprid in water samples (1.0–60.0 ng ml\(^{-1}\)) is presented. We emphasise the fact that the detection limit value obtained for the determination of imidacloprid by this PIF-FIA method is very low, and comparable to or better than those reported for other, generally very sensitive, methods, such as GC–MS. It was applied to natural waters from Cuenca and Granada (Spain) with good recovery rates.

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References