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# LC-MS/MS determination of acetamiprid residues in sweet cherries

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## Abstract

*A neonicotinoid insecticide acetamiprid has been used for control of major sweet cherry pests. In the late sweet cherry variety orchard, efficacy of acetamprid in the control of a cherry fly and its content residues in sweet cherry fruits after pre-harvest interval were studied. Applied insecticide acetamiprid expressed a high efficacy (92.9%) in control of cherry fly. For the determination of acetamprid residues, the QuEChERS method coupled with LC/MS/MS carried out. Validation of the method was performed in accordance with SANCO/12571/2013. The results showed that the average recoveries at the concentration range of 0.1-2.25 mg/kg ranged from 80.12 to 98.04%; LOD and LOQ were established at 0.5 μg/kg and 1.5 μg/kg, respectively; within-laboratory reproducibility of 0.5%. Validated method was applied to control of acetamiprid content in sweet cherry fruit obtained in orchard of late sweet cherries in which acetamprid were applied. During observing period, the concentration of acetamiprid decreased from 0.31 mg/kg to 0.09 mg/kg. Content of acetamiprid in sweet cherry samples after pre-harvest interval (PHI) were below maximum permissible level specified by Serbian (0.2 mg/kg) and EU MRLs (1.5 mg/kg). Acetamprid quantities found in the analysed sweet cherry samples per days after the application, indicated half-life of 3.65 days.*

**Keywords**: neonicotinoid insecticide, efficacy, dissipation, pre-harvest interval

## 1. Introduction

Pesticides are a numerous and diverse group of chemical compounds, which are used to eliminate pests in agriculture and households. Pesticides have many advantages, but they can also be environmentally harmful. Due to their widespread use, stability, selective toxicity and bioaccumulation, pesticides are among the most toxic substances contaminating the environment. The widespread use of pesticides not only contaminates water, soil, and air, but also causes their accumulation in crops. This is especially important for fruits and vegetables that are mostly consumed fresh. One of such fruit species is sweet cherry, with only 15% of its overall production planned for processing (COMMISSION OF THE EUROPEAN COMMUNITIES [1]).

One of the most important pests of sweet cherry is cherry fly (*Rhagoletis cerasi* L.) (KOVANCI & al. [2]). Management of pests in sweet cherry orchards is largely depended on the use of conventional, neurotoxic, broad-spectrum, synthetic chemical pesticides, such as organophosphates. However, good agricultural practice requires use of products with shorter pre-harvest interval and more convenient ecotoxicological properties than previously used insecticides (LAZIĆ & al. [3]), such as those from neonicotionid insecticide class. Neonicotinoid insecticides are relatively new group of insecticides with novel modes of

action. They act as agonists at the insect nicotinic acetylcholine receptors (nAChRs), which play an important role in synaptic transmission in the central nervous system (SEKULIĆ & al. [4]). A neonicotinoid insecticide acetamiprid ((E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine) has been introduced as an alternative to organosphosphate insecticides for control of major sweet cherry pests.

These insecticides provided many obvious benefits in agriculture however their inappropriate use can result in unacceptably high levels of these compounds in fruits and vegetables. Although in cases when applied in accordance with good agricultural practices, pesticides may leave residues (GRAHOVAC & al. [5], LAZIĆ & al. [6]. Since the presence of pesticide residues in fruits and vegetables can affect consumer health, the regulatory authorities have established maximum residue levels of pesticides for most common vegetables and fruits. The MRLs are established by each country. The European Union by No. 978/2011 has specified a maximum residue level (MRL) for acetamiprid in sweet cherries of 0.5 mg/kg by (COMMISSION REGULATION (EU) No. 978/2011 [7]), while it is 1.5 mg/kg under the EU Reg. No. 500/2013 (COMMISSION REGULATION (EU) [8]). The maximum permissible level for acetamiprid in sweet cherries set by the Serbian legislation was 0.2 mg/kg (THE OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA, No. 25/2010, [9]), and nowadays the value is harmonized with MRL in the European Union (OFFICIAL GAZETTE 29/2014 [10]).

