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# Determination of imidacloprid residues on tomatoes by High-Performance Liquid Chromatography.

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**Abstract**

A simple method for the determination of imidacloprid residues in tomatoes, grown in greenhouses, has been developed. Two procedures for extraction (acetone/ethyl acetate; acetonitrile/methanol) of the analyte from the sample matrix are suggested. Glass wool and Florisil column chromatography were used for purification of sample solution. The technique used for detection was Liquid Chromatography equipped with UV detector. LCMS was used as a confirmatory method. The recoveries ranged from 92.4 - 98.8 % for acetone/ethyl acetate extraction and from 97.5- 99.1% for acetonitrile/methanol extraction. Tomatoes treated with imidacloprid using commercial insecticide formulation - Confidor were analyzed using both procedures. There are differences between the test results obtained by the two procedures at 5% significance level. The acetonitrile/methanol extraction is recommended for use at determination of imidacloprid in tomatoes.

**Key words:** HPLC analysis, Imidacloprid, Tomato, Pesticide Residues.

## 1. Introduction

Imidacloprid is a neonicotinoid insecticide in the chloronicotinylnitroguanidine chemical family. The International Union of Pure and Applied Chemistry (IUPAC) name is 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine and the Chemical Abstracts Service (CAS) registry number is 138261-41-3. According to technical datasheet;Imidacloprid is used to control sucking insects, some chewing insects including termites, soil insects, and fleas on pets. In addition to its topical use on pets, imidacloprid may be applied to structures, crops, soil, and as a seed treatment[4].

Imidacloprid applied to soil is taken up by plant roots and translocated throughout the plant tissues [10]. Researchers grew tomato plants in soil treated with 0.333 mg active ingredient per test plot, and monitored the plants and fruits for 75 days. Plants absorbed a total of 7.9% of the imidacloprid over the course of the experiment, although absorption of imidacloprid declined with time since application. [2]

More than 85% of the imidacloprid taken up by the tomato plants was translocated to the shoots, and only small quantities were found in the roots. Shoot concentrations declined towards the top of the plant. The tomato fruits also contained imidacloprid, although tissue concentrations were not related to the position of the fruits on the plant. Although tomato fruits contained primarily unmetabolizedimidacloprid, the plants' leaves also included small quantities of the guanidine metabolite, a tentatively identified olefin metabolite, and an unidentified polar metabolite in addition to the parent compound [2].

### *1.1 Mode of action as insecticides*

Both nicotine and neonicotinoid insecticides bind to nicotinic acetylcholine receptors (nAChRs) and mimic the action of acetylcholine by opening the ion channels which allow the entry of $Na^+$ and $Ca^{2+}$ into cells. These compounds vary in their affinity for different nAChR subtypes, with nicotine showing selective toxicity for vertebrates whereas neonicotinoids are highly selective for insect nAChRs. The binding of neonicotinoids to insect nAChRs is virtually irreversible[4].

### *1.2 Metabolism*

Mammals metabolize imidacloprid in two major pathways discussed below. Metabolism occurs primarily in the liver [3].In the first pathway, imidacloprid may be broken by oxidative cleavage to 6-chloronicotinic acid and imidazolidine. Imidazolidine is excreted in the urine, and 6-chloronicotinic acid undergoes further metabolism via glutathione conju-

gation to form mercaptonicotinic acid and a hippuric acid [3,4].Imidacloprid may also be metabolized by hydroxylation of the imidazolidine ring in the *second major pathway* [3,4]. Metabolic products from the second pathway include 5-hydroxy and olefin derivatives [5].

*1.3 Food residue*

Methods for pesticide residue determinations for the qualification of food products are under rapid development. The residue limits given by the World Health Organization are becoming even lower creating an ever-increasing demand for more selective and sensitive methods. As the concentration of the pesticide residues in food material is in the nanogram, picogram or sometimes femtogram per gram range, their evaluation can be carried out only by extremely selective and sensitive detection methods[17].

