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# Simple and Sensitive Method for Determination of Imidacloprid Residue in Soil and Water by HPLC

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**Abstract** A rapid, sensitive and reliable HPLC method was developed and validated for the determination of imidacloprid residue in water at different pH and in soil. Quantification was performed by reversed phase HPLC system equipped with UV detector. The limit of quantification (LOQ) was found to be 0.02 mg/kg in soil and 0.02 mg/L in water. The limit of detection (LOD) was 0.006 mg/kg in soil and 0.006 mg/L in water. Recoveries for imidacloprid were 95.18%, 94.66%, 95.27% and 94.78% in black, red, sandy loam and clay soils, respectively. Recoveries for imidacloprid from water were 96.86%, 86.14%, and 92.34% at pH values of 4, 7, and 9, respectively.

**Keywords** HPLC · Imidacloprid · Residues · Soil · Water

Chemical pesticides are the most important component of any pest management strategy. Soil, an important component of the environment, act as a sink for the pesticides used in agriculture. The pesticides present in soil sometimes act as a source of contamination for succeeding crop. From soil, the pesticides residues can reach water bodies by leaching and runoff. Pesticides are inherently toxic molecules. Once they reach the water bodies, they start adversely affecting the aquatic environment. Presence of pesticides residue in ground water is extremely hazardous to human beings as ground water is major source of drinking water. Imidacloprid is a chloronico-tinylnitroguanidine insecticide. Its IUPAC name is 1-[(6-chloropyridin-3-yl)–n-nitro-4,5-dihydroimidazol-2-amine. Imidacloprid is a systemic chloronicotinyl insecticide that enters the target pest via ingestion or direct contact. It acts by disrupting nicotinic acetylcholine receptors in the insect central nervous system. It is used for controlling sucking insects, soil insects, and some chewing insects. It is applied to seeds, soil, and crop and used as a topical flea control treatment on domestic pests. It is also used as a seed dressing as well as for foliar, soil and stem treatment (Ishaaya and Degheele 1998). Imidacloprid is rapidly translocated across plant tissues after application, and can be present in detectable concentrations in tissues such as leaves, vascular fluids and pollen. Due to its extensive use in agriculture, its presence is expected in soil as well. When applied to the soil or as a seed treatment, imidacloprid is metabolized more or less completely depending on plant species, time, soil type and other environmental conditions (Araki et al. 1994). Due to greater solubility of the pesticide in water, it is expected to be present in water systems due to agricultural run offs. In water, degradation of the pesticide depends on pH and other organic and inorganic species present in (Pal et al. 2006).

Determination of imidacloprid residues in soil and in water is necessary so that frequency of crop cycle can be established and harmful effects of the pesticide minimized. This study therefore reports validation of an HPLC method for determination of imidacloprid residue in different soils in Gujarat and in water at different pH.

## Materials and Methods

Analytical reference standards of imidacloprid (98.5% purity) were obtained from Dr. Ehrenstorfer, Germany. All

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the other chemicals and solvents used were analytical and HPLC grade.

The soil characterization was performed to determine different physico-chemical properties viz., pH, organic carbon, water holding capacity (WHC) and clay content (particle size distribution) of soils collected from different parts of Gujarat, India. The methods followed were as per Walkley and Black (1934) and Baruah and Barthakur (1997).

The test soils were collected from different parts of Gujarat, India; viz. (a) Vadu, (b) Bardoli, (c) Umarsadi, (d) Vikram Farm, Valvada and were coded as Soil-1 (sandy loam soil), Soil- 2 (clay soil), Soil-3 (red soil), and Soil-4 (black soil), respectively. Based on organic carbon (%), pH and clay content, the soils i.e. Soil-1, Soil- 2, Soil-3, and Soil-4, were classified (OECD N° 106, 2006). The soil characteristics data is shown in Table 1.

