


Article

# Plant Protection Products Residues Assessment in the Organic and Conventional Agricultural Production

Vojislava Bursić <sup>1</sup>, Gorica Vuković <sup>2</sup>, Magdalena Cara <sup>3</sup>, Marija Kostić <sup>4</sup>, Tijana Stojanović <sup>1</sup>, Aleksandra Petrović <sup>1</sup>, Nikola Puvača <sup>5</sup> , Dušan Marinković <sup>1,\*</sup> and Bojan Konstantinović <sup>1,\*</sup>

<sup>1</sup> Department for Phytomedicine and Environmental Protection, Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia; bursicv@polj.uns.ac.rs (V.B.); tijana.stojanovic@polj.edu.rs (T.S.); aleksandra.petrovic@polj.uns.ac.rs (A.P.)

<sup>2</sup> Center for Hygiene and Human Ecology, Institute of Public Health of Belgrade, 11000 Belgrade, Serbia; gorica.vukovic@zdravlje.org.rs

<sup>3</sup> Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, 1029 Tirana, Albania; mcara@ubt.edu.al

<sup>4</sup> Faculty of Hotel Management and Tourism, University of Kragujevac, 36210 Vrnjacka Banja, Serbia; marija.kostic@kg.ac.rs

<sup>5</sup> Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, 21000 Novi Sad, Serbia; nikola.puvaca@fimek.edu.rs

\* Correspondence: dusan.marinkovic@polj.uns.ac.rs (D.M.); bojan.konstantinovic@polj.uns.ac.rs (B.K.)

**Abstract:** The organic food is progressively enticing purchasers' attention, as it is recognized to be better than the food produced by the conventional agriculture and more sustainable for the natural environment. Pesticides and their metabolites can enter the human body via food and water. In the food production, over 60 thousand chemical agents are applied, while 90% of the harmful substances are consumed. The organic production is based on the qualitative and healthy food using the natural resources in an ecologically sustainable way. The European Regulations set the maximum pesticide levels (MRLs) in the organic products, which are also regulated by The United States Department of Agriculture in their National program supported by The United States Environmental Protection Agency. It is imperative to bear in mind that in the products from the organic production, the multiple detections cannot be tolerated, i.e., that one product cannot contain more than two detected pesticide residues. In this paper, a multi-residue pesticide method has been developed to determine the pesticides in the agricultural products from the organic and conventional production. In this work, 60 pesticides were analyzed using a simple QuEChERS sample preparation procedure, followed by LC-MS/MS. The tomato, potato, apple, and carrot samples from the organic and conventional products were collected from the market and the pesticide residues assessment comparing the organic to the conventional was done.

**Keywords:** plant protection product residues; organic and conventional agriculture; LC-MS/MS



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## 1. Introduction

The organic food is increasingly attracting the interest of the consumers, as it is perceived to be healthier than the food produced by the conventional agriculture and more sustainable for the environment [1].

It is well-known that the pesticides and their metabolites can be brought into the human body through food and water. There are many efforts from the EU to achieve sustainable use of these compounds to avoid the increase of pesticide levels in the environment and food [2]. Today, in the food production, over 60 thousand chemical agents are used, whereas 90% of the harmful substances are taken with food. However, the increased use of pesticides is of concern to the agricultural workers and food consumers and threatens the environment [3]. That is why, in the last decades, there have been the

growing interest and demand for the organic production [4–8]. The organic production is based on the qualitative and healthy food through the use of natural resources in an ecologically sustainable way [9–11]. This way of the agricultural production, different from the conventional, eliminates the application of the pesticides, growth regulators, synthetic minerals, hormones, fertilizers, antibiotics and additives [1,11]. Additionally, the use of genetically modified organisms is forbidden [12,13]. The ban on the synthetic chemical formulations, which are frequently used in the conventional production for the control of weeds, pests and diseases, represents the greatest problem for the organic food producers [14]. The pesticide contamination of organic products can be induced due to the use of water and soil with pesticide residues [15].

The occurrence of pesticide residues in the organic fruit and vegetables is not enough stated in the scientific literature [16–18]. Tobin et al. [19] detected one or more pesticide residues in 15 out of 27 tested organic samples, with one pesticide being above the LOQ (imazalil in organic onion, 0.11 mg/kg). In the case of the conventional samples, the pesticide residues were present in 17 out of 27 samples in total, with 12 of them being above the LOQ with the concentrations between 0.01 and 0.154 mg/kg. Out of 136 tested organic samples, the authorized pesticide residues were detected in 4 samples, while the non-authorized pesticides were discovered in 61 samples, which was in accordance with the study from Ireland. Namely, the authors detected the pesticide residues in 15 out of the 27 tested organic samples [2].

The studies conducted in Belgium (1995–2001) determined the presence of the pesticide residues in 12% of organic food samples and 49% of the conventional food samples. The monitoring of the German market from Baden-Württemberg (2002–2009) showed that 88% of the conventional raw materials and 27% of organic product samples contained the pesticide residues. The contamination of the organic crops in some European countries is determined as follows: the Czech Republic 14%, Ireland 11%, Finland 5%, Denmark 3% and New Zealand 22% [20,21]. This statistic is also shown in the last EFSA report [22], where organic food encompassed 6.5% of the total samples [23].