The determination of low concentrations of this pesticide in matrices such as fruits and vegetables requires an effective extraction followed by chromatographic determination. Neonicotinoids are usually determined by liquid chromatography (HPLC) coupled with diode array (DAD) detection (WANG & [11]), because direct analysis by gas chromatography (VILCHEZ & [12], (ZHANG [13]) proved unsuitable due to their low volatility and high polarity. However, some analytical methods have been published for determining of acetamiprid in crops and soil by GC/ECD (TOKIEDA & al. [14], TOKIEDA & al. [15]). Furthermore, they are determined by HPLC coupled to either electrochemical detector, or mass spectrometry (LC/MS/MS) (LAZIĆ & al. [16]).

For the analysis of neonicotinoid insecticides residues in various food matrices like milk, vegetables, honey, honey liqueur, etc., LLE, solid-phase extraction (SPE) and combinations of LLE and SPE are the most commonly used pretreatment procedures techniques (JOVANOV & al. [17], JOVANOV & al. [18]. In recent decades the QuEChERS method, which is a combination of liquid-liquid extraction (LLE) and SPE, proved very popular because it is simple, has a low cost per sample and in fact has all the advantages defined by its name. QuEChERS method of acetamiprid residues analysis in sweet cherry samples is fast and relatively simple. This method involves liquid partitioning with acetonitrile followed by a dispersive SPE clean-up with primary secondary amine (PSA) and with or without graphitized carbon black (GCB) (LEHOTAY & al. [19]). The QuEChERS method proved widely accepted because it is simple, costs per sample are low and justifies all the advantages defined by its name. Today there are two commonly used buffered methods, European committee for standardization method 15662 (EN version 2.2, 2008 [20]) and AOAC official method 2007.01 [21].

In recent years, a number of researches have dealt with the behavior of acetamiprid in mustard plant (PRAMANIK & al. [22]), tea (GUPTA & al. [23]), chili (SANYAL & al.

[24]), zucchini (PARK & al. [25]), tomato, cucumber (SHAMS EL DIN & al. [26]), watermelon (WU & al. [27]) and eggplant leaves and fruits (ROMEH & al. [28]).
In this study residues of acetamiprid in sweet cherry were extracted by QuEChERS method with detection and quantification by LC-MS/MS. The present work was carried out to study the persistence of neonicotinoid insecticide acetamiprid in sweet cherry fruits under field conditions, as well as determination of $DT_{50}$ check of designated pre-harvest interval for acetamprid in sweety cherries in Serbia.

## 2. Materials and Methods

### 2.1 Field trial and sampling

The field trials were carried out in the orchard at Kać village, near Novi Sad. The trials were designed according to standard OEPP methods for experimental design and data analysis ( [29]), as well as for the insecticide efficacy against cherry fly [30]). Acetamiprid was applied as aqueous solutions of commercial formulation Mospilan 20 SP with 200 g/kg a.i., with a hand sprayer at the manufacturer's recommended concentration of 0.025%.
Sampling was performed by randomly collecting from various places of the experimental plots according to the FAO/WHO recommendations [31]. Around 0.5 kg of the sweet cherries fruit was collected from each replicate and brought to the laboratory. Samples were collected immediately before and after acetamiprid application (when the spraying mixture has dried), and 2, 4, 6, 8, 10, 12 and 14 days after the application. Every single analytical sample was considered in triplicates. The untreated sweet cherry trees were the sources of the blank sweet cherry samples, for the study of the matrix effects and to the acetamiprid recovery measurements.

### *2.2. Chemicals and solutions*

The certified standard of acetamiprid (purity 98.1%) was obtained from Dr Ehrenstorfer (Augsburg, Germany). Acetonitrile (ACN, HPLC grade), and formic acid were purchased from J.T. Baker (Germany). In order to ensure satisfactory water quality for LC-MS/MS measurements water was purified with a water purification system (TKA, Germany). For the sweet cherry sample extractions a ready-to-use QuEChERS sample preparation system, consisting of dispersive SP extraction (Cat. No. 5982-5650) and clean-up (Cat. No. 5982-5056) kits was purchased (Agilent Technologies, USA). A stock solution of acetamiprid (100 μg/ml) was prepared from appropriate amount of solid analytical standard and ACN, and it was stored at -10 °C. Calibration solutions for recording the solvent supported calibration curve (SSC) were prepared by further dilution with ACN, covering the concentration range from 0.10 to 2.25 μg/ml.
To establish whether the extraction recoveries are influenced by the sweet cherry matrix, matrix-matched calibration (MMC) standards were prepared by adding appropriate volumes of acetamiprid working standard solution to blank sweet cherry samples at the final reconstitution step, reaching acetamiprid concentrations of from 0.1 to 2.50 μg/ml, immediately before the LC/MS/MS measurements. Each solution was injected in triplicates.