The specificity of the analytical method was studied by injecting solvent, reference standard solution, control soil and water sample extracts injected onto HPLC. There was no interference of the components with each other. The linearity was established by injecting five different concentrations, viz. 0.02–5.00 mg/L, and determining the response of the compound; these were fitted by linear regression to assess the linearity. Detection Limit (signal-to-noise ratio = 3 ± 0.5:1) was established. The linear dynamic range of imidacloprid is shown in Fig. 1.

The precision (% RSD) of the analytical method was determined by five replications in duplicate injection of fortified substrate soil and water extracts at LOQ level. The accuracy (% recovery) of the method was determined by five replications in duplicate injection of fortified substrate soil and water extracts at LOQ and 10 × LOQ levels. Precision (% RSD) should not exceed 20%.

A representative sample (50 g) of a particular soil (black, red, sandy loam or clay) was transferred to 250 mL Erlenmeyer flask. The soil sample was fortified with imidacloprid at two different fortification levels: LOQ and 10 × LOQ, separately. A volume of 0.5 and 5.00 mL imidacloprid solution was transferred to each conical flask for 0.02 and 0.20 ppm fortification levels. In case of water, samples at pH 4, 7 and 9 (25 mL) were transferred into volumetric flask 50 mL capacity and fortified with imidacloprid at LOQ and 10 × LOQ levels separately. A Volume of 0.25 and 2.5 mL imidacloprid was transferred to each volumetric flask for 0.02 and 0.20 ppm fortification levels. The control samples were processed similarly where in 0.25 and 2.5 mL acetonitrile was added.

A volume of 100 mL methanol was transferred into the Erlenmeyer flask containing (50 g) fortified soil sample and allowed to stand for 2 h. The Erlenmeyer flask was placed onto orbital shaker for 30 min. After shaking, the solutions were filtered into the round bottom flask of 500 mL capacity through Whatman filter paper No.1 bearing a bed of anhydrous sodium sulphate. Solvent was removed using vacuum evaporator. The residual cake was re-extracted twice with additional volume of 50 mL methanol. The methanol extracts were collected, pooled and concentrated to smaller volume (5–10 mL) using vacuum evaporator at ≤40°C. The concentrated extract was subjected to further clean up by column chromatography. A glass column packed with florisil as adsorbent placed in between two layer of anhydrous sodium sulphate was employed. The column was pre-conditioned with methanol and concentrated extracts were loaded onto top of the column and eluted with 100 mL acetonitrile @ 2 mL/min. Eluate was concentrated to dryness using rotary vacuum evaporator at ≤40°C and residue re-dissolved in 5 mL acetonitrile. The samples were transferred into volumetric flask 10 mL capacity using Whatman No. 1 filter paper and final volume was made up to the mark with acetonitrile.

The fortified water samples (25 mL) at different pH viz. 4, 7 and 9 were transferred separately into a separating funnel of 250 mL capacity and a volume of 50 mL ethyl acetate was added into it. The separating funnel was shaken manually for 5 min with frequent vent. The contents of the separating funnel were allowed to stand for 10 min for

**Table 1** Soil characteristics

| Soil sampling site (location) | Soil code | Characteristics | | | | | | | | Soil type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Organic carbon (%) | pH (0.01 M $CaCl_2$) | pH Distilled water | Particle size | | | | | |
| | | | | | Coarse sand (%) | Fine sand (%) | Silt (%) | Clay (%) | Water holding capacity | |
| Vadu, Baroda | Soil-1 | 0.84 | 7.00 | 7.25 | 1.67 | 59.96 | 5.06 | 15.20 | 47.51 | Sandy loam soil |
| Bardoli | Soil-3 | 1.37 | 6.60 | 6.91 | 2.42 | 9.18 | 10.73 | 53.68 | 67.06 | Clay soil |
| Umarsadi | Soil-4 | 0.42 | 4.32 | 4.82 | 10.13 | 7.47 | 7.98 | 42.55 | 65.74 | Red soil |
| Vikarm Farm Valvada | Soil-2 | 0.86 | 7.15 | 7.47 | 3.64 | 10.64 | 19.01 | 38.00 | 60.06 | Black soil |

