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Multiresidual gas chromatography-tandem mass spectrometry method for determination of plant protection product residues in fruit

Multirezidualna metoda s plinsko kromatografijo sklopljeno s tandemsko masno spektrometrijo za določanje ostankov fitofarmacevtskih sredstev v sadju

Helena BAŠA ČESNIK (✉)

Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

✉ Corresponding author: helena.basa@kis.si

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ABSTRACT

A multiresidual method for the determination of 29 active substances was developed and validated. Acetone, petroleum ether and dichloromethane were used for extraction, and a gas chromatograph coupled with a tandem mass spectrometer was used for determination. A survey was conducted to test the method in practice. Twenty-two samples of apples, apricots, blueberries, grapes, nectarines, oranges, pears, raspberries and strawberries were analysed. The active substances found in the samples were fungicides: boscalid, cyprodinil, fludioxonil, fluopyram and pyrimethanil. Finally, a consumer risk assessment was conducted.

Keywords: apples, grapes, oranges, pesticide residues, gas chromatograph coupled with tandem mass spectrometer, consumer exposure

IZVLEČEK

Razvili in validirali smo multirezidualno metodo za določanje 29 aktivnih substanc. Za ekstrakcijo smo uporabljali aceton, petroleter in diklorometan, za določitev pa plinski kromatograf sklopljen s tandemskim masnim spektrometrom. Za testiranje metode v praksi smo izvedli raziskavo. Analizirali smo 22 vzorcev jabolk, marelic, borovnic, grozdja, nektarin, pomaranč, hrušk, malin in jagod. V vzorcih smo določili fungicide: boskalid, ciprodinil, fludioksonil, fluopiram in pirimetanil. Na koncu smo izvedli oceno tveganja za potrošnika.

Ključne besede: jabolka, grozdje, pomaranče, ostanki pesticidov, plinski kromatograf sklopljen s tandemskim masnim spektrometrom, izpostavljenost potrošnikov

INTRODUCTION

Fruit is part of people`s daily menu because it contains vitamins and fiber that contribute to a healthy diet. But fruit is attacked by numerous diseases and insects as it grows. In order to produce fruit in sufficient quantities, farmers must protect it from mold, rot and insects. Therefore, plant protection products (PPPs) must be used. In the past, it has been observed that fruit generally has the highest number of different active substances (pesticides) compared to vegetables (Baša Česnik et al., 2009). To ensure safe products on the market, monitoring of PPP residues must be carried out. Therefore, reliable multiresidual methods are required, which enable the determination of numerous active substances at the same time.

In the past three main solvents were used for extraction of pesticide residues from fruit: acetone (Luke et al., 1975, 1981), ethyl acetate (Mol et al., 2003; Berrada et al., 2006; Mol et al., 2007) and acetonitrile (Anastassiades et al., 2003, Lehotay et al., 2005). Nowadays, the QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method with acetonitrile is mainly used (Mozzaquatro et al., 2022; Osaili et al., 2022; Sahyoun et al., 2022; Tankiewicz and Berg, 2022; Kottadiyil et al., 2023; Zhang et al., 2024). This method reduces the amount of solvent used and uses buffering for pH-sensitive matrices. Our laboratory has used the acetone method in the past, to which dichloromethane and petroleum ether were added, so that active substances of a wide range of polarity could be extracted (Baša Česnik et al., 2006). In the present paper, we present this method modernized by reducing the amount of solvent fourfold and using a buffer similar to the QuEChERS method.

