GAS-CHROMATOGRAPHIC DETERMINATION OF IMIDACLOPRID IN WATER

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ABSTRACT

A simple and sensitive method for the analysis of imidacloprid in water has been developed using Gas Chromatography (GC). imidacloprid converted into a volatile imidacloprid-urea by alkaline hydrolysis. Water spiked with eight different concentrations of imidacloprid was mixed with NaOH and heated to obtain imidacloprid-urea. The hydrolyzed product was extracted with dichloromethane and finally taken into 1 ml mixture of acetone and n-hexane (1:9 v/v). The separation of peaks was done on gas chromatography coupled to Electron Capture Detector (ECD) and Nitrogen Phosphorous Detector (NPD) and further it was confirmed by Gas Chromatography - Mass Spectrometry (GC-MS). The mean recovery of imidacloprid-urea in water was found to be 92%. The percent Relative Standard Deviation (%RSD) of imidacloprid was found to be below 5% for different concentration levels. The limit of detection of imidacloprid-urea was found to be 20 mg L⁻¹ with a signal to noise ratio of 3:1 and limit of quantification was found to be 75 mg L⁻¹ with a signal of noise ratio 10:1. The developed method is sensitive, quick and easy to perform and could be utilized for regular monitoring of imidacloprid residue in water.

Key Words : Imidacloprid, Imidacloprid-urea, GC-ECD-NPD, GC-MS, Water

INTRODUCTION

Pesticides constitute one of the most hazardous groups of contaminants to human health, fauna and environment in general. The majority of such substances are applied directly to soil or sprayed over crop fields and hence released directly to the environment. Therefore, pesticides can contaminate surface water directly as spray drift or run-off and also via drainage through the soils of treated farmland. The amount and type of pesticides in the water of a particular area depends largely on the intensity of production and type of crops being cultivated. However, the transport of pesticides beyond the area of application results in the presence and accumulation of these compounds in many parts of the hydrosphere. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimida-zolidin-2-ylidenamine] neonicotinoids is a chloronicotinyl insecticide first introduced by Bayer Agricultural Products (Leverkusen, Germany). They are relatively new class of insecticides with a distinct mode of action. It has high insecticidal activity and is used for the protection of various crops. As insecticide its sale is fast growing globally. It is safe, to a large extent for human but it also shown adverse effects at higher dose when 90 days oral study in performed in rat. Its selective toxicity results from its high affinity to insect nicotinic acetylcholine receptors (nAChR) compared to mammals. No more literature on analytical methods for imidacloprid further use of NaOH has any advantages over previously reported method. Earlier it was detected through HPLC-PDA (High-performance liquid chromatography-Photo diode array detector) to the detection level of 0.01-0.60 mg kg⁻¹ in water. Since the detection level of imidaclopride in water through HPLC is very high. Therefore, a sensitive analytical method for measuring low
quantity of imidaclopride is needed. Imidacloprid is thermo labile and low volatile. Due to its n-nitroguanidinylne group it was not possible to analyze on gas chromatography. Therefore, to render its molecule to be more volatile it could be substituted by N-NO₂ group at the fourth position of imidazoladin ring.

![Diagram of Imidacloprid and Imidacloprid-Urea]

**Fig. 1**: Hydrolysis of imidacloprid in alkaline medium

### MATERIAL AND METHODS

All HPLC grade solvents like n-hexane, acetone, dichloromethane (DCM) and ethyl acetate were purchased from Spectrochem Pvt. Ltd. and Merk Pvt. Ltd, India and glass distilled before use. HPLC grade water was obtained from Milli-Q (Elix-10, Billerica, USA) Millipore purifier. Acetone was refluxed over potassium permanganate for 4 hr and then distilled. Sodium chloride (NaCl), sodium hydroxide (NaOH), anhydrous sodium sulphate (Na₂SO₄) and magnesium sulphate (MgSO₄) was procured from Hi-media Pvt. Ltd. India. Before use, anhydrous sodium sulphate (Na₂SO₄) and magnesium sulphate (MgSO₄) were purified with acetone and baked for 4 hr at 600°C in muffle furnace to remove possible phthalate impurities. Technical Imidacloprid (purity > 96%) was received from Bharat Rashayan Chemicals, New Delhi, India.

**Imidacloprid stock solution**

A solution of imidacloprid 400 µg ml⁻¹ was prepared in HPLC grade water from technical imidacloprid which is stable for two weeks at 4°C in dark. Working standard solutions were prepared by appropriate dilutions.

