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Validation of the multiresidual GC-MS method for determining plant protection product residues in strawberries

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ABSTRACT

Gas chromatography coupled with mass spectrometry was used for the introduction and validation of the multiresidual method for determining of plant protection product residues in strawberries. During the validation procedure, limits of quantification were set and the method was checked for its recovery, linearity, repeatability, reproducibility and measurement uncertainty. An interlaboratory comparison was also performed to check the accuracy of the method. The method was proven to be fit for purpose. Afterwards 19 strawberry samples were analysed for the presence of plant protection product residues using the validated method. In the strawberries 5 active substances, all fungicides, were found: chlorothalonil, cyprodinil, fludioxonil, metalaxyl+metalaxyl-M and pyrimethanil. Residues of these active substances were in range 0.01 – 0.44 mg/kg. No cases exceeding the maximum residue levels were measured.

Key words: pesticide residues; GC-MS; strawberries; plant protection product residues; multiresidual method

IZVLEČEK

VALIDACIJA MULTIREZIDUALNE GC-MS METODE ZA DOLOČEVANJE OSTANKOV FITOFARMACEVTSKIH SREDSTEV V JAGODAH

Plinsko kromatografijo sklopljeno z masno spektrometrijo smo uporabili za vpeljavo in validacijo multirezidualne metode za določanje ostankov fitofarmacevtskih sredstev v jagodah. Med validacijo smo postavili meje kvantitativne določitve metode in preverili izkoristek, linearnost, ponovljivost, obnovljivost in merilno negotovost metode. Sodelovali smo tudi v medlaboratorijski primerjavi, da smo preverili točnost metode. Za metodo se je izkazalo, da ustreza namenu. Nato smo z validirano metodo ugotavljali prisotnost ostankov fitofarmacevtskih sredstev v 19 vzorcih jagod. V njih smo določili 5 aktivnih spojin: klorotalonil, ciprodinil, fludioksonil, meatalaksil + metalaksil-M in pirimetanil. Ostanki teh aktivnih snovi so se gibali v območju 0,01 – 0,44 mg/kg. Preseganja maksimalnih dovoljenih količin ostankov nismo izmerili.

Ključne besede: ostanki pesticidov; GC-MS; jagode; ostanki fitofarmacevtskih sredstev; multirezidualna metoda

1 INTRODUCTION

Fruit is an important part of our diet for its nutrition and health properties. To prevent the destruction of food crops by agricultural pests and to improve plant quality, plant protection products (PPPs) must be used in fruit production. While monitoring the PPP residues in fruit, vegetables and cereals, we noticed (Baša Česnik et al., 2009) that fruit contains the highest number of active compounds. Farmers need to protect fruit against rot, mould and insects, otherwise the fruit would not grow. Strawberries are mainly attacked by the diseases *Botrytis cinerea* (Persoon), *Colletotrichum acutatum* (J.H. Simmonds), *Oidium fragariae* (Harz) and *Mycospharella fragariae* ((Tul.) Lindau) and by the pests *Steneotarsonemus fragariae* (Banks, 1901), *Anthonomus rubi* (Herbst, 1795), and *Tetranychus urticae (*C. L. Koch, 1836*)* (Sójka et al., 2015). Therefore, the use of PPPs during strawberry growth is inevitable.

Unfortunately, PPP residues can have a negative impact on consumer health when they exceed the Maximum Residue Levels (MRLs). Therefore, the monitoring of PPP residues is necessary. For proper monitoring, efficient analytical methods are required, which enable analysis of large number of active substances and their residues at the same time.

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For determining the PPP residues in strawberries, a number of analytical methods were published. The first step in the methods is usually performed by liquid-liquid extraction, with three main solvents being used: ethylacetate (Berrada et al., 2006; Ferrer et al., 2005), acetonitrile –also known as the QuEChERS method (Bakirci et al., 2014; Lehotay et al., 2007) or acetone (Jardim et al., 2012; Stan, 2000). Our laboratory used acetone because of its high volatility and miscibility with the water present in strawberry matrices. For the better extraction of active substance residues, we added dichloromethane and petroleum ether to the acetone. In this way, a wide range of active substances from medium polar (e.g. diazinon and dimethoate) to non-polar (e.g. chlorpyrifos and cyhalothrin-lambda) were extracted. The extraction of PPP residues from the strawberry matrix is complicated because of its acidity. Therefore, in our laboratory, pH adjustment was used for better extraction efficiency, similar to the in QuEChERS method. CH_3COONa and acetic acid were added to the strawberry matrix, which enhanced the extraction efficiency of pH sensitive active compounds (e.g. pirimicarb and pyrimethanil).