It is important that no specific MRLs are established for the organic food produced in accordance with Regulation (EC) No 2018/848 [24]. The MRLs set in Regulation (EC) No 396/2005 [25] apply to the conventional foodstuff.

The maximum residue limits (MRL) in the foodstuff, which represent the maximum residue concentration allowed in the food agricultural commodity, are being controlled by the established legislative framework. Being in accordance with the MRLs is now an obligatory norm for the food security. Depending on the country and the particular commodity, the MRLs can vary, which can be noted in the online databases that contain the summary of their regulatory status in the world [26–28].

We are not able to claim that the organic crops do not contain pesticide residues, as well as that they are truly produced according to the good agricultural practice in the organic production, since the products which authenticity of organic origin cannot be confirmed may be found everywhere throughout the market. There is no doubt that the organic products lack the certification, the continuous supply and a proper retail space, while the consumers rightfully expect the certification, quality and product attributes according to their price. Therefore, the aim of this case study was to compare the detected pesticide residues in organic fruit and vegetable samples with those from the conventional production. For this purpose, a monitoring study was conducted based on 92 commercial samples from the conventional (50) and the organic (42) products from 4 different commodity groups (tomato, potato, apple and carrot). The pesticide residues were analyzed using a simple QuEChERS sample preparation procedure, followed by the liquid chromatography coupled with tandem quadrupole mass spectrometry (LC-MS/MS).

## 2. Materials and Methods

Chemicals and reagents: Acetonitrile and methanol (HPLC grade) were purchased from J.T.Baker (Deventer, Netherlands), acetone was purchased from Merck (Kenilworth,

NJ, USA). The QuEChERS extract tubes (Par No. 5982-5650), as well as the dispersive SPE 15 mL kits for fruits and vegetables, EN (Part No. 5982-5056), were purchased from Agilent Technologies (Santa Clara, CA, USA). The water was purified by Mili-Q plus system from Millipore (18.2 M $\Omega$ -cm, A10 FOCN53824k, USA). The pesticides (60 active substances) and internal standards (IS, carbofuran-D3 and acetamipride-D3) were obtained from Dr. Ehrenstorfer (Munich, Germany) and Sigma Aldrich (Schnelldorf, Germany) and were prepared in acetone, methanol, or acetonitrile (depending on the solubility of the compound) at the concentration nearest to 1.0 mg/mL. Stock solutions were used to prepare working standard solutions (the mix of 60 pesticide active substances in acetonitrile at 1 and 10  $\mu$ g/mL) for the calibration. The calibration curves were prepared in the mobile phase as well as matrix-matched calibration (MMC) used in order to minimize the matrix effects because matrix constituents may increase or decrease the analytical signal. MMC was prepared for each matrix separately, namely for tomato, potato, apple and carrot. For obtaining the analytical curves in the solvent and matrix (recovery calibration) the concentration ranged from 0.005 to 0.10  $\mu$ g/mL.

**Sample collection:** Tomato, potato, apple and carrot samples from the organic and conventional production for multi-pesticide residues quantification were collected from the Serbian largest cities open markets (Belgrade, Novi Sad, Subotica, Niš, Kragujevac and Čačak) (Table 1) according to SANTE/12682/2019. Randomly sampled units in the amount of 1 kg were rapidly (within one day) transported in the polypropylene bags in the clean containers to the laboratory for the homogenization. In case of each sample the information considering the market location, purchase date and variety has been recorded. Until the moment of the preparation and the analysis, which were carried out within 3 days from the purchase date, the samples were stored at 4 °C.

**Table 1.** Number of samples from organic and conventional production.

Commodity Group	Organic Production	Conventional Production
Tomato	10	10
Potato	9	11
Apple	18	21
Carrot	5	8
Total	42	50

**Samples extraction and clean-up procedures:** The agricultural samples were extracted by the QuEChERS method described by Anastassiades et al. [15] and Bursić et al. [29]. For the extraction, the homogenized samples (10.0 g) were weighed into a polypropylene centrifuge tube (50 mL) and spiked with 100  $\mu$ L of ISs. Next, 10 mL of acetonitrile were added, and the mixture was shaken vigorously for 1 min using a vortex mixer. A liquid-liquid partitioning step was performed by adding the QuEChERS extraction kit to the tube and the solution was stirred again for 1 min. After that the mixture was centrifuged for 5 min (at 4000 rpm–1900 g). After the centrifugation, the clean-up step was done based on which an aliquot of 6 mL was transferred to a 15 mL polypropylene centrifuge tube containing dispersive SPE kits for fruits and vegetables. The extract was vigorously shaken for 1 min and centrifuged for 5 min at 4000 rpm (1900 g). Finally, an aliquot of supernatant was filtrated through a PTFE 0.45  $\mu$ m filter and transferred to a vial followed by injecting into the LC-MS/MS.