**Sample**

HPLC-water sample were made alkaline by adding 0.4 g NaOH in 250 ml HPLC water and filtered through a cellulose acetate filter (Millipore sterile millex-GV, pore size 0.22 µm), collected in a cleaned glass volumetric flask and stored at 4°C.

**Standardized procedures for determination of imidacloprid in water extraction, cleanup and GC analysis**

Water sample 250 ml containing 5 and 20 µg ml⁻¹ of imidacloprid was stirred well with 0.4g NaOH. The mixture was heated in water bath at 85°C for 20 min, cooled at 4°C for 10 min and then neutralized (pH-7) with of 50% HCl solution. It was transferred to 1000 ml separating funnel and added 75 ml of dichloromethane (DCM) and shaken the mixture for 5 min, collected the organic phase. The extraction was repeated two times with 50 ml of dichloromethane. The extracts were pooled and passed through activated anhydrous sodium sulfate (Na₂SO₄) and concentrated near to dryness in a nitrogen atmosphere.

### AIMS AND OBJECTIVES

In the present study an attempt has been made to develop the method for analyzing imidacloprid using GC-ECD/NPD by converting it into a volatile derivative-imidacloprid-urea [1-(6-chloro-3-pyridylmethyl)-imidazolien-2-one] in water. (Fig. 1)
flash concentrator. Added 1.0 ml mixture of acetone: n-hexane (1:9 v/v) and again evaporated. Finally added 1.0 ml mixture of acetone: n-hexane (1:9 v/v) and collected in GC vial. 1.0 µl extract was injected in GC and register each chromatogram on the GC-ECD and GC-NPD. Calibration graphs were thus constructed using different known concentrations of Imidacloprid.

Analysis

**GC-ECD**

The final extracts were analyzed on (Perkin Elmer Clares-500) GC equipped with fused silica capillary column DB-1 (30 mt. × 0.25mm. id) coated with 1% phenyl-methylpolysiloxane (0.25 µm film thickness) using 63Ni electron-capture detector (ECD). General operating condition were as follows: Column temperature program: initially 170ºC for 5 min, increase at 4ºC/min to 240ºC hold for 15 min then 280ºC increase 7ºC/min hold for 37 min, injection volume: 1 µl, nitrogen flow rate 0.79 ml/min and makeup 30 ml/min with split ratio 1:10, using carrier gas (N$_2$) 99.5%, injector port temperature: 280ºC, detector temperature 300ºC.

**GC-NPD**

The final extracts was also analyzed on (Perkin Elmer Clarus-500) equipped with fused silica capillary column, DB-1 (30 mt. × 0.25 mm. id.) coated with 1% phenyl-methylpolysiloxane (0.25 µm film thickness) using Nitrogen Phosphorus Detector (NPD). General operating conditions were as follows, Injector port temperature: 250ºC, detector temperature 280ºC, using carrier gas nitrogen (N$_2$), flow 1.46 ml/min, hydrogen (H$_2$) makeup is 30 ml/min and zero air 60 ml/min, column temperature program: initially 95ºC for 4 min, increase at 2.5ºC/min to 170ºC hold for 7 min, then increase 225ºC/min hold for 10 min, injection volume: 1 µl, split ratio 1:5.

**Extraction and cleanup for HPLC**

The extractions were performed using liquid-liquid extraction in 1000 ml separating funnels for 250 ml water sample. Water samples containing the two spiking level of imidacloprid (50 and 100 µg L$^{-1}$) in triplicate was prepared. Each sample was extracted three times with subsequent volume of dichloromethane (75, 50 and 50 ml). The solvent fractions were combined and concentrated on turbovap LV and turbovap II nitrogen flash evaporator (40ºC) near to dryness. The residues were taken in 1 ml mixture of acetonitrile : water (20:80 v/v) for HPLC analysis. HPLC (Waters Milford, USA) system was equipped with waters 515 binary pump, diode array detector, temperature control module and on line degasser system. Analysis were performed on RP C-18 ODS (Octadeca Silaxane) column (250 mm X 4.5 mm I.D., 5 µm particle size) at 25ºC using mobile phase of acetonitrile-water (20:80, v/v) at a flow-rate of 1.5 ml/min. It was detected at 270 nm and 0.02 AUFS. Sample injection volume was 50 µl.

**GC-MS**

A Perkin Elmer GC-MS consisting of auto system XL Gas Chromatograph with a Turbo Mass Spectrometer was used for analysis. The column used is Elite-5MS fused-silica capillary column (30 m X 0.32 mm I.D., 0.25 mm film thickness). Carrier gas : helium (purity 99.999%) with a flow rate of 1.6 ml/min. A 1µl aliquot of the final extract was injected using the split less mode. The oven temperature program is 100ºC for 1 min and then at 20ºC min$^{-1}$ to 210ºC and hold for 1 min, then at 45ºC min$^{-1}$ to 300ºC and hold for 1 min. The total runtime of the GC is 19.5 min. 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) mode for analysis of hydrolyzed imidacloprid. The injector temperature was set at 300ºC.