For determining active substance residues, chromatography is usually used. Gas chromatographs (GC), used for non-polar to medium polar and volatile compounds, can be connected to a flame ionisation detector (FID), electron capture detector (ECD), nitrogen phosphor detector (NPD), flame photometric detector (FPD) or mass spectrometer (MS). In our laboratory, an MS was used as this is the only system that enables unequivocal qualitative and quantitative detection of active substance residues based on chromatographic retention time and mass spectra.

The purpose of this paper is to present the introduced, modified (pH adjustment) and then validated gas chromatography-mass spectrometry (GC-MS) method, which enables the qualitative and quantitative determination of a wide range of active compounds in strawberries and their residues in one chromatographic run. Statistical analyses for the obtained data were used: for linearity using the F test, for accuracy by checking recoveries and cooperation in interlaboratory comparisons, for precision according to ISO 5725 standard and for measurement uncertainty by multiplying the standard deviation by Student's t factor for 9 degrees of freedom and a 95% confidence level. Finally, method implementation in practice was performed.

2 MATERIALS AND METHODS

2.1 Materials

Chemicals:
Acetone (Merck), dichloromethane (Merck), ethyl acetate (Merck), cyclohexane (Merck) and petroleum ether (Merck) with p.a. grade and GC grade were used as solvents in our experiment. Similarly, only active substances (dr. Ehrenstorfer, Pestanal) with the highest available purity on the market (a minimum of 95 %) were used.

Preparation of the solutions:
Stock solutions in a mixture of ethyl acetate and cyclohexane in a ratio of 1 to 1 of the individual active substances were prepared in 25 ml volumetric flasks with concentrations of 625 μg pesticide ml^{-1}. From 53 stock solutions, two mixed solutions of all 53 active substances were prepared in 500 ml volumetric flasks: one at a concentration of 5 μg ml^{-1} and the other at the limit of quantification (LOQ) of the active substances. All the solutions used to determine the linearity and LOQs were prepared from the 5 μg ml^{-1} mixed solution with proper dilutions. For other validation parameters, both mixed solutions (5 μg ml^{-1} concentration and the concentration at LOQ) were used. For standard solutions, solvents of GC grade were used.

2.2 Procedure

To 20 g of homogenised blank matrix (milled strawberries, which contain no PPP residues) or homogenised sample, 2 g of anhydrous CH_3COONa was added. Afterwards 40 ml of acetone p.a. and 0.4 ml 100 % acetic acid were added. The mixture was homogenised for 2 minutes with mixer (Ultra-turrax T 25, Ika-Werke). Then 80 ml mixture of petroleum ether p.a. and dichloromethane p.a. at a ratio of 1:1 was added and mixed for another 2 minutes with a mixer. This mixture was filtered into the separatory funnel, containing 3 g of NaCl. The vessel was rinsed with 80 ml of a mixture of petroleum ether p.a. and dichloromethane p.a. at a ratio of 1:1 (v/v). The solvent was added to the separatory funnel, which was shaken for 1 minute. The upper organic phase was filtered through 15 g anhydrous Na_2SO4 in 500 ml Soxhlet flask. The lower water phase was re-extracted twice using the same procedure. Solvents were evaporated to approximately 2 ml on a rotavapor and dried with a nitrogen flow.

8 ml of a mixture of cyclohexane p.a. and ethyl acetate p.a. at a ratio 1:1 (v/v) were added to dry extract. After filtration through a 0.2 μm pore size filter, 5 ml of the extract was cleaned using a gel permeation

chromatograph, containing a column filled with bio-beds SX3. The flow of the mobile phase (ethyl acetate p.a. and cyclohexane p.a., v/v = 1:1) through the GPC column was 5 ml min^{-1}. The 90-200 ml of the eluate was collected into a Soxhlet flask, evaporated to approximately 2 ml on a rotavapor and dried with a nitrogen flow. To the dry eluate, 2 ml of the mixture of ethyl acetate p.a. and cyclohexane p.a. at a ratio of 1:1 (v/v) was added in case of sample preparation. In the case of the matrix match standards, 2 ml of the working solutions with proper concentrations were added.