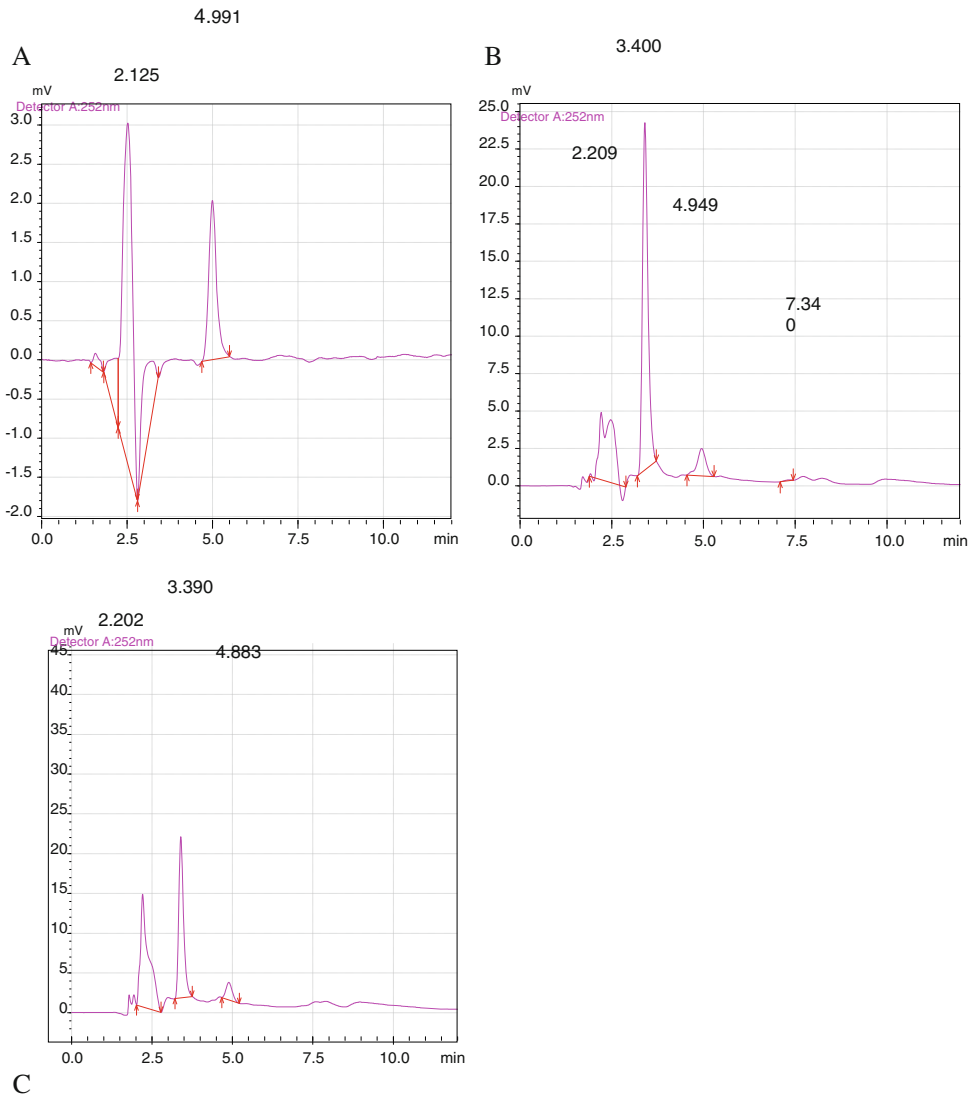


**Fig. 1** (**a**–**c**) Chromatograms of reference standard of imidacloprid 1 mg/L, soil and water samples indicating recovery in sandy loam soil and water pH 4 respectively

layer separation. The ethyl acetate organic layer was collected into a round bottom flask of 500 mL capacity. The aqueous layer was re-extracted twice with additional volume of 50 mL ethyl acetate and collected in the same round bottom flask. The combined extract was concentrated to dryness using rotary vacuum evaporator at ≤40°C temperature. The residue was re-dissolved in 5 mL acetonitrile. The samples were transferred into volumetric flask of 10 mL capacity through Whatman No. 1 filter paper and final volume was made up to the mark with acetonitrile.