### 2.3. Validation of the analytical method

The validation of the studied analytical methods was conducted by spiked control samples of sweet cherries and was evaluated according to the European SANCO/12571/2013 guidelines [32]. The evaluation of the proposed method was performed taking into consideration the following performance characteristics: linearity, recovery, intra-day precision, matrix effect, limits of detection (LOD) and quantification (LOQ). All these parameters were obtained in sweet cherry matrix supported system.

### 2.4. Sample extraction

The extraction procedure includes a widely used QuEChERS-based method (ANASTASSIADES & al. [33]) for sweet cherry samples. The homogenized sweet cherry sample (blank, spiked or treated one) (10 g) was weighed into polypropylene tubes (50 ml volume), 10 ml of ACN was added and the tube was shaken (1 min). Buffered salts (1000 mg sodium citrate, 500 mg sodium hydrogen citrate sesquihydrate, 4000 mg magnesium sulfate and 1000 mg sodium chloride) were added, the content of the tube was mixed, and centrifuged (1 min and 5 min at 3000 rpm); 6 ml from ACN phase was transferred to a tube with primary-secondary amine sorbent (150 mg) and magnesium sulfate (900 mg), mixed (1 min) and centrifuged (5 min at 3000 rpm). Finally, the appropriate aliquot of the obtained upper layer was evaporated to dryness, dissolved in 1 ml of acetonitrile, filtered through a 0.45 µm membrane filter and transferred into an autosampler vial for LC-MS/MS analyses.

### 2.5. The LC–MS/MS system and operating conditions

The measurements were performed on an Agilent 1200 Series LC system (Agilent Technologies Inc., USA) in combination with an Agilent 6410 Triple Quad LC/MS mass spectrometer under Mass Hunter work stations software version B.03.01. The reversed phase chromatographic separation was performed on an ZORBAX Eclipse XDB-C18 (50mm, 4.6 mm i.d., 1.8 µm) column in isocratic working regime based on the mobile phase with ratio of ACN/water (0.1% formic acid) 30:70, v/v. The flow rate was 0.4 ml/min, injector volume 5 µl, and the column temperature 25 $^{o}$C. The MS/MS conditions were optimized in previous work [18]. Briefly: the temperatures of heater gas and vaporization were 325 °C and 200 °C, respectively. The nebulizer gas was nitrogen at 50 psi, and a flow rate was 5 l/min. The capillary and charging voltages were 2500 V and 2000V. The multiple reaction monitoring (MRM) transitions used a dwell time of 20 ms (ms) in positive working mode, and the main parameters for acetamiprid determination are elaborated in Table 1.

Table 1. The retention time (tr), molecular weight (MW), m/z of precursor ion (Q1), m/z of monitored product ions (Q3), fragmentor voltage (FV) and collision energy (CE) of acetamiprid

| Mean $t_r$ (min) (n=15) | RSD (%) of $t_r$ | MW (g/mol) | Q1 m/z of precursor ion | Q3 m/z of monitored product ion | FV (V) fragmentor voltage | CE (V) collision energy |
|---|---|---|---|---|---|---|
| 1.40 | 0.01 | 222.68 | 223.1 | 126.0 | 124.00 | 12.00 |
| | | | | 56.1 | 124.00 | 20.00 |

## 3. Results and Discussion

### 3.1. Analytical method validation

Linearity of the calibration curve was examined at a concentration range between 0.1 and 2.25 μg/ml using six calibration solutions prepared in acetonitrile (SSC). Calculations were based on peak areas of the quantifier ions obtained for the appropriate target analyte concentrations. These peaks of acetamipirid standard solutions, obtained using the described LC-MS/MS method, are presented in Figure 1. The calibration curve was linear over the investigated concentration range with correlation coefficient of 0.999 and indicates high susceptibility of acetamiprid determination achieved by this method.

