Pesticide residues in vegetables-validation of the gas chromatography-tandem mass spectrometry multiresidual method and a survey of vegetables on Slovenian market

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Abstract: An analytical method for determining pesticide residues in vegetables was introduced and validated. The extraction was conducted using acetone, dichloromethane and petroleum ether, to enable extraction of active substances with a wide range of polarity. Determination was conducted using gas chromatography coupled with tandem mass spectrometry. Method is according to extraction efficiency and determination sensitivity comparable to other methods for determination of pesticide residues such as QuEChERS. The method was applied in practice. A total of 35 active substances (pesticides) were sought in 50 vegetable samples gathered from Slovenian stores. The active substances sought were not determined in 86.0 % of the samples analysed. Among positive samples in carrot boscalid and fluopyram were found. 21.4 % of carrot samples were positive. In lamb`s lettuce boscalid and fludioxonil were determined. 50 % of lamb`s lettuce samples were positive. In pepper boscalid, fluopyram and pyraclostrobin were found. 16.7 % of pepper samples were positive. In tomato flonicamid, fluopyram and tebuconazole were determined. 11.1 % of tomato samples were positive. The results were compared with those from the literature and the outcome was that vegetables from Slovenia contained boscalid, fluopyram, pyraclostrobin and tebuconazole, which were found also in China, Italy and Turkey.

Key words: vegetables. GC-MS/MS, pesticide residues. multiresidual method

Ostanki fitofarmacevtskih sredstev v zelenjavi - validacija multirezidualne metode s plinsko kromatografijo sklopljeno s tandemsko masno spektrometrijo in preiskava zelenjave na trgu v Sloveniji

Izvleček: Uvedli in validirali smo analizno metodo za določanje ostankov fitofarmacevtskih sredstev v zelenjavi. Ekstrakcijo smo izvedli z acetonom, diklormetanom in petroletrom, ter s tem omogočili ekstrakcijo aktivnih snovi s širokim razponom polarnosti. Določitev smo izvedli s plinsko kromatografijo sklopljeno s tandemsko masno spektrometrijo. Metoda je glede na učinkovitost ekstrakcije in občutljivost pri določitvi, primerljiva z drugimi metodami za določevanje ostankov fitofarmacevtskih sredstev kot je QuEChERS metoda. Metodo smo uporabili v praksi. V 50 vzorcih zelenjave iz slovenskih trgovskih polic smo določali skupno 35 aktivnih spojin (pesticidov). Iskanih aktivnih snovi nismo določili v 86,0 % analiziranih vzorcev. Med pozitivnimi vzorci smo v korenju našli boskalid in fluopiram. 21,4 % vzorcev korenja je bilo pozitivnih. V motovilcu smo določili boskalid in fludioksonil. 50 % vzorcev motovilca je bilo pozitivnih. V papriki smo našli boskalid, fluopiram in piraklostrobin. 16,7 % vzorcev paprike je bilo pozitivnih. V paradižniku smo določili flonikamid, fluopiram in tebukonazol. 11,1 % vzorcev paradižnika je bilo pozitivnih. Rezultate smo primerjali z literaturnimi podatki in ugotovili, da je zelenjava v Sloveniji vsebovala boskalid, fluopiram, piraklostrobin in tebukonazol, ki so jih določili tudi na Kitajskem, v Italiji in Turčiji.

Ključne besede: zelenjava, GC-MS/MS, ostanki fitofarmacevtskih sredstev, multirezidualna metoda

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1 INTRODUCTION

Vegetable is important source of nutrients, vitamins and fibers and is consumed daily by human population. To produce it in quantities large enough for whole population, farmers have to use plant protection products (PPPs) to protect it against numerous diseases and insects attacking vegetables. But demanding consumers require not only healthy but also safe food. Therefore it is important to monitor PPPs residues in food products on the market.

Numerous analytical methods have been developed to analyse PPPs residues in food. In the past there were three main routes for extraction procedure: acetone (Díez et al., 2006, Pizzutti et al., 2009), ethyl acetate (Sharif et al., 2006) and acetonitrile (Anastassiades et al., 2003, Lehotay and Maštovska, 2005, Lehotay, 2007). Nowadays most of laboratories use Quick Easy Cheap Effective Rugged and Safe method also called QuEChERS method, where acetonitrile is used (Calderon et al., 2022, Ngabirano and Birungi, 2022, Sahyoun et al., 2022, Tankiewicz and Berg, 2022). The advantage of this method is, that it is less time consuming and needs lower volumes of organic solvent. In our laboratory we are using method with acetone, to which dichloromethane and petroleum ether were added so that active substances of wide range of polarity can be extracted (Baša Česnik and Gregorčič, 2003, Baša Česnik et al., 2006) from very polar (for instance, flonicamid) to non-polar (for instance, cyhalothrin-lambda). In this paper we present simplified extraction procedure with the same three solvents, which is similarly as the QuEChERS method less time consuming and needs lower volumes of organic solvents as previous one.

Determination of PPPs residues is nowadays usually performed using gas chromatography coupled with mass spectrometry (GC-MS) (Knežević and Serdar, 2009, Santarelli et al., 2018), gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) (Calderon et al., 2022, Ngabirano and Birungi, 2022, Sahyoun et al., 2022, Tankiewicz and Berg, 2022) and/or liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Balkan and Yilmaz, 2022, Qin et al., 2021). The most sensitive is tandem mass spectrometry, which was also used in our laboratory.

Numerous authors have analysed pesticide residues in vegetables with GC-MS/MS. Calderon et al. (2022) analysed 22 active substances in vegetables from Chile and Mexico. Ngabirano and Birungi (2022) analysed 1 active substance in vegetables from Uganda. Sahyoun et al. (2022) tested vegetable samples from France and Lebanon for 14 active substances. Tankiewicz and Berg (2022) introduced a method for determining 31 active substances in Polish vegetables. Up to 11 of active substances sought in these studies were introduced in our study as well. Our selection of active substances was based on both, those authorised for use in Slovenia (94.3 %) and those not authorised for use in Slovenia, but authorised in previous years (5.7 %), the latter to cover misuse of PPPs Of those selected, 57.2 % were fungicides, 25.7 % were acaricides and/or insecticides and 17.1 % were herbicides.

