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Short communication

# Determination of the insecticide imidacloprid in water and soil using high-performance liquid chromatography

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**Abstract**

We describe an analytical technique for measuring residues of imidacloprid, a relatively new and highly active insecticide, in water and soil using high-performance liquid chromatography (HPLC). All analyses were performed on reversed-phase HPLC with UV detection at 270 nm using a mobile phase of acetonitrile–water (20:80, v/v). Fortified water samples were extracted with either solid-phase extraction (SPE) or liquid–liquid extraction methods. A detection limit of 0.5 μg/l was achieved using the SPE method. The imidacloprid residues in soils were extracted with acetonitrile–water (80:20, v/v), and the extract was then evaporated using a rotary evaporator. The concentrated extract was redissolved in 1 ml of acetonitrile–water (20:80, v/v) prior to analysis by reversed-phase HPLC. A detection limit of 5 μg/kg was obtained by this method which is suitable for analysis of environmental samples. Accuracy and precision at 10 and 25 μg/kg soil samples were 85±6% and 82±4%, respectively. © 1997 Elsevier Science B.V.

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

## 1. Introduction

Imidacloprid, 1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine (Fig. 1 insert), is a nitromethylene insecticide recently developed by Bayer. Due to its high insecticidal activity at very low application rates (0.3 mg/l), imidacloprid is regarded as a promising insecticide [1–3]. For this reason, sensitive analytical method is needed for measuring the low levels of imidacloprid residues in soil and water. It has been shown that direct determination of imidacloprid by gas chromatography (GC) is not possible due to its thermolabile and polar N-nitroguanidinyl moiety. Moreover, the substitution of the acidic hydrogen of the NH at the 3-position of the imidazolidine ring (Fig. 1, insert) may render the molecule even more volatile [4]. In contrast to GC, high-performance liquid chromatography (HPLC) utilises mild conditions for the separation and detection of analytes. Recently a HPLC method has been developed for the measurement of imidacloprid residues in crops [4–6] and the method was also tested for one soil [4]. However, no HPLC method for analysis of imidacloprid residue in water is available in the literature. The method reported by Ishii et al. [4] involves extraction of imidacloprid residue in crops and soil with acetonitrile–water (80:20, v/v), followed by a sequence of clean-up

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steps using liquid–liquid extraction with cyclohexane and dichloromethane and silica gel column chromatography. As part of our current investigation on the degradation of imidacloprid in soil, we required a rapid analytical technique with minimal use of toxic organic solvents. This paper reports a rapid and sensitive HPLC method that was developed and tested for imidacloprid residues in soil and water samples.

## 2. Experimental

All solvents were of HPLC grade from Mallinckrodt (Paris, KY, USA). Water was glass-distilled and further purified through a Millipore Milli-Q water purifier. Imidacloprid, technical grade (98.8%) was obtained from Bayer Australia. The solid-phase extractions were performed using Extract-Clean $C_{18}$ (500 mg×2.8 ml) disposable columns from Alltech Associates. Anatop syringe filters and Anatop membranes from Alltech Associates were used for filtration. A primary standard solution (100 mg/l) was prepared by dissolving imidacloprid (5.06 mg) in 50 ml of methanol. This stock standard solution was diluted (1:10) with methanol to obtain a working standard solution (10 mg/l). Analytical standards (0.1–10 mg/l) for HPLC calibration were prepared from aliquots of the working standard solution by evaporating the solvent to dryness with a stream of nitrogen and dissolving the residue in acetonitrile–water (20:80, v/v).

All standard samples and soil and water extracts were analysed on a Varian HPLC equipped with a Star 9012 ternary gradient pump, Polychrom 9065 diode array detector, Star 9050 programmable variable-wavelength UV detector, an autoinjector, column oven and a Star 9100 autosampler with electric sample valve. Data were collected and processed on the Star HPLC data system. All analysis were performed on a ODS2-$C_8$ (25 cm×4.6 mm I.D., 5 μm particle size) reversed-phase column kept at 25°C using a mobile phase of acetonitrile–water (20:80, v/v) at a flow-rate of 1.5 ml/min. The detection was performed at 270 nm and 0.02 a.u.f.s. Sample injection volume was 50 μl. External standards of imidacloprid were run after every ten samples.

Water samples were extracted using both liquid–liquid extraction and SPE methods. Liquid–liquid extraction was performed with 50- and 250-ml separatory funnels for 10 and 100 ml water samples, respectively. Triplicate water samples containing the two spike levels of imidacloprid (100 and 10 μg/l) were extracted three times with each of dichloromethane, ethyl acetate and cyclohexane. The volumes of solvent used were 5 and 25 ml for the 10 and 100 ml water samples, respectively. The solvent fractions were combined and dried on a rotary evaporator (40°C) and the residue was redissolved in 1 ml of acetonitrile–water (20:80, v/v) for HPLC analysis.