2.3 Determination

Table 1: Chromatographic conditions of the GC (HP 6890)-MS (HP 5973) system:

Liner:	HP 5181-3316
Temperature of injector:	250 ºC
Injection type:	Pulsed Splitless
Precolumn:	2 m * 0,25 mm
Column:	HP 5 MS, 30 m * 0.25 mm, 0.25 µm film
Temperature gradient of column:	55 ºC 2 min 55 ºC – 130 ºC 25 ºC/min 130 ºC 1 min 130 ºC – 180 ºC 5 ºC/min 180 ºC 30 min 180 ºC – 230 ºC 20 ºC/min 230 ºC 16 min 230 ºC – 250 ºC 20 ºC/min 250 ºC 13 min 250 ºC – 280 ºC 20 ºC/min 280 ºC 20 min
Temperature of ion source:	230 ºC
Temperature of connector:	280 ºC
Temperature of detector:	150 ºC
Carrier gas:	Helium 6.0, 1.2 ml/min constant flow
Volume of injection:	1 µl

Table 2: Detection (selective ion monitoring):

active substance	**T**, Q_1, Q_2, Q3 **(m/z)**
aldrin	**263**, 265, 261
azinphos-methyl	**160**, 132, 105
azoxystrobin	**344,** 388, 345
bifenthrin	**181**, 165, 166
bromopropylate	**183,** 341, 185
bupirimate	**273**, 316, 208
captan	**79**, 107, 119, 149
chlorothalonil	**266**, 264, 268
chlorpropham	**213**, 127, 154
chlorpyriphos	**314**, 316, 197
chlorpyriphos-methyl	**286**, 288, 125
cyhalotrin-λ	**181**, 197, 208
cypermethrin (four isomers)	**181,** 163, 165
cyprodinil	**224,** 225, 210
DDT (5 isomers)	DDD-o,p: **235**, 237, 165
	DDD-p,p and DDT-o,p: **235**, 237, 165
	DDE-p,p: **318**, 246, 248
	DDT-p,p: **235**, 237, 165

active substance	**T**, Q_1, Q_2, Q3 **(m/z)**
deltamethrin	**181**, 251, 255
diazinon	**179**, 304, 199
dichlofluanid	**226,** 123, 167
dimethoate	**87**, 229, 143
diphenylamine	**169,** 167, 168
endrin	**263**, 261, 265
fenitrothion	**277**, 260, 109
fenthion	**278**, 279, 280
fludioxonil	**248**, 154, 127
folpet	**260**, 262, 130
HCH-alpha	**219**, 181, 183
heptachlor	**272**, 274, 270
heptenophos	**124**, 215, 250
iprodione	**314**, 316, 187
kresoxim-methyl	**116,** 206, 131
lindane	**183,** 219, 181
mecarbam	**131**, 159, 329
metalaxyl+metalaxyl-M	**249,** 206 , 234
methidathion	**145**, 85, 125
myclobutanil	**179,** 288, 150
parathion	**291**, 292, 235
penconazole	**248,** 159, 161
permethrin (2 isomers)	**183**, 163, 165
phosalone	**182**, 367, 121
pirimicarb	**166,** 238, 167
pirimiphos-methyl	**290**, 305, 276
propyzamide	**173,** 175, 145
pyridaphenthion	**199,** 340, 188
pyrimethanil	**198,** 199, 200
quinalphos	**146,** 298, 157
spiroxamine (2 isomers)	**100,** 126, 198
tolclofos-methyl	**265,** 267, 250
tolylfluanid	**238,** 137, 240
triadimefon	**208,** 210, 181
triadimenol (2 isomers)	**112,** 168, 128
triazophos	**161**, 162, 285
trifloxystrobin	**116,** 222, 186
vinclozolin	**285**, 124, 187

2.4 Sampling

Strawberry samples were randomly taken in May and June 2007 directly in the field after the expiration of pre-harvest interval. Samples originated from 6 production areas in Slovenia: Celje, Kranj, Ljubljana, Maribor, Murska Sobota and Novo mesto.

