

# Application of Liquid Chromatography with Diode-Array Detector for Determination of Acetamiprid and 6-chloronicotinic Acid Residues in Sweet Cherry Samples

Sanja Lazić<sup>1</sup>, Dragana Šunjka<sup>1</sup>, Nada Grahovac<sup>2</sup>, Valéria Guzsány<sup>3</sup>, Ferenc Bagi<sup>1</sup> and Dragana Budakov<sup>1</sup>

<sup>1</sup>University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia (*draganas@polj.uns.ac.rs*)

<sup>2</sup>Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

<sup>3</sup>University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

Received: December 4, 2012

Accepted: January 17, 2013

## SUMMARY

A rapid and simple method for simultaneous determination of acetamiprid and its metabolite 6-chloronicotinic acid in sweet cherry samples has been developed. This residue analysis method is based on the reversed phase separation on C<sub>18</sub> column with gradient elution. Analytes' determination and quantification were performed by high performance liquid chromatography (HPLC) with diode-array detector and chromatograms were extracted at 230 nm. Extraction efficiency experiments demonstrated the ability of this method to extract neonicotinoids from sweet cherry samples. These insecticides were extracted with a mixture of acetonitril/0.1N ammonium-chloride (8/2, v/v). The average recoveries of acetamiprid and 6-chloronicotinic acid from sweet cherry samples were in the range of 95-101% and 73-83%, respectively, with the associated relative standard deviations (RSDs) <5%. Expanded measurement uncertainties for the analyzed compounds were 2.7 and 3.01%. The limit of quantification (LOQ) was 10 µg/kg and 30 µg/kg for acetamiprid and 6-chloronicotinic acid, respectively. Thus, the developed HPLC/DAD method can be considered a useful tool for sensitive and rapid determination of acetamiprid and 6-chloronicotinic acid. Hence, the method may find further application in the analysis of real sweet cherry samples contaminated with these insecticides at a ppb level.

**Keywords:** Acetamiprid; 6-chloronicotinic acid; HPLC/DAD; Sweet cherry; Pesticide residues

## INTRODUCTION

In modern agriculture, pesticides have been broadly employed in order to protect agricultural products against harmful insects and weeds, to improve their quality and increase their yields (Kim et al., 2008; Lee et al., 2008). Pesticide application which is not in accordance with GAP (Good Agricultural Practice) and ignoring the pre-harvest interval, leads to consequences for human health, beneficial insects and animals. For this reason there is a constant need to develop new and more sensitive analytical methods for quantitative determination and monitoring of pesticides in food and the environment. This is particularly true of fruits and vegetables which are mainly consumed fresh, such as sweet cherries.

In order to reduce the use of organophosphates for protection of cherries, alternative compounds have been recommended, such as those from the class of neonicotinoids. Over the past 15 years neonicotinoids have gained increasing interest in the agricultural sector across Europe (Council Directive 91/414/EEC). These insecticides are the fastest growing class of insecticides introduced to the market since the launch of pyrethroids (Muccio et al., 2006).

Neonicotinoids are used for foliar and soil application but imidacloprid also has a very high usage as a seed treatment (Roberts and Hutson, 1999). Neonicotinoid insecticides are strong selective agonists of insect nicotinic acetylcholine receptors (nAChRs), they exhibit specific activity against the insect nervous system. This unique mode of action makes these pesticides highly applicable for controlling the biological effect of insects in cases when they have developed resistance to organophosphate, carbamate and pyrethroid insecticides (Jeschke et al., 2001).

Besides their positive effects, neonicotinoid pesticides also pose various health risks to consumers. Due to a growing use of insecticides from the family of neonicotinoids, their increased presence in the environment is evident. For this reason, the concentration of neonicotinoid residues, including their metabolites, in agricultural products should be monitored. This requires appropriate methods of extraction and determination, such as the already existing methods for imidacloprid in potato and onion (Mandić et al., 2002, 2005; Lazić et al., 2002, 2003, 2004), or thiamethoxam in pepper (Lazić et al., 2002a).

Acetamiprid with the IUPAC name (E)-N'-[(6-Chloro-3-pyridyl)methyl]-N<sup>2</sup>-cyano-N'-methylacetamidine,

CAS number 135410-20-7 and molecular weight of 222.7 g/mol is a systemic insecticide. It is used to control Hemiptera, especially aphids, Thysanoptera and Lepidoptera on a wide range of crops, especially vegetables, fruits and tea (Roberts and Hutson, 1999). Its chemical structure is shown in figure 1.