The purpose of this paper is to present the multiresidual GC-MS/MS method introduced for identifying 35 active substances in vegetables using acetone, dichloromethane and petroleum ether as the extraction solvent. The validation parameters for lettuce, potato and tomato are summarised, as well as the practical use of the method on 50 samples of vegetables gathered from Slovenian stores. The most problematic were carrot, where boscalid and fluopyram were found, lamb`s lettuce where boscalid and fludioxonil were determined, pepper where boscalid, fluopyram and tebuconazole were found and tomato where flonicamid, fluopyram and tebuconazole were determined. Concentrations of active substances were in range 0.005–0.060 mg kg^{-1}. All concentrations were below valid Maximum Residue Levels (MRLs). The contents of pesticide residues were compared with those from the literature. Finally, a risk assessment for consumers was conducted.

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

The certified standards were supplied by Dr. Ehrenstorfer (Augsburg, Germany). The acetone - p.a. grade, dichloromethane – p.a. grade, petroleum ether – p.a. grade (used for the extraction procedure) and acetone

HPLC-grade (used for preparation of standards) were supplied by J.T.Baker (Deventer, Netherlands). All other chemicals used were supplied by Sigma-Aldrich (Steinheim, Germany). The water used was MilliQ deionised water.

2.1.2 Preparation of the solutions

Stock solutions in acetone of individual active substances were prepared with the concentrations of 625 µg

Table 1: The active substances sought, their activity type, MRM transitions, dwell time and collision energy

Active substance	Activity type[a]	MRM transitions (Q1, Q2, Q3, Q4)[b]	Dwell (ms)	CE (V)[c]
azoxystrobin	F	**344→329**.1, 344→171.9, 344→155.8	40	10, 40, 40
benthiavalicarb-isopropyl	F	**181→180**, 181→126.9, 181→83.1	20.3, 17.6	20, 40, 40
boscalid	F	**140→112**, 140→76	45.7	10, 30
clomazone	H	204→107, **125→99**	87.2	20, 20
cyflufenamid	F	412→118.1, 412→89.9, **118→90.1**, 118→63	8.2, 8.6	30, 40, 10, 40
cypermethrin	A, I	181→152.1, **181→126.9**, 181→76.9	24.2, 19.7, 19.1, 22.1	30, 40, 40
cyprodinil	F	**225→223.7**, 224→208.1	17.3	20, 20
deltamethrin	I	253→171.9, **253→93.1**, 253→77	26.9	10, 20, 40
fenhexamid	F	301→176.9, **301→97**, 301→54.8	13.5	10, 10, 40
flonicamid	I	**174→146**, 174→126, 174→69	77.6	10, 20, 40
fluazifop-p-butyl	H	383→282.1, **254→146**	8.2	10, 20
fludioxonil	F	248→182.1, 248→154.1, **248→127.1**	9.7	10, 20, 30
flufenacet	H	**151→136.1**, 151→95.1	30.2	10, 30
fluopicolide	F	347→172, **209→182**, 173→145	14.5	30, 20, 10
fluopyram	F	**173→145**, 173→95.1	15.3	20, 30
flutolanil	F	**172.8→145**, 172.8→95, 172.8→75	12.6	15, 35, 55
indoxacarb	I	**264→176**, 264→147.9, 264→112.9	23.7	10, 30, 40
iprovalicarb	F	158→98, **158→72.1**, 158→55.1	8.6, 8.1	10, 10, 20
kresoxim-methyl	F	206→131.1, **206→116.1**	12.7	10, 10
lambda-cyhalothrin	I	**181→152.1**, 181→127.1, 181→77.1	18.6, 17.6	20, 30, 40
metazachlor	H	**209→132.1**, 209→117.1, 133→131.7	14	20, 40, 20
metrafenone	F	408→393, 393→378, **379→364**	29.9	10, 10, 10
myclobutanil	F	**179→125**, 179→90, 179→63	8.6	10, 40, 40
penconazole	F	248→206.1, 248→192.1, **248→157.1**	12.7	10, 10, 30
pendimethalin	H	252→191.1, **252→162.1**, 252→106.1	12.2	10, 10, 40
pirimicarb	I	**238→166.1**, 166→96.1	33.4	10, 10
proquinazid	F	**288→245**, 288→217, 272→216	13.5	10, 30, 20
prosulfocarb	H	**251→128.1**, 162→91.1, 162→65	32.5	10, 10, 40
pyraclostrobin	F	**164→132.1**, 164→104, 132→104	34.1	10, 30, 10
pyrimethanil	F	198→183.1, **198→118**	63.4	20, 40
pyriproxyfen	I	226→186.1, **226→77.1**	21.1	10, 40
tebuconazole	F	250→153, **250→125**, 250→70	10.2	10, 30, 10
tebufenpyrad	A	335→319.9, 333→318.2, **333→276.1**	21.3	10, 10, 10
tefluthrin	I	177→137, **177→127**, 177→87.1	36.6	20, 20, 40
tetraconazole	F	**336→218.1**, 336→164	24.7	20, 30

a A = acaricide, I = insecticide, F = fungicide, H = herbicide
b Q = qualifier ion, bold qualifier was used for integration
c CE = collision energy

pesticide ml⁻¹. From 35 stock solutions, three mixed solutions of all 35 active substances were prepared with a concentration of 5 µg ml⁻¹, 1 µg ml⁻¹ and 0.1 µg ml⁻¹.

2.2 EXTRACTION PROCEDURE

To 20 g of sample in the beaker, 30 ml of acetone : dichloromethane : petroleum ether = 1 (v) : 2 (v) : 2 (v) was added. The mixture was homogenised for 2 minutes with a mixer. 10 g of anhydrous Na_2SO_4 was added. The mixture was homogenised for 2 minutes with a mixer. The whole content was filtered through filter paper black ribbon, which contained 20 g of anhydrous Na_2SO_4, into a 500 ml Soxhlet flask. Matrix was returned to the same beaker, 30 ml of acetone: dichloromethane: petroleum ether = 1 (v): 2 (v): 2 (v) was added, mixture was homogenised for 2 minutes with a mixer and afterwards filtered through the same filter paper as previously. Last step was repeated twice. Then solvent solution in Soxhlet flask was evaporated to approximately 2 ml on a rotavapor and dried with nitrogen flow. The dry eluate was dissolved in 2 ml of acetone for HPLC using ultrasound in order to prepare a sample. Extract was filtered with 0.2 µm pore size filter.