For determination of active substances mainly gas chromatograph coupled with mass spectrometer (GC-MS) (Bibi et al., 2022; Wang et al., 2022; Asl et al., 2023; Balkan and Karaağaçli, 2023) or gas chromatograph coupled with tandem mass spectrometer (GC-MS/MS) (Mozzaquatro et al., 2022; Osaili et al., 2022; Sahyoun et al., 2022; Tankiewicz and Berg, 2022; Kottadiyil et al., 2023; Zhang et al., 2024) or liquid chromatograph coupled with tandem mass spectrometer (LC-MS/MS) (Concha-meyer et al., 2019; Naik et al., 2022; Liu et al., 2023; Qi et al., 2023; Gkountouras et al., 2024) are nowadays used. Mass spectrometry enables unequivocal identification of substances sought based on the compounds' mass spectra. In our laboratory, GC-MS/MS was used, a sensitive technique, which enables low limits of quantification (LOQs).

Various authors have developed multiresidual methods for determination of PPP residues in fruit using GC-MS/MS: Sahyoun et al. (2022) presented a method for determination of 14 active substances, Zhang et al. (2024) presented a method for determination of 12 active substances, Tankiewicz and Berg (2022) presented a method for determination of 31 active substances, Mozzaquatro et al. (2022) presented a method for determination of 30 active substances and Kottadiyil et al. (2023) presented a method for determination of 32 active substances. Up to 10 active substances reported by these authors were the same as those introduced by our laboratory.

This paper aims to present the validation of the GC-MS/MS multiresidual method for the determination of 29 active substances on apples, grapes and oranges, so that it is suitable for the analysis of fruit samples. The method was tested in practice. 22 samples of apples, apricots, blueberries, grapes, nectarines, oranges, pears, raspberries and strawberries sampled in Slovenian stores were analyzed. The areas of parts of Slovenia from which the samples originated are unknown. Sampling was conducted according to the EU (2002) directive by the author. Finally, consumer risk assessment was conducted for the active substances found.

MATERIALS AND METHODS

Sampling

22 fruit samples were collected in various Slovenian stores in Ljubljana in May and June 2024. The sampling distribution is presented in Table 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 deionized water.

Preparation of solutions

Stock solutions in acetone of individual active substances were prepared with the concentrations of 625 µg pesticide/mL. From 29 stock solutions, three mixed solutions of all 29 active substances were prepared with a concentration of 5 mg/L, 1 mg/L and 0.1 mg/L.

Table 1. Fruit samples collected from stores in Slovenia in 2024

No. of sample	Crop	Type of production	Origin	State of crop	Sample weight (kg)
1	Apples	Conventional	Slovenia	Fresh	2
2	Apples	Conventional	Slovenia	Fresh	1.8
3	Apples	Conventional	Slovenia	Fresh	1.5
4	Apples	Organic	Slovenia	Fresh	1.8
5	Apricots	Organic	Spain	Fresh	1
6	Apricots	Organic	Italy	Fresh	1
7	Apricots	Conventional	Italy	Fresh	1
8	Blueberries	Conventional	Croatia	Fresh	1
9	Blueberries	Organic	Spain	Fresh	1
10	Grapes	Conventional	India	Fresh	2
11	Grapes	Organic	Italy	Fresh	2
12	Nectarines	Conventional	Italy	Fresh	1
13	Nectarines	Organic	Spain	Fresh	1
14	Oranges	Conventional	Spain	Fresh	2.3
15	Oranges	Organic	Italy	Fresh	2
16	Oranges	Conventional	Spain	Fresh	2
17	Pears	Conventional	Netherlands	Fresh	1
18	Pears	Organic	Argentina	Fresh	2
19	Raspberries	Conventional	Slovenia	Fresh	1
20	Raspberries	Conventional	Spain	Fresh	1
21	Strawberries	Organic	Italy	Fresh	0.9
22	Strawberries	Conventional	Slovenia	Frozen	0.9

