

## DETERMINATION OF CAPTAN, CHLORPYRIPHOS AND CYPERMETHRIN RESIDUES IN CHERRIES\*

VOJISLAVA BURSIĆ, SANJA LAZIĆ, MIRA PUCAREVIĆ,  
FERENC BAGI, SLAVICA VUKOVIĆ<sup>1</sup>

*SUMMARY: The paper deals with the determination of the residues of captan, chlorpyrifos and cypermethrin in the samples of cherry species Aida, Margit and Šandor grown in the orchards of Vojvodina according to Good Agricultural Practice. By the analysis of cherry samples with the application of GC-ECD, the detected values of captan and cypermethrin range from 0.39 to 1.44 mg/kg i.e. 0.01 to 0.08 mg/kg and do not exceed the MRLs of 5.0 i.e. 3.0 mg/kg respectively. The detected values of chlorpyrifos range from 0.21 to 0.29 mg/kg but taking into account the relative standard deviation the detected values may be above the MRLs of 0.3 mg/kg.*

**Key words:** cherry, pesticide residues, captan, chlorpyrifos, cypermethrin, GC-ECD.

### INTRODUCTION

The production of fruit in Serbia is economically important due to the favourable climatic and soil conditions for growing numerous fruit species among which cherries tend to be more and more significant. Serbia is 16th in the world for cherry production and in the total production it participates with only 2.7% (Ognjanov et al., 2011; FAO, 2010). Cherry fruits are predominantly used for table consumption and to a lesser extent for home-made or industrial processing into: stewed fruit, jam, fruit salad and juices (Milatović and Nikolić, 2011). In order to prevent the cherry production from being endangered by plant diseases agents *Blumeriella jaapii*, *Monilinia spp.*, *Stigmina carpophila*, phytopathogenic bacteria, viruses and pests *Rhagoletiscerasi* (cherry fly),

---

Original scientific paper / *Originalni naučni rad*

<sup>1</sup>Vojislava Bursić, MSc, assistant, Sanja Lazić, PhD professor, Ferenc Bagi, PhD, docent, Slavica Vuković, PhD, assistant, Faculty of Agriculture, Novi Sad. Mira Pucarević, PhD, professor, EDUCONS University, Vojvode Putnika bb, Sremska Kamenica.

Corresponding author: Vojislava Bursić, Poljoprivredni fakultet, Trg D. Obradovića 8, Phone: +381214853346, e-mail: bursicv@polj.uns.ac.rs

\*The authors acknowledge the financial support of the Ministry of Science and Technological Development, Republic of Serbia for Project Ref. TR31038 "Selection of sweet and sour cherry dwarfing rootstocks and development of intensive cultivation technology based on the sustainable agriculture principles", 2011-2014.

*Myzus cerasi* (black cherry louse), *Aculus fockeui* (sour cherrypink rust mite) and *Rynchites auratus* (sour cherry weevil), it is necessary to use, besides agrotechnical measures, the chemical measures of protection as well (Miletić and Tamaš, 2011).

It is known that in the conditions of the contemporary agricultural production over 30% of the produce does not find its way to the consumers, whereas with the application of the chemical agents the loss will be doubled (Bursić et al., 2006; Bursić et al., 2009). There have been eight insecticide, one acaricide, eleven fungicide and one herbicide active compounds registered in our country (Sekulić and Jeličić, 2011). The pesticides affect the quality of the product and the yield increase but on the other hand they are dangerous because of the possible pesticide residues in products and as such a potential threat to consumers (Pucarević et al., 2010). The residues of pesticides in fruit are the consequence of their direct application in the agricultural production (Lazić et al., 2009). Due to their biocide activity and potential risk to the consumers, the concentration of pesticides must be kept at the minimum in fruits and has to be below the maximum residue limits (MRLs).

