ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2017, *62*(6-7), 525-530

COMPARATIVE METHODS FOR MONITORING OF IMIDACLOPRID RESIDUES IN MAIZE AND WHEAT CULTIVATED IN BANAT COUNTY

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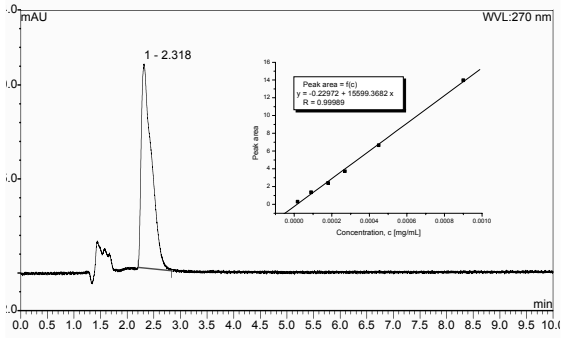
Received October 28, 2016

The aim of this paper is the detection of Imidacloprid in maize and wheat cultivated in Banat County. Extraction was performed by ultrasonic technique using acetonitrile and methanol as solvents, at 59 kHz, 30±2°C, during 30 minutes. Three highly sensitive and rapid methods: gas chromatography-mass spectrometry (GC-MS), high performance chromatography (HPLC-PAD) and cyclic voltammetry were used for quantification of insecticide residues. The limit of imidacloprid detection (LOD) was found to be 4.5 mg · L^{-1} in GC-MS method, 0.009 mg · L^{-1} in HPLC method and 0.45 mg · L^{-1} in cyclic voltammetry. The developed methods were sensitive, quick and easy to use.



INTRODUCTION

There are more than one thousand pesticides in worldwide used to protect cereal crops, in different combinations and at different stages of plants growth, but pollution due to the uncontrolled use of these compounds triggered an alarm signal for health.[1,2] Nowadays, the chain: soil-plants-pesticides-environment-human and animals health is very strictly controlled.[1-3] Productivity in Banat, Cărpiniş - Jimbolia farming zone has been greatly enhanced by expanding cultivation areas and developing technical works, use of the best seeds and better water management, and also by the efficient administration of pesticides. Use of organo-phosphoric and organo-chlorine pesticides (OPs, OCls) for pest control in agriculture has caused serious environmental problems worldwide.[1, 3-7] OPs and OCls are highly toxic, with many opportunities to cause neurological disorders in humans because of the many ways to enter into human body: drinking water, farm and sea animals, crops, soil and surface water, air. Following the increasing application of these compounds in various agricultural activities, it has become imperative to accurately monitor the concentration levels for the protection of ecological systems and food supplies. Although, there are many conventional methods available for detecting pesticides, the trend is to develop portable sensors to facilitate the routine analyses with several advantages. These techniques are based on powerful alternative functional materials, including sensors based on nano-materials (graphene, gold nanoparticles, nanocomposites with carbon nanotubes coated with Fe_3O_4, etc.), fluorescent sensors, printed molecular sensors (MIP), and very performing electrochemical sensors and biosensors.[5] Imidacloprid is a systemic chloronicotinyl insecticide with soil, seed and foliar

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uses, which causes blockage of a type of nicotinergic neuronal receptors, which are more abundant in insects than in warm-blooded animals. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventual death. Imidacloprid is used to control the sucking insects such as rice hoppers, aphids, ticks, white flies, termites, and turf insects. The aim of this research was to improve some highly sensitive and rapid methods: GC-MS, HPLC-PAD and cyclic voltammetry, in order to monitor imidacloprid content in crops at farmers or intermediate buyer demand.

RESULTS

Maize and wheat cultivated in Carpinis-Jimbolia zone, in 2015, and stored in silos were analyzed. Ultrasonic extracts in acetonitrile and methanol of maize and wheat were analyzed using three rapid methods: GC-MS, HPLC and cyclic voltammetry.[8-11] The GC-MS spectra, HPLC-PAD spectra and cyclic voltammograms for maize and wheat were presented in Figure 1, 2 and 3, respectively. Time of analyses varied between 2 and 20 minutes. The best results for identification and quantification of imidacloprid were obtained in the case of HPLC-PAD method.

