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BRIEF REPORT: Extraction of Imidacloprid from Human Biological Samples Using the QuEChERS Method and Its Determination by High-Performance Liquid Chromatography

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ABSTRACT

A Simple and fast QuEChERS method has been applied for extraction of imidacloprid from human biological samples. Imidacloprid the was extracted from blood and liver of a deceased body admitted to the legal Medicine of Mazandaran. Factors affecting the extraction procedure such as type of the organic solvent and its volume, amount of salt, amount of sorbent, and pH were examined and optimized for the maximum recovery. The appropriate condition for extraction of imidacloprid was as follows: sample pH=5, 3mL of acetonitrile, 0.1 g of NaCl, and 0.4 g of MgSO4. The maximum recovery of imidacloprid at these conditions was 96%. The LOD and LOQ were 0.02 and 0.06 ppm, respectively. Finally, the blood and liver samples were extracted under the appropriate condition and determined using HPLC which is equipped with a photodiode array detector. The amounts of Imidacloprid in blood and liver samples were 11 and 1.8 ppm, respectively.

Introduction

Because of the vast use of pesticides in agricultural activities human poisoning with these toxic compounds is investable. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] belongs to a relatively new class of neuroactive neonicotinoids that are synthetic nicotine derivatives (***Figure 1***). Most neonicotinoids are much less toxic for mammals than insects and thus, they have almost replaced other organophosphate insecticides and more toxic carbamates. Within a decade, such pesticides have gained a 77% share of the market (1). Nowadays, in order to extract the toxins and the residue analysis of the pesticides, a procedure is known as QuEChERS is utilized for extraction as a general method. The method

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was primarily developed by Anastassiades et al. in 2003 for analyzing the residue of several pesticides. To survey the number of pesticides in biological samples, the studies indicate that blood is the highest applied sample which is analyzed and evaluated and most of the reports about the therapeutic concentration and toxic concentration of drugs have been obtained by analyzing blood samples (2, 3). In case of lack of blood sample, usually, liver is picked for analysis and among the reported results, the maximum data are related to the liver for comparing the concentration of drugs (3). This method would be useful for the analysis of pesticides in a small volume of clinical or forensic samples to support toxicological applications (4). in the forensic field, one research based on the QuEChERS method has been reported by Plössl et al (5).

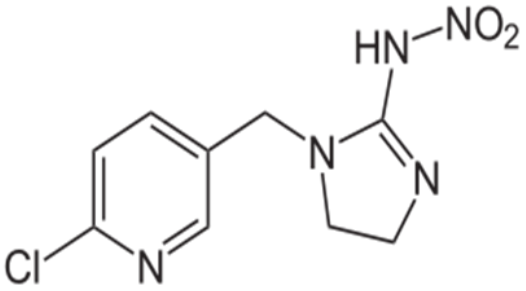


Figure 1. Imidacloprid Molecular Structure

Methods

Materials

Imidacloprid of purity 98%, dehydrated magnesium sulfate, and sodium chloride were Purchased from (Sigma-Aldrich, USA), acetonitrile, water, and methanol of chromatographic degree were prepared from Merck (Germany based Merck), The HPLC system was Knaver equipped with a PDA detector.

Chromatographic conditions

All HPLC analysis was carried out using an HPLC system equipped with a PDA detector and 7125i manual injector with a 20 _L sample loop (Knaver, GmbH, Germany). Chromatographic separation was performed using the C_{18} Column (250 mm ×4.6 mm, 5 _m particle size) from Waters (Milford, MA, USA). The mobile phase used for the determination of Imidacloprid was of phosphate buffer with pH=2.3 and acetonitrile in the ratio (70: 30 v/v) with a flow rate of 1.0 mL/min at the ambient temperature.

Extraction of standard solutions

The standard solution of Imidclopride was prepared in acetate buffer with pH=5. 3 ml of acetonitrile as an organic solvent, 0.4 g of $MgSO_4$ adsorbent salt, and 0.1 g of NaCl were added to the vials containing Imidacloprid standard. Finally, the vials underwent vortex at 1000 rpm and were centrifuged for 3 min at 3000 rpm. After removing the tubes, the supernatant or acetonitrile was separated and 20 µl of it was injected into the HPLC (***Figure 2A***).