**LC-MS/MS analysis:** The detection and quantification were performed by the liquid chromatography tandem mass spectrometry equipped with the electrospray ionization (LC(ESI)-MS/MS), 6410B Agilent Technologies. In terms of chromatographic conditions, a Zorbax Eclipse XDBC18 column (50 mm  $\times$  4.6 mm id 1.8  $\mu$ m) was used and kept at 25 °C. The mobile phase consisted of the gradient using methanol with 0.1% formic acid (solvent A) and 0.1% formic acid in water (solvent B), with the following gradient: 0 min–90% B; 2 min–90% B; 15 min 20% B; 20 min–15% B; 25 min–5% B and then returning to the initial conditions in 5 min. The total run time was 30 min. The flow rate of

the mobile phase was 0.4 mL/min and the volume of 5 µL of sample extract was injected into the column. In terms of mass spectrometry, the MS source temperature was set at 350 °C, nitrogen gas flow 10 L/min and nebulizer pressure 40 psi. The data acquisition in the multiple reaction monitoring mode (MRM) was optimized after direct infusion of each pesticide. The instrument uses MassHunter software (vB.06.00, Agilent Technologies, Santa Clara, CA, USA) for the acquisition and quantification [30].

Method validation: All the validation parameters were evaluated following the Document N° SANTE/12682/2019 [31]. The analytical curves linearity was evaluated by injecting the analytical solutions prepared in the solvent and the matrix (tomato, potato, apple and carrot–matrix match calibration-MMC) at 0.005, 0.01, 0.05 and 0.1 µg/mL. The recovery was obtained by spiking the samples with a known amount of the mixture solution in the concentration range at 0.005 and 0.1 mg/kg. For each concentration five replicates were performed. The limit of detection (LOD) was approximated in the MRM mode analysis as the lowest concentration level that yielded a signal-to-noise ratio S/N ratio greater than 5. The limit of quantification (LOQ) of the method was set on 0.005 mg/kg as the most common default LOQ value for pesticide residues, i.e., which is below the MRLs for most pesticides in food [32].

### 3. Results and Discussion

The fragmentation of the protonated molecular ion obtained by LC-MS/MS in the positive electrospray ionization (ESI+) of the examined pesticides is given in Table 2. The selected reaction monitoring mode (SRM) was carried out to obtain the maximum sensitivity for each pesticide detection, while the confirmation of pesticides, two SRM transitions and a correct ratio between the optimized SRM transitions abundance were used taking into account the matching of the Rt (pesticide retention time).

**Table 2.** MRM transitions, fragmentation, and collision energies.

Pesticide	Molecular Formula	M g/Mol	Precursor ion m/z	Product ion m/z	Frag (V)	CE (V)	Rt (min)
Acetamiprid	C <sub>10</sub> H <sub>11</sub> ClN <sub>4</sub>	223	223.0	125.8	120	10	11.45
			223.0	55.7	120	10	
Azoxystrobin	C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub>	403	404.1	372.0	100	9	13.17
			404.1	344.1	100	25	
Aldicarb	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S	190	213	116	120	10	13.90
			213	89	120	15	
Azinphos-ethyl	C <sub>12</sub> H <sub>16</sub> N <sub>3</sub> O <sub>3</sub> PS <sub>2</sub>	346	346	132	120	16	6.20
			346	77.1	120	16	
Bitertanol	C <sub>20</sub> H <sub>23</sub> O <sub>2</sub> N <sub>3</sub>	337.4	338	145	120	20	7.68
			338	117	120	30	
Dimethomorph	C <sub>21</sub> H <sub>22</sub> ClNO <sub>4</sub>	388.1	388.1	301.1	120	30	17.30
			388.1	165	120	20	
Epoxiconazole	C <sub>17</sub> H <sub>13</sub> ClFN <sub>3</sub> O	329.7	330.1	121	130	21	18.13
			330.1	101	130	50	
Ethiofencarb	C <sub>11</sub> H <sub>15</sub> NO <sub>2</sub> S	225.3	226.1	164.1	80	5	14.95
			226.1	107	80	5	
Fenarimol	C <sub>17</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>2</sub> O	331.2	331	268	80	10	18.40
			331	81	80	25	
Fenoxycarb	C <sub>17</sub> H <sub>19</sub> NO <sub>4</sub>	301.4	302.1	116.1	100	5	18.30
			302.1	88	100	20	
Fenpropathrin	C <sub>22</sub> H <sub>23</sub> NO <sub>3</sub>	349.4	350.1	125	135	24	7.51
			350.1	97	135	34	
Fenpropimorph	C <sub>20</sub> H <sub>33</sub> NO	303.5	304.2	147.1	120	30	5.53
			304.2	57.2	100	28	

Table 2. Cont.