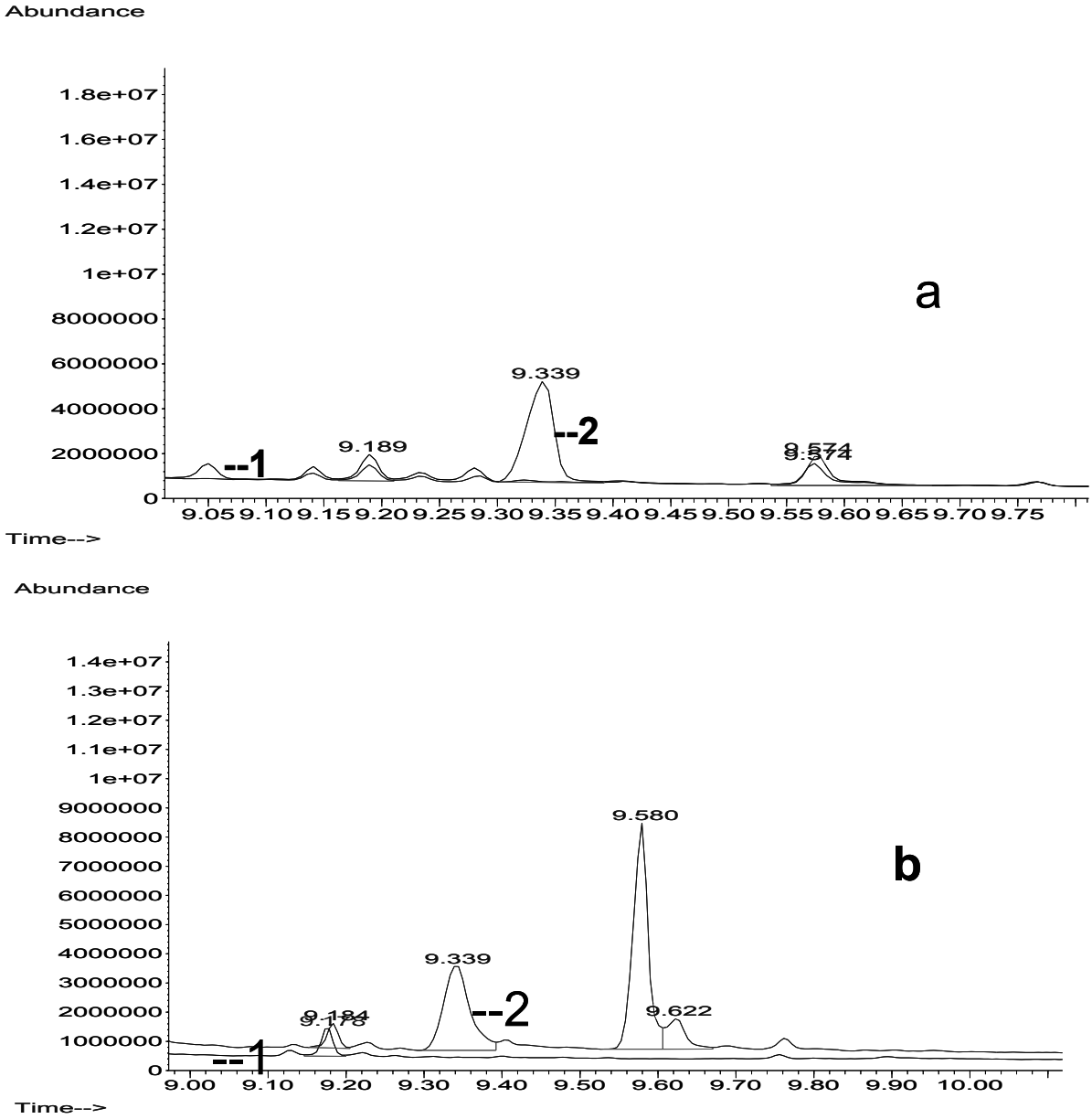

Fig. 1 – GC-MS spectra (fragment 9÷10 min.) of: a. maize extract in MeOH (1); maize extract contaminated with imidacloprid (2); b. wheat extract in MeOH (1); wheat extract contaminated with imidacloprid (2).