The transfer line and source temperature was set at 280ºC and 230ºC respectively. Solvent delay for MS is 5 min.
RESULTS AND DISCUSSION

Validation and application of the method

Quality control

Each congener was identified by matching the retention time of the sample with standard. Procedural blank, consisting of all reagents and glassware’s used during the analysis were periodically determined to check the cross contamination. Since, no compound were detected that interfere with the sample values were not corrected for procedural blank. Recovery studies with fortified sample have indicated that overall recovery exceeded 75 to 100% (Table 1). For 250 ml\(^{-1}\) water samples the limit of detection (LOD) was about 0.02 µg/ L\(^{-1}\), whereas limit of quantification (LOQ) was about 0.08 to 0.1µg/L\(^{-1}\)(Table 1). In all cases, it is highly desirable to improve the accuracy and precision for imidaclopride-urea as the quantity increases in water samples.

Table 1: Calibration of imidacloprid-urea on GC-ECD/NPD in water

<table>
<thead>
<tr>
<th>Spiking level (µg/L(^{-1}))</th>
<th>Recovery % (±SD)</th>
<th>LOD (µg L(^{-1}))</th>
<th>LOQ (µg L(^{-1}))</th>
<th>RSD%</th>
<th>RT</th>
<th>(R^2)</th>
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<tr>
<td>ECD 0.1</td>
<td>80.12±0.002</td>
<td>0.03</td>
<td>0.08</td>
<td>4.5</td>
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<td>0.25</td>
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<td>0.5</td>
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<td>0.08</td>
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<td>95.08±0.012</td>
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<td>2.0</td>
<td>21.67</td>
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<tr>
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<td>0.04</td>
<td>0.10</td>
<td>1.5</td>
<td>21.67</td>
<td>0.999</td>
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<tr>
<td>5</td>
<td>97.70±0.010</td>
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<td>0.07</td>
<td>1.2</td>
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<td>0.10</td>
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<td>4.5</td>
<td>20.13</td>
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<td>0.09</td>
<td>4.2</td>
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<td>20.07</td>
<td>0.999</td>
</tr>
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</table>

LOD= Limit of Detection, LOQ = Limit of quantification, RSD= Relative standard deviation, RT= Real Time, \(R^2\) =Correlation coefficient.

Linearity

Imidacloprid-urea calibration curve is generated and the linear relationship is evaluated across the range of the expected water sample concentrations. Standard of imidacloprid fortified into control water are processed through the extraction procedure.

Linearity is obtained by a linear regression plot of known concentration vs. area response with minimum of 8 different concentrations of imidacloprid (Fig. 6(a) and Fig. 6(b)). The linear relationship is calculated by un weighted linear regression, but may be fit to a weighted linear regression with weighting factors of
1/concentration (1/X) or 1/concentration² (1/X²), if justified. Acceptability of the weighting factors is determined by evaluation of the imidacloprid-urea across three runs of the 8 different concentrations (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 µg L⁻¹). The criteria of recommended acceptance for a standard curve is dependent upon the of the standard curve format. Calibration of standard curves generated by fortification of control water samples and processed through the procedure are subject to the same acceptance criteria as the samples. Calibration standard curves (r² = 0.978 for ECD and r² = 0.986 for NPD) generated by standards in solvent by fortification of control water.

Accuracy (recovery)

Accuracy refers to the closeness of agreement between the true value of the imidacloprid-urea concentration and the mean results that is obtained by applying the experimental procedure (Table 1). It is closely related to systematic error (analytical method bias) and percent recovery of imidacloprid-urea (Table 1). Accuracy of the imidacloprid-urea residue varies depending upon its concentration.

Precision (repeatability)

Precision of a validated method is the closeness of agreement between independent test results obtained from spiking water test sample under stipulated conditions of use. In this study repeatability difference is less than 5%. However, recovery variability in different concentration of imidacloprid-urea (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 µg L⁻¹) in water samples ranges from 75 to 100% and RSD is less than 5% (Table 1). Hence, the determination of the intra and inter-day precision of the analytical method as part of the validation procedure was also carried out. Instead of establishing reproducibility of the assay an inter-day precision can be determined. Inter-day precision can also be referred to as between-day precision whereas repeatability is defined as within-day (intra-day) precision. Intra and inter-day precision were determined by the evaluation of a minimum of three replicates at eight different concentrations which is represented the intended validation range across three days of analysis. Acceptable variability is dependent upon the concentration of the imidacloprid-urea for the purpose of the residue method validation.