2.3 DETERMINATION

The samples were analysed using a gas chromatograph (Agilent Technologies 8890, Shanghai, China) coupled with tandem mass spectrometer (Agilent Technologies 7010B, Santa Clara, USA), equipped with a Gerstel 20PRE0795 multipurpose sampler (Gerstel, Sursee, Switzerland) and a HP-5 MS UI column (Agilent Technologies, 30 m, 0.25 mm i.d., 0.25 µm film thickness) with a constant flow of helium at 1.2 ml min⁻¹. The GC oven was programmed as follows: 55 °C for 2 min, from 55 °C to 100 °C at 20 °C min⁻¹, from 100 °C to 280 °C at 4 °C min⁻¹, held at 280 °C for 19.75 min. The temperature of the ion source was 230 °C, the auxiliary temperature was 280 °C and the quadrupoles temperature was 150 °C. For qualitative and quantitative determination, the MRM transitions were used. For each active substance two to four transitions, presented in Table 1, were used. The calibration was performed to matrix match standards.

2.4 VALIDATION OF METHODS

Method was validated on three representatives of vegetables: lettuce, which contains a lot of chlorophyll, potato, which contains a lot of starch and tomato which is acidic matrix.

2.4.1 LOQ and linearity

The linearity was verified using the matrix match standards (two repetitions for one concentration level, four to eight concentration levels for the calibration curve). The linearity and range were determined by linear regression, using the F test.

LOQs were estimated from the chromatograms of matrix match standards. LOQs were chosen at a minimum of S/N = 10.

2.4.2 Precision

Blank lettuce, potato and tomato were bought in store and analysed to prove that they contain no pesticide residues. For the determination of precision (ISO 5725), i.e. repeatability and reproducibility, the extracts of spiked blank lettuce, potato and tomato were analysed at LOQ. Within a period of 10 days, two parallel extracts were prepared each day for each concentration level. Each one was injected once. Then the standard deviation of the repeatability of the level and the standard deviation of reproducibility of the level were both calculated.

2.4.3 Uncertainty of repeatability and uncertainty of reproducibility

The uncertainty of repeatability and the uncertainty of reproducibility were calculated by multiplying the standard deviation of repeatability and the standard deviation of reproducibility by the Student's t factor, for nine degrees of freedom and a 95 % confidence level ($t_{95;9}$ = 2.262).

$$U_r = t_{95;9} \times s_r \; ; \; U_R = t_{95;9} \times s_R$$

The measurement uncertainty for PPPs residues should be 50 %, as proposed in SANTE/11312/2021. When validating, analysts must prove that their measurement uncertainty is below or equal to the proposed measurement uncertainty.

Table 2: Vegetable samples collected from stores in Slovenia in 2023 .

No. of sample	Crop	Type of production	Origin	State of crop	Sample mass (kg)
1	brussels sprouts	conventional	Netherlands	fresh	1.1
2	carrot	conventional	Slovenia	frozen	0.9
3	carrot	conventional	Slovenia	frozen	0.9
4	carrot	conventional	Slovenia	fresh	1.3
5	carrot	conventional	Austria	processed	1.2
6	carrot	conventional	Slovenia	processed	2
7	carrot	organic	Italy	fresh	1
8	carrot	organic	Italy	fresh	1
9	carrot	conventional	Slovenia	fresh	1
10	carrot	conventional	Slovenia	fresh	1.1
11	carrot	conventional	Slovenia	fresh	1.2
12	carrot	conventional	Slovenia	fresh	2.5
13	carrot	conventional	Slovenia	fresh	2.5
14	carrot	conventional	Slovenia	fresh	2.5
15	carrot	organic	Italy	fresh	2
16	cauliflower	conventional	unknown	frozen	1
17	cauliflower	conventional	Netherlands	fresh	1.7
18	cauliflower	organic	Italy	fresh	1
19	cauliflower	conventional	Croatia	fresh	1.8
20	kale	conventional	Croatia	fresh	1.2
21	lamb's lettuce	conventional	Italy	fresh	0.5
22	lamb's lettuce	organic	Italy	fresh	0.5
23	lamb's lettuce	conventional	Italy	fresh	0.5
24	lamb's lettuce	conventional	Croatia	fresh	0.5
25	lettuce	conventional	Slovenia	fresh	1
26	lettuce	conventional	Slovenia	fresh	1
27	pepper	organic	Italy	fresh	1.6
28	pepper	organic	Italy	fresh	1.2
29	pepper	organic	Italy	fresh	1.8
30	pepper	conventional	Macedonia	fresh	1.3
31	pepper	conventional	Poland	fresh	1.5
32	pepper	conventional	Italy	fresh	1.2
33	spinach	organic	Italy	frozen	1.2
34	spinach	conventional	Slovenia	frozen	0.9
35	spinach	organic	Italy	fresh	0.5
36	spinach	conventional	Croatia	fresh	0.5
37	spinach	organic	Italy	fresh	1
38	tomato	organic	Italy	processed	1
39	tomato	conventional	Italy	processed	1
40	tomato	conventional	Italy	processed	1
41	tomato	conventional	Croatia	fresh	1.5
42	tomato	conventional	Slovenia	fresh	1.1
43	tomato	conventional	Croatia	fresh	1.2
44	tomato	conventional	Croatia	fresh	1
45	tomato	conventional	Croatia	fresh	1.1
46	tomato	organic	Italy	fresh	1
47	zucchini	conventional	Italy	fresh	1.3
48	zucchini	organic	Italy	fresh	1
49	zucchini	conventional	Croatia	fresh	1.5
50	zucchini	organic	Italy	fresh	1

2.4.4 Accuracy

The accuracy was verified by checking the recoveries. The average of the recoveries from the tests for precision (10 days, 2 parallel samples each day) was calculated. According to the requirements for method validation procedures (SANTE/11312/2021), acceptable mean recoveries are those within the range of 70 % to 120 %, with an associated repeatability of RSDr ≤ 20 %.

According to the guidelines for single-laboratory validation (Alder et al. 2000), acceptable mean recoveries at level > 0.001 mg kg^{-1} ≤ 0.01 mg kg^{-1} are those within the range of 60 % to 120 %, with an associated repeatability RSDr ≤ 30 %.

2.5 CONSUMER RISK ASSESSMENT

Long-term exposure was calculated using the EFSA PRIMo model revision 3.1 (EFSA, 2024). Input values were supervised trial median residues (STMRs) and Acceptable Daily Intakes (ADIs). Chronic consumer exposure was expressed in % of the ADI. The acceptable limit for long-term exposure is 100 % of the ADI.

Short-term exposure was calculated using the EFSA PRIMo model revision 3.1. Input values were the highest residues (HRs) and Acute Reference Doses (ARfDs). Where ARfDs were not allocated, ADIs were used instead. Acute consumer exposure was expressed in % of the ARfD. The acceptable limit for short-term exposure is 100 % of the ARfD.