Pesticide	Molecular Formula	M g/Mol	Precursor ion m/z	Product ion m/z	Frag (V)	CE (V)	Rt (min)
Fluroxypyr-meptyl	C <sub>15</sub> H <sub>21</sub> Cl <sub>2</sub> FN <sub>2</sub> O <sub>3</sub>	367.2	367	254.9	80	11	7.44
			367	181	80	32	
Flusilazole	C <sub>16</sub> H <sub>15</sub> F <sub>2</sub> N <sub>3</sub> Si	315.4	316.1	247.1	110	12	18.21
			316.1	165	110	20	
Flutriafol	C <sub>16</sub> H <sub>13</sub> F <sub>2</sub> N <sub>3</sub> O	301.2	302.1	70.2	100	18	5.24
			302.1	123.1	100	29	
Phoxim	C <sub>12</sub> H <sub>15</sub> N <sub>2</sub> O <sub>3</sub> PS	298.3	299	129	80	10	17.52
			299	77	80	20	
Hexaconazole	C <sub>14</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O	314.2	314.1	159	100	20	18.80
			314.1	70.1	130	17	
Imazalil	C <sub>14</sub> H <sub>14</sub> Cl <sub>2</sub> N <sub>2</sub> O	297.1	297.1	255	100	15	14.80
			297.1	159	100	23	
Imidacloprid	C <sub>9</sub> H <sub>10</sub> ClN <sub>5</sub> O <sub>2</sub>	255.7	256	208.7	100	15	11.60
			256	174.6	100	20	
Indoxacarb	C <sub>22</sub> H <sub>17</sub> ClF <sub>3</sub> N <sub>3</sub> O <sub>7</sub>	527.8	528.1	203	120	36	18.80
			528.1	150	120	16	
Isoproturon	C <sub>12</sub> H <sub>18</sub> N <sub>2</sub> O	206.3	207	78	135	17	12.40
			207	123	135	17	
Carbaryl	C <sub>12</sub> H <sub>11</sub> NO <sub>2</sub>	201.2	202.1	145	100	10	15.50
			202.1	127	100	35	
Carbendazim	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	191.1	192.1	160.1	104	18	9.35
			192.1	132	104	34	
Carbofuran	C <sub>12</sub> H <sub>15</sub> NO <sub>3</sub>	221.2	222.1	165.1	90	20	15
			222.1	123	90	15	
Carboxin	C <sub>12</sub> H <sub>13</sub> NO <sub>2</sub> S	235.3	236	87	120	20	6.19
			236	143	120	20	
Carbosulfan	C <sub>20</sub> H <sub>32</sub> N <sub>2</sub> O <sub>3</sub> S	380.5	381.2	118.1	31	33	5.52
			381.2	160.1	31	22	
Clothianidin	C <sub>6</sub> N <sub>5</sub> H <sub>8</sub> SO <sub>2</sub> Cl	249.6	250	169.1	90	10	11.80
			250	132.1	90	15	
Kresoxim-methyl	C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub>	313.3	336.2	246.2	120	15	18.40
			336.2	229.2	120	15	
Quintozene	C <sub>6</sub> Cl <sub>5</sub> NO <sub>2</sub>	295.3	237	143	30	10	13.61
			237	119	30	10	
Myclobutanil	C <sub>15</sub> H <sub>17</sub> ClN <sub>4</sub>	288.7	289.2	125.1	150	20	17.78
			289.2	70.2	150	15	
Linuron	C <sub>9</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	249.0	249	182	70	18	9.72
			249	160	70	18	
Malathion	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub>	330.3	331.1	127	90	5	17.6
			331.1	99	90	21	
Metalaxyl	C <sub>15</sub> H <sub>21</sub> NO <sub>4</sub>	279.3	280.2	220.1	120	10	16.3
			280.2	192.1	120	15	
Metamitron	C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O	202.2	203.1	175	115	14	12.78
			203.1	104	115	22	
Methidathion	C <sub>6</sub> H <sub>11</sub> N <sub>2</sub> O <sub>4</sub> PS <sub>3</sub>	302.3	303	165	120	10	12.76
			303	127	120	20	
Methiocarb	C <sub>11</sub> H <sub>15</sub> NO <sub>2</sub> S	225.3	226.1	169	62	6	17.36
			226.1	121	62	18	
Metconazole	C <sub>17</sub> H <sub>22</sub> ClN <sub>3</sub> O	319.8	320	125	100	20	18.88
			320	70	100	20	
Methoxyfenozide	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub>	368.5	369.2	149.1	100	20	17.2
			369.2	133	90	25	
Methomyl	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> S	162.2	163.1	106	80	5	9.8
			163.1	88	80	5	
Nicosulfuron	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O <sub>6</sub> S	410.4	411	182	100	32	4.57
			411	106	100	32	

Table 2. Cont.