The method for the determination of pesticide residues in food materials consists of two steps: sample preparation and analysis. Chromatography can be used as a preliminary concentrating procedure, which can be continued with column chromatography as a part of the clean up. Moreover, chromatography also plays a very important role in the determination step [16]. We have established a method for single residue analysis of imidacloprid in tomatoes, by High-Performance Liquid Chromatography with UV detection.

## 2. Material and methods

*2.1 Reagents and Aparatus*

*Pesticide standard***.**Pesticide standard reference material was purchased from Fluka analytical. The purity of imidacloprid was more than 95 % by liquid chromatography (LC).

*Pesticide working solutions.*Pesticide standard solutions (1000 µg/ml) were prepared by dissolving the pesticide standard in methanol HPLC grade and diluting to suitable concentrations with the same solvent.

*Organic solvents and reagents.*Acetone, ethyl acetate, acetonitrile, methanol were of special grade forpesticide residue analysis. Anhydrous sodium sulfate ($Na_2S0_4$) was ofanalytical grade. These reagents were used without prewashing. Florisil (60-100 mesh) was obtained from Varian, U.S.A. Florisil and anhydrous $Na_2S0_4$ were heated overnight at 130°C and desiccated before use.

*Samples.*Tomatoes were obtained from greenhouses, treated with commercial insecticide formulation – Confidor 200 SC.

*ApparatusLiquid chromatograph*Shimadzu equipped with an UV detector was used fordetermination ofimidacloprid. Shim-pack VP-ODS (250mm.×4.6 mm i.d.) columns were used for pesticide content determination.

*Chromatographic tube for column chromatography.*A glass column of40 cm x 22 mm i.d. wasused in Florisil column chromatographyforpurification ofsample solution.

*Rotary evaporator* Equipped withwater bath and vacuum pump were used to concentrate the organic solvents. A water bath was set at 35-40 $^0$C.

*2.2 Extraction*

*2.2.1.Acetone/ethyl acetate extraction.*

Approximately 1000 g ofwhole tomatoes were mechanically minced to provide a homogeneous tomato mix, from which sub-samples were taken. A subsample of10 g was placed in a blender cup. One hundred milliliters ofan Acetone/ethyl acetatemixture (1: 1 v/v) was added. The homogenate was filtered through No. 5A filter paper, and the residue was re homogenized with 100 ml ofthe same mixture and then filtered again. The filtrate was combined, than was transferred completely to a Florisil column along with 10 g florisil and 20 g of anhydrous $Na_2SO_4$Imidacloprid was eluted with 100 ml of this mixture. The eluate was concentrated using a rotary evaporator. The residue was re dissolved in acetonitrile and made up to final volume of 3 ml (the sample solution).

*2.2.2 Acetonitrile/methanol extraction*

A subsample of 10 g oftomatoes was transferred to a blender cup, to which 60 ml ofAcetonitrile/methanolhad been added. The mixture was filtered through No. 5A filter paper, and the residue was re homogenized with the same amount of Acetonitrile /methanoland then filtered again. The filtrate was combined, than was transferredcompletely to a Florisil column along with 10 g of florisiland 20 g of anhydrous $Na_2S0_4$.Imidacloprid was eluted with 100 ml of this mixture. The eluate was concentrated using a rotary evaporator. The residue was re dissolved in acetonitrile and made up to final volume of 3 ml (the sample solution).

*2.3 Determination of pesticides.*

Test solution prepared was subjected to HPLC for imidacloprid content determination under the following conditions:

**Table 1**.HPLC conditions

| | |
|---|---|
| *Column:* | *Shim-pack VP-ODS (250mmL.×4.6mm i.d.)* |
| Flow Rate: | 0.8 mL/min |
| Detection; | SPD-10AVVP at 252nm |
| Mobile Phase: | Water/Acetonitrile 10/90(v/v) |
| Temperature: | 25˚C |

## 3. Results and discussion

*3.1 Extraction*

Basically, most procedures for the determination of pesticide residues could be subdivided into three steps;extraction, clean-up and instrumental determination. With a view to improving efficiency, improvements were made in these three aspects as to achieve a simple and rapid determination of imidacloprid residues in tomatoes.