Extraction procedure

After collection in stores, the quantity of samples with peel provided in Table 1 was homogenized with a mill (Stephan) and frozen until analysis. To 20 g of homogenized sample at room temperature in the beaker, 30 mL of acetone: dichloromethane: petroleum ether = 1:2:2 (V/V/V) and 2 g anhydrous CH_3COONa and 0.4 mL 100% acetic acid were added. The mixture was homogenized for two minutes with a mixer. 10 g of anhydrous Na_2SO_4 was added. The mixture was homogenized for two minutes with a mixer again. The whole content was filtered through filter paper black ribbon (Whatman), which contained 20 g of anhydrous Na_2SO_4, into a 500 mL Soxhlet flask. The sample was returned to the same beaker, and 30 mL of acetone: dichloromethane: petroleum ether = 1:2:2 (V/V/V) was added. The mixture was homogenized for two minutes with a mixer and afterwards filtered through the same filter paper as previously. The last step was repeated twice. The solvent solution in the 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 to prepare a

sample. The extract was filtered with a 0.2 µm pore size filter (Sartorius).

Samples were analysed in one replicate.

Determination

The samples were analyzed 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 an HP-5 MS UI column (Agilent Technologies, 30 m, 0.25 mm i.d., 0.25 µm film thickness) with a constant helium flow at 1.2 mL/min. The GC oven was programmed as follows: 55 °C for 2 min, from 55 °C to 100 °C at 20 °C/min, from 100 °C to 280 °C at 4 °C/min, 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 2, were used. The calibration was performed to matrix-matched standards.

Validation

Limit of quantification, linearity

The limit of quantification (LOQ) was set using matrix-matched standards. The minimum S/N ratio had to be 10. Linearity was checked using matrix-matched standards (two repetitions for one concentration level, three to six concentration levels for the calibration curve). The linearity and range were determined by linear regression, using the F test.

Uncertainty

A blank apple, a grape and oranges were bought in a store and analyzed to prove that they contain no pesticide residues. For the determination of precision (ISO, 2019), i.e. repeatability and reproducibility, the extracts of the spiked blank apple, grape and oranges were analyzed at LOQ. Within 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. 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 PPP residues should be 50%, as proposed in SANTE (2021). When validating, analysts must prove that their measurement uncertainty is below or equal to the proposed measurement uncertainty.

Accuracy

The accuracy was verified by checking the recoveries at the LOQs. The average recoveries and RSDs were calculated from the analyzed extracts used for determination of precision (20 measurements per active substance). According to SANTE (2021), acceptable mean recoveries are those within the range of 70% to 120%, with an associated repeatability of RSD ≤ 20%. In exceptional cases, recoveries can be 30 – 140% if RSDs are ≤ 20%. According to Alder et al. (2000), acceptable mean recoveries at levels> 0.001 mg/kg and ≤ 0.01 mg/kg are those within the range of 60% to 120%, with an associated repeatability RSD ≤ 30%. The same requirement as set by Alder et al. (2000) is set in SANTE (2020).

Consumer risk assessment

Long-term exposure was calculated using the EFSA PRIMo model revision 3.1. Input values were the supervised trial median residues (STMR) 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.

Table 2. 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
Cyprodinil	F	225→223.7, 224→208.1	17.3	20, 20
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
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
Myclobutanil	F	179→125, 179→90, 179→63	8.6	10, 40, 40
Napropamide	H	271→72, 128→100.1, 128→72.1	17.7	20, 10, 10
Penconazole	F	248→206.1, 248→192.1, 248→157.1	12.7	10, 10, 30
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
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
[c] CE = collision energy

Short-term exposure was calculated using the EFSA PRIMo model revision 3.1. Input values were the highest residues (HR) 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.

RESULTS AND DISCUSSION

Comparison of the previous and present extraction methods)

The previous extraction method described by Baša Česnik and Gregorčič (2003) and Baša Česnik et al. (2006) used the same solvents but in four times larger quantities than in the present method. The separation between water and the organic phase was with the old method using separatory funnels. In the present method, anhydrous Na_2SO_4 was added directly to the mixture of sample and organic solvent, thus eliminating the step of separation in separatory funnels, as described by Baša Česnik and Velikonja Bolta (2024) for vegetables. Buffering of matrix with anhydrous CH_3COONa and 100% acetic acid was added in the present method, similarly to the QuEChERS method at the beginning of the extraction procedure, resulting in mainly 10-20% higher recoveries in apple and grape matrices.

