sciendo

Contemporary Agriculture
The Serbian Journal of Agricultural Sciences
University of Novi Sad, Faculty of Agriculture
Trg Dositeja Obradovića 8, 21000 Novi Sad, R. Serbia
Tel.: +381 21 485-3477; Fax : +381 21 6350-019;
www.contagri.info contagri@polj.edu.rs

Original scientific paper

UDC: : 582.711.713:632.95
DOI:10.1515/contagri-2018-0033

ARTICLE ENTITLED: DETERMINATION OF MULTI-CLASS PESTICIDE RESIDUES IN SOUR CHERRIES BY LC-MS/MS *

Vojislava BURSIĆ [1], , Gorica VUKOVIĆ[2], Marina ĐUKIĆ[2], Aleksandra PETROVIĆ[1], Magdalena CARA[3], Dušan MARINKOVIĆ[1], Rada ĐUROVIĆ- PEJČEV[4]

Summary: During 2018 a total of 42 sour cherry samples were collected and analysed for pesticide residues by LC-MS/MS with the carbofuran–D3 and acetamiprid-D3 as internal standards. The study showed that 42.86% of the analysed samples contained pesticide residues. In percentage terms, it seems to be high, but most of the detections were below the recommended MRL values, which is encouraging. The analysis indicated that three of the analysed sour cherry samples (7.14%) contained pesticide residues above the MRLs (propiconazole, methomyl, dimethoate and prochloraz). In spite of the low concentrations, ten samples contained multiple detections, i.e. 55.56% of the analysed samples contained more than one pesticide residue.

Key words: *sour cherries, pesticide residues, LC-MS/MS.*

INTRODUCTION

The Republic of Serbia has exceptionally favourable natural and climatic conditions for growing both cherries and sour cherries. The average number of fertile sour cherry trees in Serbia amounts to 8.6 million. Sour cherries are known to be very adaptive, growing at altitudes of even up to 1000 meters but the most suitable grounds are at altitudes of 400-800 meters. The yield of sour cherries in Serbia is relatively low and amounts to 2.5 tons per hectare, whereas the European average yield is 4.2 tons per hectare. In sour cherry production, Serbia is at the seventh place in the world, accounting for 7% of the total world production (Sredojević, 2011).
In the Republic of Serbia there are 95 plant protection products (PPPs) registered for protection of sour cherry (18 insecticides, 5 acaricides, 56 fungicides, 15 herbicides and one plant growth regulator) (Petrović and Sekulić, 2017).

[1]Vojislava Bursić, PhD, associate professor, Aleksandra Petrović, PhD, assistant professor, Dušan Marinković, PhD, assistant professor, University of Novi Sad, Faculty of Agriculture,
[2]Gorica Vuković, PhD, research assistant, Marina Đukić, MSc, Institute of Public Health, Belgrade, Serbia,
[3]Magdalena Cara, PhD, professor, University of Agriculture, Tirana, Albania,
[4]Rada Đurović-Pejčev, PhD, research assistant, Institute of Pesticides and Environmental Protection Belgrade, Serbia.
* 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".

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

Such a high number of active ingredients (22 fungicide active substances, 5 insecticides, one herbicide and one plant growth regulator) could be present as residues in this fruit, especially taking into account that sour cherries are used as unprocessed food for human consumption (Bursić et al., 2011).

Chromatographic pesticide determination in such a complex matrix presents a unique analytical challenge, considering the high number of planar molecules which could present a problem in determining pesticide residues in sour cherries (Vuković et al., 2016).

Considering all the data, this paper provides an insight into health safety of the sour cherry samples collected from different open-air markets on the territory of Novi Sad, by the validated multi-class liquid chromatography with tandem mass spectrometry (LC-MS/MS) method, used in the positive mode with Electrospray Ionization (+ESI) in accordance with DG SANTE/11812/2017 guidelines (Stojanović et al., 2018). The validated method will be applied for detection and quantification of 70 pesticide residues in 42 sour cherry samples.

The quality of the analysed sour cherries will be determined by the data processing. The degree of health safety of the analysed sour cherry samples will be measured by comparing the results with the established national (Off. Gazz. No 22/2018) and EU maximum pesticide residual levels (EC No 395/2006).