Polar solvents, such as acetone or methanol, are most commonly used to extract pesticide residues from food samples. As these solvents are miscible with water, they can penetrate the food matrices more effectively [6]. In this study, an *Acetone/ethyl acetate* mixture (extraction 1) and *Acetonitrile/methanol*(extraction 2) was directly employed in the extraction of the samples [17]. Thus, an additional solvent partitioning step was not necessary. These solvents were chosen owing to their proven applicability in the determination of organophosphorus insecticides [8]. To enhance the extraction efficiency, a sufficient amount of anhydrous sodium sulfate was added to absorb the water originally present in the sample [10]. After filtration, a measured volume of the extract was taken out, for column chromatographic clean up and concentrated [14].

*3.2 Clean-up*

Vegetables contain a wide variety of compounds that are extractable and may give rise to interferences or other problems. More insidious effects arise when co-extractives cause adsorption or decomposition of pesticides [11]. Lipids, chlorophyll, or other high molecular materials accumulating in the injection region of gas chromatographs are particularly problematic. Vegetables are generally low in lipids [12], but can have relatively high levels of pigments and phenols. Thus, *clean up* by Florisil column chromatography was necessary in the quantitation of pesticides [13].

*3.3 Chromatographic determination*

Gas and liquid chromatography remains the basis for determination of residues in most single or multiresidue methods. According to the applied program, the retention time of imidacloprid was 1.89 minutes. Figure 1, shows the standard solution chromatogram of imidacloprid (0.5 µg/ml) using the Shim-pack VP-ODS (250mm×4.6mmi.d.)column.

As indicated by the high correlation coefficient, responses is linear within the concentration ranges

The identity of the peak was confirmed by LC-MS (Fig. 4). The electron ionization mass spectrum (70 eV) of isolated imidacloprid was compared with its mass spectrum from the NIST Library (Fig. 5).

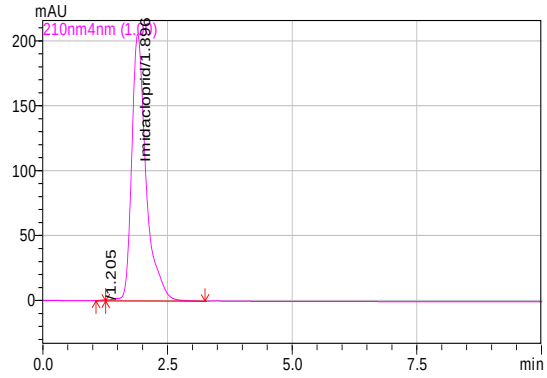


**Figure 1.**Chromatogram of the standard solution *of* Imidacloprid*(0.5* µg $ml^{-1}$) in methanol.Rtin proposed conditionsis 1.89 min.