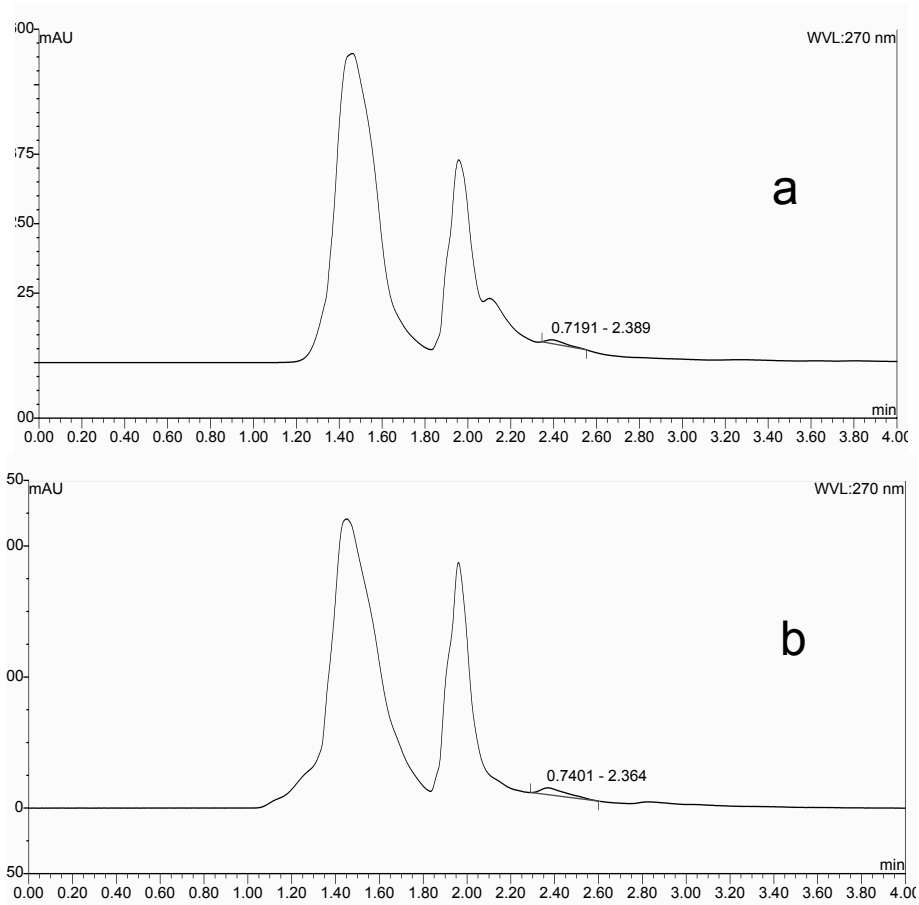

Fig. 2 – HPLC-PAD spectra of: a. methanolic maize extract and b. methanolic wheat extract.