Limit of detection

The Limit of Detection (LOD) is the smallest measured concentration of an imidacloprid-urea from which it is possible to deduce the presence of the imidacloprid-urea in the test sample with acceptable certainty. The LOD for imidacloprid-urea in the present study was calculated as 0.02 µg L⁻¹ (Table 1).

Limit of quantification

The Limit of Quantification (LOQ) is the smallest measured content of imidacloprid-urea above which the determination can be made with the specified degree of accuracy and precision. The LOQ for imidacloprid-urea in the present study was calculated as 0.08 µg L⁻¹ (Table 1).

The results of HPLC analysis of parent compound-imidacloprid and its hydrolyzed product- imidacloprid-urea have been shown in Fig. 2 and Fig. 3 respectively.

The GC analysis of imidacloprid in term of imidacloprid-urea has been shown in chromatograms on GC-ECD and GC-NPD in Fig. 4(a) and Fig. 4(b). The volatile Imidacloprid-urea[1-(6-chloro-3Pyridyl methyl)-Imidazolien-2-one] was further confirmed by GC-MS. (Fig. 5). Its suitable volatility and thermal stability allows us to expect its usefulness in GC. The hydrolysis rate of imidacloprid in alkaline medium is temperature dependent and low at ambient temperature, therefore it was heated at 85°C for 20 min. Addition of 0.4g NaOH was found an adequate amount for the conversion of 200 µg imidacloprid into equal amount of its derivative imidacloprid-urea. The GC chromatographic peak areas of imidacloprid-urea remain constant for at least 24 hrs.
Fig. 2: HPLC chromatogram of imidacloprid

Fig. 3: HPLC chromatogram of hydrolysed product-imidacloprid-urea

Fig. 4(a): GC-ECD chromatogram of imidacloprid-urea

Fig. 4(b): NPD chromatogram of imidacloprid-urea
The calibration graph for the samples treated according to the procedure described above as shown in (Table 1 and Fig. 6(a) and Fig. 6(b)). GC-ECD/NPD peak area of imidaclopride-urea was linear for the concentration range 0.1-20 μg L\(^{-1}\). To check the linearity of the calibration graph, applied for two replicates and three injections of each spiking concentration shows the results for the correlation coefficients range is \(R^2\) ECD(0.9780.999) and NPD(0.986-0.999). The data yield a good linearity within the range 0.1-20 μg L\(^{-1}\) (Fig. 6(a) and 6(b)). Imidacloprid can be analyzed on both the detector. It is detected on ECD due to the presence of chlorine ion (Cl). However detection of imidaclopride-urea on NPD is due to presence of four nitrogen in the imidazoladin ring. Though ECD appears to be more sensitive than NPD but unwanted peak are more on ECD. However NPD there is a single sharp peak. Limit of detection (LOD) and limit of quantification (LOQ) on GC-ECD and GC-NPD was worked out and calculated LOD through this method on both detector is 0.02 μg ml\(^{-1}\) which is much lower than the LOD (0.16 μg ml\(^{-1}\)) on HPLC.

Validation of this method for water samples was carried out by using the standard addition methodology.\(^{15-16}\) Three experiments are required to obtain set data necessary for statistical calculation. The validation for water sample was carried out by using a recovery test through a series of different amount of water samples spiking with different level of imidacloprid (Table 1). Standard addition calibration (AC) obtained by addition of standard...
imidacloprid to spiked 250ml water (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 μg L⁻¹) By applying linear regression analysis, the slope, the intercept and the R.S.D. for each curve of samples were estimated.

CONCLUSION

Hydrolysis of imidacloprid-[1-(6-chloro-3-pyridylmethyl)-N-nitroimida-zolidin-2-ylidene amine] occurs in alkaline medium. Under alkaline condition hydrolysis of imidacloprid is highly dependent on pH and temperature. High pH values and temperatures increase of the rate of hydrolysis.

The hydrolysis of imidacloprid yield the main product: imidacloprid-urea,[1-(6-chloro-3pyridylmethyl)-imidazolien-2-one] which is highly volatile and could be analyzed on GC-ECD/NPD using this reaction. A quick, simple and sensitive GC-ECD/NPD method for the determination of imidacloprid is developed in water samples with good percentage recovery (75-100%) in all cases. The limit of detection was 0.02 μg L⁻¹.

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Save the nature