Blood and liver sampling

The blood and liver samples were obtained from a deceased body admitted to the Legal Medicine of Mazandaran. The body was found hanging in his own house while the residues of an unknown white powder were observed around his mouth.

Extraction procedure

One mL of blood sample and 2 ml of the homogenized liver tissue were added to the tube and then 2.5 ml of acetate buffer with pH=5, 3 ml of acetonitrile, 0.4 g of $MgSO_4$, 0.1 g of NaCl were weighed, and added to each tube. After vortex for a minute, the mixture was centrifuged at 3000 rpm for 3 minutes, the supernatant was collected, filtered, and injected into the HPLC system.

Results and Discussion

Selection of organic extraction solvent

In order to select a suitable solvent for extraction, acetonitrile and acetone were compared. Results showed that 81% and 96% of imidacloprid recovery were obtained using acetone and acetonitrile respectively. Moreover, since the polarity of acetonitrile was more than acetone, the recovery percentage of the pesticides with moderate to

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high polarity will be higher with acetonitrile (6). the results showed that the maximum

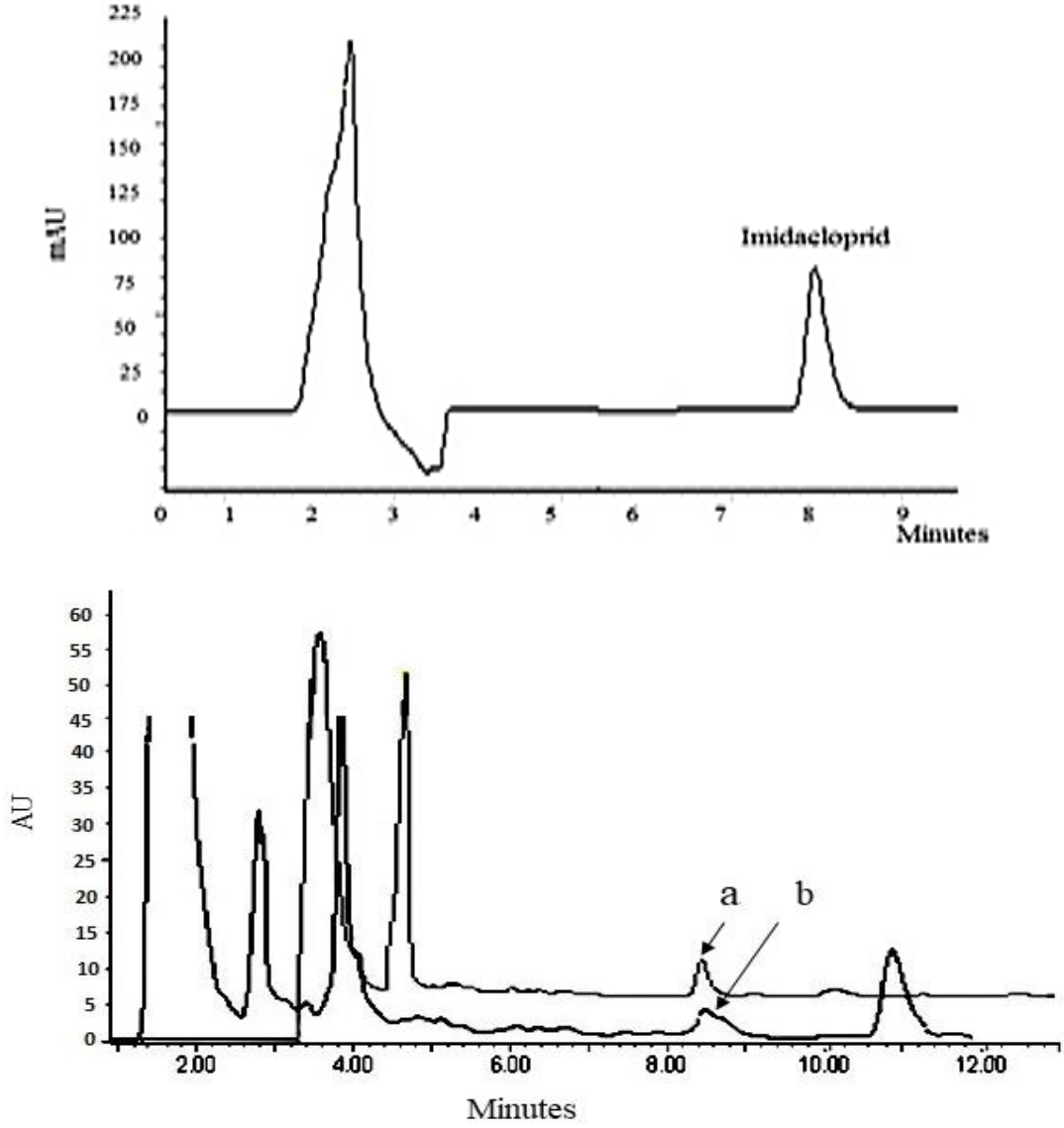


Figure 2. Chromatograms of Imidacloprid standard (A) blood (a) and liver (b) samples (B)

recovery was obtained using the 3 ml of acetonitrile.

Effect of pH

To evaluate the pH-induced effect on Imidacloprid extraction, the pH in the range of 2-7 was analyzed. According to the results, pH=5 provided the highest recovery (96%) and it was selected as the appropriate pH for the acceptor phase.

Effect of sorbent addition

In order to find the appropriate amount of sorbent different amounts of $MgSO_4$ were examined (0.1, 0.2, 0.3, and 0.4) and the results showed that the maximum recovery was obtained using the 0.4 g of $MgSO_4$.

Effect of salt addition

The effect of salt addition on the recovery of Imidacloprid extraction was evaluated by the addition of NaCl from 0.0 to 0.3 g in a sample solution. The highest recovery (96%) was obtained for 0.1 g NaCl.

Determination of Imidacloprid pesticide in the blood and liver tissue sample

In order to assess the applicability of the proposed extraction method for quantification of Imidacloprid in real samples

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the blood and liver tissue samples, were extracted and analyzed using this method under the optimum conditions. Determination of imidacloprid was performed using the standard addition method and its concentration in blood and liver samples were 11 and 1.8 ppm, respectively (***Figure 2B***).