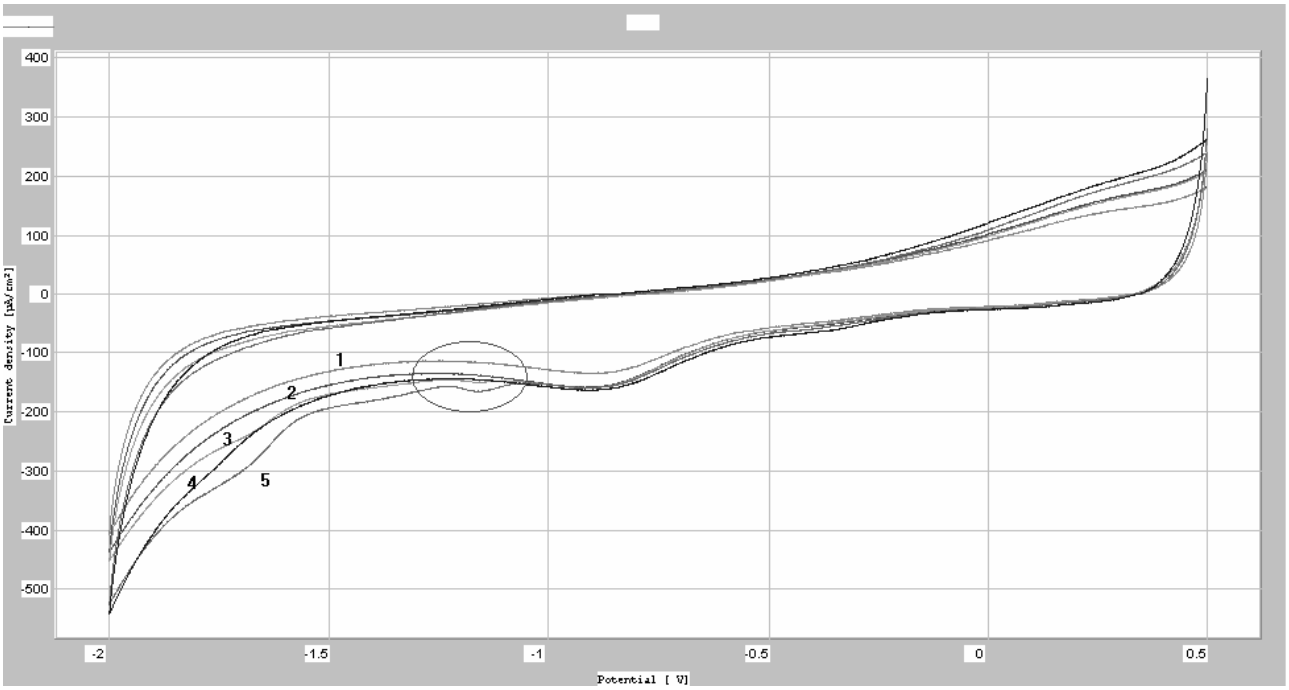

Fig. 3 – Cyclic voltammograms of natural and contaminated maize and wheat extracts in methanol: 1 - Support electrolyte Britton - Robinson buffer solution (pH=7); 2 - natural wheat extract in buffer solution; 3 - wheat extract in buffer solution contaminated with 9 µg imidacloprid; 4 - natural maize extract in buffer solution; 5 - maize extract in buffer solution contaminated with 9 µg imidacloprid.

The GC-MS method did not allow to detect small quantities of contaminant in samples.[9] We deliberately contaminated the extracts in order to show where imidacloprid (t_R = 9.339 min.) will be on chromatograms (Figure 1 a, b).

Both maize and wheat extract presented a characteristic peak for imidacloprid. For quantification, the first calibration curve was used. In maize, peak area was 0.7191 and this value corresponded to 0.0608 mg·L^{-1} imidacloprid (0.122 µg/g maize or 0.122ppm). In wheat, peak area was 0.7401 and this value corresponded to 0.0622 mg·L^{-1} imidacloprid (0.124 µg/g wheat or 0.124ppm). These values were slightly higher than the admissible limits of imidacloprid in crops. The European Directive 491/2014 established the maximum residues limits for imidacloprid, MRLs: 0.1 mg/kg in maize and 0.1 mg/kg in wheat.[12]

The voltammograms for ultrasonic methanolic extract of maize and wheat were presented in Figure 3 and did not show the presence of imidacloprid residues. This method was not enough sensitive. We deliberately contaminated the samples with 9 µg insecticide and the curves 3 and 5 showed the characteristic peak at (-1200) mV (vs. SCE) for imidacloprid.