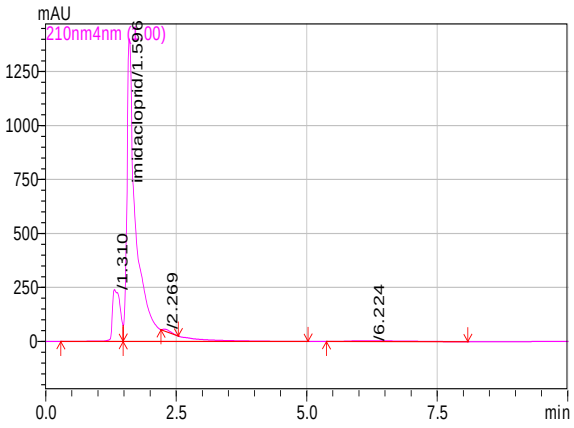


**Figure 2.**Chromatogram *of* the test solution *of* tomato in methanol after extraction with Acetonitrile /methanol

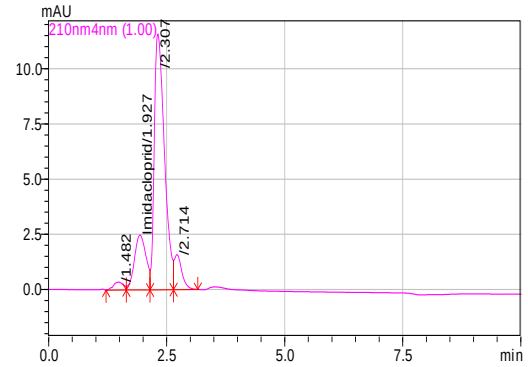


**Figure 3.**Chromatogram *of* the test solution *of* tomato in methanol after extraction with acetone /ethyl acetate. Method specificity is lower then the Acetonitrile/methanol extraction.

**Table 2. Linear regression output for the calibration curves of Imidacloprid.**

| *Insecticide* | *Concentration range* $\mu g\ ml^{-1}$ | *Correlation coefficient* (r) | *Slope* ($\mu g\ ml^{-1}$ *per unit area)* | *Intercept(unit area)* |
|---|---|---|---|---|
| Imidacloprid | 0.05 - 0.5 | 0.9996 | 2194.1 | 11.281 |