Pesticide	Molecular Formula	M g/Mol	Precursor ion m/z	Product ion m/z	Frag (V)	CE (V)	Rt (min)
Oxadixyl	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	278.3	279.1	219.1	80	10	14.35
			279.1	133.3	80	15	
Oxamyl	C <sub>7</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	219.2	237.1	90	60	5	9
			237.1	72	60	10	
Pencycuron	C <sub>19</sub> H <sub>21</sub> ClN <sub>2</sub> O	328.8	329.1	125.1	120	38	17.62
			329.1	99.1	130	35	
Pymetrozine	C <sub>10</sub> H <sub>11</sub> N <sub>5</sub> O	217.2	218	105	120	30	3.61
			218	78	100	20	
Pyraclostrobin	C <sub>19</sub> H <sub>18</sub> ClN <sub>3</sub> O <sub>4</sub>	387.8	388.1	194	100	10	18.6
			388.1	163	100	10	
Pyrimethanil	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub>	199.2	200.1	107.1	136	26	16
			200.1	82.1	136	30	
Pirimiphos-methyl	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>3</sub> PS	305.3	306	164	20	20	7.49
			306	108	20	39	
Pirimicarb	C <sub>11</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>	238.2	239.2	182.1	120	15	12
			239.2	72	120	20	
Pyriproxyfen	C <sub>20</sub> H <sub>19</sub> NO <sub>3</sub>	321.3	322.1	227.1	120	10	20
			322.1	185.1	120	10	
Prochloraz	C <sub>15</sub> H <sub>16</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	376.6	376	308	80	10	18.39
			376	266	80	10	
Propamocarb	C <sub>9</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	188.2	189.1	102	120	20	1.82
			189.1	144	100	20	
Propiconazole	C <sub>15</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	342.2	342.1	159	120	20	18.60
			342.1	69	120	20	
Propyzamide	C <sub>12</sub> H <sub>11</sub> Cl <sub>2</sub> NO	236.3	256.1	190	120	23	5.98
			256.1	173	120	31	
Propoxur	C <sub>11</sub> H <sub>15</sub> NO <sub>3</sub>	209.2	210.1	168.1	60	5	15.10
			210.1	111	60	10	
Spiroxamine	C <sub>18</sub> H <sub>35</sub> NO <sub>2</sub>	297.4	298	144	120	32	5.44
			298	100	100	20	
Tebufenpyrad	C <sub>18</sub> H <sub>24</sub> ClN <sub>3</sub> O	333.8	334.2	145.1	175	24	19.70
			334.2	117	175	32	
Tebuconazole	C <sub>16</sub> H <sub>22</sub> ClN <sub>3</sub> O	307.8	308.1	125	100	25	18.58
			308.1	70	100	25	
Tefluthrin	C <sub>17</sub> H <sub>14</sub> ClF <sub>7</sub> O <sub>2</sub>	418.7	177	137	10	15	14.99
			177	127	10	15	
Thiodicarb	C <sub>10</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> S <sub>3</sub>	354.4	355.1	108	80	10	15.50
			355.1	88	80	15	
Thiacloprid	C <sub>10</sub> H <sub>9</sub> ClN <sub>4</sub> S	252.7	253	186	110	10	13.40
			253	126	110	20	
Trifloxystrobin	C <sub>20</sub> H <sub>19</sub> F <sub>3</sub> N <sub>2</sub> O <sub>4</sub>	408.3	409.1	206.1	120	10	18.95
			409.1	186.1	120	15	

The obtained results indicate a good response linearity in the range of 0.005 to 0.1 µg/mL for all the investigated analytes. Therefore, the method is selective, showing good linearity, expressed by the values of determination coefficient ( $r^2$ ) > 0.99 for all 60 pesticides. The matrix effect (ME) was estimated on matrix and solvent calibration graph slopes and it indicated that tomato, potato, apple and carrot matrix have a strong influence on 60 pesticides. The ME was compensated with MMC.

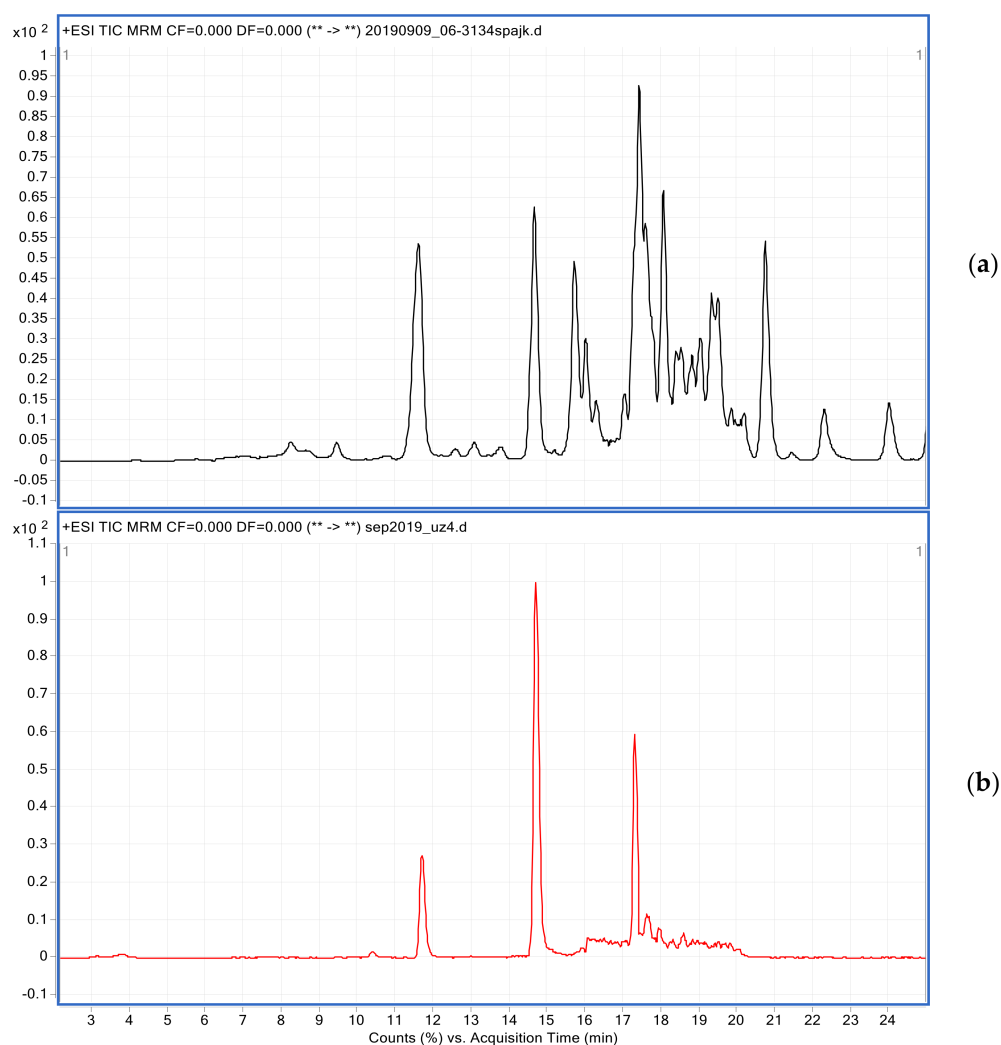
The LOQ as the lowest concentration that will be detected and quantified by an outstanding analytical method with sufficient precision and accuracy was established on 0.005 mg/kg for every pesticide and was confirmed experimentally. The LODs were calculated by MassHunter software and all the values were in the range of 0.001 to 0.003 mg/kg.