DISCUSSION

Imidacloprid standard was dissolved in 10 mL mixture water: acetonitrile (6:4 v/v) to obtain the stock solution. Stock solution was kept at 4°C in dark. Calibration curves were registered for each method. Results were processed with OriginPro 6 software. GC-MS was the longer method, retention time for imidacloprid was t_R = 9.3 min. and work conditions permitted only a detection limit LOD = 4.5 mg·L^{-1} (Figure 4). Calibration curve was realised in range of 4.5 – 13.5 mg·L^{-1} (R = 0.94535, $p<0.01$).

HPLC-PAD was the highest sensitive method. The imidacloprid presented an very intense signal at λ = 270 nm and a retention time t_R = 2.3 min. (Figure 5). The intense signal allowed to work with very small quantity of insecticide. A value LOD = 0.009 mg·L^{-1} was detected. Two calibration curves were realised: the first in the range of 0.000018 – 0.001 mg·L^{-1} (R=0.99989, $p<0.001$) and the second in the range 0.001-0.01 mg·L^{-1} (R = 0.99763, $p<0.01$).

The LOD for this method was 0.45 mg ·L^{-1} which makes it an easy and efficient method for larger quantities of imidacloprid in cereals than those detected in maize and wheat (0.122ppm in maize and 0.124ppm in wheat, respectively).

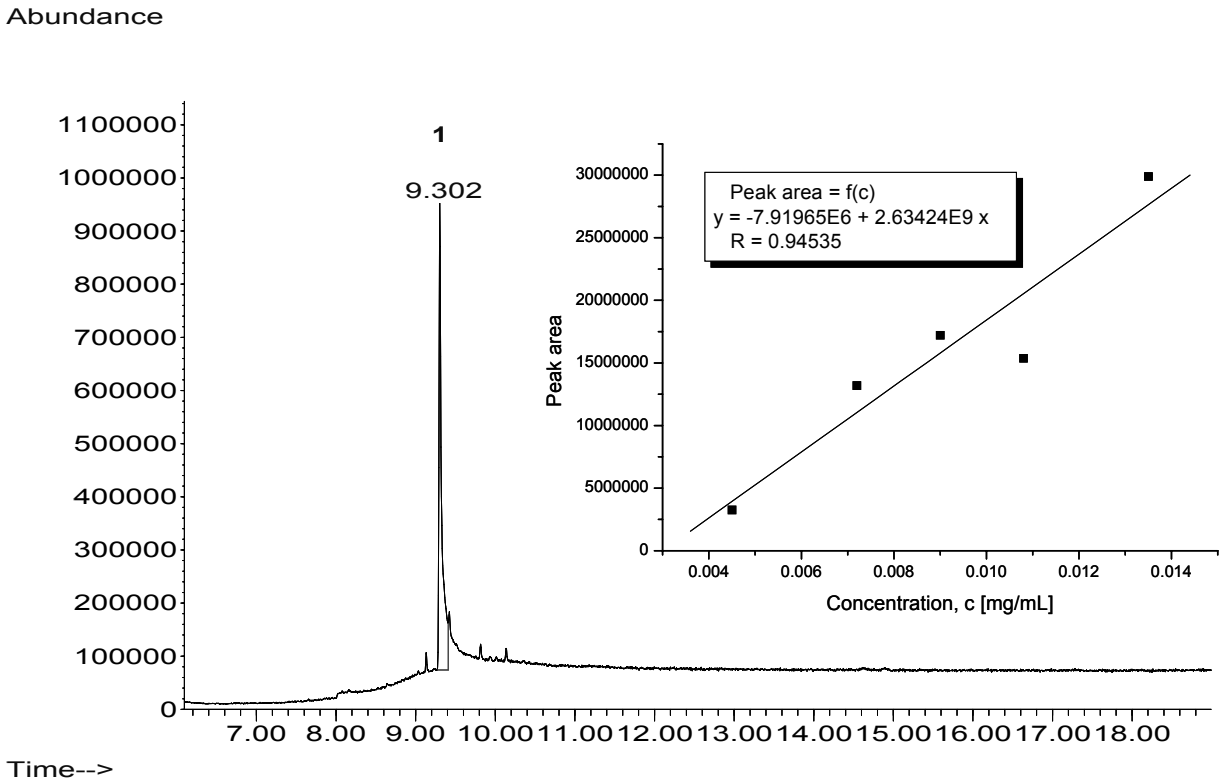

Fig. 4 – Calibration curve of imidacloprid by GC-MS method.