For the analysis of pesticides in fruit, simple treatment is required in order to isolate the compounds from the complex matrices (Vuković et al., 2010). Liquid-liquid extraction with organic solvents such as acetone, ethyl acetate, cyclohexane, acetonitrile and dichloromethane (Sannino, 2007; Wang et al., 2006) or solid phase extraction – SPE (Zroslikova et al., 2003; Topuz, 2005) were used to determine captan, chlorpyrifos and cypermethrin in fruit.

This paper will present the insight into the health safety of the analyzed cherry samples of the species Aida, Alex, Margit and Šandor, based on the residues of captan, chlorpyrifos and cypermethrin and it will certify whether the pesticide residues are within the regulated MRLs, which for captan is 5.0 mg/kg, for chlorpyrifos 0.3 mg/kg and for cypermethrin 1.0 mg/kg (Gazzete RS 25/2010). The pesticide residues determination will be carried out by gas chromatography with electron capture detector, GC-ECD.

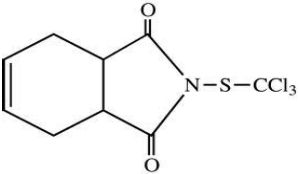
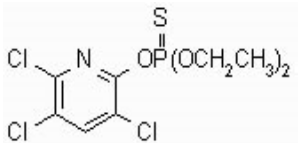
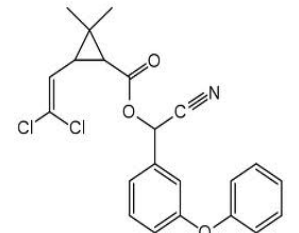
## MATERIAL AND METHODS

**Chemicals and Apparatus.** The analytical standards of captan (99.1%), chlorpyrifos (99.5%) and cypermethrin (95.8%) by Dr. Ehrenstorfer GmbH, Germany, were used. Table 1 shows the chemical characteristics of the compounds studied. The stock standard solutions in the concentration of 100 µg/ml of captan were prepared in acetone, chlorpyrifos in acetonitrile (ACN) (J.T. Baker) and cypermethrin in petroleum ether (PE) (J.T. Baker). The working standard solutions (0.02, 0.03, 0.1, 0.25, 0.5, 0.75, 1.0, 1.25 µg/ml) were obtained by dilution with acetone, ACN and PE. The pesticide determination was carried out by the use of a GC-ECD (Hewlett Packard Gas Chromatograph 5890 Series II) with splitless injection (2 µl) and capillary column SPB-5 (30mx0.32mmx0.25µm, Supelco, No 18441-03A). The carrier gas flow was 1 ml/min. The temperature of the injector was 250 °C and of the detector 300 °C. The temperature programme of captan and chlorpyrifos determination: the column temperature of 160 °C maintained for 1 minute, the rise of 7 °C/min to 250 °C; of cypermethrin determination: the column temperature of 150 °C maintained for 1 minute, the rise of 20 °C/min to 260 °C hold for 20 min. The total run time for captan was 7 min, for chlorpyrifos was

15 min, and for cypermethrin was 20 min. The retention time of captan was 3.37 min, of chlorpyrifos 12.95 min and of cypermethrin 10.82 min.

Table 1. Chemical characteristics of the pesticides studied

Tabela 1. Hemijske karakteristike ispitivanih pesticida

Name <i>Ime</i>	Chemical structure <i>Hemijska struktura</i>	Molecular weight <i>Molekulska težina</i>	Molecular formula <i>Molekulska formula</i>	$K_{ow}^*$ $K_{ow}^*$	ADI* (mg/kg b.w. daily) ) ADI (mg/kg t.m. dnevno)	Oral LD* (mg/kg) <i>Oralna LD*</i> (mg/kg)
Captan <i>Kaptan</i>		300.6	C <sub>9</sub> H <sub>8</sub> Cl <sub>3</sub> NO <sub>2</sub>	2.8	0.1	9000, rats
Chlorpyrifos <i>Hlorpirifos</i>		350.6	C <sub>9</sub> H <sub>11</sub> Cl <sub>3</sub> NO <sub>3</sub> PS	4.7	0.01	490, duck
Cypermethrin <i>Cipermetrin</i>		416.3	C <sub>22</sub> H <sub>19</sub> Cl <sub>2</sub> NO <sub>3</sub>	6.6	0.05	>10000, duck

\*Tomlin (2006).