The quantitative analysis of imidacloprid in soil and water extracts was conducted by reverse phase HPLC technique. A Shimadzu LC-2010 AHT equipped with UV detector, Phenomenex C-18 column (250 mm length × 4.6 mm i.d. and 0.5 µm particle size) and LC-solution software was used.

Mobile phase A: 0.01% (v/v) acetic acid in water (60). Add 0.1 mL acetic acid and dilute to 1 L with water. Mobile phase B: acetonitrile (40). The mobile phase was delivered to mode of low pressure gradient at 1 mL flow rate and detector set a 252 nm $\lambda_{max}$ was used for analysis. Imidacloprid standard showed sharp peak at 4.93 min under the described HPLC conditions. Figure 1a–c depicts a typical chromatogram of the separation of imidacloprid reference standard and recovery in soil and water samples.

## Results and Discussion

Soil characterization: Table 1 shows that the mean pH (0.01 M $CaCl_2$ suspension) of the different soils as determined (soil solution of (1:2.5)) for Soil-1, Soil- 2, Soil-3, and Soil-4 were 7.15, 6.60, 4.32, and 7.00, respectively. The corresponding mean pH (distilled water suspension) of the different soils determined (soil solution 1:2.5) were 7.47, 6.91, 4.82 and 7.25, respectively. The percent organic

carbon for soils i.e. Soil-1, Soil- 2, Soil-3, and Soil-4 were 0.86, 1.37, 0.72 and 0.84, respectively. The particle size fractions of the test soils were determined by international pipette method. The coarse sand content of the test soils i.e. Soil-1, Soil- 2, Soil-3, and Soil-4 were 3.64%, 2.42%, 10.13% and 1.63%, respectively. The fine sand content was 10.64, 9.18, 7.47 and 59.96, respectively. The silt content of the test soils were 19.01%, 10.73%, 7.98% and 5.06%, respectively. The percent clay content of the test soils were 38.00, 53.68, 42.55 and 15.20, respectively. The water holding capacity of the test soils i.e. Soil-1, Soil- 2, Soil-3, and Soil-4 were 60.06%, 67.06%, 65.74% and 47.51%, respectively.

The linearity of the detector response was tested for imidacloprid, in solvent and in matrix (soil) over the concentration range of 0.02–5.00 mg/kg. A very precise linear relation between the injected amount and the resulting peak area was observed over the entire concentration range with correlation coefficient value of 0.999 as seen in Table 2.

**Table 2** Data for linearity determination of imidacloprid in solvent

| Concentration (mg/L) | Mean peak area | % Variation |
|---|---|---|
| 0.02 | 647.00 | 0.62 |
| 0.05 | 1628.50 | 0.06 |
| 0.25 | 8172.00 | 0.05 |
| 1.00 | 32523.00 | 0.11 |
| 5.00 | 165401.50 | 0.11 |

Regression parameters imidacloprid standard in solvent

Slop: (b) = 33096.66

Y-axis intercept: (a) = −159.78

Correlation coefficient: (r) = 0.999

Ishii et al. (1994) has also reported an HPLC method for determination of imidacloprid residue in 9 kinds of crops and soil. The method consisted of extraction with acetonitrile/water (80:20 v/v), pre-washing of the concentrated extracts with cyclohexane and alkaline solution, silica gel column chromatography, and finally reversed-phase HPLC. The recoveries of imidacloprid were 75%–109%. The limits of detection were 0.005, 0.01 and 0.02 mg/kg for crops, rice straw and soil, respectively. (Bonmatin et al. 2003) also developed a method for determination of imidacloprid in soils, plants (leaves and flowers), and pollens by using HPLC coupled to tandem mass spectrometry (APCI-MS/MS). Extraction, separation, and detection were performed. The linear range of application was found to be 0.5–20 μg/kg imidacloprid in soils, in plants, and in pollens, with a relative standard deviation of 2.9% at 1 μg/kg. The limits of detection and of quantification were LOD = 0.1 μg/kg and LOQ = 1 μg/kg, respectively.