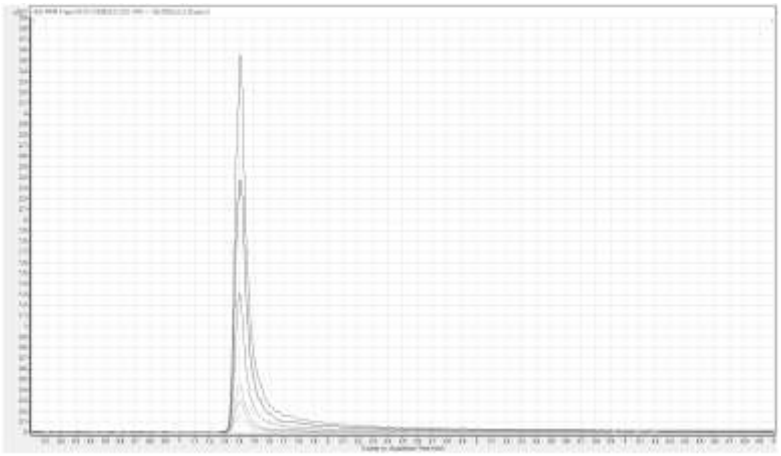

Figure 1. Extracted MRM ion chromatograms of acetamiprid standard solutions in concentration range from 0.1 to 2.25 μg/ml

Influence of matrix (SSE, signal suppression/enhancment) after QuEChERS sample preparation was determined for whole linear measurement range, by comparison of slope ratio of MMC and SSC calibration curves for acetamiprid (FERRER & al. [34]). The values obtained for the analytical curves with solutions prepared in solvent and in matrix extract demonstrated satisfactory linearity with linear regression equation y = 105660x – 3482, y = 98724x + 141,3 and correlation coefficients of 0.999 for the pesticide in solvent and matrix. Matrix-effect of 93.4% for the applied sample preparation method based on QuEChERS sample clean up showed that the sweet cherry matrix did not significantly affect the response of acetamiprid.

The limit of detection (LOD) was determined at signal-to-noise ratio of three, whereas the limit of quantification (LOQ) was determined considering a signal-to-noise ratio of 10, using the sweet cherry matrix-matched calibration curve. The LOD and LOQ of acetamiprid in this study were 0.5 μg/kg and 1.5 μg/kg, respectively. This method provided a detection limit which is lower than the MRLs for acetamiprid residues in sweet cherries, established by Serbian legislation [9] and the European Commission [8] as well.

According to the previously mentioned, the accuracy of the proposed method was evaluated as recovery, using blank sample spiked with a solution of acetamipid insecticide at six levels (0.10, 0.20, 0.30, 0.75, 1.50 and 2.25 mg/kg). The recoveries calculated by matrix-matched calibration curves of investigated neonicotinoid are given in Table 2. As it can be seen, the

determined amounts of acetamiprid obtained by LC-MS/MS agreed well with the added amounts. The obtained accuracy of the method is between 80.12% and 98.04%. The highest RSD was 6.61%.

Table 2. Accuracy of the acetamiprid in sweet cherries

| Spiking level | Recovery Mean % | RSD% |
|---|---|---|
| **0.10** | 86.04 | 5.04 |
| **0.20** | 80.12 | 0.69 |
| **0.30** | 96.19 | 6.36 |
| **0.75** | 88.10 | 6.61 |
| **1.50** | 95.22 | 4.39 |
| **2.25** | 98.04 | 1.50 |

### 3.2. Acetamiprid efficacy

Results of the product Mospilan 20 SP efficacy in control of cherry fly (*R. cerasi*) in the sweet cherry orchard at locality Kać are presented in Table 3. Cherry fly number in cherry fruits after application of the studied products was on significantly lower level in relation to the control. The efficacy the product Mospilan 20 SP was 92.9 %.