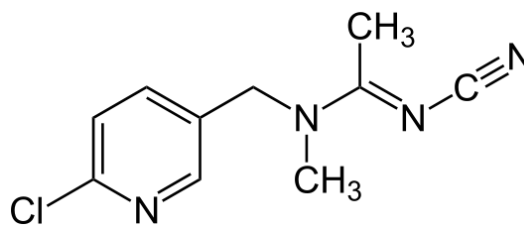


Figure 1. Chemical structure of acetamiprid

Following the legislation for food safety in vegetables and fruits, only parent insecticides (e.g. organophosphorus or neonicotinoid) are monitored and there is no control over the presence of metabolites or transformation products forming after application (Žabar et al., 2011). Based on a few published papers, a common transformation product of acetamiprid is 6-chloronicotinic acid (6CNA) (Marin et al., 2004). Its chemical structure is shown in figure 2.

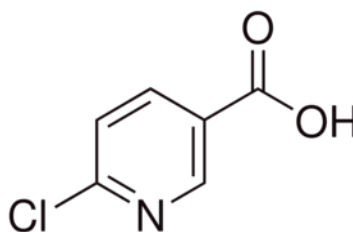


Figure 2. Chemical structure of 6-chloronicotinic acid (6CNA)

Determination of low concentrations of these pesticides in different matrices requires an effective fruit extraction procedure, followed by final chromatographic determination, in order to separate as much analyte as possible from the matrix interference substances. Gas chromatography (GC) combined with a selective and sensitive electron capture detector (ECD) and nitrogen phosphorus detector (NPD) has become a routine technique for the analysis of pesticides in fruits. Until now,

several analytical methods have been published for determining acetamiprid in crops and soils by GC/ECD (Tokieda et al., 1998, 1999), by high-performance liquid chromatography (HPLC) (Tokieda et al., 1998a) and by enzyme-linked immunosorbent assay (ELISA) (Wanatabe et al., 2000). However, the high sensitivity of these detectors contrasts with their lack of identification power. Relative retention-time-based identification and determinations of pollutants are no longer sufficient. An additional confirmatory technique is necessary, and detection by mass spectrometry (MS) is frequently used because of its identification capability. Acetamiprid and its metabolite 6CNA can also be determined using first-order derivative spectrometry (Gaál et al., 2005; Guzsvány et al., 2012; Guzsvány et al., 2012a).

There are also some alternative analytical approaches to the conventional methods of pesticides determination (Guzsvány, 2006; Papp et al., 2009; Guzsvány et al., 2012).

A number of papers have so far focused on determination of acetamiprid residues in cotton, vegetables, fruits, milk, honey and fine airborne particulate matter (Obananet et al., 2002; Jansson et al., 2004; Ferrer et al., 2005; Fidente et al., 2005; Guzsvány et al., 2006a; Lesueur et al., 2008; Seccia et al., 2008; Zhang et al., 2008; Coscollà et al., 2009; Lee et al., 2009). In order to track the pesticide's metabolites in the environment and in different matrices, sensitive techniques for their monitoring should be developed. In literature, the most widely applied method is the mass spectrometry technique, coupled with GC or LC, because of its intrinsic characteristics, such as selectivity, sensitivity and identification-confirmation capability (Martínez Vidal et al., 2009; Hernández et al., 2008b; del Mar Gómez-Ramos et al., 2011). 6CNA has been determined in samples such as water, soil, air, honeybee, apple, potato, grape, bananas and maize (Gil García et al., 2007; Kamel, 2010; Žabar et al., 2012).

The principal objective of this study was to develop a simple and sensitive method for extraction and determination of acetamiprid and 6-chloronicotinic acid in sweet cherry samples using HPLC/DAD.

## MATERIAL AND METHODS

### Standards and reagents

Analytical standards of acetamiprid (98.1%) and 6-chloronicotinic acid (99.5%) produced by Dr Ehrenstorfer (Augsburg, Germany) were used. Acetonitrile