MATERIAL AND METHODS

Chemicals and apparatus. All the used solvents were of chromatography grade and obtained from J.T. Baker (Netherlands). The certified pesticide analytical standard was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and the internal standards carbofuran–D3 and acetamiprid-d3 were purchased from Sigma Aldrich (Cat.No.34019 and 39246). The pesticide stock standard solutions were in the concentration of 1 mg/mL, while the working standards (mix of all investigated pesticides) had the final mass concentration of 10 and 1 μg/mL in acetonitrile (MeCN). Magnesium sulphate, disodium hydrogencitrate sesquihydrate, trisodium citrate dihydrate, sodium chloride and formic acid, primary secondary amine and graphitized black carbon (GBC) were purchased from Fisher Scientific UK (Loughborough, UK). For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system with a binary pump was used. Liquid chromatograph was equipped with a reversed-phase C18 analytical column of 50×4.6 mm and 1.8 μm particle size (Zorbax Eclipse XDB-C18, Agilent). The mobile phase was methanol (solvent A) and Milli-Q water (solvent B), both containing 0.1% formic acid in gradient mode, with the flow rate of 0.4 mL/min. The elution program was started with 90% B and held for 2 min. It was linearly decreased to 20% B in 15 min, 10% B in 20 min, 5% B in 25 min and held constantly for 3 min. The stop time was 28 min with the post run of 5 min. The injection volume was 5 μL. For the mass spectrometric analysis, Agilent 6410B Triple-Quad LC/MS system was applied. Agilent MassHunter B.06.00 software was used for the data acquisition and processing. The analysis was performed in the positive ion modes. The ESI source values were as follows: drying gas (nitrogen) temperature 350 °C, drying gas flow rate 10 L/min, nebulizer pressure 40 psi and capillary voltage 3500 V. The detection was performed using the multiple reactions monitoring mode (MRM).

Validation parameters. The analytical method based on a simple QuEChERS solvent-based extraction was validated according to DG SANTE/11813/2017. The LOD was estimated from the chromatogram of the lowest level of calibration using the Agilent MassHunter software (Agilent Technologies, B.06.00) for the concentrations that provided a signal to noise ratio of 3:1. The LOQ was defined as the lowest validated spike level which meets the requirements of a recovery within the range of 70–120% and a RSD≤20%. The LOQ was determined at 0.01 mg/kg in accordance with Reg. 396/2005. Recovery studies were done at two spiking levels (0.010 and 0.10 mg/kg). The method precision is expressed as the repeatability (RSDr) based on the recovery data.

Sample collection. During 2018 a total of 42 sour cherry samples were collected for pesticide residue analysis. The fruit samples were purchased at open-air markets on the territory of Novi Sad. The sampling was performed in accordance with the general principles and methods of the European Commission (EC) directive 2002/63/EC for establishing MRLs in food commodities. Each representative fruit sample was a composite of 1 kg commodity collected through random sampling. All the samples were placed in polythene bags, labeled, and transported to the laboratory for processing. A representative portion (200 to 250 g) of the samples was chopped into small pieces and blended in order to get a homogenized sample. The homogenized samples were analyzed immediately or stored at -8 ◦C.

Sample preparation. The pesticides were extracted from the cherry samples using an extraction procedure based on the QuEChERS methodology: 100 μL of IS solutions (10 μg/mL) and 10 mL of MeCN were added to 10 g of a fine homogenised sample. After extracting on a vortex mixer for 1 min, 6.0 g of magnesium sulfate anhydrous, 1.5 g of sodium chloride, 1.5 g of trisodium citrate dehydrate and 0.75 g of disodium hydrogencitrate sesquihydrate were added and the mixture was shaken vigorously for 1 min and afterwards centrifuged for 5 min at 4000 rpm. After the centrifugation, 6 mL of supernatant was transferred into a clean-up tube containing 900 mg of $MgSO_4$, 150 mg of

PSA and 150 mg of GBC and shaken vigorously for 2 minutes. After the centrifugation for 5 min at 4000 rpm, 5 μL of supernatant was injected into LC-MS/MS.