Validation. The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average of baseline (SHI, 2009; SANCO, 10232/2006). The limit of quantification (LOQ) was determined as the lowest amount of a given pesticide giving a response of ten times the average of baseline. For the determination of LOD and LOQ untreated cherry samples spiked with 0.03 µg/ml of captan, 0.001 µg/ml of chlorpyrifos and 0.01 µg/ml of cypermethrin were used. The linearity in the response was studied with a standard solution of captan ranging from 0.05 to 5.5 µg/ml, for chlorpyrifos from 0.05 to 0.5 µg/ml and for cypermethrin from 0.05 to 1.25 µg/ml. The recovery assays were performed by spiking untreated cherry samples with captan, chlorpyrifos and cypermethrin, each (separately) with the concentration of 0.05, 0.25, 0.5 and 1.0 µg/ml in three replicates.

Sampling. The treatment of cherries with the compounds based on captan, chlorpyrifos and cypermethrin was carried out at the end of April 2011 in accordance with GAP (Good Agricultural Practice) and the sampling was carried out at the end of June on the expiry of the pre-harvest interval of all the pesticides (PHI for captan is 21 days, for chlorpyrifos is 28 days and for cypermethrin is 28 days as well). The basic samples

of cherry species Aida, Alex, Margit and Šandor, 2 kg each, were collected from various orchards in Vojvodina. All the samples were kept in polyethylene black bags in deep-freeze until being analyzed.

## RESULTS

Analytical method. By means of the ANOVA Single Factor and Regression Statistics all the validation parameters were calculated and shown in Table 2.

Table 2. Validation parameters

Tabela 2. Parametri validacije

	LOD (mg/kg) <i>LOD (mg/kg)</i>	LOQ (mg/kg) <i>LOQ (mg/kg)</i>	Prosečna vrednost prinosa ekstrakcije±RSD (%) <i>Average Recovery±RSD (%)</i>
Kaptan/ <i>Captan</i>	0.030	0.090	94±3.57
Hlorpirifos/ <i>Chlorpyrifos</i>	0.001	0.003	97±3.45
Cipermetrin/ <i>Cypermethrin</i>	0.010	0.030	85±7.29

By checking the linearity of the detector response, the correlation coefficients of 0.994 for the captan, 0.998 for the chlorpyrifos and 0.991 for the cypermethrin were obtained. By meeting the basic validation parameters, the conditions for the reliable chromatographic analysis of the pesticides of interest in the samples of cherry species Aida, Alex, Margit and Šandor were checked and defined.

The obtained LOD and LOQ were low enough for the adequate determination of the captan, chlorpyrifos and cypermethrin residues in cherry samples.

