A rapid spectrophotometric determination of imidacloprid in selected commercial formulations in the presence of 6-chloronicotinic acid

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Abstract: A simple first-order derivative spectrophotometric method was developed for the simultaneous determination of imidacloprid and 6-chloronicotinic acid (6-CNA). By using the zero-crossing approach, imidacloprid was determined at 249 nm and 6-CNA at 236 nm with detection limits of 0.32 and 0.17 µg mL⁻¹, respectively, and relative standard deviations not exceeding 1.2 % in the case of model systems. The proposed method was applied for the determination of imidacloprid and 6-CNA in commercial formulations. A conventional spectrophotometric method (at 270 nm) was also employed for the determination of the content of imidacloprid in the same commercial formulations. The results of the developed spectrophotometric methods were in good agreement with those obtained by the high-performance liquid chromatographic method.

Keywords: derivative spectrophotometry; imidacloprid; 6-chloronicotinic acid; insecticide formulations.

INTRODUCTION

Imidacloprid ((EZ)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylidenamine, Fig. 1a) belongs to the most efficient class of insecticides nowadays, called neonicotinoids, which account for about 17 % of the total insecticide market.¹,² Since its launch in 1991, products containing imidacloprid have gained registration in about 120 countries and are marketed for use in agriculture (for over 140 agricultural crops), on turf, on pets and for household pests.² The mechanism of imidacloprid action has been extensively studied and is relatively well known.

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It acts as an agonist by binding to nicotinic acetylcholine receptors in the nervous system of insects. This leads to an accumulation of acetylcholine, resulting in the paralysis and death of insects.\textsuperscript{1–3} Imidacloprid is marketed under a variety of names, including Gaucho, Merit, Admire, Confidor, Macho and Winner. Although imidacloprid has been in use for a relatively short period compared to other common pesticides, it is now considered to be the most widely used insecticide globally.\textsuperscript{1,2}

One of the synthetic precursors, and also an intermediate of imidacloprid decomposition, is 6-chloronicotinic acid (6-CNA), Fig. 1b.\textsuperscript{4,5} Recent investigations showed positive effects of imidacloprid on the stress resistance of several plants, which was probably due to 6-CNA. This substance is known to stimulate the defense systems of plants and thus protect them against disease.\textsuperscript{5}

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}

Fig. 1. Structure of imidacloprid (1) and 6-CNA (2).

All the above facts impose the necessity for reliable analytical methods for the determination of these two compounds in their mixtures. Analytical techniques used for imidacloprid determination include high-performance liquid chromatography (HPLC) with diode array (DA),\textsuperscript{6–8} mass spectrometric (MS)\textsuperscript{9,10} and thermal lens spectrometric\textsuperscript{11} detection. Some alternative techniques, such as an enzyme-linked immunosorbent assay,\textsuperscript{12,13} fluorimetry,\textsuperscript{14} Fourier transform infrared spectroscopy\textsuperscript{15} and voltammetry,\textsuperscript{16–20} have also been employed to analyze different imidacloprid-containing samples.

Several methods were developed to determine both imidacloprid and 6-CNA. Thus, capillary electrophoresis with DA\textsuperscript{21} and LC with DA,\textsuperscript{22,23} pulsed reductive amperometric\textsuperscript{24} and MS\textsuperscript{25} detection are used for this purpose. Matrix solid phase dispersion combined with LC–APCI–MS\textsuperscript{26} and column switching LC with post-column photochemical fluorescence detection\textsuperscript{27} was also applied for the simultaneous determination of imidacloprid and 6-CNA. Monitoring of 6-CNA in human urine by GC–MS, as indication of exposure to the pesticide imidacloprid, was also described.\textsuperscript{28}

Due to common availability of the instrumentation, simplicity of procedures, speed, precision and accuracy, spectrophotometric methods enjoy wide popularity. In addition, they are more economic and simpler, compared to methods such as chromatography and electrophoresis.\textsuperscript{29} Hitherto, no spectrophotometric me-
Several techniques have been proposed for the treatment of spectrophotometric data, with the objective of extracting a largest amount of analytical information from spectra composed of unresolved bands. Undoubtedly, a major success was achieved by derivative treatment of the absorbance curves, in which the first- or a higher-order mathematical derivative of the absorbance is plotted against the wavelength ($dA/d\lambda$). Derivative spectrophotometry offers a convenient solution to a number of analytical problems, such as resolution of multi-component systems, removal of sample turbidity, matrix background and enhancement of spectral details. Due to this, it was applied in the analysis of different pharmaceuticals, foods, cosmetics, and environmental samples.\textsuperscript{29,30} The same method was also applied for the simultaneous determination of pesticides or pesticides and their degradation products.\textsuperscript{31–37}

In this work, a rapid, environmentally acceptable and inexpensive first-order derivative spectrophotometric method was developed for the determination of imidacloprid and 6-CNA in their mixtures, both in model solutions and commercial formulations (Macho 200 SL and Confidor 200 SL). The conventional spectrophotometric method was also tested for the determination of imidacloprid in these two formulations. The results of the spectrophotometric methods developed were compared with those obtained by HPLC with DA detection, HPLC–DAD.