Method Validation

The figures of merit including; linearity, the limit of detection (LOD), repeatability, reproducibility, Limit of Quantification (LOQ), Relative Standard Deviation (% RSD), and recovery were obtained to evaluate the practical applicability of the proposed QuEChERS technique. The linearity in the samples was verified at concentrations ranging from 0.5 to 65 µg/mL by analyzing each concentration in triplicate. The analyte exhibited good linearity with the correlation of determination of $R2 > 0.9931$, in the studied range. Based on the signal-to-noise ratio of 3 (S/N = 3), LOD of 0.02 µg/mL was obtained and, in order to determine Sb, the mobile phase was passed through the chromatographic column for 20 min and the peak height was recorded. Under the optimum conditions, the Limit of Quantification (LOQ) and the extraction recovery (R%) was 0.06 µg/mL and 96 % respectively. To find out the intraday and interday RSD, 3 Imidacloprid standard solutions at concentrations 0.5, 20, and 55 µg/ml were prepared (3 separate solutions from each concentration) and each of the solutions was injected into HPLC during 3 consecutive days. The repeatability (intra-day) and reproducibility (inter-day) were obtained as RSD = 1.4-1.6% and 1.5-6.5% respectively.

Conclusion

The QuEChERS method reported in this work proved to be simple, easy, fast with a little use of an organic solvent to extract imidacloprid from biological samples. material consumption and the need for the cleaning step are reduced due to the presence of less disturbing compounds in the acetonitrile phase. This method presented a high recovery percentage and results in efficient extraction of the sample. HPLC offers a large linear range and low limit of detection (0.02 µg/mL). Overall, the advantages of the method allow its potential application for imidacloprid analysis at low levels from the blood and liver tissue sample.

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Ethical standards statement

This article was approved by the research ethics committee of Mazandaran University of Medical Sciences with the code number IR.MAZUMS.REC.1398.262.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

M. Shokrzadeh designed, interpreting the results and supervised the work. Z. Karimi contributed to the data collection, writing the first draft of proposal and the article. V. Sharifi was the advisor of the article. A. Abbasi contributed to the data analysis.

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