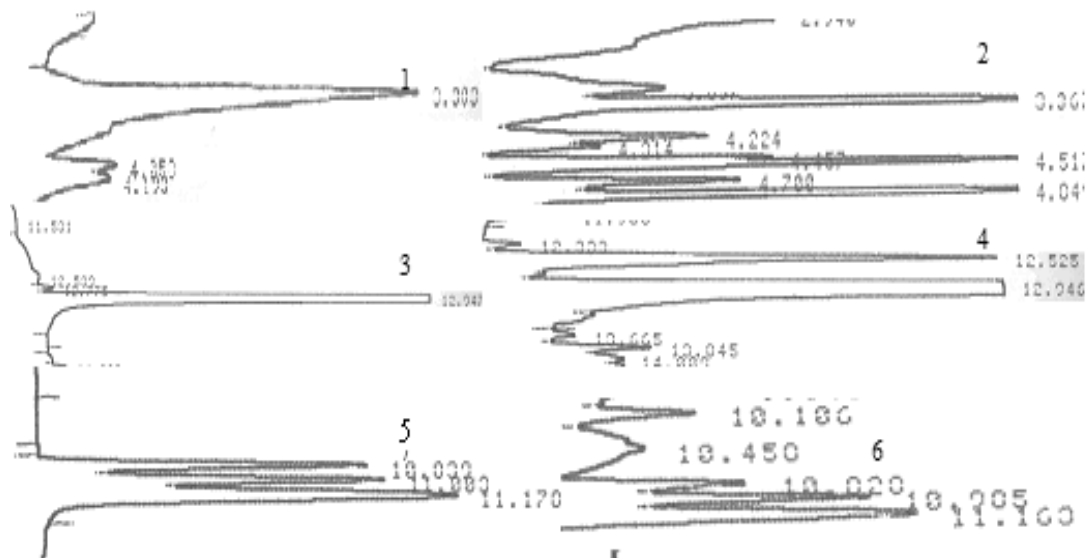


Figure 1. Chromatograms of captan (1), chlorpyrifos (3) and cypermethrin (5) standards and samples with detected captan (2), chlorpyrifos (4) and cypermethrin (6).

Slika 1. Hromatogrami standarda kaptana (1), hlorpirifosa (3) i cipermetrina (5) i uzoraka trešanja sa detekcijama kaptana (2), hlorpirifosa (4) i cipermetrina (6).

The detection and quantification of the pesticide residues were carried out based on the gas chromatographic analysis of standard captan, chlorpyrifos and cypermethrin and then by injecting cherry samples when, based on the retention times of the standards, the detection of captan, chlorpyrifos and cypermethrin in the samples was carried out.

Table 3. Captan, chlorpyrifos and cypermethrin content in cherry samples  
*Tabela 3. Ostaci kaptana, hlорpirifosa i cipermetrina u uzorcima trešanja*

Sorte <i>Species</i>	Prosečne vtrednosti detektovanih ostataka (mg/kg) ±RSD(%)* <i>Mean value of residues (mg/kg) ±RSD(%)**</i>		
	Kaptan <i>Captan</i>	Hlorpirifos <i>Chlorpyrifos</i>	Cipermetrin <i>Cypermethrin</i>
Aida	0.39 ±13.49	0.21±11.64	0.08±13.01
Alex	0.85±9.14	0.23±14.11	0.01±11.42
Šandor	1.33±7.95	0.24±12.50	0.01±7.69
Margit	1.44±5.19	0.29± 8.97	0.01±11.11

\*Svaki uzorak je analiziran tri puta u dva ponavljanja

\*Each sample was individually analyzed three times in duplicates

## DISCUSSION

By the statistical analysis of the studied validation parameters (LOD, LOQ, linearity and recovery) the LODs for captan, chlorpyrifos and cypermethrin were 0.030, 0.001 and 0.010 mg/kg with the LOQs of 0.090, 0.003 and 0.030 mg/kg, respectively. The linearity of detector response with the regression coefficient in the range of 0.991-0.998, confirms the linearity. The recovery for the analyzed pesticides ranges from 85.0-97.0% (RSD<8.0%).

Based on the presented values of captan in the analyzed cherry samples, the detected values ranged from 0.39 do 1.44 mg/kg and do not exceed the maximum residue value of 5 mg/kg. The detected values of chlorpyrifos range from 0.21 to 0.29 mg/kg but taking into account the relative standard deviation the detected values may be above the MRLs of 0,3 mg/kg. The residue values of cypermethrin are from 0.01 to 0.08 mg/kg and do not exceed the MRLs of 3.0 mg/kg, as regulated (Gazette RS 25/2010). It is interesting to note that the MRLs of captan in cherries according to Codex Alimentarius is 25 mg/kg and according to the Canadian and Australian regulations 5 and 15 mg/kg respectively (Hugh, 2011).