2.6 SAMPLING

A total of 50 vegetable samples were collected in September 2023 on Slovenian market. The sampling distribution is presented in Table 2. Processed carrot was cooked carrot in salt solution and processed tomato was tomato paste.

3 RESULTS AND DISCUSSION

3.1 COMPARISON OF PREVIOUS AND PRESENT EXTRACTION METHOD WITH ACETONE,

DICHLOROMETHANE AND PETROLEU-METHER

In our previous method (Baša Česnik and Gregorčič, 2003, Baša Česnik et al., 2006) separation of water and organic phase was conducted in separatory funnels, which is time consuming and physically demanding. In present method this phase is no longer required. Water is eliminated by adding anhydrous Na_2SO_4 directly to the mixture of matrix and solvents.

Also, in previous method (Baša Česnik and Gregorčič, 2003, Baša Česnik et al., 2006) 74 ml of acetone p.a., 148 ml of dichloromethane p.a. and 148 ml of petroleum ether p.a. were used per sample. In present method only 18 ml of acetone p.a., 36 ml of dichloromethane p.a. and 36 ml of petroleum ether p.a. were used per sample. Therefore approximately 4-times lower amounts of solvents were used.

3.2 VALIDATION OF METHOD

3.2.1 LOQ and linearity

The linear model is valid for all active substances presented in Tables 3-5. Linearity was proven in the range of 0.005 mg kg^{-1} to 0.04 mg kg^{-1} for all active substances for lettuce, potato and tomato. R^2 ranged from 0.953 to 0.999 for lettuce, from 0.970 to 0.999 for potato and from 0.960 to 0.997 for tomato. Results are presented in Tables 3-5.

3.2.2 Accuracy

The results for the recoveries are given in Tables 3-5. The recoveries at LOQs for the active substances scanned with GC-MS/MS are in the range of 73.4 % to 94.3 %, with RSDs of 11.7 % to 17.8 % for lettuce, 75.0 % to 89.0 %, with RSDs of 8.6 % to 18.3 % for potato and 81.8 % to 100.9 %, with RSDs of 9.6 % to 16.7 % for tomato.

All recoveries and RSDs are within the required ranges from the literature (Alder et al., 2000; SANTE/11813/2017).

3.2.3 Uncertainty of repeatability and uncertainty of reproducibility

Table 3: Validation parameters for lettuce

Active substance	Linearity range (mg kg^{-1})	R^2	LOQ (mg kg^{-1})	Recovery (%)	RSD (%)[a]	U$_r$ (mg kg^{-1})[b]	U$_r$ (%)[c]	U$_R$ (mg kg^{-1})[d]	U$_R$ (%)[e]
azoxystrobin	0.005-0.04	0.985	0.005	80.6	12.3	0.0008	15.9	0.0011	22.7
benthiavalicarb-isopro-pyl	0.005-0.04	0.995	0.005	84.6	16.1	0.0007	14.3	0.0016	31.5
boscalid	0.005-0.04	0.984	0.005	77.1	16.3	0.0010	19.8	0.0014	28.8
clomazone	0.005-0.04	0.953	0.005	88.7	16.7	0.0014	27.1	0.0017	33.9
cyflufenamid	0.005-0.04	0.983	0.005	81.0	13.0	0.0006	12.2	0.0012	24.2
cypermethrin	0.005-0.04	0.994	0.005	78.8	16.2	0.0009	17.5	0.0015	29.4
cyprodinil	0.005-0.04	0.999	0.005	86.5	12.2	0.0006	11.8	0.0012	24.5
deltamethrin	0.005-0.04	0.988	0.005	74.0	17.0	0.0009	18.3	0.0014	28.9
fenhexamid	0.005-0.04	0.995	0.005	84.2	14.3	0.0008	15.9	0.0014	27.7
flonicamid	0.005-0.04	0.993	0.005	93.3	12.5	0.0007	13.1	0.0013	27.0
fluazifop-p-butyl	0.005-0.04	0.995	0.005	82.7	13.1	0.0005	10.9	0.0013	25.1
fludioxonil	0.005-0.04	0.972	0.005	80.8	17.1	0.0008	15.5	0.0016	31.9
flufenacet	0.005-0.04	0.991	0.005	83.4	12.9	0.0010	20.5	0.0012	24.6
fluopicolide	0.005-0.04	0.987	0.005	85.5	13.2	0.0007	13.9	0.0013	26.1
fluopyram	0.005-0.04	0.993	0.005	89.2	11.7	0.0006	12.2	0.0012	24.1
flutolanil	0.005-0.04	0.994	0.005	89.2	11.9	0.0006	12.2	0.0012	24.6
indoxacarb	0.005-0.04	0.980	0.005	73.4	16.9	0.0008	15.1	0.0014	28.6
iprovalicarb	0.005-0.04	0.997	0.005	86.3	13.6	0.0007	13.5	0.0014	27.0
kresoxim-methyl	0.005-0.04	0.990	0.005	82.7	12.0	0.0005	10.7	0.0012	23.0
lambda-cyhalothrin	0.005-0.04	0.991	0.005	82.5	14.1	0.0007	13.8	0.0013	26.9
metazachlor	0.005-0.04	0.980	0.005	85.9	12.5	0.0008	15.6	0.0012	24.6
metrafenone	0.005-0.04	0.982	0.005	79.8	14.1	0.0007	14.7	0.0013	25.9
myclobutanil	0.005-0.04	0.989	0.005	84.5	12.9	0.0006	12.6	0.0013	25.2
penconazole	0.005-0.04	0.999	0.005	89.2	12.4	0.0006	12.9	0.0013	25.4
pendimethalin	0.005-0.04	0.997	0.005	81.1	13.7	0.0007	14.4	0.0013	25.6
pirimicarb	0.005-0.04	0.994	0.005	94.3	12.6	0.0007	13.8	0.0014	27.5
proquinazid	0.005-0.04	0.998	0.005	81.2	13.3	0.0006	12.1	0.0012	25.0
prosulfocarb	0.005-0.04	0.994	0.005	87.4	12.3	0.0006	12.1	0.0012	24.8
pyraclostrobin	0.005-0.04	0.986	0.005	73.5	17.8	0.0009	17.8	0.0015	30.2
pyrimethanil	0.005-0.04	0.988	0.005	91.9	12.4	0.0007	13.5	0.0013	26.4
pyriproxyfen	0.005-0.04	0.995	0.005	80.5	13.7	0.0006	12.4	0.0013	25.4
tebuconazole	0.005-0.04	0.996	0.005	85.0	14.0	0.0007	14.8	0.0014	27.4
tebufenpyrad	0.005-0.04	0.987	0.005	81.1	14.0	0.0007	13.5	0.0013	26.2
tefluthrin	0.005-0.04	0.996	0.005	89.7	12.3	0.0007	13.2	0.0013	25.4
tetraconazole	0.005-0.04	0.998	0.005	89.4	12.1	0.0007	13.2	0.0013	25.0

a RSD was obtained during recovery analyses
b,c Ur = uncertainty of repeatability
d,e UR = uncertainty of reproducibility