Table 3. Nuber of cherry fly (*R. cerasi*) in sweet cherry fruits and efficacy of the product Mospilan 20 SP during picking

| Insecticide (l/ha) | replications | | | | Σ | x | SD (±) | E% |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | | | | |
| Mospilan 20 SP | 1 | 0 | 2 | 1 | 4 | **1.0 b** | 0.81 | **92.9** |
| Control | 14 | 16 | 18 | 9 | 57 | **14.3 a** | 3.86 | |
| LSD (0.05%) | | | | | | 2.85 | | |

Σ – sum; x – the average number; SD± - standard deviation; E% - efficacy

### 3.3. Insecticide degradation under field conditions

The proposed LC-MS/MS method in combination with QuEChERS-based sample preparation was applied to control acetamiprid content in sweet cherry fruit obtained in orchard of late sweet cherries in which acetamprid was applied. Figure 2 presents chromatogram of a real sweet cherry sample.

Determination of the acetamiprid in samples was carried out by an eight points level matrix matched calibration, to compensate the matrix effects. Acetamiprid residue levels in samples of late sweet cherry variety, monitored for two weeks from the application are presented in Table 4.

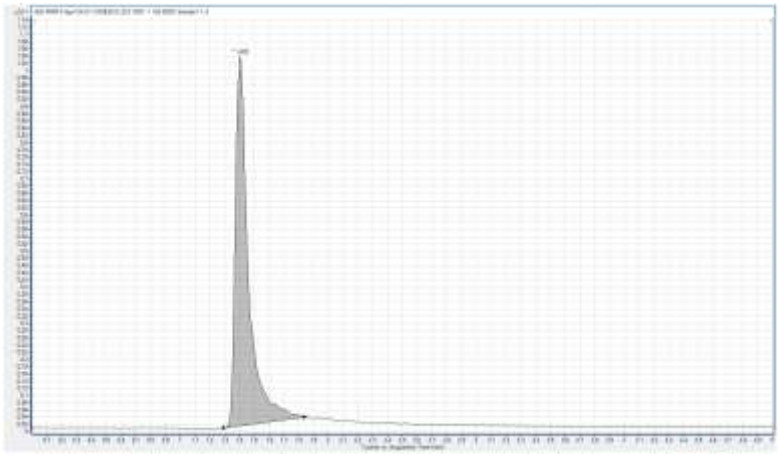

Figure 2. A real sweet cherry sample chromatogram

Table 4. Dissipation of acetamiprid residues in late variety of sweet cherry fruits

| **Interval/days** | Acetamiprid mg/kg | SD* mg/kg | Loss % | Persistence % |
|---|---|---|---|---|
| **Initial** | 0.31 | 0.01 | 0.00 | 100.00 |
| **2** | 0.30 | 0.01 | 1.14 | 98.86 |
| **4** | 0.29 | 0.02 | 7.14 | 92.86 |
| **6** | 0.22 | 0.03 | 29.74 | 70.26 |
| **8** | 0.17 | 0.00 | 45.15 | 54.85 |
| **10** | 0.16 | 0.00 | 47.26 | 52.74 |
| **12** | 0.10 | 0.00 | 66.43 | 33.57 |
| **14** | 0.09 | 0.03 | 69.85 | 30.15 |

*SD – standard deviation

The initial deposit of acetamiprid was 0.31 mg/kg. Immediately after drying deposit the concentration of acetamiprid in sweet cherries was lower than the MRL of 1.5 mg/kg, according to EU Reg. No. 500/2013 [8] and Regulations of the Republic of Serbia from 2014 (Official Gazette 29/2014) [10]. Average content of acetamiprid residues determined in samples collected on the second day after its application was 0.30 mg/kg with standard deviation of 0.01 mg/kg. Subsequently, residues decreased slowly and at the intervals of 4, 6, 8, 10, 12 and 14 days after treatments, the estimated residues were 0.29, 0.22, 0.17, 0.16, 0.10 and 0.09 mg/kg, respectively (Table 4).

Acetamiprid content below the maximum permitted level, defined by Regulations of the Republic of Serbia from 2010 (Official Gazette 25/2010) [9], was established in samples collected eight days after the insecticide application.