Validation of the method

Limit of quantification, linearity

For all 29 substances LOQ was 0.005 mg/kg. Linearity ranged from 0.005 – 0.04 mg/kg with R^2 0.985 to 1.000 for apples, from 0.005 – 0.03 or 0.045 or 0.05 mg/kg with R^2 0.953 to 0.993 for grapes and from 0.005 – 0.05 mg/kg with R^2 0.951 to 0.996 for oranges. Results are presented in Tables 3-5.

Uncertainty

The uncertainty of repeatability and the uncertainty of reproducibility were calculated at LOQ.

For apples, the uncertainty of repeatability ranged from 0.0005 to 0.0009 mg/kg and/or from 10.1 to 18.9%. For grapes, the uncertainty of repeatability ranged from 0.0005 to 0.0012 mg/kg and/or from 10.8 to 23.2%. For oranges, the uncertainty of repeatability ranged from 0.0002 to 0.0009 mg/kg and/or from 4.3 to 17.8%.

For apples, the uncertainty of reproducibility ranged from 0.0010 to 0.0015 mg/kg and/or from 20.4 to 30.6%. For grapes, the uncertainty of reproducibility ranged from 0.0011 to 0.0019 mg/kg and/or from 22.2 to 38.7%. For oranges, the uncertainty of reproducibility ranged from 0.0006 to 0.0013 mg/kg and/or from 12.4 to 26.0%.

All uncertainties were <50% as required by SANTE (2021). Results are presented in Tables 3-5.

Accuracy

For apples, the recoveries at LOQ were 78.1 to 95.8% with RSD 11.0 to 15.1%. For grapes, the recoveries at LOQ were 72.5 to 88.7% with RSD 12.1 to 19.9%. For oranges, the recoveries at LOQ were 62.3 to 86.8% with RSD 6.5 to 14.3%. All recoveries for apples and grapes and most of the recoveries for oranges are in accordance with all three guidelines (Alder et al., 2000; SANTE, 2020; SANTE, 2021). Recoveries of cyflufenamid, fluazifop-p-butyl, kresoxim-methyl, pyriproxyfen, proquinazid, prosulfocarb and tebufenpyrad in oranges are acceptable for the guidelines Alder et al. (2000) and SANTE (2020). Results are presented in Tables 3-5.

Survey of pesticide residues in fruit samples

Thirteen samples of 22 analysed (59.1%) contained pesticide residues. All residues were below the Maximum Residue Levels (MRLs). Nine samples of 22 analysed (40.9%) were pesticide-free. Pesticide residues were found in apples, blueberries, grapes, oranges, pears, raspberries and strawberries. Apricots and nectarines were pesticide-free. In organic samples, no pesticide residues were found. From six samples originating from Slovenia, four contained pesticide residues (66.7%).

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Table 3 Validation parameters for pesticides