The recovery studies were appraised at two levels, spiking blank tomato, apple, carrot and potato samples at 0.01 and 0.1 mg/kg in five replicates (Figure 1). The 53 out of 60 analyzed pesticides showed the recovery ranging from 67.4 to 118.5%. The obtained results are



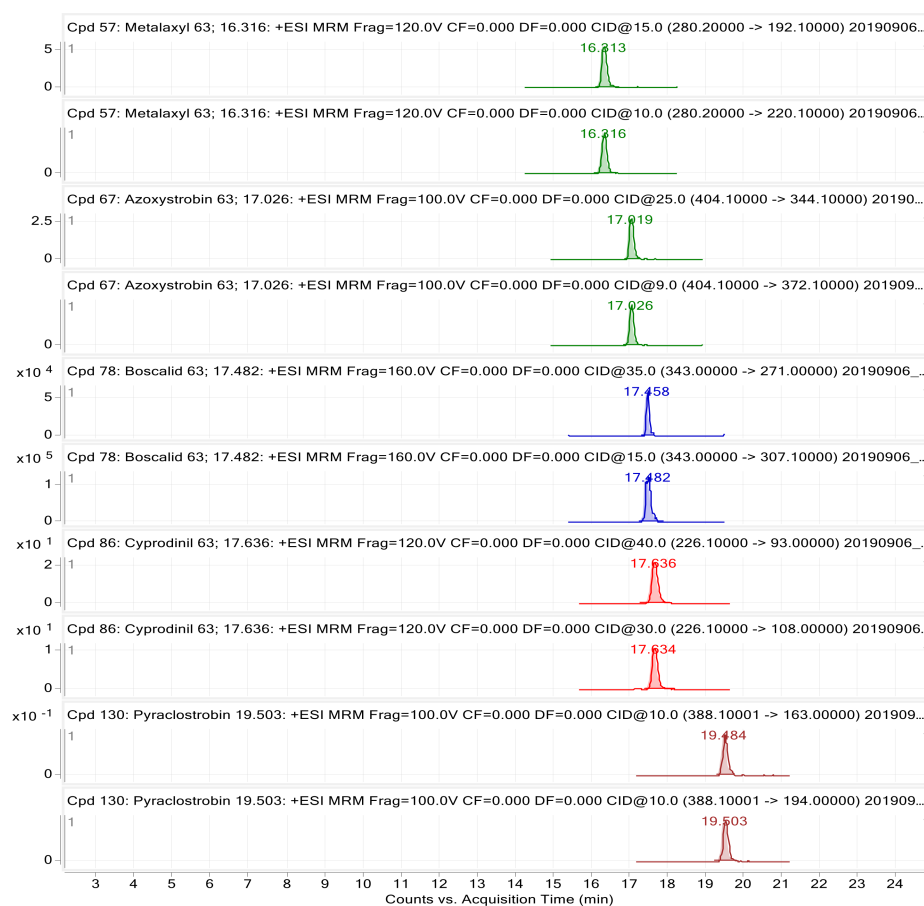
in accordance with those published by Mao et al. [18], whose values for recovery varied from 61.6 to 119.4%. The repeatability, expressed as a relative standard deviation (%RSD), was between 1.87 and 14.73%. Broadly, the accuracy and precision results were tolerable to all investigated pesticides, according to the Document N° SANTE/12682/2019 [31].

According to the validation parameters, LC-MS/MS is a suitable technique for the qualitative and quantitative analysis of 60 pesticide residues in selected matrices-samples. TIC and MRM chromatograms of the pesticides determined in the apple samples from the organic production are given in Figures 1 and 2.