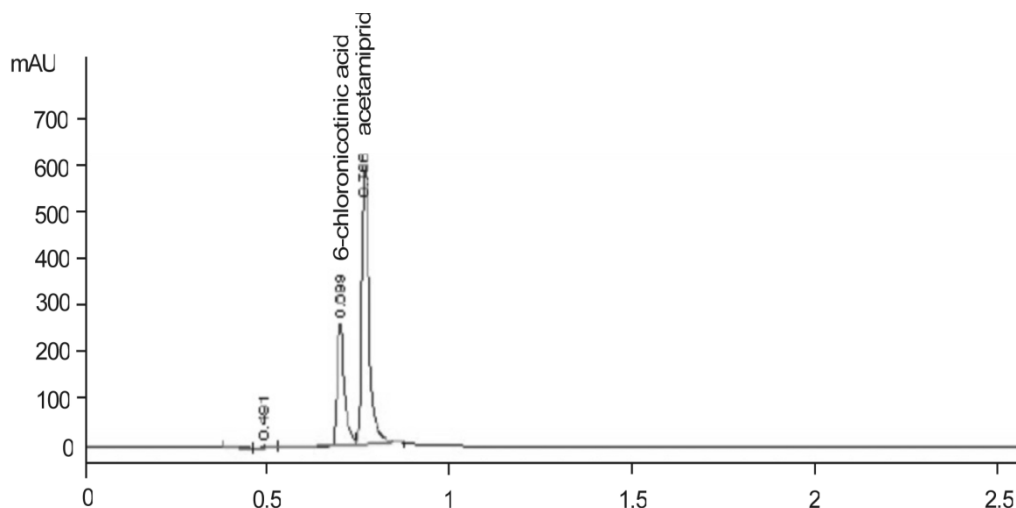
(ACN) and dichloromethane of a suitable grade for pesticide residue analysis were from J.T. Baker, Germany. Reagent-grade ammonium chloride (in crystal form) was ordered from Alkaloid, Skopje. Sodium hydroxide (NaOH) was obtained from Hemos, Beograd. Ultra-pure water was obtained from the water purification system TKA, Germany. Sweet cherry samples were purchased from orchards untreated with pesticides, and stored at -10°C.

### Instrumental and Chromatographic Conditions

An HPLC method with UV detection was selected for the method of analysis. Chromatographic separation was carried out on an Agilent HPLC 1100 system (Agilent, United States) using a reversed phase procedure that utilized an Agilent Zorbax C<sub>18</sub> column (50 mm × 4.6 mm internal diameter, 1.8 µm particle size) and UV detection at 230 nm. This wavelength was selected because it is a UV maximum and provides the sensitivity needed for quantization of low concentration in the dissolution samples. The column temperature was maintained at 30°C. The mobile phases were acetonitrile (A) and aqueous 2% acetic acid (B). The gradient program was as follows: from 40-50% A in 2 min, from 50-40% A in 1 min. The total running time was 3 min and 1 min for re-equilibration after each analysis. The flow rate was constant, 1 ml/min during the whole process and 10 µl of sample was injected in every case.

### Standard solutions

A stock solution of each pesticide (acetamipride and 6-chloronicotinic acid) was prepared dissolving pure standard in a mixture of acetonitrile/water (ACN/H<sub>2</sub>O). Working standard solutions containing a mixture of the analytes at concentration of 0.05–1 µg/ml were prepared from the above by appropriate solvent dilutions. Working standard solutions were used to calibrate the HPLC/DAD system and spike samples of sweet cherry in recovery experiments. All the solutions were protected from light and kept in a refrigerator until being used. All calibration standard solutions were prepared within 24 h of sample extract analysis. Figure 3 shows the chromatogram of the mixture of acetamipride and 6CNA.



**Figure 3.** Chromatogram of the mixture of acetamipride and 6CNA

### Extraction procedure

Fresh samples of sweet cherry were homogenized with 20 ml volume of mixture of ACN and 0.1N ammonium-chloride, and vigorously mixed for 30 min using a shaker (Promax 2020, Heildolph). The fruit puree was clarified by centrifugation at 8000 rpm for 2 min. The supernatant was filtered through celite layer (1 cm) under vacuum and evaporated to ca. 5 ml using a rotary evaporator (Laborota 4000, Heildolph) with a water bath at maximum 40°C. The total volume of 5 ml after evaporation was dissolved in deionized water (pH 7 with NaOH). As previously mentioned, the whole mixture was added to 100 ml dichloromethane in the separatory funnel and vigorously mixed for 5 min. This step was repeated three times. After separating the layers, the whole dichloromethane layer was collected and evaporated to dryness. The dry residue was dissolved in 1 ml of ACN/H<sub>2</sub>O and analyzed by HPLC/DAD. Sweet cherry samples were fortified at three levels (0.2, 0.5 and 1.0 mg/kg) with a mixture of acetamiprid and 6CNA.