**EXPERIMENTAL**

**Chemicals and solutions**

All employed chemicals were of the analytical reagent grade. The analytical standard of imidacloprid and 6-CNA was of Pestanal quality (Riedel de Haën, Germany). Stock solutions were prepared by dissolving the compounds in doubly distilled water to obtain a concentration of 0.50 mg mL$^{-1}$, which did not change over a long period when the solutions were kept in the dark at 4 °C. Britton–Robinson buffer solutions were prepared from a stock solution containing 0.040 mol L$^{-1}$ phosphoric (Merck, Darmstadt, Germany), boric (Merck) and acetic (Merck) acids by adding 0.20 mol L$^{-1}$ sodium hydroxide (Merck) to the required pH values, covering the pH range of approx. 2.0–10. Commercial formulations of imidacloprid were Confidor 200 SL (Bayer CropScience, Germany) and Macho 200 SL (Hemovet, Serbia), both with a declared imidacloprid content of 200±12 g L$^{-1}$. The amount of 6-CNA in spiked commercial formulations was 115.0 g L$^{-1}$.

**Apparatus**

The spectrophotometric measurements were performed on an Anthelie Data UV–visible single-beam spectrophotometer (SECOMAM, France) with a fixed slit width (2 nm) operated via Anthelie Data software. The chromatograms were recorded on an Agilent 1100 liquid chromatograph (Agilent Technologies Inc., USA) furnished with an Agilent Hypersil ODS-C18 column (2.0 mm×250 mm, 5 μm). A digital pH-meter (PHM 62, Radiometer, Denmark) and a combined glass electrode were used for pH measurements.
Procedures

Spectrophotometry. Characterization of the individual optical behavior of imidacloprid and 6-CNA was performed at the same molar concentration (1×10^{-4} mol L^{-1}), i.e., 25.57 µg mL^{-1} and 15.76 µg mL^{-1}, respectively, in the pH range 2.0–10 and in the wavelength range 200–400 nm. Standard solutions for the calibration curves were prepared by the stepwise dilution of the stock solution to obtain concentrations in the 1.6–22.5 µg mL^{-1} for both compounds. The conventional spectrophotometric determination of imidacloprid was performed at a working wavelength of 270 nm, while the simultaneous derivative spectrophotometric determination was realized at 249 nm (imidacloprid) and 236 nm (6-CNA).

Chromatography. The mobile phase was 80:20 v/v water (containing 0.2 % phosphoric acid):acetonitrile. The wavelength of the DA-detector was 270 nm for imidacloprid and 224 nm for 6-CNA, with a reference wavelength of 360 nm. Other parameters were: flow rate 0.8 mL min^{-1}, column temperature 25 °C and injection volume 20.0 µL. The linearity of the detector response was checked in the concentration range 1.61–22.5 µg mL^{-1} for both analytes.

Procedure for the commercial formulations. For both the spectrophotometric and HPLC measurements, 0.25 mL of the commercial formulation was diluted stepwise to 1:50000 with doubly distilled water. The standard addition method was used for the determination in order to eliminate the matrix effect. In the case of the HPLC measurements, the solutions were filtered through Millex 0.22 µm syringe filters.

Validation of the analytical method. The linearity of both the spectrophotometric and comparative chromatographic method was checked in the concentration range 1.6–22.5 µg mL^{-1}. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following equations:

$$LOD = \frac{3s}{m} \quad \text{and} \quad LOQ = \frac{10s}{m},$$

where $s$ is the standard deviation of the blank and $m$ is the slope of the calibration curve.

RESULTS AND DISCUSSION

Optimization of the conventional and derivative spectrophotometric methods

To study the optical characteristics of the investigated compounds, the corresponding spectra were recorded in Britton–Robinson buffers (pH 2.0–10.0) in the wavelength range 200–400 nm. Representative spectra of imidacloprid and 6-CNA obtained at pH 7.0 are shown in Fig. 2a. The spectra of imidacloprid have two discrete absorption bands with maxima at 212 and 270 nm, whereby the latter is much more intense. No significant changes in the absorption spectra were observed in dependence on the pH of the solution. The spectra of 6-CNA also have two discrete, well-defined absorption bands with maxima at 224 and 269 nm, the former band being more intense. The shape of the spectra and their maxima depended significantly on the pH, especially at pH < 4.0. At higher pH values, no significant changes were observed. In this context, pH 7.0 was selected for the further investigations. As can be seen from Fig. 2a, the strong overlapping of the spectra of the investigated compounds hindered their conventional spectrophotometric determination in the mixture. Hence, derivative spectrophotometry was investigated to develop a method for their simultaneous determination. The derivative spectra of solutions containing the individual analytes were investigated in order to optimize the derivative order. As can be seen from Fig. 2, the first-
-order derivative spectrum (b) showed a high sensitivity and a good resolution for the simultaneous determination. Higher derivative orders (c, d) were discarded because the noise attenuation was less effective and the signal became distorted.