Table 4: Validation parameters for potato

Active substance	Linearity range (mg kg⁻¹)	R²	LOQ (mg kg⁻¹)	Recovery (%)	RSD (%)[a]	U_r (mg kg⁻¹)[b]	U_r (%)[c]	U_R (mg kg⁻¹)[d]	U_R (%)[e]
azoxystrobin	0.005-0.04	0.981	0.005	87.9	16.9	0.0009	17.7	0.0017	34.2
benthiavalicarb-isopropyl	0.005-0.04	0.981	0.005	86.4	11.7	0.0007	14.6	0.0012	23.3
boscalid	0.005-0.04	0.984	0.005	86.3	12.4	0.0007	13.9	0.0012	24.8
clomazone	0.005-0.04	0.988	0.005	88.5	14.9	0.0009	18.0	0.0015	30.4
cyflufenamid	0.005-0.04	0.990	0.005	79.2	10.6	0.0006	12.6	0.0010	19.3
cypermethrin	0.005-0.04	0.988	0.005	75.0	17.2	0.0009	17.1	0.0015	29.7
cyprodinil	0.005-0.04	0.997	0.005	82.2	9.3	0.0006	13.0	0.0009	17.4
deltamethrin	0.005-0.04	0.976	0.005	78.1	16.4	0.0009	18.0	0.0015	29.4
fenhexamid	0.005-0.04	0.987	0.005	85.7	12.2	0.0008	16.0	0.0012	24.0
flonicamid	0.005-0.04	0.991	0.005	81.2	9.1	0.0007	14.0	0.0008	16.9
fluazifop-p-butyl	0.005-0.04	0.994	0.005	78.7	11.8	0.0007	13.6	0.0011	21.4
fludioxonil	0.005-0.04	0.980	0.005	82.0	12.2	0.0008	16.4	0.0011	22.9
flufenacet	0.005-0.04	0.992	0.005	82.7	9.3	0.0007	13.2	0.0009	17.5
fluopicolide	0.005-0.04	0.970	0.005	83.6	9.2	0.0007	13.4	0.0009	17.6
fluopyram	0.005-0.04	0.991	0.005	82.6	10.1	0.0006	12.2	0.0010	19.1
flutolanil	0.005-0.04	0.995	0.005	81.5	10.8	0.0006	12.5	0.0010	20.3
indoxacarb	0.005-0.04	0.974	0.005	85.6	18.3	0.0009	18.7	0.0018	36.2
iprovalicarb	0.005-0.04	0.991	0.005	84.4	10.2	0.0006	12.2	0.0010	19.8
kresoxim-methyl	0.005-0.04	0.990	0.005	81.5	9.9	0.0006	12.3	0.0009	18.5
lambda-cyhalothrin	0.005-0.04	0.984	0.005	77.2	13.1	0.0007	14.7	0.0012	23.3
metazachlor	0.005-0.04	0.995	0.005	83.2	9.4	0.0007	13.2	0.0009	17.9
metrafenone	0.005-0.04	0.980	0.005	83.1	12.3	0.0007	14.9	0.0012	23.4
myclobutanil	0.005-0.04	0.988	0.005	83.2	9.7	0.0006	12.9	0.0009	18.5
penconazole	0.005-0.04	0.991	0.005	82.5	9.6	0.0007	13.6	0.0009	18.2
pendimethalin	0.005-0.04	0.992	0.005	75.0	10.9	0.0006	12.4	0.0009	18.7
pirimicarb	0.005-0.04	0.997	0.005	82.1	9.7	0.0007	14.5	0.0009	18.1
proquinazid	0.005-0.04	0.987	0.005	80.9	10.3	0.0007	13.2	0.0010	19.2
prosulfocarb	0.005-0.04	0.996	0.005	79.9	9.2	0.0006	12.8	0.0008	16.8
pyraclostrobin	0.005-0.04	0.977	0.005	89.0	17.7	0.0009	18.4	0.0018	36.4
pyrimethanil	0.005-0.04	0.995	0.005	82.5	8.6	0.0006	12.9	0.0008	16.2
pyriproxyfen	0.005-0.04	0.980	0.005	81.5	11.6	0.0007	14.2	0.0011	21.7
tebuconazole	0.005-0.04	0.984	0.005	84.6	10.2	0.0007	13.9	0.0010	19.9
tebufenpyrad	0.005-0.04	0.980	0.005	81.8	11.4	0.0007	14.2	0.0011	21.4
tefluthrin	0.005-0.04	0.999	0.005	75.0	9.3	0.0006	13.0	0.0008	16.0
tetraconazole	0.005-0.04	0.991	0.005	82.4	10.3	0.0007	14.3	0.0010	19.5

a RSD was obtained during recovery analyses
b,c Ur = uncertainty of repeatability
d,e UR = uncertainty of reproducibility