### 2.1. SPE

The $C_{18}$ column was preconditioned with 5 ml of methanol and washed with 5 ml of water. Immediately after the wash triplicate water samples (10 and 100 ml) containing three spike levels of imidacloprid (10, 1 and 0.5 μg/l) were aspirated through the $C_{18}$ columns (flow-rate 10 ml/min) without allowing the column bed to dry and the eluate discarded. The $C_{18}$ columns were then dried by pulling air through for 5 min before the adsorbed chemical was eluted with methanol (2 ml). The methanol eluate was evaporated under $N_2$ and then redissolved in 1 ml of acetonitrile–water (20:80, v/v) for HPLC analysis.

Moist soil materials (25 g on oven-dry basis) spiked with different concentrations of imidacloprid (50, 25, 10 and 5 μg/kg dry soil) were extracted with 40 ml of acetonitrile–water (80:20, v/v). The suspension was stirred and equilibrated for 2 h using an end-over-end shaker. Following centrifugation (20 min, 1164 *g*), the aqueous solutions were filtered through glass fibre filter membranes (0.22 μm) and the operation of shaking and filtration was repeated. The combined extract was transferred to a 100-ml round-bottomed flask and concentrated using a rotary evaporator at 45°C. The residue was redissolved in 1 ml of acetonitrile–water (20:80, v/v) for HPLC analysis. Five replicates at each fortification level, including unspiked controls, were extracted and analysed by HPLC.

For comparison, we also used the published extraction method for crops and soil [4] using acetonitrile–water as extractant followed by a se-

quence of clean-up steps using liquid–liquid extraction and silica-gel column chromatography.

## 3. Results and discussion

An absorption maxima with least interferences from the soil matrix (see Fig. 1) was observed at 270 nm. Consequently, the variable-wavelength UV detector was set at 270 nm to measure the imidacloprid in water and soil extracts. A UV detector response curve at 270 nm was obtained by injecting triplicate standard solutions ranging from 0.1–10 mg/l. The response of imidacloprid was linear in the concentration range studied and the correlation coefficient determined was 0.992 ($P<0.001$). Under the conditions used the minimum detectable concentration was 0.01 mg/l.

### 3.1. Reproducibility and recovery

The day-to-day reproducibility of the retention time and peak height were examined by using a 1 mg/l standard and 50 μg/kg spiked soil extracts over a 10-day period; i.e., a total of 30 injections of each over 10 days. The results obtained showed that the imidacloprid peak height variabilities for both the 1 mg/l standard and the spiked soil extract were within 5.4% R.S.D. Retention time fluctuations showed the maximum R.S.D. value of 6.1%.

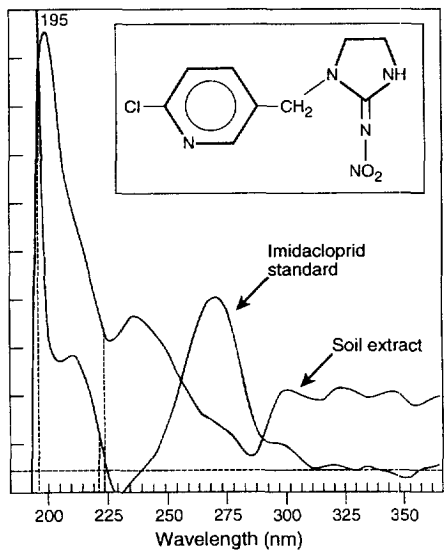


Fig. 1. Spectral scan for soil extract and imidacloprid standard. Insert shows the structure of imidacloprid.

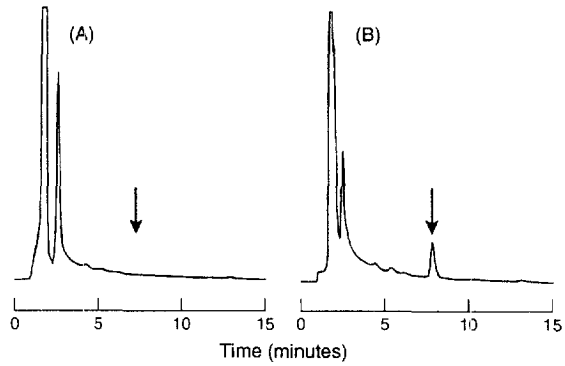


Fig. 2. HPLC chromatograms of (A) unspiked soil extract and (B) soil spiked with 10 μg/kg of imidacloprid. The arrow indicates the retention time of imidacloprid in either (A) unspiked soil or (B) spiked soil.