Active substance	Linearity range (mg/kg)	R^2	LOQ (mg/kg)	Recovery (%)	RSD[a] (%)	Ur[b] (mg/kg)	Ur[c] (%)	UR[d] (mg/kg)	URe (%)
Azoxystrobin	0.005-0.04	0.997	0.005	95.8	12.9	0.0009	17.8	0.0014	28.5
Benthiavalicarb-isopropyl	0.005-0.04	0.999	0.005	94.6	14.0	0.0008	16.2	0.0015	30.6
Boscalid	0.005-0.04	1.0	0.005	94.1	11.3	0.0008	16.9	0.0012	24.4
Clomazone	0.005-0.04	1.0	0.005	86.7	15.1	0.0007	13.9	0.0015	30.3
Cyflufenamid	0.005-0.04	0.999	0.005	80.6	11.0	0.0005	10.7	0.0010	20.4
Cyprodinil	0.005-0.04	0.999	0.005	84.8	12.4	0.0007	14.1	0.0012	24.2
Flonicamid	0.005-0.04	0.999	0.005	88.2	13.8	0.0008	16.0	0.0014	28.0
Fluazifop-p-butyl	0.005-0.04	0.999	0.005	81.1	12.5	0.0005	10.1	0.0012	23.4
Fludioxonil	0.005-0.04	0.999	0.005	92.2	13.9	0.0009	18.9	0.0015	29.4
Flufenacet	0.005-0.04	0.985	0.005	78.1	14.5	0.0006	12.7	0.0013	26.1
Fluopicolide	0.005-0.04	0.999	0.005	88.8	11.2	0.0007	13.9	0.0011	22.9
Fluopyram	0.005-0.04	1.000	0.005	83.6	12.6	0.0007	13.2	0.0012	24.2
Flutolanil	0.005-0.04	0.999	0.005	84.5	13.0	0.0005	10.9	0.0013	25.4
Iprovalicarb	0.005-0.04	1.0	0.005	88.0	13.3	0.0009	17.1	0.0013	26.0
Kresoxim-methyl	0.005-0.04	0.999	0.005	84.3	11.9	0.0006	12.7	0.0012	23.2
Lambda-cyhalothrin	0.005-0.04	1.0	0.005	88.1	12.9	0.0006	11.9	0.0013	26.2
Metazachlor	0.005-0.04	0.999	0.005	87.0	12.6	0.0008	15.1	0.0013	25.3
Myclobutanil	0.005-0.04	0.998	0.005	86.5	11.2	0.0007	14.0	0.0011	22.3
Napropamide	0.005-0.04	0.999	0.005	83.6	12.6	0.0005	10.7	0.0012	24.4
Penconazole	0.005-0.04	0.998	0.005	84.5	12.3	0.0007	14.3	0.0012	23.9
Pirimicarb	0.005-0.04	0.997	0.005	86.0	13.1	0.0007	14.7	0.0013	26.0
Proquinazid	0.005-0.04	0.998	0.005	84.3	12.8	0.0007	14.8	0.0012	24.8
Prosulfocarb	0.005-0.04	0.997	0.005	82.6	12.6	0.0007	14.2	0.0012	24.0
Pyrimethanil	0.005-0.04	1.0	0.005	84.8	12.4	0.0007	13.2	0.0012	24.2
Pyriproxyfen	0.005-0.04	1.0	0.005	85.9	12.0	0.0009	17.8	0.0012	23.5
Tebuconazole	0.005-0.04	1.0	0.005	89.6	11.1	0.0007	13.0	0.0011	23.0
Tebufenpyrad	0.005-0.04	0.995	0.005	86.5	11.1	0.0008	15.7	0.0011	21.9
Tefluthrin	0.005-0.04	0.999	0.005	78.2	14.7	0.0007	13.3	0.0013	26.5
Tetraconazole	0.005-0.04	0.998	0.005	84.3	12.5	0.0006	12.7	0.0012	24.4

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.

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Table 4 Validation parameters for