Figure 3 shows the dissipation curve of acetamiprid in sweet cherry sprayed at the recommended rate (0.025%). The experimental data were subjected to statistical analysis using Microsoft Excel (Windows 2000). The dissipation kinetic of the acetamiprid in sweet cherry was determined by plotting residue concentration against time. The half-life of acetamiprid in different matrices was calculated using the first order rate equation:

$$C_t=C_0e^{-kt}$$

where Ct represents the concentration of the pesticide residue at time t, $C_0$ represents the initial concentration and k is the rate constant per day. The half lives ($t_{1/2}$) were determined from the k value, $t_{1/2}=\ln 2/k$ (GUPTA & al. [23]).

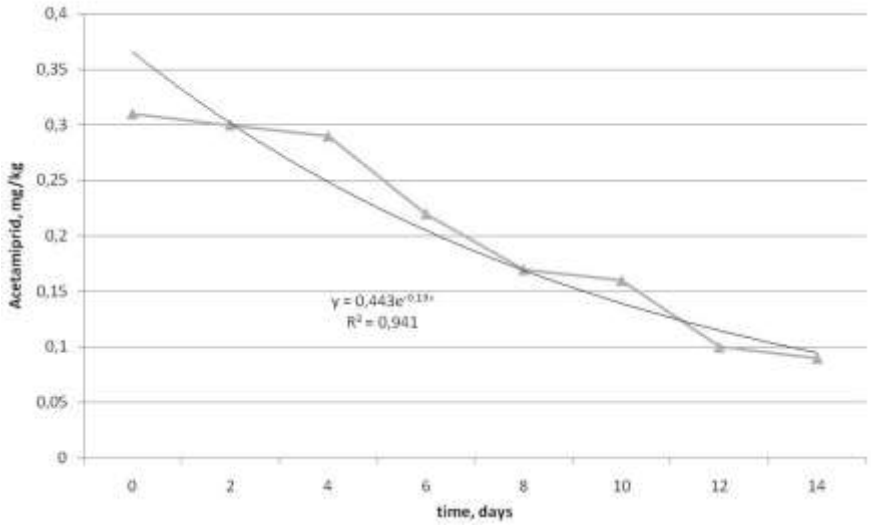


Figure 3. Dissipation curve of acetamiprid in late variety of sweet cherries

In this study the first-order kinetic equations determination coefficient ($R^2$) was 0.941. The change in acetamiprid residue concentrations in analyzed variety of sweet cherry in this study indicated half-life of 3.65 days. In recent years, a number of researches have dealt with the behavior of acetamiprid in plant products. PRAMANIK & al. [22] established that acetemiprid half-life in mustard plants is 1.02 days, while acetamiprid half-life in made tea was 1.84–2.33 days (GUPTA & al. [23]). Acetamiprid half-life determined in tomato and cucumber fruits was 1.04 and 1.18 days, respectively (SHAMS EL DIN & al. [26]). Analysis of acetamiprid residues in zucchini grown under greenhouse conditions were performed by PARK & al. [25]. $DT_{50}$ of acetamiprid achieved in this experiment was 1.9 days.
Obtained half-life of acetamprid degradation in sweet cherries of 3.65 days is longer in comparison to $DT_{50}$ in mustard plants, tea, tomato and cucumber fruits, as well as in zucchini.

## 4. Conclusions

Applied insecticide acetamiprid expressed high efficacy (92.9%) in control of cherry fly in sweet cherry fruits. The validated method described in this study is suitable for determination of acetamiprid residues in sweet cherry. Acetamprid quantities found in the analysed sweet cherry samples per days after its use, indicate acetamiprid half-life of 3.65 days. The established half-life of acetamprid degradation in sweet cherries is longer in comparison to $DT_{50}$ obtained in mustard plants, tomato and cucumber fruits, as well as in tea and zucchini. Nonetheless, the residues in sweet cherry were lower than the MRL of 1.5 mg/kg immediately after deposit drying. This suggests that the use of acetamiprid at the

recommended rate can be considered quite safe from the point of view of health hazards to consumers and a PHI of 14 days of acetamiprid in sweet cherry can be shortened.

## 4. Acknowledgements

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