According to the available data in Slovenija out of the total 391 analyzed samples (10 cherries), 3.3% exceeded the MRLs for acetamiprid, captan, chlorothalonil, cyprodinil, fludioxonil and folpet (Česnik et al., 2008). Based on the results of the study of pesticide residues in fruit carried out by Pucarević et al. (2010), the most frequently detected pesticide residues in different fruit samples were exactly captan, chlorpyrifos and cypermethrin.

## CONCLUSION

By the statistical analysis of the studied limit of detection, limit of quantification, linearity and recovery, as the basic validation parameters needed for this kind of labora-

tory certification of the method, the LODs for captan, chlorpyrifos and cypermethrin were 0.030, 0.001 and 0.010 mg/kg with the LOQs of 0.090, 0.003 and 0.030 mg/kg, respectively, which enabled the detection of very low amounts, below the MRLs, of the studied analytes. The linearity of detector response with the regression coefficient in the range of 0.991-0.998, confirms the linearity. The recovery for the analyzed pesticides ranges from 85.0 to 97.0% (RSD < 8.0%). The obtained validation parameters prove that the qualitative analysis of the treated samples of cherry species Aida, Alex, Margit and Šandor, grown in various orchards throughout Vojvodina according to Good Agricultural Practice – GAP. In the analysis of cherry samples with the application of GC-ECD, the detected values of captan range from 0.39 to 1.44 mg/kg and do not exceed the maximum residue level of 5 mg/kg. The detected values of chlorpyrifos range from 0.21 to 0.29 mg/kg but taking into account the relative standard deviation the detected values may be above the MRLs of 0.3 mg/kg. The values of cypermethrin are from 0.01 to 0.08 mg/kg and do not exceed the MRLs of 3.0 mg/kg, as regulated (Gazette RS 25/2010).

## REFERENCES

- BURSIĆ, V., LAZIĆ S., STOJŠIN, V., FERENC, B., BALAŽ, F.: Određivanje ostataka azoksistrobina u plodovima krastavca. Zbornik radova sa Eko-konferencije, Novi Sad, Srbija, 20-23. 09. 2006, pp. 291-296.
- BURSIĆ, V., LAZIĆ S., VUKOVIĆ, S., ŠUNJKA, D., PUCAREVIĆ, M.: Disappearance of azoxystrobin in cucumber. *Savremena poljoprivreda*, 58(3-4)81-86, 2009.
- ČESNIK, B.H., GREGORČIĆ, A., VELIKONJA BOLTA, Š.: Pesticide residues found in agricultural products of Slovene origin in the years 2006 to 2007. Book of abstracts from the 7<sup>th</sup> EPRW, Berlin, Germany, 1-5 June, 2008. pp. 252.
- FAO. 2010. <http://faostat.fao.org>.
- HUGH, G.P.: Domestic and foreign maximum residue limits for pesticides recommender use on sweet cherries grown in BC. *IPM*, 2011, 260.
- LAZIĆ, S., PUCAREVIĆ, M., BURSIĆ, V., OSTOJIĆ, N., VUKOVIĆ, S.: Pesticide residues in apple samples on the market of the Republic of Serbia 2004-2007. *Plant Science*, 46:306-310, 2009.
- MILATOVIĆ, D., NIKOLIĆ, D.: Oplemenjivanje trešnje i višnje u svetu. Zbornik radova sa III savetovanja "Inovacije u voćarstvu", Beograd, Srbija, 10.02.2011. pp. 21-48.
- MILETIĆ, N., TAMAS, N.: Zaštita višnje i trešnje od prouzrokovala biljnih bolesti i štetočina. Zbornik radova sa III savetovanja "Inovacije u voćarstvu", Beograd, Srbija, 10.02.2011. pp. 133-144.
- OGNJANOV, V., LJUBOJEVIĆ M., PEČURICA A., ČALIĆ, M., MLADENOVIĆ, E., ČUKANOVIĆ, J.: Vegetativne i generativne karakteristike novih sorti trešnje. Zbornik radova sa III savetovanja "Inovacije u voćarstvu", Beograd, Srbija, 10.02.2011. 153-162.
- PRAVILNIK o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja, Službeni glasnik RS broj 25/2010, 2010.
- PUCAREVIĆ, M., LAZIĆ, S., MOJAŠEVIĆ, M., BURSIĆ, V., VASILJEVIĆ, I., ŠPIROVIĆ, B., ŠUNJKA, D.: Pesticide residues in fruit and vegetables on the market

of the Republic of Serbia during 2009. Book of abstracts of the 8<sup>th</sup> EPRW, Strasbourg, France, 20-24 June, 2010. pp. 234.