Fig. 2. Absorption spectra (a), first- (b), second- (c) and third-order (d) derivative spectra of imidacloprid (1) and 6-CNA (2). Measurement parameters: $c(1) = 16.9 \, \mu g \, mL^{-1}, c(2) = 11.6 \, \mu g \, mL^{-1}, \text{pH} \, 7.0$.

The main disadvantage of the derivative technique is that the signal-to-noise ratio worsens with increasing order of the derivative. Therefore, the practical derivative technique includes a certain degree of low-pass filtering or smoothing, to control the noise increase, which is an inevitable consequence of the noise signal differentiation. The effect of smoothing a peak-type signal is that the noise is reduced, which is desirable. However, it distorts the signal, which is undesirable but unavoidable. Thus, optimization of the smoothing factor is very important in order to obtain appropriate signals. In the present study, the adjacent averaging method was tested, using smoothing factors of 2, 5 and 8 (Fig. 3). The obtained curves were then compared with unsmoothed ones (Fig. 2b). The smoothing factor 5 was selected because this yielded good sensitivity without a significant sacrifice of the signal to noise ratio.
Fig. 3. Effect of smoothing on the first-order derivative spectra of imidacloprid (1) and 6-CNA (2). Measurement parameters: $c(1) = 16.9 \, \mu g \, mL^{-1}$, $c(2) = 11.6 \, \mu g \, mL^{-1}$, pH 7.0, smoothing factors: 2 (a), 5 (b) and 8 (c).
The smoothed first derivative spectrum of both compounds have more zero-crossings, of which those at 236 nm in case of imidacloprid and 249 nm in case of 6-CNA offer better sensitivity for the determination of the second compound (Fig. 3b). At these wavelengths, all the absorption is attributed to a single compound. The effect of the concentration of the analytes on both zero-crossing points was studied in the concentration range 1.61–22.5 µg mL⁻¹. The selected zero-crossing values were independent of the concentration.

In the case of the conventional spectrophotometric determination of imidacloprid, the absorbance was measured at the absorption maximum (270 nm).

**Determination of imidacloprid in a model solution and commercial formulations**

The simultaneous determination of imidacloprid and 6-CNA in the model solution is demonstrated in Fig. 4a. Using the selected conditions, linear graphs of \(\frac{dA}{d\lambda}\) versus the analyte concentration were obtained in the concentration range of 1.6–22.5 µg mL⁻¹ for both analytes. The calculated values of the LOD were 0.32 and 0.17 µg mL⁻¹ for imidacloprid and 6-CNA, respectively. The relative standard deviations (RSDs) did not exceed 1.2 %. The results of the first-order spectrophotometric method were compared with those of the HPLC–DAD method. The retention times of 6-CNA and imidacloprid were 7.85 and 9.04 min, respectively. The repeatability of the retention times and peak areas were checked by injecting the standard mixture solution six times and the RSD of the retention times and that of the peak areas were less than 0.1 and 1.1 %, respectively. The analytical parameters for both methods are presented in Table I.

Preliminary HPLC analysis did not confirm the presence of 6-CNA in the investigated commercial formulations (Confidor 200 SL, and Macho 200 SL). Hence, before applying the developed derivative spectrophotometric method for the determination of imidacloprid and 6-CNA (Fig. 4c), the formulations were spiked with a defined amount of 6-CNA. The standard addition method was used for the determination in order to eliminate the matrix effect. As can be seen from Table II, the determined amounts of imidacloprid and 6-CNA agreed well with the supplier’s data (imidacloprid) or with the added amount (6-CNA). The HPLC–DAD measurements confirmed the results of the spectrophotometric measurements. On the other hand, the conventional spectrophotometric analysis of imidacloprid in commercial formulations by the standard addition method (205.0 g L⁻¹, 2.27 % RSD in case of Confidor 200 SL (Fig 4b) and 194.5 g L⁻¹, 3.93 % RSD in case of Macho 200 SL) agreed well with the derivative spectrophotometric and HPLC data (Table II), confirming that simple conventional spectrophotometry can also give valuable insight into the content of the active compound of some commercial formulations.