## RESULTS AND DISCUSSION

### Method validation

Analytical parameters related to linearity, repeatability, accuracy and limit of quantification (LOQ) were investigated to evaluate the viability of the proposed method. The linearity of the method was evaluated by

constructing five-point calibration curves (each level in triplicate) with a wide concentration range (0.05-1 µg/ml). As shown in Table 1, good linearity was observed for both compounds at concentrations within the tested interval, with correlation coefficients ( $R^2$ ) of 0.995 for acetamiprid and 0.947 for 6CNA.

The method's repeatability was tested by determining the RSD of chromatographic signals obtained from a mixture of standard solution of acetamiprid and 6CNA (concentration 0.1 µg/ml) analyzed five times. The repeatability of the retention times and peak areas of both compounds were tested. Retention time ( $R_t$ ) of acetamiprid and 6CNA was 0.789 min and 0.699 min, respectively. The obtained values of relative standard deviation (RSD) of the retention times are shown in Table 1, while RSD of the peak area ranged from 0.84% for acetamiprid to 2.16% for 6-chloronicotinic acid. The RSD values achieved in these linearity and repeatability tests, indicating good precision of this method, are consistent with the regulations for analysis of pesticide trace levels (SANCO/12495/2011).

Limits of quantification (LOQ) for the analyzed acetamiprid and 6CNA were estimated from the fortified samples. Signal-to-noise (S/N) ratios reported by the instrument software were used to calculate the analyte concentration that yielded a signal-to-noise ratio of 10 times. The range of LOQs, summarized in Table 2, was from 10 µg/kg for acetamiprid to 30 µg/kg for 6-chloronicotinic acid. Precision at limit of quantification was checked by analyzing six test solutions prepared at LOQ level and calculating the percentage of RSD of area.

**Table 1.** Analytical parameters for HPLC/DAD determination of acetamiprid and 6CNA

Parameter	Retention time (min), $t_r \pm \Delta t_r$	Concentration interval ( $\mu\text{g/ml}$ )	Calibration curve	Linearity ( $R^2$ )
Acetamiprid	$0.789 \pm 0.001$	0.05-1	$y = 2.79 + 7.22x$	0.995
6CNA	$0.699 \pm 0.002$	0.05-1	$y = -1.53 + 5.56x$	0.947

The European Union has specified the maximum residue level (MRL) of 0.5 mg/kg for acetamiprid in sweet cherry (Regulation No. 978/2011 of the European Parliament and Council, 2011). The permissible level for acetamiprid in sweet cherry set by Serbian legislation is 0.2 mg/kg (Official Gazette, No. 25/2010). On the other hand, there is no control over the presence of pesticide transformation products forming after application (Žabar et al., 2011). The LOQs established for identification and quantification of target analytes were below the MRLs established by the EU legislation and Serbian Regulations.

Recovery was obtained for acetamiprid and 6CNA spiked at the appropriate concentration levels (0.2, 0.5 and 1.0 mg/kg) in sweet cherry samples. The obtained values of the recovery study for each compound are shown in Table 2.

**Table 2.** Recovery values and LOQ for determination of acetamiprid and 6CNA in sweet cherry samples

Parameter	LOQ	Recovery $\pm$ RSD
Analyte	$\mu\text{g/kg}$	%
Acetamiprid	10	$97.5 \pm 3.1$
6CNA	30	$80.0 \pm 4.1$

This shows that mean recoveries ranged from 95–101% for acetamiprid and from 73–83% for 6CNA, with the associated relative standard deviations (RSDs) of 3.1% and 4.1% for acetamiprid and 6CNA, respectively. The RSDs were acceptable for analytical performance.

### Measurement uncertainty

In the method validation procedure, the estimation of uncertainty is one of the main focuses of interest due to its importance in showing the data quality. The ISO standard 17025 requires presentation of uncertainty data for analytical results. Since a typical chemical

measurement consists of a number of measurement steps, it requires a careful design of measurement procedure to keep the traceability chain to the SI unit. To make a measurement result traceable to the SI unit, it is also necessary to evaluate the uncertainty of each step in the measurement procedure and combine them to meet the principles of the internationally agreed guide (Serpil, 2006; ISO/IEC17025).

According to the requirement of ISO17025, testing laboratories shall have and apply procedures for estimating the uncertainties of measurement. When estimating the uncertainty of measurement, all uncertainty components which are of importance in the given situation shall be taken into account using appropriate methods of analysis (ISO/IEC17025).