SANCO Document No. 10232/2006, Quality control procedures for pesticide residues analysis, 2006.

SANNINO, A.: Determination of three natural pesticides in processed fruit and vegetables using high-performance liquid chromatography/handem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 21, 2079-2086, 2007.

SAVEZ HEMIJSKIH INŽINJERA, Validacija metoda u laboratorijskoj praksi, Priručnik za kurs validacija mernih metoda, 2009.

SEKULIĆ, J.S., JELIČIĆ, S.N.: Sredstva za zaštitu bilja u prometu u Srbiji (2011). *Biljni lekar*, 2-3, 113-380, 2011.

TOMLIN, C.D.S. (ed.): *The pesticide manual*. British Crop Production Council, Hampshire, UK, pp.1-1349, 2006.

TOPUZ, G., OZHAN, B., ALPERTUNGA, B., Simultaneous determination of various pesticides in fruit juice by HPLC-DAD. *Food Control*, 16, 87-92, 2005.

VUKOVIĆ, G., ŠPIROVIĆ, B., STOJANOVIĆ, Z., BURSIĆ, V., LAZIĆ, S., MOJAŠEVIĆ, M.: Comparison of different clean-up procedures for the determination of fungicides in raspberries by LC-MS/MS. *Proceedings of the Eco-confefence 2010*, Novi Sad, Serbia, 22-25 September, 2010. pp. 325-332.

WANG, J.H., ZHANG, Y.B., WHANG, X.L.: Determination of multiclass pesticide residues in apple juice by gas chromatography-mass spectrometry with large-volume injection. *Journal of separaration science*, 29, 2330-2337, 2006.

ZROSTLIKOVA, J., HAJŠLOVA, J., KOVALCZUK, T., ŠTEPAN, R., POUSTKA, J.: Determination of seventeen polar/termolabile pesticides in apples and apricots by liquid chromatography/mass spectrometry. *Journal of AOAC International*, 86(3)612-622, 2003.

# ODREĐIVANJE OSTATAKA KAPTANA, HLORPIRIFOSA I CIPERMETRINA U UZORCIMA TREŠANJA

VOJISLAVA BURSIĆ, SANJA LAZIĆ, MIRA PUCAREVIĆ,  
FERENC BAGI, SLAVICA VUKOVIĆ

## Izvod

U radu je prikazano određivanje ostataka kaptana, hlorspirifosa i cipermetrina u uzorcima trešanja sorti Aida, Alex, Margit i Šandor, gajenih u voćnjacima u Vojvodini u skladu sa dobrom poljoprivrednom praksom. Analizom uzoraka trešanja primenom GC-ECD, detektovane vrednosti kaptana i cipermetrina se kreću u intervalu od 0,39 do 1,44 mg/kg, odnosno 0,01 do 0,08 mg/kg i ne prelaze MDK od 5,0, odnosno 3,0 mg/kg. Detektovane vrednosti hlorspirifosa, kreću se u intervalu od 0,21 do 0,29 mg/kg, ali uzimajući u obzir relativnu standardnu devijaciju, detektovane vrednosti mogu biti iznad MDK od 0,3 mg/kg.

**Ključne reči:** trešnje, ostaci pesticida, kaptan, hlorspirifos, cipermetrin, GC-ECD.

Received / *Primljen*: 06.11.2011.

Accepted / *Prihvaćen*: 05.12.2011.