3 RESULTS AND DISCUSSION

The previous protocol for the determination of PPP residues in fruit and vegetables was published before (Baša Česnik et al., 2006). The disadvantage of this procedure was, that when it was used for strawberries, some active substances were not extracted at all. Recoveries of previous procedure were compared to recoveries of new procedure (the one that includes pH adjustment) for two parallel samples of blank strawberries (strawberries that contained no PPP residues) spiked at level 0.2 mg kg^{-1}. The new procedure differs from old procedure only in step where the anhydrous CH_3COONa and the 100 % acetic acid are added to the sample. pH adjustment enabled extraction of bupimirate, pirimicarb, pyrimethanil and spiroxamine, where recoveries were 0 without pH adjustment.

3.1 Linearity and limits of quantification

Linearity was verified using the matrix match standards (five repetitions for one concentration level, three to seven concentration levels for the calibration curve). The linearity and range were determined by linear regression using the F test. The linear model is fit and remains linear throughout the range presented in Table 1. The limits of quantification (LOQs) were estimated from chromatograms of the matrix match standards. LOQs were chosen at S/N = 10. The LOQ is the lowest value of the linearity range for particular active substance presented in Table 3.

Table 3: Linearity

active substance	linearity range (mg kg^{-1})	R^2	active substance	linearity range (mg kg^{-1})	R^2
aldrin	0.005-0.2	0.997	heptenophos	0.01-0.2	0.997
azinphos-methyl	0.01-0.2	0.989	iprodione	0.01-0.2	0.995
azoxystrobin	0.04-0.2	0.985	kresoxim-methyl	0.02-0.2	0.995
bifenthrin	0.01-0.2	0.997	lindane	0.01-0.2	0.997
bromopropylate	0.01-0.2	0.997	mecarbam	0.04-0.2	0.995
bupirimate	0.02-0.2	0.995	metalaxyl+metalaxyl-M	0.01-0.2	0.998
captan	0.1-0.2	0.994	methidathion	0.01-0.2	0.995
chlorothalonil	0.01-0.2	0.995	myclobutanil	0.05-0.2	0.996
chlorpropham	0.01-0.2	0.997	parathion	0.03-1.0	0.992
chlorpyriphos	0.01-0.2	0.997	penconazole	0.01-0.2	0.996
chlorpyriphos-methyl	0.02-0.2	0.997	permethrin	0.02-0.2	0.994
cyhalotrin-lambda	0.01-0.5	0.977	phosalone	0.01-0.2	0.993
cypermethrin	0.03-0.2	0.991	pirimicarb	0.01-0.2	0.997
cyprodinil	0.01-0.2	0.996	pirimiphos-methyl	0.01-0.2	0.998
DDT	0.05-1.0	0.997	propyzamide	0.01-0.2	0.997
deltamethrin	0.03-0.2	0.989	pyridaphenthion	0.01-1.0	0.991
diazinon	0.01-0.2	0.998	pyrimethanil	0.01-0.2	0.997
dichlofluanid	0.01-0.2	0.997	quinalphos	0.01-0.2	0.996

active substance	linearity range (mg kg^{-1})	R^2	active substance	linearity range (mg kg^{-1})	R^2
dimethoate	0.01-0.2	0.995	spiroxamine	0.02-1.0	0.993
diphenylamine	0.01-0.2	0.996	tolclofos-methyl	0.01-0.2	0.997
endrin	0.01-0.2	0.996	tolylfluanid	0.01-0.2	0.996
fenitrothion	0.01-1.0	0.991	triadimefon	0.02-0.2	0.997
fenthion	0.005-0.2	0.996	triadimenol	0.02-0.2	0.994
fludioxonil	0.01-0.2	0.992	triazophos	0.01-0.2	0.992
folpet	0.02-1.0	0.988	trifloxystrobin	0.03-0.2	0.996
HCH-alpha	0.005-0.2	0.997	vinclozolin	0.01-0.2	0.997
heptachlor	0.005-0.2	0.998			

3.2 Accuracy

Accuracy was verified by checking the recoveries. Ten extracts of spiked blank strawberry homogenate (milled strawberries that contained no PPP residues) were prepared for each spiking level in the shortest period possible. Each extract was injected twice. The average of the recoveries was calculated. According to the requirements for the method validation procedures (Document N° SANTE/11945/2015), acceptable mean recoveries are those within the range of 70-120 %, with an associated repeatability $RSD_r \leq 20$ %. Our recoveries of the spiking level at LOQ ranged from 96.6 % to 105.4 % with $RSD_r \leq 15$ %, except for HCH-alpha were the RSD_r was 23 %. At spiking level 0.2 mg kg^{-1}, recoveries ranged from 96.8 % to 99.9 % with $RSD_r \leq 13$ %.