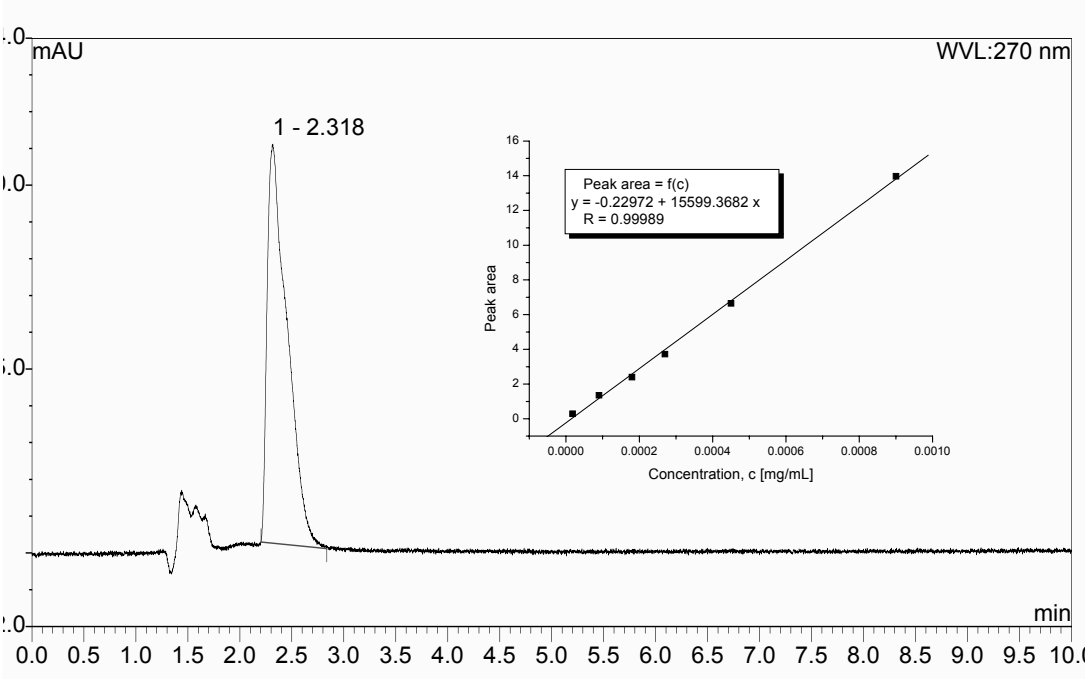

Fig. 5 – Calibration curve 1 (0.00001-0.001 mg/mL) of imidacloprid by HPLC-PAD method.