Measurement uncertainty is a quantitative indicator of confidence in the analytical data and describes the range around a reported or experimental result within which the true value can be expected to lie within a defined probability (confidence level). Uncertainty ranges must take into consideration all sources of error (SANCO/12495/2011).

The main sources of uncertainty were identified as uncertainty associated with the method of calibration, trueness of the method (recovery) and overall repeatability of the procedure. Uncertainty sources were further divided to uncertainty components: uncertainty of volumetric operations, uncertainty of chromatographic operations, purity of reagents and repeatability of operations. Measurement uncertainty evaluation was based on method validation data, assuming that they comprise the total analytical procedure. Trueness of the method was determined by a recovery study. The precision of the procedure represents a substantial source of measurement uncertainty and therefore requires detailed consideration in order not to lead to overestimation or underestimation of the combined uncertainty. With a combination of recovery, within-laboratory reproducibility and repeatability, all the relevant uncertainty sources were covered. The relative uncertainty of the recovery of the analyte was independent of concentration level. The uncertainty of standard addition

to a sample was calculated from the uncertainty of added volume and uncertainty arising from the concentration of acetamiprid and 6-chloronicotinic acid solution. The limits of accuracy of the glassware were declared by the manufacturer. There were no data on distribution, so a triangular distribution was assumed. Therefore, to obtain the standard deviation, values were divided by  $\sqrt{6}$ . Purity of the reagents acetamiprid and 6-chloronicotinic acid were evaluated according to the manufacturer's certificate. Since there were no data on distribution, a rectangular distribution was assumed, and therefore the stated uncertainty was divided by  $\sqrt{3}$  for conversion to one standard deviation.

Standard uncertainty associated to bias (B) was calculated using the equation:

$$u(B) = \sqrt{B^2 + \frac{S_B^2}{\sqrt{n}} + u(C_R)}$$

where  $B$  is deviation from true value,  $S_B$  is standard deviation of the bias,  $n$  is the number of measurements,  $u(C_R)$  is uncertainty of the recovery. Uncertainty originating from method bias, within-laboratory reproducibility and repeatability was calculated to be 1.7, 0.2 and 0.6%, respectively. For the sweet cherry matrices studied, the combined relative uncertainty  $u(CIMS)$  for acetamiprid and 6-chloronicotinic acid was 1.35% and 1.50%, respectively. The expanded uncertainty ( $U_c$ ) was calculated as  $U_c = k \cdot u_c$ , where  $k$  is the coverage factor with a level of confidence of approximately 95% considering a coverage factor of 2 (Ellison et al., 2000). Expanded uncertainty ( $U_c$ ) for acetamiprid and 6-chloronicotinic acid were calculated to be 2.7% and 3.01%, respectively.

In this paper a possibility of simultaneous determination of acetamiprid and 6-chloronicotinic acid using HPLC/DAD in sweet cherry samples was developed. Considering the obtained values of analytical parameters, the proposed method proved to be an efficient and sensitive method for determination of these compounds in carry samples. Bearing in mind that the maximum residue levels of acetamiprid in cherries are 0.2 mg/kg and 0.5 mg/kg, the method is sensitive enough for determination of pesticides and their metabolites at concentrations well below permissible levels.

We proved that the method was specific for determination of acetamiprid and 6-chloronicotinic acid in the relevant matrices. After validation and measurement

uncertainty evaluation steps, the results obtained showed that the method can be efficiently applied for monitoring of these compounds in sweet cherry samples.

## ACKNOWLEDGEMENT

The authors would like to acknowledge financial support of the Ministry of Education and Science of the Republic of Serbia (Project No. TR31038).

## REFERENCES

- COMMISSION REGULATION (EU) No 978/201** of 3 October 2011 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid, biphenyl, captan, chlorantraniliprole, cyflufenamid, cymoxanil, dichlorprop-P, difenoconazole, dimethomorph, dithiocarbamates, epoxiconazole, ethephon, flutriafol, fluxapyroxad, isopyrazam, propamocarb, pyraclostrobin, pyrimethanil and spirotetramat in or on certain products.
- Coscolla, C., Yusa, V., Beser, M.I. and Pastor, A.:** Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM 2.5) by microwave-assisted extraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1216: 8817-8827, 2009.
- Council Directive 91/414/EEC**, of 15 July 1991 concerning the placing of plant protection products on the market (OJ L 230, 19.8.1991, p. 1).
- del Mar Gómez-Ramos, M., Pérez-Parada, A., García-Reyes, J.F., Fernández-Alba, A.R. and Agüera, A.:** Use of an accurate-mass database for the systematic identification of transformation products of organic contaminants in wastewater effluents. *Journal of Chromatography A*, 1218: 8002-8012, 2011.
- Directorate of General Health and Consumer Protection Document No. SANCO/12495/2011**, Method validation and quality control procedures for pesticide residues analysis in food and feed.
- Ellison, S.L.R., Rosslein, M. and Williams, A.:** Quantifying Uncertainty in Analytical Measurement (Second Edition). EURACHEM/CITAC, 2000.
- Ferrer, I., Thurman, E.M. and Fernandez-Alba, A.R.:** Quantitation and accurate mass analysis of pesticides in vegetables by LC/TOF-MS. *Analytical Chemistry*, 77: 2818-2825, 2005.
- Fidente, P., Seccia, S., Vanni, F. and Morrica, P.:** Analysis of nicotinoid insecticides residues in honey by solid matrix