Active substance	Linearity range (mg/kg)	R^2	LOQ (mg/kg)	Recovery (%)	RSD[a] (%)	Ur[b] (mg/kg)	Ur[c] (%)	UR[d] (mg/kg)	URe (%)
Azoxystrobin	0.005-0.045	0.964	0.005	82.2	14.5	0.0009	18.7	0.0014	27.4
Benthiavalicarb-isopropyl	0.005-0.045	0.959	0.005	86.3	19.5	0.0011	22.8	0.0019	38.7
Boscalid	0.005-0.045	0.993	0.005	83.4	17.8	0.0005	10.8	0.0017	34.4
Clomazone	0.005-0.05	0.961	0.005	72.5	16.1	0.0008	15.3	0.0013	26.9
Cyflufenamid	0.005-0.045	0.962	0.005	73.7	16.8	0.0007	14.0	0.0014	28.6
Cyprodinil	0.005-0.045	0.969	0.005	80.6	15.2	0.0006	11.4	0.0014	28.4
Flonicamid	0.005-0.05	0.960	0.005	83.1	18.6	0.0007	13.5	0.0018	35.7
Fluazifop-p-butyl	0.005-0.045	0.973	0.005	81.9	14.4	0.0008	15.7	0.0014	27.2
Fludioxonil	0.005-0.03	0.961	0.005	88.7	19.0	0.0012	23.2	0.0019	38.7
Flufenacet	0.005-0.045	0.960	0.005	75.2	19.9	0.0009	18.7	0.0017	34.5
Fluopicolide	0.005-0.045	0.963	0.005	83.8	16.8	0.0007	14.4	0.0016	32.5
Fluopyram	0.005-0.045	0.975	0.005	83.6	16.0	0.0006	11.6	0.0015	31.0
Flutolanil	0.005-0.045	0.960	0.005	80.1	13.1	0.0007	13.1	0.0012	24.1
Iprovalicarb	0.005-0.045	0.975	0.005	86.1	14.6	0.0007	13.4	0.0015	29.0
Kresoxim-methyl	0.005-0.045	0.980	0.005	79.4	15.0	0.0007	14.1	0.0014	27.5
Lambda-cyhalothrin	0.005-0.045	0.973	0.005	80.7	17.6	0.0009	17.2	0.0016	32.7
Metazachlor	0.005-0.045	0.954	0.005	77.6	16.5	0.0006	11.6	0.0015	29.6
Myclobutanil	0.005-0.045	0.968	0.005	79.8	13.8	0.0007	14.7	0.0013	25.4
Napropamide	0.005-0.045	0.975	0.005	80.1	12.1	0.0007	14.8	0.0011	22.2
Penconazole	0.005-0.045	0.973	0.005	80.6	15.5	0.0007	13.7	0.0014	28.9
Pirimicarb	0.005-0.03	0.960	0.005	82.1	13.3	0.0007	13.6	0.0013	25.2
Proquinazid	0.005-0.045	0.966	0.005	77.2	15.4	0.0008	16.6	0.0014	27.3
Prosulfocarb	0.005-0.05	0.960	0.005	76.6	16.7	0.0008	15.7	0.0015	29.4
Pyrimethanil	0.005-0.05	0.959	0.005	81.5	15.1	0.0006	12.3	0.0014	28.6
Pyriproxyfen	0.005-0.045	0.965	0.005	75.7	18.4	0.0009	18.2	0.0016	32.1
Tebuconazole	0.005-0.045	0.953	0.005	83.6	19.2	0.0007	14.2	0.0019	37.2
Tebufenpyrad	0.005-0.045	0.962	0.005	75.9	17.5	0.0008	16.4	0.0015	30.7
Tefluthrin	0.005-0.05	0.974	0.005	73.7	14.0	0.0007	13.6	0.0012	23.8
Tetraconazole	0.005-0.045	0.982	0.005	81.9	17.0	0.0007	13.8	0.0016	32.1

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.

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Table 5 Validation parameters for pesticides