We have developed and validated the analytical method for the determination of low amounts of imidacloprid in/on different soils viz. sandy loam soil, clay soil, red soil and black soil and in water at different pH values, viz. 4, 7 and 9 using HPLC. The accuracy and precision of the method was evaluated on the basis of the recoveries obtained for fortified soil and water samples. The limit of quantitation (LOQ) was found to be 0.02 mg/kg for imidacloprid in soil and 0.02 mg/L in water. The limit of detection (LOD) was 0.01 mg/kg for imidacloprid in soil and 0.01 mg/L in water. Recoveries for imidacloprid were 95.18%, 94.66%, 95.27% and 94.78% in black soil red soil sandy loam and clay soil, respectively. The recoveries for imidacloprid were 96.86%, 96.14% and 92.34% in water at pH 4, 7and 9, respectively. The accuracy (% recovery) data in soil and

**Table 3** Precision (%RSD and accuracy (% Recovery) of imidacloprid in soil and water

| Substrates | Fortification of LOQ and 10 × LOQ levels in mg/kg | % Recovery | Mean % recovery | SD | % RSD |
|---|---|---|---|---|---|
| Black soil | 0.02 | 97.20 | 95.18 | 1.18 | 1.21 |
| | 0.20 | 93.16 | | 2.24 | 2.40 |
| Red soil | 0.02 | 97.25 | 94.66 | 1.86 | 1.91 |
| | 0.20 | 92.07 | | 3.10 | 3.37 |
| Sandy loam soil | 0.02 | 97.00 | 95.27 | 1.97 | 2.03 |
| | 0.20 | 93.54 | | 3.01 | 3.22 |
| Clay soil | 0.02 | 96.35 | 94.78 | 1.31 | 1.36 |
| | 0.20 | 94.60 | | 2.23 | 2.36 |
| Water pH 4 | 0.02 | 97.60 | 96.86 | 1.63 | 1.67 |
| | 0.20 | 96.11 | | 2.21 | 2.30 |
| Water pH 7 | 0.02 | 96.30 | 96.14 | 1.60 | 1.66 |
| | 0.20 | 95.98 | | 3.10 | 3.23 |
| Water pH 9 | 0.02 | 95.00 | 92.34 | 1.25 | 1.31 |
| | 0.20 | 89.67 | | 2.34 | 2.61 |

water is depicted in Table 3. Schoning and Schmuck (2003) has reported a gradient HPLC – tandem MS method for imidacloprid recovery. The repeatability of the method was determined for the analyte by running a set of five recoveries each at two different fortification levels for selected matrices. The resulting mean recovery rates ranged from 79% to104% with relative standard deviations between 0.8% and 15.3%. Similar results were found in present method validation for different soil and waters. The repeatability of the method in the present study was determined for each fortification levels by running a set of five recoveries each different fortification levels for selected matrices. The % RSD was the resulting mean recovery rates ranged from 94.66% to 95.27% in soil with relative standard deviations between 1.21% and 3.37%. The % RSD was the resulting mean recovery rates ranged from 92.34% to 96.86% in water with relative standard deviations between 1.66 and 3.23%. These data demonstrate the excellent sensitivity, selectivity and precision of the method (Table 3).

We have developed and validated a rapid, simple, sensitive and specific method for the determination of imdacloprid residues in/on different soil viz. sandy loam, clay, red and black soil and waters viz. pH4, 7 and 9 through HPLC. A simple clean-up procedure using column chromatography was found to yield sufficiently clean samples.

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