Table 5: Validation parameters for tomato

Active substance	Linearity range (mg kg⁻¹)	R²	LOQ (mg kg⁻¹)	Recovery (%)	RSD (%)[a]	U_r (mg kg⁻¹)[b]	U_r (%)[c]	U_R (mg kg⁻¹)[d]	U_R (%)[e]
azoxystrobin	0.005-0.04	0.960	0.005	97.1	16.0	0.0009	19.0	0.0018	35.8
benthiavalicarb-isopropyl	0.005-0.04	0.965	0.005	88.7	12.5	0.0010	19.5	0.0013	25.3
boscalid	0.005-0.04	0.963	0.005	90.3	11.2	0.0006	11.7	0.0012	23.4
clomazone	0.005-0.04	0.997	0.005	89.3	16.2	0.0011	21.1	0.0017	33.2
cyflufenamid	0.005-0.04	0.996	0.005	84.1	11.8	0.0009	18.0	0.0011	22.8
cypermethrin	0.005-0.04	0.981	0.005	91.8	14.6	0.0012	23.3	0.0015	30.7
cyprodinil	0.005-0.04	0.995	0.005	84.1	12.6	0.0010	19.6	0.0012	24.2
deltamethrin	0.005-0.04	0.970	0.005	95.1	14.5	0.0008	15.2	0.0016	31.8
fenhexamid	0.005-0.04	0.986	0.005	90.2	12.9	0.0011	22.4	0.0013	26.5
flonicamid	0.005-0.04	0.995	0.005	86.4	13.6	0.0011	22.5	0.0013	26.9
fluazifop-p-butyl	0.005-0.04	0.995	0.005	83.4	12.3	0.0010	20.1	0.0012	23.3
fludioxonil	0.005-0.04	0.991	0.005	87.3	15.2	0.0010	20.1	0.0015	30.6
flufenacet	0.005-0.04	0.996	0.005	89.3	13.7	0.0011	22.2	0.0014	28.0
fluopicolide	0.005-0.04	0.990	0.005	84.7	10.5	0.0009	18.0	0.0010	20.2
fluopyram	0.005-0.04	0.996	0.005	84.4	12.7	0.0010	20.4	0.0012	24.4
flutolanil	0.005-0.04	0.996	0.005	85.1	12.4	0.0010	19.2	0.0012	24.2
indoxacarb	0.005-0.04	0.972	0.005	97.6	15.5	0.0009	17.4	0.0017	34.9
iprovalicarb	0.005-0.04	0.994	0.005	85.4	12.6	0.0010	20.8	0.0012	24.6
kresoxim-methyl	0.005-0.04	0.996	0.005	84.0	12.2	0.0010	19.1	0.0012	23.3
lambda-cyhalothrin	0.005-0.04	0.973	0.005	83.3	11.1	0.0009	17.9	0.0011	21.0
metazachlor	0.005-0.04	0.996	0.005	87.0	13.0	0.0009	18.5	0.0013	26.0
metrafenone	0.005-0.04	0.970	0.005	85.1	10.3	0.0009	17.8	0.0010	19.9
myclobutanil	0.005-0.04	0.994	0.005	84.4	11.2	0.0009	18.3	0.0011	21.6
penconazole	0.005-0.04	0.996	0.005	84.9	12.3	0.0010	19.4	0.0012	23.8
pendimethalin	0.005-0.04	0.993	0.005	83.6	13.5	0.0010	20.3	0.0013	25.9
pirimicarb	0.005-0.04	0.995	0.005	85.7	13.6	0.0011	22.7	0.0013	26.5
proquinazid	0.005-0.04	0.981	0.005	81.8	11.5	0.0009	17.9	0.0011	21.4
prosulfocarb	0.005-0.04	0.996	0.005	84.8	13.6	0.0011	21.6	0.0013	26.2
pyraclostrobin	0.005-0.04	0.977	0.005	100.9	16.7	0.0009	17.1	0.0020	39.0
pyrimethanil	0.005-0.04	0.997	0.005	85.2	13.0	0.0011	21.2	0.0013	25.3
pyriproxyfen	0.005-0.04	0.975	0.005	83.5	9.6	0.0009	17.3	0.0009	18.2
tebuconazole	0.005-0.04	0.981	0.005	85.2	10.2	0.0009	17.6	0.0010	19.8
tebufenpyrad	0.005-0.04	0.980	0.005	84.1	9.8	0.0008	16.3	0.0009	18.7
tefluthrin	0.005-0.04	0.997	0.005	84.8	13.5	0.0011	21.6	0.0013	26.1
tetraconazole	0.005-0.04	0.996	0.005	85.1	12.8	0.0010	19.3	0.0012	25.0

a RSD was obtained during recovery analyses
b,c Ur = uncertainty of repeatability
d,e UR = uncertainty of reproducibility

Table 6: Concentrations and MRLs (mg kg-1) (EC, 2005) of pesticide residues found in 50 vegetable samples

no of sample / active substance	boscalid	flonicamid	fludioxonil	fluopyram	pyraclostrobin	tebuconazole
CARROT						
MRL	2			0.4		
sample no. 4	0.018	-	-	-	-	-
sample no. 9	0.006	-	-	0.006	-	-
sample no. 10	0.005	-	-	0.009	-	-
LAMB`S LETTUCE						
MRL	50		20			
sample no. 21	-	-	0.011	-	-	-
sample no. 23	0.005	-	-	-	-	-
PEPPER						
MRL	3			2	0.5	
sample no. 32	0.060	-	-	0.008	0.027	-
TOMATO						
MRL		0.5		0.5		0.9
sample no. 44	-	0.024	-	0.009	-	0.009

Table 7: Input values for chronic and acute risk assessment

boscalid	carrot		lamb`s lettuce		pepper	
ADI = 0.04 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)	STMR (mg kg-1)	HR (mg kg-1)	STMR (mg kg-1)	HR (mg kg-1)
ARfD = not applicable	0.006	0.018	0.005	0.005	0.06	0.06
flonicamid	tomato					
ADI = 0.025 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)				
ARfD = 0.025 mg kg-1 bw	0.024	0.024				
fludioxonil	lamb`s lettuce					
ADI = 0.37 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)				
ARfD = not applicable	0.011	0.011				
fluopyram	carrot		pepper		tomato	
ADI = 0.012 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)	STMR (mg kg-1)	HR (mg kg-1)	STMR (mg kg-1)	HR (mg kg-1)
ARfD = 0.5 mg kg-1 bw	0.008	0.009	0.008	0.008	0.009	0.009
pyraclostrobin	pepper					
ADI = 0.03 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)				
ARfD = 0.03 mg kg-1 bw	0.027	0.027				
tebuconazole	tomato					
ADI = 0.03 mg kg-1 bw/d	STMR (mg kg-1)	HR (mg kg-1)				
ARfD = 0.03 mg kg-1 bw	0.009	0.009				

ADI = Acceptable daily intake
ARfD = Acute reference dose
HR = Highest residue
STMR = Supervised trial median residue

The uncertainty of repeatability and uncertainty of reproducibility were determined at contents equal to the LOQs. The results are presented in Tables 3-5. Uncertainty of repeatability ranged for lettuce, potato and tomato from 0.0005 mg kg⁻¹ to 0.0014 mg kg⁻¹, which is 10.7 % to 27.1 % of LOQ, from 0.0006 mg kg⁻¹ to 0.0009 mg kg⁻¹, which is 12.2 % to 18.7 % of LOQ and from 0.0006 mg kg⁻¹ to 0.0012 mg kg⁻¹, which is 11.7 % to 23.3 % of LOQ, respectively. Uncertainty of reproducibility ranged for lettuce, potato and tomato from 0.0011 mg kg⁻¹ to 0.0017 mg kg⁻¹, which is 22.7 % to 33.9 % of LOQ, from 0.0008 mg kg⁻¹ to 0.0018 mg kg⁻¹, which is 16.0 % to 36.4 % of LOQ and from 0.0009 mg kg⁻¹ to 0.0020 mg kg⁻¹, which is 18.2 % to 39.0 % of LOQ, respectively.