RESULTS

The summary of MRM transitions and LC-MS/MS operating parameters selected for the analysis of pesticides and carbofuran–D3 and acetamiprid-D3, as internal standard, in ESI positive mode are given in Table 1.

Table 1. MRM transitions of pesticides, retention times and fragment energies (Frag. (V)) (part of investigated pesticides)

Pesticide	Rt (min)	Transitions, *Q*	Transitions, *q*	Frag.(V)
Acetamiprid-d3	11.13	226.1 -> 125.9		100
Acetamiprid	11.13	223.1 -> 125.8	223.1 -> 55,7	100
Azoxystrobin	16.01	404.1 -> 372.1	404.1 -> 344,1	100
Carbendazim	7.93	192.1 -> 160.1	192.1 -> 132	104
Carbofuran	13.95	222.1 -> 123.0	222.1 -> 165,1	90
Carbofuran-d3	13.93	225.1 -> 165.0	225.1 -> 123	94
Clothianidin	10.35	250.0 -> 132.1	250.0 -> 169,1	90
Cyproconazole 1	16.71	292.1 -> 70.1	292.1 -> 125	120
Cyprodinil	16.75	226.1 -> 93.0	226.1 -> 108	120
Cyromazine	2.21	167.1 -> 85.0	167.1 -> 125,1	100
Dimethoate	11,03	230-> 171	167.1 -> 199	80
Difenconazol	18.25	406.0 -> 251.0	406.0 -> 337	100
Dimethomorph	16.55	388.1 -> 301.1	388.1 -> 165	120
Fenhexamide	17.03	302.1 -> 97.1	302.1 -> 55,1	110
Fenoxycarb	17.43	302.1 -> 88.0	302.1 -> 116,1	100
Fenpropimorph	15.04	304.3 -> 147.1	304.3 -> 130	120
Fluroxypyr-methyl	19.43	367.1 -> 209.0	367.1 -> 255	100
Flusilazole	17.36	316.1 -> 247.1	316.1 -> 165	110
Flutriafol	15.14	302.1 -> 123.0	302.1 -> 70	110
Imazalil	13.99	297.1 -> 159.0	297.1 -> 255	100
Imidacloprid	10.26	256.0 -> 208.7	256.0 -> 174,6	100
Indoxacarb	18.19	528.1 -> 203.0	528.1 -> 150	120
Metalaxyl-M	15.42	280.2 -> 220.1	280.2 -> 192,1	90
Metconazol	18.02	320.0 -> 70.0	320.0 -> 125	100
Methomyl	8.41	163.1 -> 88.0	163.1 -> 106	80
Methoxyfenozide	17.03	369.2 -> 149.1	369.2 -> 133	100
Metsulfuron-methyl	13.96	382.1 -> 167.1	382.1 -> 141,1	120
Myclobutanil	16.71	289.2 -> 70.2	289.2 -> 125,1	150
Oxadixyl	13.14	279.1 -> 219.1	279.1 -> 72	80
Pencycuron	18.18	329.1 -> 125.1	329.1 -> 99,1	120
Phoxim	18.03	299.1 -> 77.0	299.1 -> 129	80
Pirimifos-methyl	18.02	306.2 -> 164.2	306.2 -> 108,2	150
Propoxur	13.79	210.1 -> 168.1	210.1 -> 111	60
Propyconazol	18.23	342.1 -> 159.0	342.1 -> 69	120
Pymetrozin	4.84	218.1 -> 104.9	218.1 -> 78,9	100
Pyraclostrobin	17.96	388.1 -> 194.0	388.1 -> 163	100
Pyrimethanil	15.19	200.1 -> 107.1	200.1 -> 82,1	136

Pyriproxifen	19.33	322.1 -> 227.1	322.1 -> 185,1	120
Spiroxamine	15.42	298.3 -> 144.1	298.3 -> 100.1	130
Tebuconazol	18.02	308.1 -> 125.0	308.1 -> 70	100
Tebufenpyrad	18.92	334.2 -> 145.1	334.2 -> 117	175
Thiamethoxam	8.85	292.0 -> 211.0	292.0 -> 181	80

All the validated parameters were according to DG SANTE/11813/2017 guidelines testing the recovery at two fortification levels. For all the investigated pesticides the recovery was between 70 and 120% (except for acetamipride 64.8% and spiridiclofene 54.3%) with precision lower than 20%. The limit of quantification (LOQ) was 0.01 mg/kg (Stojanović et al., 2018; Bursić et al., 2018).
LC-MS/MS chromatographyc separation of the obtained sour cherries extracts from 42 samples detected ten pesticide residues in eighteen samples, as presented in Table 2.