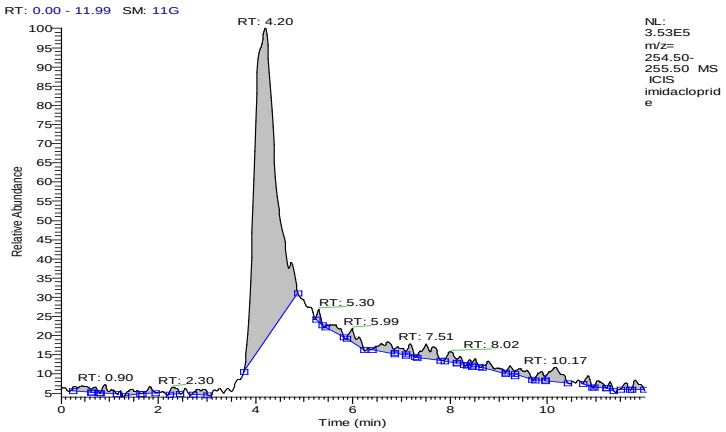


**Figure 4**. LC-MS Chromatogram of isolated imidaclopridfrom tomato

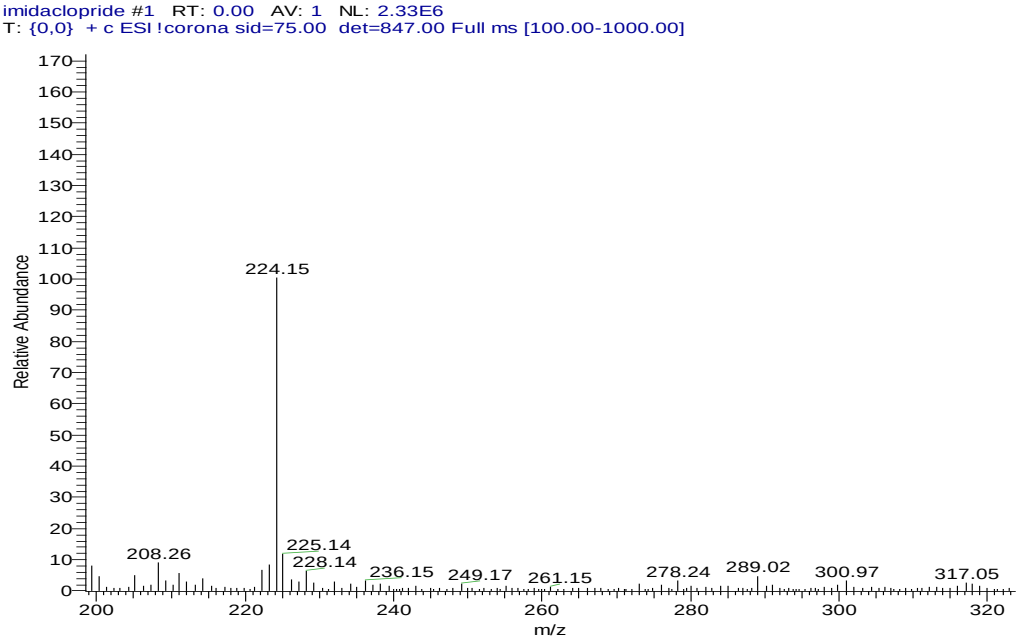


**Figure 5.** LC-MS mass spectrum of isolated imidacloprid from tomato.

For the estimation of detection limits, sample extracts were spiked with imidacloprid at low concentration levels so that they gave rise to marginally identifiable peaks in the chromatogram [14,15]. Chromatographic determination was then repeated 10 times [19]. The standard deviation (s) of the determination was calculated. The detection limit was defined as 3 s after taking into account the concentration factor achieved in the sample preparation steps[18]. The detection limit for the imidacloprid achieved by the present method is 0.012 mg/kg. This value is well below the maximum residue levels published by EU pesticide database (0.5 mg $kg^{-1}$) [20]. In the recovery studies, solutions of imidacloprid standard were spiked to comminuted tomatoes, which were previously tested to be free of this insecticide. The spiked concentrations imidacloprid were 0.1 mg /kg, 0.2 mg /kg and 0.3 mg /kg [18]. The spiked samples were processed according to the two newly developed procedures and the results are summarized in Table 3. Recoveries ofimidacloprid were 92.4 - 98.8 % (with acetone/ethyl acetate) and 97.5 - 99.1 % (with acetonitrile/methanol)

**Table 3.** Recovery of imidacloprid from spiked tomatoes

| *No.* | *Added (µg g-1)* | *Calculated (µg g-1)* | *Determined (µg g-1)* | *R (%)* |
|---|---|---|---|---|
| | Acetonitrile/methanol extraction | | | |
| 1 | - | - | 0.143 | - |
| 2 | 0.1 | 0.243 | 0.237 | 97.5 |
| 3 | 0.2 | 0.343 | 0.340 | 99.1 |
| 4 | 0.3 | 0.443 | 0.439 | 99.1 |
| | Acetone/ethyl acetate extraction | | | |
| 1 | - | - | 0.192 | |
| 2 | 0.1 | 0.292 | 0.270 | 92.4 |
| 3 | 0.2 | 0.392 | 0.386 | 98.5 |
| 4 | 0.3 | 0.492 | 0.486 | 98.8 |

**Table 4.**Results of analysis of tomatoes treated with Confidor 20 EC.

| | ***Concentration*** | | |
|---|---|---|---|
| | Extraction 1* | Extraction 2** | ***t*** |
| ***Imidacloprid*** | 0.143 | 0.192 | 40.12 |

*Extraction 1: Acetone/ethyl acetate extraction
**Extraction 2: Acetonitrile/methanol extraction,
n=5

These two procedures ofextraction were also verified by the analysis oftomatoes, grown in greenhouses, treated with imidacloprid using commercial formulation – Confidor 20 EC. These treated tomatoes were analyzedusing both procedures. The results obtained are summarized in Table 4. A *t*-test was performed [21]. There are differences between the test results obtained by the two procedures at 5 % significance level.

## 4. Conclusions

A simple and rapid analytical procedure for the determination of *imidacloprid* in tomatoes was developed. The *acetonitrile/methanol* extraction gave the better recoveries of imidacloprid then the acetone/ethyl acetate extraction, it is recommended for use at determination of imidacloprid in tomatoes.

The speed, accuracy and sensitivity of this method are its advantages, which couldwarrant its introduction for routine residue analysis of *imidacloprid* in tomatoes. With minor adaptationsto the extraction procedure, *imidacloprid* could also be determinate in a wide range of other produce.

## 5. References

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