According to the guidelines for single-laboratory validation (Alder et al., 2000), the acceptable mean recoveries:

- at level > 0.1 mg kg^{-1} ≤ 1 mg kg^{-1} are within the range 70-110 %, with an associated repeatability RSDr ≤ 15 %,
- at level > 0.01 mg kg^{-1} ≤ 0.1 mg kg^{-1} are within the range 70-120 %, with an associated repeatability RSDr ≤ 20 % and
- at level > 0.001 mg kg^{-1} ≤ 0.01 mg kg^{-1} are within the range 60-120 %, with an associated repeatability RSDr ≤ 30 %.

These requirements were achieved for all 53 active compounds. The results are given in Table 4.

Table 4: Recoveries for spiked strawberry blank matrix

active substance	spiking level (mg kg^{-1})	recovery (%)	RSD (%)	spiking level (mg kg^{-1})	recovery (%)	RSD (%)
aldrin	0.005	99.1	6.8	0.2	97.7	7.8
azinphos-methyl	0.01	98.9	11.4	0.2	99.9	12.2
azoxystrobin	0.04	98.8	14.5	0.2	99.8	12.2
bifenthrin	0.01	101.0	12.1	0.2	97.7	9.2
bromopropylate	0.01	101.3	13.8	0.2	97.6	9.3
bupirimate	0.02	103.2	13.6	0.2	97.5	9.6
captan	0.1	101.5	9.9	0.2	97.4	9.4
chlorothalonil	0.01	96.6	9.3	0.2	97.8	9.2
chlorpropham	0.01	100.6	8.6	0.2	97.6	8.2
chlorpyriphos	0.01	102.6	12.4	0.2	97.1	8.0
chlorpyriphos-methyl	0.02	102.1	9.6	0.2	97.5	7.8
cyhalotrin-lambda	0.01	99.8	8.4	0.2	97.3	10.6

active substance	spiking level (mg kg^{-1})	recovery (%)	RSD (%)	spiking level (mg kg^{-1})	recovery (%)	RSD (%)
cypermethrin	0.03	97.3	6.9	0.2	98.8	12.5
cyprodinil	0.01	103.1	12.1	0.2	97.5	9.3
DDT	0.05	101.6	9.8	1.0	97.4	9.1
deltamethrin	0.03	99.9	10.4	0.2	98.9	12.4
diazinon	0.01	104.2	12.0	0.2	97.8	7.4
dichlofluanid	0.01	100.1	8.8	0.2	97.4	8.1
dimethoate	0.01	102.7	10.9	0.2	97.9	8.9
diphenylamine	0.01	99.5	7.3	0.2	98.0	7.5
endrin	0.01	97.9	9.2	0.2	97.5	8.7
fenitrothion	0.01	100.1	8.3	0.2	97.0	10.2
fenthion	0.005	101.8	13.9	0.2	97.4	8.3
fludioxonil	0.01	99.3	11.8	0.2	99.3	11.3
folpet	0.02	101.7	11.2	0.2	97.6	10.7
HCH-alpha	0.005	100.9	23.0	0.2	97.8	7.5
heptachlor	0.005	99.8	7.0	0.2	97.9	7.5
heptenophos	0.01	101.2	8.4	0.2	97.9	7.9
iprodione	0.01	99.1	11.6	0.2	98.2	10.3
kresoxim-methyl	0.02	103.3	12.2	0.2	97.5	9.6
lindane	0.01	99.4	8.4	0.2	97.9	7.4
mecarbam	0.04	103.1	11.0	0.2	97.7	8.8
metalaxyl+metalaxyl-M	0.01	103.2	11.1	0.2	97.6	8.1
methidathion	0.01	103.5	12.0	0.2	98.0	9.8
myclobutanil	0.05	104.5	14.6	0.2	97.8	9.7
parathion	0.03	98.3	7.7	0.2	96.8	10.1
penconazole	0.01	104.9	10.2	0.2	97.7	9.1
permethrin	0.02	100.3	12.6	0.2	98.0	11.3
phosalone	0.01	101.3	11.5	0.2	98.1	10.9
pirimicarb	0.01	101.5	10.8	0.2	97.8	8.0
pirimiphos-methyl	0.01	103.6	12.1	0.2	97.9	8.2
propyzamide	0.01	102.1	9.0	0.2	97.4	8.4
pyridaphenthion	0.01	103.6	10.7	0.2	97.8	11.0
pyrimethanil	0.01	100.8	9.4	0.2	97.6	8.2
quinalphos	0.01	104.5	13.7	0.2	97.3	9.3
spiroxamine	0.03	102.2	10.9	0.2	97.4	8.1
tolclofos-methyl	0.01	101.6	8.3	0.2	97.8	7.8
tolylfluanid	0.01	100.1	9.7	0.2	97.2	8.7
triadimefon	0.02	101.5	10.6	0.2	97.3	8.8
triadimenol	0.02	105.4	10.6	0.2	97.6	9.9
triazophos	0.01	102.1	12.4	0.2	97.6	11.5
trifloxystrobin	0.03	102.9	13.4	0.2	97.8	9.9
vinclozolin	0.01	100.3	8.6	0.2	97.5	8.6