partition clean-up and liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A*, 1094: 175-178, 2005.

**Gaal, F., Guzsvány, V., Lazić, S. and Vidaković, N.:** Determination of acetamiprid and 6-chloronicotinic acid by derivative spectrophotometry and HPLC methods. Proceedings of the 12<sup>th</sup> Symposium on Analytical and Environmental Problems-SZAB, Szeged, Hungary, 2005, pp. 88-92.

**Gil García, M.D., Martínez Galera, M., Santiago Valverde, R., Galanti, A. and Girotti, S.:** Column switching liquid chromatography and post-column photochemically fluorescence detection to determine imidacloprid and 6-chloronicotinic acid in honeybees. *Journal of Chromatography A*, 1147: 17-23, 2007.

**Guzsvány, V.:** Prilog karakterizaciji i određivanju nekih neonikotinoida. Doktorska disertacija. Univerzitet u Novom Sadu. Prirodno-matematički fakultet, Novi Sad, 2006.

**Guzsvány, V., Lazić, S. and Gaal, F.:** Determination of neonicotinoid insecticides in honey by solid-phase extraction and high-performance liquid chromatography. 6th European Pesticide Residue Workshop-EPRW, Corfu, Greece, 2006a, p. 148.

**Guzsvány, V., Lazić, S., Vidaković, N. and Papp, Zs.:** Derivative spectrophotometric determination of acetamiprid in the presence of 6-chloronicotinic acid. *Journal of Serbian Chemical Society*, 77(1): 1-11, 2012.

**Guzsvány, V.J., Rajić, Lj.M., Jović, B., Orčić, D.Z., Csanadi, J.J., Lazić, S.D. and Abramović, B.F.:** Spectroscopic monitoring of photocatalytic degradation of the insecticide acetamiprid and its degradation product 6-chloronicotinic acid on TiO<sub>2</sub> catalyst. *Journal of Environmental Science and Health part A—toxic/hazardous substances and environmental engineering*, 47(12): 1919-1929, 2012a.

**Hernández, F., Sancho, J.V., Ibañez, M. and Grimalt, S.:** Investigation of pesticide metabolites in food and water by LC-TOF-MS. *TrAc – Trends in Analytical Chemistry*, 27: 10, 2008.

**ISO/IEC 17025** International Standard (Second Edition), General requirements for the competence of testing and calibration laboratories, 2005.

**Jansson, C., Pihlstrom, T., Osterdahl, B.G. and Markides, K.E.:** A new multiresidue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection. *Journal of Chromatography A*, 1023: 93-104, 2004.

**Jeschke, P., Moriya, K., Lantzsch, R., Seifert, H., Lindner, W., Jelic, K., Göbrt, A., Beck, M.E. and Etzel, W.:** Triacloprid (Bay YRC 2894) - A new member of the chloronicotinyl insecticide (CNI) family. *Pflanzenschutz-Nachrichten Bayer*, 54: 147-160, 2001.

**Kamel, A.:** Refined Methodology for the Determination of Neonicotinoid Pesticides and Their Metabolites in Honey Bees and Bee Products by Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS). *Journal of Agricultural and Food Chemistry*, 58: 5926-5931, 2010.

**Kim, M.R., Na, M.A., Jung, W.Y., Kim, C.S., Sun, N.K., Seo, E.C., Lee, E.M., Pak, Y.G., Byun, J.A., Eom, J.H., Jung, R.S. and Lee, J.H.:** Monitoring of pesticide residues in special products. *Korean Journal of Pesticide Science*, 12: 323-334, 2008.