Active substance	Linearity range (mg/kg)	R^2	LOQ (mg/kg)	Recovery (%)	RSD[a] (%)	Ur[b] (mg/kg)	Ur[c] (%)	UR[d] (mg/kg)	URe (%)
Azoxystrobin	0.005-0.05	0.980	0.005	78.8	14.3	0.0007	13.9	0.0013	26.0
Benthiavalicarb-isopropyl	0.005-0.05	0.966	0.005	85.8	11.2	0.0009	17.2	0.0011	21.9
Boscalid	0.005-0.05	0.977	0.005	76.8	10.9	0.0003	6.0	0.0010	19.4
Clomazone	0.005-0.05	0.981	0.005	84.6	7.8	0.0005	9.8	0.0008	15.2
Cyflufenamid	0.005-0.05	0.992	0.005	65.5	13.3	0.0005	9.0	0.0010	20.1
Cyprodinil	0.005-0.05	0.981	0.005	70.8	11.8	0.0004	7.2	0.0010	19.4
Flonicamid	0.005-0.05	0.978	0.005	82.6	7.3	0.0003	5.7	0.0007	14.0
Fluazifop-p-butyl	0.005-0.05	0.988	0.005	64.9	11.9	0.0006	11.2	0.0009	17.7
Fludioxonil	0.005-0.05	0.957	0.005	80.7	8.7	0.0004	8.1	0.0008	16.3
Flufenacet	0.005-0.05	0.951	0.005	81.7	10.7	0.0006	11.8	0.0010	20.2
Fluopicolide	0.005-0.05	0.967	0.005	72.9	8.7	0.0003	6.9	0.0007	14.7
Fluopyram	0.005-0.05	0.981	0.005	74.5	9.7	0.0003	5.7	0.0008	16.7
Flutolanil	0.005-0.05	0.995	0.005	73.5	10.8	0.0003	6.3	0.0009	18.4
Iprovalicarb	0.005-0.05	0.987	0.005	78.4	10.2	0.0003	5.6	0.0009	18.5
Kresoxim-methyl	0.005-0.05	0.983	0.005	63.7	13.0	0.0003	5.6	0.0010	19.2
Lambda-cyhalothrin	0.005-0.05	0.959	0.005	81.7	12.7	0.0009	17.8	0.0012	23.7
Metazachlor	0.005-0.05	0.987	0.005	86.8	6.8	0.0006	11.7	0.0007	13.4
Myclobutanil	0.005-0.05	0.985	0.005	78.3	8.9	0.0003	6.7	0.0008	16.2
Napropamide	0.005-0.05	0.996	0.005	75.8	13.8	0.0003	5.0	0.0012	24.3
Penconazole	0.005-0.05	0.984	0.005	74.8	10.0	0.0003	6.8	0.0009	17.2
Pirimicarb	0.005-0.05	0.966	0.005	84.9	6.5	0.0002	4.3	0.0006	12.8
Proquinazid	0.005-0.05	0.960	0.005	64.5	11.7	0.0002	4.9	0.0009	17.5
Prosulfocarb	0.005-0.05	0.975	0.005	63.7	13.3	0.0004	7.1	0.0010	19.6
Pyrimethanil	0.005-0.05	0.978	0.005	79.0	8.4	0.0005	9.8	0.0008	15.2
Pyriproxyfen	0.005-0.05	0.959	0.005	64.3	13.6	0.0006	12.8	0.0010	20.1
Tebuconazole	0.005-0.05	0.985	0.005	77.8	9.2	0.0004	7.3	0.0008	16.6
Tebufenpyrad	0.005-0.05	0.963	0.005	62.3	12.7	0.0005	10.5	0.0009	18.2
Tefluthrin	0.005-0.05	0.970	0.005	70.1	7.7	0.0003	6.5	0.0006	12.4
Tetraconazole	0.005-0.05	0.976	0.005	73.5	12.0	0.0005	10.2	0.0010	20.4

[a] RSD was obtained during recovery analyses.
[b,c] U_r = uncertainty of repeatability.
[d,e] U_R = uncertainty of reproducibility.

From 16 samples originating from other countries, five contained pesticide residues (31.3%). In all fruit samples, only fungicides were determined. Apples contained fludioxonil, blueberries contained boscalid, grapes contained fluopyram, oranges contained fludioxonil and pyrimethanil, pears and raspberries contained boscalid, cyprodinil and fludioxonil, strawberries contained fluopyram and pyrimethanil. In apples of Slovenian origin, active substances were found which are authorised for use on apples in Slovenia. In the Slovenian strawberry sample, pyrimethanil was found, which is authorised for use on strawberries in Slovenia. In the same sample, fluopyram was also found, which is not authorised for use on strawberries in Slovenia. Results are presented in Table 6.