The sufficiently good recoveries and low RSDs reflect the high accuracy and precision of the proposed derivative spectrophotometric method. The method is sen-
Figure 4. Simultaneous derivative spectrophotometric determination of imidacloprid and 6-CNA in a model solution (a), conventional spectrophotometric determination of imidacloprid in Confidor 200 SL (b) and derivative spectrophotometric determination of 6-CNA in spiked Confidor 200 SL (c).
SPECTROPHOTOMETRIC DETERMINATION OF IMIDACLOPRID

Sensitive, simple, relatively rapid and inexpensive, thus making it a convenient alternative tool for the fast determination of imidacloprid in commercial formulations, even in the presence of 6-CNA.

**TABLE I.** Analytical parameters for the derivative spectrophotometric and HPLC–DAD determination of imidacloprid and 6-CNA in model systems

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of determination</th>
<th>Imidacloprid</th>
<th>6-CNA</th>
<th>Imidacloprid</th>
<th>6-CNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration interval, µg mL(^{-1})</td>
<td>Derivative spectrophotometry</td>
<td>1.1–22.5</td>
<td>0.58–22.5</td>
<td>0.19–22.5</td>
<td>0.24–22.5</td>
</tr>
<tr>
<td>Slope(^a)</td>
<td>HPLC–DAD</td>
<td>0.0024</td>
<td>–0.0031</td>
<td>0.688</td>
<td>0.528</td>
</tr>
<tr>
<td>Intercept(^a)</td>
<td></td>
<td>0.001</td>
<td>–0.0006</td>
<td>0.032</td>
<td>–0.07</td>
</tr>
<tr>
<td>Correlation coefficient(^a)</td>
<td></td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>LOD / µg mL(^{-1})</td>
<td></td>
<td>0.32</td>
<td>0.17</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>LOQ / µg mL(^{-1})</td>
<td></td>
<td>1.07</td>
<td>0.58</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>RSD / %</td>
<td></td>
<td>1.2</td>
<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\(^a\)Y = a + bc, where c is concentration in µg mL\(^{-1}\) and Y is d\(\Delta A\)/d\(\lambda\).

**TABLE II.** The content of imidacloprid and 6-CNA in commercial formulations spiked with 6-CNA (n = 6)

<table>
<thead>
<tr>
<th>Commercial formulation</th>
<th>Method of determination</th>
<th>Imidacloprid</th>
<th>6-CNA</th>
<th>Imidacloprid</th>
<th>6-CNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Derivative spectrophotometry</td>
<td>202.14</td>
<td>3.22</td>
<td>116.60</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>HPLC–DAD</td>
<td>205.15</td>
<td>2.13</td>
<td>117.91</td>
<td>1.75</td>
</tr>
<tr>
<td>Confidor 200 SL</td>
<td></td>
<td>196.68</td>
<td>2.54</td>
<td>115.81</td>
<td>3.12</td>
</tr>
<tr>
<td>Macho 200 SL</td>
<td></td>
<td>194.30</td>
<td>1.40</td>
<td>115.80</td>
<td>2.85</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

A simple and rapid derivative spectrophotometric method, based on the zero-crossing approach, was developed for the simultaneous determination of imidacloprid and 6-CNA at pH 7.0. The first derivatives of the absorption spectra were used in the case of both compounds. Imidacloprid was determined at 249 nm, and 6-CNA at 236 nm. The method, tested by determining imidacloprid and 6-CNA in commercial formulations of imidacloprid, requires no sample clean-up, which saves time, money and the environment. Conventional spectrophotometry was successfully employed for the determination of imidacloprid in commercial formulations. The results of both the conventional and derivative spectrophotometric methods were in good agreement with the comparative HPLC–DAD procedure, and also with the composition declared by the manufacturer.

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ИЗВОД
БРЗО СПЕКТРОФОТОМЕТРИСКО ОДРЕЂИВАЊЕ ИМИДАКЛОПРИДА У ОДАБРАНИМ КОМЕРЦИЈАЛНИМ ПРЕПАРАТИМА У ПРИСУСТВУ 6-ХЛОРНИКОТИНСКЕ КИСЕЛИНЕ

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Предложена је једноставна спектрофотометријска метода на бази првог извода за истовремено одређивање имидаклоприда и 6-хлорникотинске киселине (6-ХНК). Примењујући приступ нултог пресека имидаклоприд је одређиван у модел систему на 249 nm a 6-ХНК нa 236 nm, са границама детекције од 0,32 и 0,17 µg mL⁻¹, респективно и релативном стандардном девијацијом мањом од 1,2 %. Предложена метода је примењена за одређивање имидаклоприда и 6-ХНК у комерцијалним препаратима. Конвенционална спектрофотометријска метода (на 270 nm) је такође примењена за одређивање садржаја имидаклоприда у истим комерцијалним препаратима. Резултати предложене спектрофотометријске методе су у доброј сагласности са результатима добијеним методом течне хроматографије високе ефикасности.

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