**Lazić, S., Mandić, A., Kelemen-Mašić, Đ., Ekres, S. and Indić, D.:** Ostaci insekticida imidakloprida u krompiru i luku. XII Simpozijum o zaštiti bilja i savetovanje o primeni pesticida, Zlatibor, Srbija, 2002, p. 151.

**Lazić, S., Mandić, A. and Klokočar-Šmit, Z.:** Optimizacija metoda određivanja ostataka insekticida tiametoksama u paprici. XII Simpozijum o zaštiti bilja i savetovanje o primeni pesticida, Zlatibor, Srbija, 2002a, p. 150.

**Lazić, S., Mandić, A., Dedić, B. and Djelmiš, A.:** Determination of the insecticide imidacloprid residues in onion. 3<sup>rd</sup> Symposium of Mediterranean Group of Pesticide Research-«Crop Protection», Aix en Provence, France, 2003, p. 45.

**Lazić, S., Mandić, A., Indić, D., Delmiš, A. and Dedić, B.:** Usage of Different SPE Columns for the Imidacloprid Residues Determination in Tobacco Leaves and Onion. International Conference on Sustainable Agriculture and European Integration Processes, Novi Sad, Serbia, 2004, p. 130.

**Lee, Y.E., Nob, H.H., Park, Y.S., Kang, K.W., Jo, S.Y., Lee, S.R., Park, I.Y., Kim, T.H., Jin, Y.D. and Kyoung, K.S.:** Monitoring of pesticide residues in agricultural products collected from market in Cheongju and Jeonju. *Korean Journal of Pesticide Science*, 12: 357-362, 2008.

**Lee, S.J., Park, S., Choi, J.Y., Shim, J.H., Shin, E.H., Choi, J.H., Kim, S.T., Abd El-Aty, A.M., Jin, J.S., Bae, D.W. and Shin, S.C.:** Multiresidue analysis of pesticides with hydrolyzable functionality in cooked vegetables by liquid chromatography tandem mass spectrometry. *Biomedical Chromatography*, 23: 719-731, 2009.

**Lesueur, C., Knittl, P., Gartner, M., Mentler, A. and Fuerhacker, M.:** Analysis of 140 pesticides from conventional farming foodstuff samples after extraction with the modified QuEChERS method. *Food Control*, 19: 906-914, 2008.

**Mandić A., Lazić S., Gaal F., Okresz S. and Kelemen-Mašić, Đ.:** Determination of the insecticide imidacloprid residues in potato. Proceedings of the 9<sup>th</sup> Symposium of Analytical and Environmental Problems, Szeged, Hungary, 2002, pp. 5-9.