Fig. 2A and B show the chromatograms for unspiked soil and for the soil spiked at 10 μg imidacloprid/kg, respectively. The chromatograms for unspiked soil showed no interference in the retention region for imidacloprid. The recovery data for soil materials spiked with imidacloprid is presented in Table 1. The mean recoveries were 82–85% over the spiked range (5–50 μg/kg) with excellent reproducibility, even at low levels (mean R.S.D. 4.8%). The detection limit was 5 μg/kg, limited principally by the signal-to-detector-noise ratio of the analyte. The detection was further improved by larger injection volumes (100 μl).

### 3.2. Water extraction

Imidacloprid was extracted effectively by both the

Table 1
Recovery of imidacloprid from spiked soil materials as determined by HPLC

| Amount spiked (μg/kg) | Mean recovery (%) | R.S.D. (%) |
|---|---|---|
| 50 | 83 | 4.2 |
| 25 | 82 | 4.0 |
| 10 | 85 | 5.5 |
| 5 | 82 | 5.3 |
| Mean | 83 | 4.8 |

Table 2
Mean recovery of imidacloprid from spiked water samples by liquid–liquid extraction and solid-phase extraction methods

| Extracting medium | Imidacloprid recovered (%)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 ml sample | | | | 100 ml sample | | | |
| | 0.5 μg/l | 1.0 μg/l | 10 μg/l | 100 μg/l | 0.5 μg/l | 1.0 μg/l | 10 μg/l | 100 μg/l |
| Liquid–liquid extraction | –[b] | – | 83 (4.1) | 87 (4.4) | – | – | 82 (4.3) | 88 (5.0) |
| Solid-phase extraction | 82 (4.2) | 90 (5.3) | 87 (3.7) | – | 84 (3.8) | 95 (4.5) | 87 (5.0) | – |

[a] Values in parentheses indicates the standard error of mean.
[b] Samples not evaluated.

liquid–liquid extraction and SPE methods (Table 2). The detection limit (signal-to-noise ratio of 3:1) for imidacloprid in water samples by the SPE method described was 0.5 μg/l for 100 ml samples using detector settings of 0.02 a.u.f.s at 270 nm. These limits could potentially be lowered either by increasing the volume of injection or by reducing the volume of solvent used to redissolve the extract residue. The detection limit established in this study is comparable to those for other pesticides using SPE methods [7–9]. In addition to the lowest detection limit, the $C_{18}$ columns could be reused for 4–5 times by washing after use with an extra 2 ml of methanol in addition to the 5 ml of methanol used for preconditioning.

Several advantageous result from the use of the SPE method including, reduced cost, higher sample throughput, low use of solvent and safer to use. With cleaner samples the disposable $C_{18}$ columns can be used more than once. There is also a reduction in solvent disposal cost with SPE. Also several SPE extractions could be carried out simultaneously resulting in an increase in the number of samples processed in a given time. Advantages of SPE over liquid–liquid extraction are well recognised [10–12].

### 3.3. Soil extraction

The recoveries with the extraction method for the soils used in this study are given in Table 1. These recoveries were obtained without the sequence of clean-up steps suggested by Ishii et al. [4] for the measurement of imidacloprid in soil. However, for comparison, we also followed their clean-up sequence and found that the recoveries (e.g. 87% at 20 μg/kg) of imidacloprid were comparable with those obtained with the method described in this study (see Table 1). The published extraction method using the sequence of clean-up procedures with different solvents may be required for analyses in crops, due to the presence of interfering plant materials such as, chlorophyll, oily constituents, acidic substances and resinous tissue of the plants. However, the clean-up steps may not be required for all soils, especially those which contain lower organic matter. The detection limit for imidacloprid in the soil materials was 5 μg/kg. In addition to the improved sensitivity, the extraction method reported in this study uses lower solvent volumes than that reported in the Ishii et al. [4] method.

The analytical technique reported in this communication has several advantageous over the liquid–liquid extraction method. The work-up of the crude extracts was not complicated using the extraction method described above. In addition, the extraction system applied in this method to concentrate the extracts was rapid, used less solvent, less glassware and comparable recoveries than the liquid–liquid extraction procedure published [4]. The recoveries were highly reproducible in the residue range of 10–50 μg/kg and were suitable for studies on degradation of imidacloprid in soil.

## 4. Conclusion

A rapid and reliable analytical method has been described for analysis of residues of the nitromethylene insecticide, imidacloprid in water and soil using reversed-phase HPLC with UV detection at 270 nm. Determination of imidacloprid in water samples by the SPE method uses solvent volumes smaller than the liquid–liquid extraction method. For soil extraction, the method described in this study is

rapid, does not need extensive clean-up sequence and therefore allows more efficient operation. Reproducible recovery data for imidacloprid in the concentration range 5–50 μg/kg soil showed that the method is adequate for measurement of the residues in soils during degradation studies. In the soils we studied, a detection limit of 5 μg/kg imidacloprid was established.

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