Table 5: Interlaboratory comparison results (in mg kg^{-1}) (BIPEA, 2015)

active substance	reference	tolerance	maximum	minimum	our result	z
azoxystrobin	0.053	0.027	0.080	0.026	0.056	0.22
bifenthrin	0.022	0.011	0.033	0.011	0.018	-0.73
cyhalotrin-lambda	0.064	0.032	0.096	0.032	0.062	-0.13
deltamethrin	0.166	0.076	0.242	0.090	0.164	-0.05
diphenylamine	0.129	0.062	0.191	0.067	0.112	-0.55
dimethoate	0.066	0.033	0.099	0.033	0.061	-0.3
fenitrothion	0.044	0.022	0.066	0.022	0.050	0.55
phosalone	0.163	0.075	0.238	0.088	0.158	-0.13
kresoxim-methyl	0.023	0.012	0.035	0.011	0.020	-0.5
lindane	0.146	0.068	0.214	0.078	0.140	-0.18
metalaxyl+metalaxyl-M	0.036	0.018	0.054	0.018	0.028	-0.89
myclobutanil	0.032	0.016	0.048	0.016	0.031	-0.13
pirimicarb	0.169	0.078	0.247	0.091	0.152	-0.44

Accuracy was also checked with participation in a proficiency testing scheme organised by BIPEA (Bureau interprofessionnel d´études analytiques). All the results were within the required range ($-2 \geq z \leq 2$). The results are presented in Table 3.

3.3 Precision

For the determination of precision (ISO 5725), i.e. repeatability and reproducibility, the extracts of spiked blank strawberry matrix were analysed at two concentration levels. Within the 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 repeatability of the level and the standard deviation of reproducibility of the level were both calculated. The results are given in Table 6.

Table 6: Standard deviation of repeatability (s_r) and reproducibility (s_R) of the method