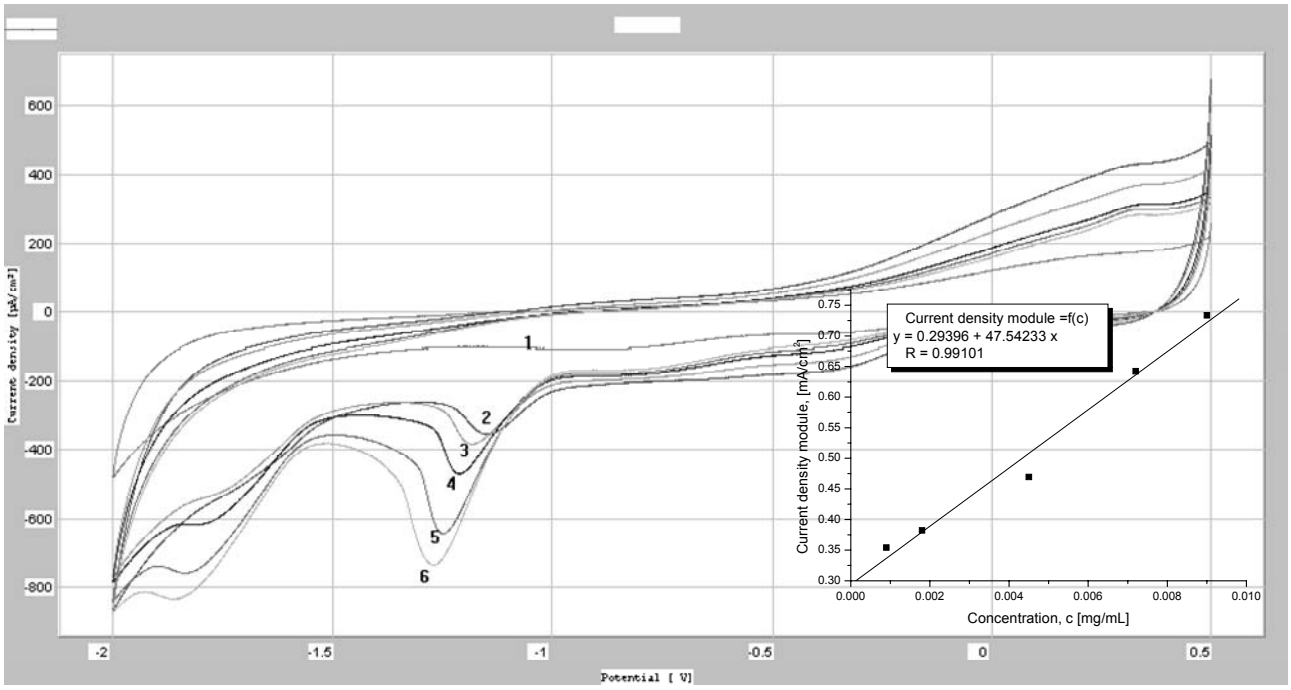

Fig. 6 – Calibration curve of imidacloprid by electrochemical method: 1 - Support electrolyte Britton - Robinson buffer solution (pH=7); 2 - c = 0.0009 mg ·cm^{-3}; 3 - c = 0.0018 mg ·cm^{-3}; 4 - c = 0.0045 mg ·cm^{-3}; 5 - c = 0.0072 mg ·cm^{-3}; 6 - c = 0.009 mg ·cm^{-3}.

EXPERIMENTAL

Materials

Maize and wheat cultivated in Carpinis-Jimbolia zone, in 2015, and preserved in silos were obtained directly from farmers. Imidacloprid - Pestanal standard (10 mg) was purchased from Sigma-Aldrich. Methanol, acetonitrile and water HPLC grade were purchased from Sigma-Aldrich. Reagents p.a. acetic acid, phosphoric acid, boric acid, and natrium hydroxide were procured from Sigma-Aldrich. Britton - Robinson buffer solutions were prepared in lab (0.04M phosphoric acid, 0.04M boric acid, 0.04M acetic acid and 0.02M natrium hydroxide).

Extraction and samples preparation

Samples of maize and wheat were grinded and sieved. Humidity of samples was determined by gravimetric method and was between 11.49-11.52%. The particles with dimensions

≤ 1mm were treated with acetonitrile and methanol (finally, methanol, cheaper, was preferred) as solvents, at 59 kHz and 30±2°C, during 30 min., in an ultrasonic bath (Falc Instruments, Italy) in order to extract the pesticides residues. In extraction process ratio of liquid: solid was 2:1 v/w (10 ml solvent: 5g sample). The extracts were filtered on paper (MN 640 Ø125 mm) and centrifuged during 30 min. at 3000g (EBA20 Hattech Zentrifugen, Germany). Then, the samples were evaporated under vacuum (30–35°C) in a rotary evaporator (Laborota 4000 Efficient-Heidolph, Germany), to dryness. The samples were dissolved in 1 mL acetonitrile or methanol and were filtered using 0.45 µm PTFE membranes. The residue of imidacloprid insecticide from the commercial product Nuprid AL 600 was determined.