3.3 SURVEY OF PESTICIDE RESIDUES IN VEGETABLE SAMPLES

In 50 vegetable samples gathered from stores in Slovenia, 35 active substances were sought. Only 14 % of samples analysed contained pesticide residues. 6 active substances were determined at LOQ (0.005 mg kg⁻¹) and up to 0.06 mg kg⁻¹ in carrot, lamb`s lettuce, pepper and tomato. Brussels sprouts, cauliflower, kale, lettuce, potato, spinach and zucchini contained no pesticide residues. Concentrations of all active substances found, were below MRLs. 28 % of samples was of Slovene origin. 21.4 % of samples of Slovene origin and 11.4 % of samples of foreign origin contained pesticide residues. One active substance found is insecticide (flonicamid), the rest 5 are fungicides. Organically produced commodities contained no pesticide residues. 2 active substances (boscalid and fluopiram) were determined in fresh carrot of Slovenian origin. Both of them are authorised for use on carrot in Slovenia. 2 active substances (boscalid and fludioxonil) were determined in fresh lamb`s lettuce of Italian origin. 3 active substances (boscalid, fluopiram and pyraclostrobin) were determined in fresh pepper of Italian origin. 3 active substances (flonicamid, fluopyram and tebuconazole) were determined in fresh tomato of Croatian origin. Results are presented in Table 6.

A consumer risk assessment was performed using the EFSA PRIMo model rev. 3.1, in which 36 national diets from EU countries are included. This model was used since Slovenia has not created a model of its own. The same model is used in the process of registration of PPPs in Slovenia. Input values for chronic (STMRs) and acute risk assessment (HRs) are presented in Table 7. Where ARfD was not allocated, ADI value was used instead. Results of risk assessment are presented in Table 9. The highest chronic exposure was < 1% and the highest acute exposure < 10%. Based on these calculations, the conclusion was that the analysed vegetable samples are of no cause for concern for consumers.

Our results were compared with the results from other scientific papers. Santarelli et al. (2018) found in raw green vegetables marketed in Italy boscalid in 22.67 % of samples, cyprodinil in 6.00 % of samples, deltamethrin in 3.33 % of samples, fludioxonil in 2.33 % of samples, azoxystrobin, lambda-cyhalothrin and fenhexamid each in 1.33 % of samples, and fluopicolide in 0.33 % of samples. In comparison to our study, boscalid was found in 10 % of vegetable samples, fluopyram in 8 % of samples, flonicamid, fludioxonil, pyraclostrobin and tebuconazole each in 2 % of samples. In these two studies considering the same active substances sought, only boscalid and fludioxonil were found in both of them.

Fluopyram was found in the Turkey lettuce up to a concentration of 0.03 mg kg⁻¹ by Balkan and Yilmaz (2022). Balkan and Yilmaz (2022) also reported that pyraclostrobin was found in Turkey lettuce and spinach at a maximum concentration of 0.24 and 0.01 mg kg⁻¹, respectively. Qin et al. (2021) wrote that tebuconazole was found in 14.63 % of the China vegetable samples analysed, with a maximum concentration of 0.36 mg kg⁻¹. Tebuconazole was also found by Balkan and Yilmaz (2022) in Turkey lettuce at a maximum concentration of 0.01 mg kg⁻¹. In Slovenia, fluopyram, pyraclostrobin and tebuconazole were found up to concentration 0.009, 0.027 and 0.009 mg kg⁻¹, respectively. Maximum concentrations found in

Table 8: Input values for chronic and acute risk assessment

active substance	% ADI	% ARfD
boscalid	0.1	9.0
flonicamid	0.1	6.0
fludioxonil	0.0003	0.01
fluopyram	0.3	0.1
pyraclostrobin	0.1	5.0
tebuconazole	0.1	2.0

Slovenia are lower than maximum concentrations from literature.

Other active substances analysed in our laboratory, namely cypermethrin, deltamethrin, kresoxim-methyl, metrafenone, pyrimethanil and lambda-cyhalothrin were not detected in Slovenian vegetables, but were found in samples originating from Chile, China, France, Lebanon, Marocco, Mexico, Turkey and Uganda. Concentrations and/or ratio of positive samples are reported in Table 9.

4   CONCLUSIONS

In our research, a method for determining pesticide residues in vegetables was introduced and validated. The limit of quantification was 0.005 mg kg^{-1} for all active substances. The calibration curves gave a linear response with R^2 0.953 to 0.999. The recoveries ranged from 73.4 % to 100.9 % with RSDs from 8.6 % to 18.3 %. The measurement uncertainty of repeatability ranged from 10.7 to 27.1 % and the measurement uncertainty of reproducibility from 16.0 to 39.0 %. The method was found to be fit for purpose of measuring possible breaches of MRL for 35 active substances.

The method was used to analyse 50 vegetable samples gathered from Slovenian stores from organic and conventional production. A total of 35 active substances were sought, but only the insecticide flonicamid and fungicides boscalid, fludioxonil, fluopyram, pyraclostrobin and tebuconazole were found in 7 of these samples (14.0 %). In 86.0 % of the samples analysed, the active substances sought were not determined. A risk assessment revealed that the Slovenian vegetable samples are no cause for concern for consumers.