- Mandić, A., Lazić, S., Okresz, S. and Gaal, F.:** Determination of the insecticide imidacloprid in potato (*Solanum tuberosum* L.) and onion (*Allium cepa*) by high-performance liquid chromatography with diode-array detection. *Journal of Analytical Chemistry*, 60(12): 1134-1138, 2005.
- Marin, A., Martinez Vidal, J.L., Egea Gonzalez, F.J., Garrido Frenich, A., Glass, C.R. and Sykes, M.:** Assessment of potential (inhalation and dermal) and actual exposure to acetamiprid by greenhouse applicators using liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B*, 804: 269-275, 2004.
- Martinez-Vidal, J.L., Plaza-Bolaños, P., Romero-González, R. and Garrido-Frenich, A.:** Determination of pesticide transformation products: A review of extraction and detection methods. *Journal of Chromatography A*, 1216: 6767-6788, 2009.
- Muccio, A.D., Fidente, P., Barbini, D.A., Dommarco, R., Seccia, S. and Morrica, P.:** Application of solid-phase extraction and liquid chromatography–mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables. *Journal of Chromatography A*, 1108: 1-6, 2006.
- Obana, H., Okihashi, M., Akutsu, K., Kitagawa, Y. and Hori, S.:** Determination of acetamiprid, imidacloprid, and nitenpyram residues in vegetables and fruits by high-performance liquid chromatography with diodearray detection. *Journal of Agricultural and Food Chemistry*, 50: 4464-4467, 2002.
- Papp, Z.J., Svancara, I., Guzsavany, V.J., Vytras, K., Gaal, F.F.:** Voltammetric determination of imidacloprid insecticide in selected samples using a carbon paste electrode. *Microchimica Acta*, 166 (1-2): 169-175, 2009.
- Roberts, T. and Huston, D.:** Metabolic Pathways of Agrochemicals, Insecticides and fungicides, part two. The Royal Society of Chemistry, Cambridge, UK, 1999.
- Seccia, S., Fidente, P., Montesano, D. and Morrica, P.:** Determination of neonicotinoid insecticides residues in bovine milk samples by solidphase extraction clean-up and liquid chromatography with diodearray detection. *Journal of Chromatography A*, 1214: 115-120, 2008.
- Serpil, Y.:** Validation and Uncertainty Assessment of Rapid Extraction and Clean-up Methods for the Determination of 16 Organochlorine Pesticide Residues in Vegetables. *Analytica Chimica Acta*, 571: 298-307, 2006.
- Službeni list Republike Srbije, Pravilnik br. 25/2010 i 28/2011:** Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja.
- Tokieda, M., Ozawa, M., Kobayashi, S. and Gomyo, T.:** Method for determination of total residues of the insecticide acetamiprid and its metabolites in crops by gas-chromatography, *Nippon Noyaku Gakkaishi*, 23: 94-94, 1998.
- Tokieda, M., Tanaka, T., Ozawa, M. and Gomyo, T.:** High-performance liquid-chromatographic determination of acetamiprid and its degradation products in soil, *Nippon Noyaku Gakkaishi*, 23: 296-299, 1998a.
- Tokieda, M., Ozawa, M. and Gomyo, T.:** Methods of determination of acetamiprid and its degradation products in soil by gas chromatography. *Journal of Pesticide Science*, 24: 181-185, 1999.
- Wanatabe, S., Ito, S., Kamata, Y., Omoda, N., Yamazaki, T., Munakata, H., Kaneko, T., Yuasa, Y.:** Development of competitive enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies for chloronicotinoid insecticides imidacloprid and acetamiprid. *Analytica Chimica Acta*, 427: 211-219, 2001.
- Žabar, R., Dolenc, D., Jerman, T., Franko, M. and Trebše, P.:** Photolytic and photocatalytic degradation of 6-chloronicotinic acid. *Chemosphere*, 85: 861-868, 2011.
- Žabar, R.:** Persistence, degradation and toxicity of transformation products of selected insecticides. Dissertation. University of Nova Gorica, Graduate school, Nova Gorica, Slovenia, 2012.
- Zhang, B., Pan, X., Venne, L., Dunnun, S., McMurry, S.T., Cobb, G.P. and Anderson, T.A.:** Development of a method for the determination of 9 currently used cotton pesticides by gas chromatography with electron capture detection. *Talanta*, 75: 1055-1060, 2008.



# Primena tečne hromatografije sa DAD detektorom za određivanje ostataka acetamiprida i 6-hlornikotinske kiseline u uzorcima trešanja

## REZIME

U radu je predstavljena jednostavna metoda za određivanje acetamiprida i njegovog metabolita, 6-hlornikotinske kiseline, u uzorcima trešanja. Metoda je bazirana na primeni reverzno-faznog razdvajanja na C18 koloni primenom gradijentnog eluiranja. Određivanje i kvantifikacija analita je vršena tečnom hromatografijom (HPLC) sa DAD detektorom, pri čemu je korišćena talasna dužina od 230 nm. Tačnost metode je ocenjena procenom merne nesigurnosti. Ekstrakcija acetamiprida i 6-hlornikotinske kiseline iz uzoraka trešanja je vršena smešom acetonitril/amonijum-hlorid (0,1N) u odnosu 80:20 (v/v). Sva merenja su vršena u tri ponavljanja, pri čemu su dobijeni prinosi određivanja acetamiprida i 6-hlornikotinske kiseline u rasponima 95-101% i 73-83%, respektivno. Relativne standardne devijacije (RSD) merenja su u svim slučajevima bile ispod 5%. Limiti kvantifikacije za acetamiprid i 6-HNK iznosili su 10 i 30 µg/kg, respektivno. Kombinovana merna nesigurnost rezultata analize acetamiprida i njegovog metabolita procenjena je na 1,35, odnosno 1,50%, a proširena na 2,7 i 3,01%, upotrebom faktora pokrivanja ( $k=2$ ) koji odgovara nivou poverenja od 95%, za normalnu raspodelu. Nakon validacije i procene merne neizvesnosti dobijeni rezultati pokazuju da se razvijena HPLC/DAD metoda može primeniti za određivanje sadržaja acetamiprida i 6-hlornikotinske kiseline u uzorcima trešanja i relevantnim matriksima kontaminiranim ovim jedinjenjima.

**Ključne reči:** Acetamiprid; 6-hlornikotinska kiselina; HPLC/DAD; trešnje; ostaci pesticida