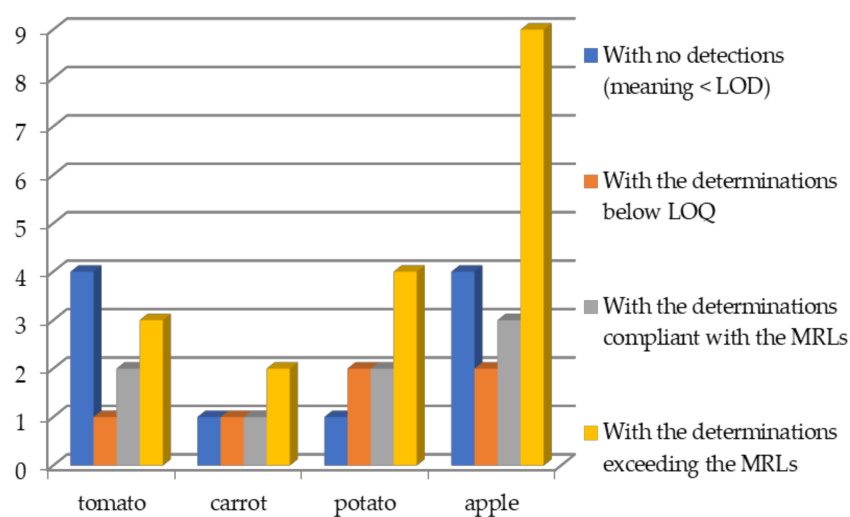


**Figure 1.** TIC chromatograms of (a)—spiked apple sample (0.01 mg/kg) and (b)—analyzed apple sample from organic production.

The results presented in Figures 3 and 4 show the pesticide residues in the investigated samples from the organic (Figure 3) and conventional production (Figure 4) with no detections (meaning  $< \text{LOD}$ ), the samples with the determinations below LOQ, the determinations compliant with the MRLs and the determinations exceeding the MRLs.



**Figure 2.** MRM chromatograms of determined pesticides in apple samples from organic production.



**Figure 3.** Samples from organic production.



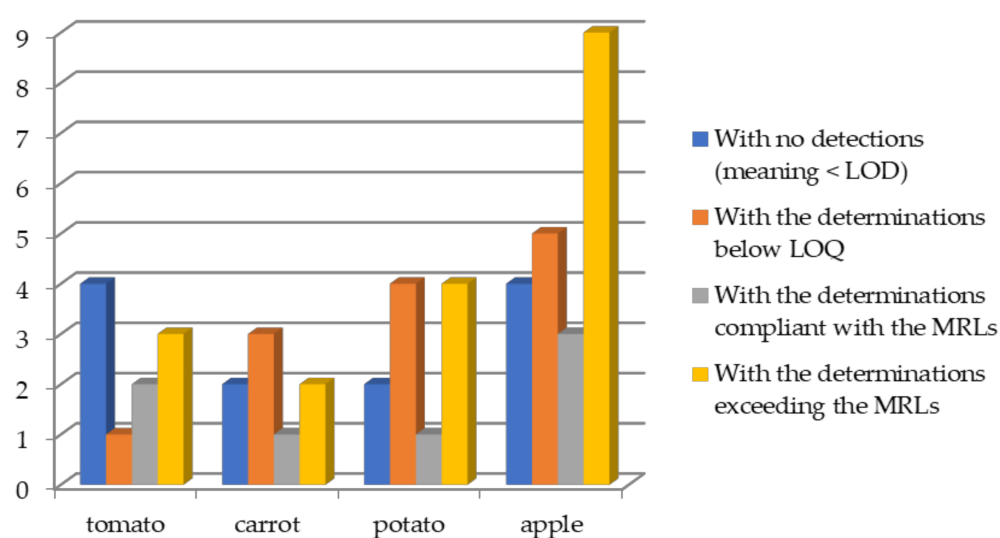


Figure 4. Samples from conventional production.

The EU-harmonized MRLs are set for more than 500 pesticides covering 370 food products/food groups. A default MRL of 0.01 mg/kg is applicable for pesticides not explicitly mentioned in the MRL legislation. The Regulation (EC) No 396/2005 [25] imposes on the Member States the obligation to carry out the controls to ensure that the food placed on the market is compliant with the legal limits. For the organic food items produced following Regulation (EC) No 834/2007 [33] no specific MRLs are established. However, in the Regulation (EC) No 396/2005 [25] in Article 18 it is stated that 0.01 mg/kg is the MRL value for those products for which no specific MRL is set out in Annexes II or III, or for the active substances not listed in Annex IV. The value of 0.01 mg/kg is the usually accepted MRL for organic products. According to Regulation (EC) No 834/2007 [33], the plant protection products should only be used if they are compatible with the objectives and principles of the organic production following the provisions laid down in Article 16(3)(c). Regulation (EC) No 889/2008 [20] lays down the detailed rules for the implementation of Council Regulation (EC) No 834/2007 [33] on organic production and labelling of the organic products. It defines the restricted list of plant protection products that may be used in the organic farming. Most of these substances are exempted from the setting of legal limits under Regulation (EC) No 396/2005 [25], as these substances are listed in Annex IV of the MRL regulation. The EOCC (European Organic Certifiers Council) is an organization of the organic certifiers in Europe. The EOCC has formed a “task force residues”, which developed the “EOCC pesticide residues guideline”, and presented it to the public in 2012. This guideline also follows the BNN (Bundesverband Naturkost Naturwaren) concept of the orientation value of 0.010 mg/kg, but the value is called ‘action level’. This guideline emphasizes the procedural aspects in which certifiers should handle pesticide residues. Together with this guideline, the ‘EOCC task force residues’ has also published a discussion paper in which the possibilities of applying a maximum pesticide level for the organic products are discussed [34]. This maximum level is called ‘critical level’. The task force proposed that the critical level might be set at the value of 10% of the MRL, but does not insist on this particular value. It is extremely important to bear in mind the fact that in the products from the organic production the multiple detections cannot be tolerated, i.e., that one product cannot contain more than two detected pesticide residues concerning the BNN.