GC-MS analyses

Analyses were made on an Agilent Technologies GC 7890A coupled with MSD 5975C system (Germany), using a Zebron ZB – 35 column, volum of sample injected 0.2µL, in splitless mode, and 1 ml/min. He gas flow rate. Oven temperature was T_{oven} = 90°C during 2 min. and then rised to 290°C, with 25°C/min. rate, during 10 min. Injection temperature was T_{inj} = 260°C, and ionization potential of MS was 70eV, at $T_{interface}$ = 280°C. Target ions were m/z 256, 209 and m/z 175. A ChemStation G1701EA software was used. All chromatograms were performed in duplicate.

HPLC-PAD analyses

A Dionex Ultimate 3000 (Dionex Corp., USA) apparatus equipped with a LPG 3400A quaternary pump, PDA 3000 photodiode array detector and thermostated column compartment Dionex TCC-3000, and a column C-18 Acclaim® 120 Silica-Based reversed-phase (4.6x150 mm, 5 µm) was used. The mobile phase was acetonitrile: water (50: 50 v/v), at 1 mL/min. flow rate. Column temperature was T_{col} = 30°C. All chromatograms were registered at 270 nm in duplicate and were processed with Chromeleon 6.8 software.

Electrochemical analyses

Electrochemical experiments were carried out with a Voltalab 80 PGZ 402 apparatus (Radiometer -Copenhagen), equipped with software VoltaMaster 4, version 7.0.8. A three-electrode electrochemical cell equipped with working electrode - glassy carbon electrode (1.02 cm^2), platinum plate auxiliary electrode (0.8 cm^2) and reference electrode $Hg/HgCl_2$/sat.KCl. Britton-Robinson buffer (pH=7) was used as supporting electrolyte and was prepared in lab.[13] Prior to each run, the electrode surface was cleaned by polishing with alumina sandpaper and was ultrasonicated 2 minutes in HCl 0.5%. Then, the electrode was pretreated *ex situ* in 10 mL buffer solution, during 10-11 cycles in the range 500-(-2000) mV. Cyclic voltammograms were recorded at room temperature, immediately after the immersion of the working electrode in the solutions, in the range 500-(-2000) mV, at 100 mV/s. A single cathodic peak at about (-1200) mV (vs. SCE) with no peak on reverse scan was registered for imidacloprid. This peak indicated the irreversible nature of reaction at electrode. Calibration curves were made using imidacloprid standard in the range 0.0009-0.009 mg/mL (R=0.99101, p<0.01). The peaks analyses were automatically calculated by the instrument. All cyclic voltammograms were recorded in duplicate.

Statistical analysis

Statistical analysis was performed using OriginPro 6 software. Data regarding calibration curves by each method were expressed as the mean ± standard deviation (SD) and linear regression analysis was carried out. Differences at $p < 0.01$ were considered statistically significant.

CONCLUSIONS

Three sensitive methods for detection of imidacloprid insecticide residues in maize and wheat were developed: GC-MS, HPLC-PAD and cyclic voltammetry. Extraction of pesticides from cereals was performed using ultrasonic method and two solvents: methanol and acetonitrile. The GC-MS method was the longest and presented values of LOD and LOQ too high for imidacloprid residues detection and quantification. The HPLC-PAD and cyclic voltammetry methods were quick and easy and the values for LOD and LOQ corresponded to the aim of this work. The HPLC-PAD was the best method: quick, efficient, and very sensitive; retention time was t_R = 2.31 min., LOD = 0.009 $mg \cdot L^{-1}$ and it permitted to establish the residual quantity of imidacloprid. Further efforts will be concentrated in acquisition of other pesticide standards according to the pesticides used by farmers and sampling and analysis of cereals immediately after harvest or after some periods of silos storage.

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