Table 6. Concentrations and MRLs (mg/kg) of pesticide residues found in 22 fruit samples

Active substance / No of sample	Boscalid	Cyprodinil	Fludioxonil	Fluopyram	Pyrimethanil
			Apples		
MRL			5		
Sample no. 1			0.008		
Sample no. 2			0.006		
			Blueberries		
MRL	15				
Sample no. 8	0.013				
			Grapes		
MRL				1.5	
Sample no. 10				0.014	
			Oranges		
MRL			10		8
Sample no. 14					0.29
Sample no. 16			0.012		0.625
			Pears		
MRL	1.5	2	5		
Sample no. 17	0.116	0.094	0.131		
			Raspberries		
MRL	10	3	5		
Sample no. 19	0.037	0.086	0.068		
			Strawberries		
MRL				2	5
Sample no. 22				0.024	0.005

A consumer risk assessment was performed using the EFSA PRIMo model rev. 3.1, which includes 36 national diets from EU countries. This model was used since Slovenia has not created a model of its own. The same model is also used in the process of registration of PPPs in Slovenia. The input values for chronic (STMRs) and acute risk assessment (HRs) are presented in Table 7. Where ARfD was not allocated, the ADI value was used instead. Results of the risk assessment are presented in Table 8. The highest chronic exposure was 1% and the highest acute exposure 43%. Based on these calculations, the conclusion was that the analysed fruit samples are of no cause for concern for consumers.

Table 7. Input values for chronic and acute risk assessment

Boscalid	Blueberries		Pears		Raspberries	
ADI = 0.04 mg/kg bw per day	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
ARfD = not applicable	0.013	0.013	0.116	0.116	0.037	0.037

Cyprodinil	Pears		Raspberries	
ADI = 0.03 mg/kg bw per day	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
ARfD = not applicable	0.094	0.094	0.086	0.086

Fludioxonil	Apples		Oranges		Pears		Raspberries	
ADI = 0.37 mg/kg bw per day	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
ARfD = not applicable	0.006	0.008	0.012	0.012	0.131	0.131	0.068	0.068

Fluopyram	Grapes		Strawberries	
ADI = 0.012 mg/kg bw per day	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
ARfD = 0.5 mg/kg bw	0.014	0.014	0.024	0.024

Pyrimethanil	Oranges		Strawberries	
ADI = 0.17 mg/kg bw per day	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
ARfD = not applicable	0.29	0.625	0.005	0.005

Table 8. Risk assessment results

Active substance	% ADI	% ARfD
Boscalid	1	40
Cyprodinil	1	43
Fludioxonil	0.2	5
Fluopyram	0.9	0.2
Pyrimethanil	0.7	23

CONCLUSIONS

The multiresidual method for the determination of pesticide residues in fruit was modernized by reducing the amount of solvent by a factor of four and buffering the samples to achieve better recoveries. The validation on apples, grapes and oranges showed that the method is suitable for the determination of 29 active substances. The LOQ for all active substances was 0.005 mg/kg. The calibration curves gave linear responses with R^2 0.951 to 1.0. The recoveries ranged from 62.3 to 95.8% with RSD 6.5 – 19.9% at LOQ. The uncertainties of repeatability ranged from 4.3 to 23.2%, and the uncertainties of reproducibility ranged from 12.4 to 38.7% at LOQ.

The survey on the Slovenian market showed that sampled apples, apricots, blueberries, grapes, nectarines, oranges, pears, raspberries and strawberries contained pesticide residues below the MRL. The active substances found were boscalid, cyprodinil, fludioxonil, fluopyram and pyrimethanil. The consumer risk assessment revealed that the sampled fruit does not represent risk for short-term or long-term exposure.

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