Table 2. Pesticide residues in sour cherry samples (mg/kg)

Pesticides	1	2	3	4	5	6	7	8	9
Acetamiprid	0.032	0.075	0.050	0.016	0.116	<0.01	0.090	0.017	<0.01
Azoxystrobin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Boscalid	<0.01	0.025	<0.01	<0.01	<0.01	0.033	0.060	<0.01	<0.01
Dimethoate	<0.01	<0.01	<0.01	0.021	<0.01	<0.01	<0.01	0.011	<0.01
Dodin	<0.01	<0.01	<0.01	<0.01	0.013	0.285	0.439	<0.01	<0.01
Carbendazime	<0.01	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Methomyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.024
Prochloraz	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propiconazole	<0.01	<0.01	0.045	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiamethoxam	<0.01	<0.01	<0.01	<0.01	0.013	0.285	0.439	<0.01	<0.01
Pesticides	10	11	12	13	14	15	16	17	18
Acetamiprid	0.011	0.011	<0.01	0.019	0.043	0.011	0.011	0.011	<0.01
Azoxystrobin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.011
Boscalid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dimethoate	<0.01	<0.01	0.204	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dodin	<0.01	<0.01	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	<0.01
Carbendazime	<0.01	<0.01	<0.01	<0.01	<0.01	0.046	<0.01	<0.01	<0.01
Methomyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prochloraz	<0.01	<0.01	0.201	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propiconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiamethoxam	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

The most frequently detected pesticides were acetamiprid with 14 detections, dodine with four and thiametoxam with three detections (Figure 1).

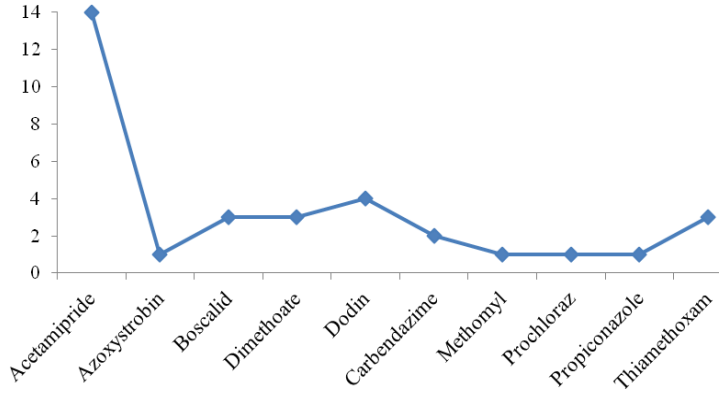

Figure 1. Frequency of pesticide detections in sour cherries samples

DISCUSSION

Based on the presented values of the detected pesticide residues in the sour cherry samples, most of the pesticide residues concentrations were below the maximum residues levels (MRLs) established by the national and European regulations (Off. Gazz. No 22/2018; Reg.EC No 395/2006), except for four detections in three samples. The concentration of propiconazole, methomyl, dimethoate and prochloraz were above the MRLs. The MRLs of the detected pesticides according to the national and EU regulations are given in Table 3.

Table 3. MRLs for detected pesticides and detected concentrations in sour cherries (mg/kg)

Pesticide	MRL mg/kg	C (detected) mg/kg
Acetamiprid	1.5	0.011-0.116
Azoxystrobin	2.0	0.011
Boscalid	4.0	0.033-0.060
Dimethoate	0.02	0.011-0.021
Dodin	3.0	0.013-0.439
Carbendazime	0.5	0.022-0.046
Methomyl	0.01	0.024
Prochloraz	0.05	0.201
Propiconazole	0.01	0.045
Thiamethoxam	0.6	0.013-0.439

Most of the detections were just under the limit of quantification (LOQ), which was set at a level of 0.01 mg/kg. The concentrations of the most frequently detected pesticide – acetamipride ranged from 0.011 to 0.116 mg/kg and did not exceed the MRL of 1.5 mg/kg.