In national monitoring program, for analyses of pesticide residues in vegetables, requirement is to analyse 1 sample per matrix from organic production. Despite the fact that we did not detect a violation in either conventional or organic vegetables, we recommend increasing the number of taken ecological samples in the

Table 9: Literature results for active substances sought, but not found in our laboratory

active substance	commodity	max content (mg kg^{-1})	ratio of positive samples (%)	country of origin	reference
cypermethrin	cucumber	1.5	not reported	Lebanon	Sahyoun et al., 2022
cypermethrin	cauliflower	0.0034	not reported	Uganda	Ngabirano e tal., 2022
cypermethrin	tomato	0.0034	not reported	Uganda	Ngabirano e tal., 2022
cypermethrin	lettuce	0.166	50	Chile	Calderon et al., 2022
cypermethrin	tomato	0.064	40	Chile	Calderon et al., 2022
cypermethrin	spinach	0.454	33.3	Mexico	Calderon et al., 2022
cypermethrin	tomato	0.061	12.5	Mexico	Calderon et al., 2022
cypermethrin	lettuce	0.2	not reported	Turkey	Balkan and Yilmaz, 2022
deltamethrin	lettuce	0.11	not reported	Turkey	Balkan and Yilmaz, 2022
kresoxim-methyl	tomato	0.0004	not reported	France	Sahyoun et al., 2022
kresoxim-methyl	lettuce	1.43	not reported	Turkey	Balkan and Yilmaz, 2022
metrafenone	lettuce	3.49	not reported	Turkey	Balkan and Yilmaz, 2022
pyrimethanil	vegetables	0.53	6.5	China	Qin et al., 2021
pyrimethanil	lettuce	0.27	not reported	Turkey	Balkan and Yilmaz, 2022
λ-cyhalothrin	cucumber	0.002	not reported	Lebanon	Sahyoun et al., 2022
λ-cyhalothrin	pepper	0.0015	not reported	Marocco	Sahyoun et al., 2022
λ-cyhalothrin	lettuce	0.028	12.5	Mexico	Calderon et al., 2022
λ-cyhalothrin	spinach	0.043	12.5	Mexico	Calderon et al., 2022

national monitoring program from 1 sample per matrix to approximately 30 % of taken samples per matrix.

6 REFERENCES

Alder L., Hill A., Holland P.T., Lantos J., Lee S.M., Mac-Neil J.D., O'Rangers J., van Zoonen P., Ambrus A. (2000). Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals, *Principles and practices of method validation* (ed.: A. Fajgelj, A. Ambrus). The Royal Society of Chemistry, pp. 179 – 252.

Anastassiades M., Lehotay S. J., Štajnbaher D., Schenck F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and »dispersive solid-phase extraction« for the determination of pesticide residues in produce. *Journal of AOAC International*, 86, 412-431. DOI: 10.1093/jaoac/86.2.412.

Balkan T. and Yilmaz Ö. (2022). Method validation, residue and risk assessment of 260 pesticides in some leafy vegetables using liquid chromatography coupled to tandem mass spectrometry. *Food Chemistry, 384*, 132516-132537. DOI: 10.1016/j.foodchem.2022.132516.

Baša Česnik H., Gregorčič A. (2003). Multirezidualna analizna metoda za določevanje ostankov pesticidov v sadju in zelenjavi. *Zbornik Biotehniške fakultete, Zootehnika, 82*, 167-180.

Baša Česnik H., Gregorčič A., Velikonja Bolta Š., Kmecl V. (2006).Monitoring of pesticide residues in apples, lettuce and potato of the Slovene origin, 2001-04. *Food Additives and Contaminants, 23*, 164-173. DOI:10.1080/02652030500401199.

Calderon R., García-Hernández J., Palma P., Leyva-Morales J.B., Zambrano-Soria M., Bastidas-Bastidas P.J., Godoy M. (2022). Assessment of pesticide residues in vegetables commonly consumed in Chile and Mex-ico: Potential impacts for public health. *Journal of Food Composition and Analysis, 108*, 104420-104430. DOI: 10.1016/j.jfca.2022.104420.

Díez C., Traag W.A., Zommer P., Marinero P., Atienza J. (2006). Comparison of an acetonitrile extraction/partitioning and"dispersive solid-phase extraction" method with classical multi-residue methods for the extraction of herbicide residues in barley samples. *Journal of Chromatography A, 1131*, 11-23. DOI: 10.1016/j.chroma.2006.07.046.

EC (2005). 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.

EFSA (2024), *Pesticide evaluation, Tools*, https://www.efsa.europa.eu/en/applications/pesticides/tools, 7.10.2024.

ISO 5725. (1994). Accuracy (trueness and precision) of measurement methods and results - *Part2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*, pp. 1-42.

Knežević Z. and Serdar M. (2009). Screening of fresh fruit and vegetables for pesticide residues on Croatian market. *Food Control, 20*, 419-422. DOI:10.1016/j.foodcont.2008.07.014.

Lehotay S. J. and Maštovska K. (2005). Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *Journal of AOAC International, 88*, 630-638. DOI:10.1093/jaoac/88.2.630.

Lehotay S. J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *Journal of AOAC International*, 90, 485-520. DOI:10.1093/jaoac/90.2.485.

Ngabirano H. and Birungi G. (2022). Pesticide residues in vegetables produced in rural south-western Uganda. *Food Chemistry, 370*, 130972-130983. DOI: 10.1016/j.foodchem.2021.130972.

Pizzutti I.R., de Kok A., Hiemstra M., Wickert C., Prestes O.D. (2009). Method validation and comparison of acetonitrile and acetone extraction for the analysis of 169 pesticides in soya grain by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1216*, 4539-4552. DOI:10.1016/j.chroma.2009.03.064.

Qin G., Chen Y., He F., Yang B., Zou K., Shen N., Zuo B., Liu R., Zhang W., Li Y. (2021). Risk assessment of fungicide pesticide residues in vegetables and fruits in the mid-western region of China. *Journal of Food Composition and Analysis, 95*, 103663-103670. DOI: 10.1016/j.jfca.2020.103663.

Santarelli G.A., Migliorati G., Pomilio F., Marfoglia C., Centorame P., D'Agostino A., D'Aurelio R., Scarpone N.B., Di Simone F., Aprea G., Iannetti L. (2018). Assessment of pesticide residues and microbial contamination in raw leafy green vegetables marketed in

Italy. *Food Control, 85*, 350-358. DOI: 10.1016/j.foodcont.2017.09.035.

Sahyoun W., Net S., Baroudi M., Ouddane B. (2022). Monitoring of pesticides residues in fruits and vegetables: Method optimization and application. *Food Bioscience, 50*, 102175-102187. DOI: 10.1016/j.fbio.2022.102175.

SANTE/11312/2021. Analytical quality control and method validation procedures for pesticide residues analysis In food and feed. *Sante 11312/2021*. DG SANTE, European Comission, 2021.

Sharif Z., Che Man Y.B., Hamid N.S.A., Keat C.C. (2006). Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using solid phase extraction clean-up cartridges. *Journal of Chromatography A, 1127*, 254-261. DOI: doi:10.1016/j.chroma.2006.06.007.

Tankiewicz M. and Berg A. (2022). Improvement of the QuEChERS method coupled with GC–MS/MS for the determination of pesticide residues in fresh fruit and vegetables. *Microchemical Journal, 181*, 107794-107806. DOI:10.1016/j.microc.2022.107794.