The most detected pesticides from the conventional production were fluopyram, difenoconazole, metalaxyl, pyrimethanil, azoxystrobin, boscalid, cyprodinil, pyraclostrobin and deltamethrin. The concentrations were in the range from 0.003 to 0.154 mg/kg. In the samples from the organic production the most frequently detected were fluopyram, difenoconazole, azoxystrobin, boscalid and cyprodinil.

According to Montiel-León et al. [35] the pesticides of great concern these days imply carbamates, neonicotinoids, organophosphates and triazines.

The similar results to those obtained in our case study were published by Mao et al. [18], where the conventional vegetable samples contained multiple pesticide residues compared with those in the organic vegetable samples and most of these residues were detected at higher levels in the conventional than in the organically produced vegetables.

According to Mansour et al. [36], the organic potato tubers sampled from the market have had higher pesticide residue levels than those collected from a specific organic farm. Therefore, along with our results, these findings may give an indication that the data obtained from a single supervised farm may not reflect the market quality where the products from the different agricultural producers could be found. Although the pesticide residues uptake from soils depended on plant variety, the preparation of the products for sale on the market could have a significant influence. For example, Zohair et al. [37] emphasized that washing and peeling carrots or potatoes removed 52–100% of the contaminant residues, which also varied with the crop type and the contaminant amount and properties.

Considering the fact that our samples were taken simultaneously during a week in April, the interesting fact that should not be neglected is the seasonal dynamic of pesticide residue levels. According to Mansour et al. [36], the highest pesticide residue peaks in the conventional potato production were noticeably raised in August, December, February and April, and for the organic potatoes in September. The total pesticide contamination level showed different arrangements: winter > summer > fall > spring in the conventional and fall > summer > winter > spring in the organic potato production.

The tomato, carrot and potato samples are considered to be the organic products based on the pesticide residues. However, the analyzed organic apple sample contained six pesticide residues, with the pyrimethanil and pyraclostrobin residues above the MRLs (for the conventional production) of 0.05 and 0.02 mg/kg, respectively. This sample cannot implement the state established in SANTE/11945/20, as well as IFOAM [38], which allows the pesticide residue detection concerning the measurement of the uncertainty of 50% because we have detections of six pesticide residues.

The apple samples from the conventional production contained four pesticide residues, with azoxystrobin concentration over MRL of 0.01 mg/kg [32]. The conventional tomato and potato did not contain pesticide residues, all detections were under the LOQs. The carrot sample contained fluopyram and difenoconazole with residues being below the MRLs.

Montiel-León et al. [39] conducted the research on 37 samples of apples and determined that 57% of the tested samples contained at least one of the studied pesticides. The most common detected pesticide was acetamiprid, with the detection frequency being 41% and the maximum concentration of 24 µg/kg in the case of the Cortland apple, which was sampled from the conventional production. They also detected carbendazim (detection frequency of 19%), carbaryl (3%) and simazine (5%), as well as some other neonicotinoids: clothianidin (detection frequency of 3%), imidacloprid (16%) and thiacloprid (5%). Their research also comprised the analysis of the tomato samples, the results of which showed that 17% of the tested samples contained at least one of the studied pesticides, all of which were classified as neonicotinoids. The acetamiprid was detected in one sample (detection frequency of 3%) at 16 µg/kg, dinotefuran was found in two samples (concentrations of 13 and 20 µg/kg), while the imidacloprid was registered in 10% of the tested tomato samples (concentrations of 7.6, 10 and 11 µg/kg).

The analysis of the pesticide residues in food is subject to constant modification owing to matrix complexity, low concentrations of the compounds of interest and the increasing number of pesticides approved for use [40]. Namely, LC coupled with a QQQ tandem mass spectrometer, working in the multiple reaction monitoring (MRM) mode is the most frequently applied platform used in the analysis of pesticide residues in food. The most important advantages of validated LC-MS/MS in this study include high sensitivity and selectivity, short duration of analysis, which enables the separation and determination of a considerable number of compounds (60 pesticides with internal standard) during a

single analytical run. The obtained results indicate good response linearity in the range of 0.005 to 0.1 µg/mL for all 60 pesticides ( $r^2 > 0.99$ ). The MMC reduces the matrix effect on the quantification results, especially taking into account that the amount of pesticide residues is in/on the trace levels. Very low LOQ set on 0.005 mg/kg for every pesticide, with the LODs values in the range of 0.001 to 0.003 mg/kg, potentiate the quantification of pesticide residues in the organic food below the 0.01 mg/kg. Additionally, the recovery studies on two spiking levels (0.01 and 0.1 mg/kg) indicate that 88.3% of the investigated pesticides have the recovery in the interval from 67.4 to 118.5%, with the RSD between 1.87 and 14.73%.

The obtained results of the present study provide an indication regarding the pesticide residues in the organic apples. However, they cannot be responsible for the de-characterization of apples as an organically-produced commodity. The amount of the analyzed samples is not high; still, the results of our results accentuate the need for the constant monitoring of the products from the organic, as well as from the conventional production.

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