CONCLUSION

The study showed that 42.86% of the analysed sour cherry samples contained pesticide residues. In percentage terms, it seems to be high, but most of the detections were below the recommended MRLs values, which is encouraging. The analysis indicated that three of the analysed sour cherry samples (7.14%) contained the pesticide residues above the MRLs (propiconazole, methomyl, dimethoate and prochloraz). In spite of the low concentrations, ten samples contained multiple detections, i.e. 55.56% of the analysed samples contained more than one pesticide residue. It should also be taken into consideration that the presence of multiple residues is especially dangerous, because it may result in appearance of different forms of combined toxicity with no predictable effects.

On the other hand, plant protection products based on azoxystrobin, methomyl and thiamethoxam are not registered for use in sour cherry protection in the Republic of Serbia.

REFERENCES

BURSIĆ, V., VUKOVIĆ, N., VUKOVIĆ, G., GVOZDENAC, S., ĐUROVIĆ-PEJČEV, R., LJUBOJEVIĆ M., CARA M.: Determination of pesticide in sour cherries using QuEChERS method by LC-MS/MS, 12th European Pesticide Residue Workshop "Pesticides in Food and Drink", 22-25th May, Munich, Germany, Programme and Book of Abstracts, 146, 2018.

BURSIĆ V., LAZIĆ, S., PUCAREVIĆ, M., BAGI, F., VUKOVIĆ, S.: Determination of captan, chlorpyriphos and cypermethrin residues in cherries, Savremena poljoprivreda, 60, 3-4, 359-366, 2011.

PETROVIĆ M., SEKULIĆ J.: Sredstva za zaštitu bilja u prometu u Srbiji (2017), Biljni lekat, 1-2, 2017.

REDOJEVIĆ, Z., Ekonomska analiza proizvodnje, prerade i plasmana trešnje i višnje u Srbiji, USAID-ov Agrobiznis projekat u Srbiji, 2011.

REGULATION (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

SANTE/11813/2017: Method validation and quality control procedures for pesticide residues analysis in food and feed.

SLUŽBENI GLASNIK RS broj 22/2018: 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.

STOJANOVIĆ, T., BURSIĆ, V. ĐUKIĆ, M., VUKOVIĆ, G., PETROVIĆ, A., MARINKOVIĆ, D., ZEREMSKI, T.: Multi-class pesticide residues validation in sour cherries by LC-MS/MS in accordance with SANTE/11813/2017, 11th International scientific/professional conference "Agriculture in nature and environment protection", Vukovar 28-30 May, Proceedings and book of abstracts, 225-228, 2018.

VUKOVIĆ, G., BURSIĆ, V., AGARSKI, M., ZEREMSKI, T., ĐUROVIĆ-PEJČEV, R., MARINKOVI, D., PETROVIĆ, M.: QuEChERS pesticides extraction using EMR sorbent, Second International Symposium of Veteinary Madicine –ISVM, 22-24 June, Belgrade, Serbia, Proceedings, 256-262, 2016.

LC-MS/MS ODREĐIVANJE OSTATAKA PESTICIDA U TREŠNJAMA

Izvod: Analizirano je ukupno 42 uzoraka višanja prikupljenih tokom 2018. godine, LC-MS/MS metodom uz korišćenje karbofurana-D3 i acetamiprida-D3 kao internih standarda. Ostatke pesticida je sadržavalo 42.86% uzoraka. Ovaj procenat je možda velik. ali treba napomenuti da je većina detekcija bilo ispod propisanih MDK vrednosti. Tri uzorka (7,14%) su sadržavali detekcije propikonazola, metomila, dimetoata i prohloraza iznad propisanih vrednosti MDK. Iako su u rezidue pesticida prisutne u niskim koncentracijama, 55,56% uzoraka u kojima su prisutni pesticidi sadrže više od jednog detektovanog pesticida.

Ključne reči: višnje, ostaci pesticida, LC-MS/MS.

Received / Primljen: 04.12.2018.
Accepted / Prihvaćen: 26.12.2018.