active substance	spiking level (mg kg^{-1})	means of the levels (mg kg^{-1})	s_r (mg kg^{-1})	s_R (mg kg^{-1})	spiking level (mg kg^{-1})	means of the levels (mg kg^{-1})	s_r (mg kg^{-1})	s_R (mg kg^{-1})
aldrin	0.005	0.0050	0.0002	0.0003	0.2	0.19	0.01	0.01
azinphos-methyl	0.01	0.010	0.001	0.001	0.2	0.19	0.02	0.02
azoxystrobin	0.04	0.038	0.005	0.006	0.2	0.19	0.02	0.02
bifenthrin	0.01	0.0098	0.0007	0.0008	0.2	0.19	0.01	0.01
bromopropylate	0.01	0.0097	0.0009	0.0009	0.2	0.19	0.01	0.01
bupirimate	0.02	0.020	0.001	0.001	0.2	0.19	0.01	0.01
captan	0.1	0.10	0.02	0.02	0.2	0.19	0.01	0.02
chlorothalonil	0.01	0.0099	0.0007	0.0007	0.2	0.19	0.01	0.01
chlorpropham	0.01	0.0098	0.0005	0.0006	0.2	0.19	0.01	0.01
chlorpyriphos	0.01	0.0098	0.0005	0.0007	0.2	0.19	0.01	0.01
chlorpyriphos-methyl	0.02	0.020	0.001	0.001	0.2	0.19	0.01	0.01
cyhalotrin-lambda	0.01	0.0095	0.0007	0.0009	0.2	0.19	0.01	0.01
cypermethrin	0.03	0.029	0.003	0.003	0.2	0.19	0.01	0.01
cyprodinil	0.01	0.0098	0.0006	0.0007	0.2	0.19	0.01	0.01
DDT	0.05	0.050	0.003	0.004	1.0	0.95	0.05	0.06
deltamethrin	0.03	0.029	0.003	0.003	0.2	0.19	0.02	0.02
diazinon	0.01	0.0098	0.0005	0.0006	0.2	0.19	0.01	0.01
dichlofluanid	0.01	0.0096	0.0006	0.0009	0.2	0.19	0.01	0.01
dimethoate	0.01	0.0097	0.0007	0.0008	0.2	0.19	0.01	0.01
diphenylamine	0.01	0.0099	0.0004	0.0005	0.2	0.19	0.01	0.01
endrin	0.01	0.0100	0.0006	0.0006	0.2	0.19	0.01	0.01
fenitrothion	0.01	0.0098	0.0007	0.0008	0.2	0.19	0.01	0.01
fenthion	0.005	0.0049	0.0003	0.0004	0.2	0.19	0.01	0.01
fludioxonil	0.01	0.010	0.001	0.001	0.2	0.19	0.01	0.02
folpet	0.02	0.020	0.004	0.004	0.2	0.19	0.01	0.02
HCH-alpha	0.005	0.0049	0.0002	0.0002	0.2	0.19	0.01	0.01
heptachlor	0.005	0.0050	0.0003	0.0003	0.2	0.19	0.01	0.01

active substance	spiking level (mg kg^{-1})	means of the levels (mg kg^{-1})	s_r (mg kg^{-1})	s_R (mg kg^{-1})	spiking level (mg kg^{-1})	means of the levels (mg kg^{-1})	s_r (mg kg^{-1})	s_R (mg kg^{-1})
heptenophos	0.01	0.0098	0.0004	0.0006	0.2	0.19	0.01	0.01
iprodione	0.01	0.0097	0.0009	0.0011	0.2	0.19	0.01	0.01
kresoxim-methyl	0.02	0.020	0.001	0.001	0.2	0.19	0.01	0.01
lindane	0.01	0.0100	0.0005	0.0005	0.2	0.19	0.01	0.01
mecarbam	0.04	0.039	0.003	0.003	0.2	0.19	0.01	0.01
metalaxyl+metalaxyl-M	0.01	0.0098	0.0004	0.0004	0.2	0.19	0.01	0.01
methidathion	0.01	0.0098	0.0009	0.0009	0.2	0.19	0.01	0.01
myclobutanil	0.05	0.049	0.004	0.004	0.2	0.19	0.01	0.01
parathion	0.03	0.029	0.002	0.002	0.2	0.19	0.01	0.01
penconazole	0.01	0.0097	0.0006	0.0007	0.2	0.19	0.01	0.01
permethrin	0.02	0.020	0.002	0.002	0.2	0.19	0.01	0.01
phosalone	0.01	0.0097	0.0009	0.0011	0.2	0.19	0.01	0.01
pirimicarb	0.01	0.0099	0.0006	0.0006	0.2	0.19	0.01	0.01
pirimiphos-methyl	0.01	0.0098	0.0005	0.0006	0.2	0.19	0.01	0.01
propyzamide	0.01	0.0098	0.0005	0.0005	0.2	0.19	0.01	0.01
pyridaphenthion	0.01	0.010	0.001	0.001	0.2	0.19	0.01	0.01
pyrimethanil	0.01	0.0098	0.0006	0.0006	0.2	0.19	0.01	0.01
quinalphos	0.01	0.0098	0.0007	0.0009	0.2	0.19	0.01	0.01
spiroxamine	0.03	0.0296	0.001	0.002	0.2	0.19	0.01	0.01
tolclofos-methyl	0.01	0.0099	0.0005	0.0005	0.2	0.19	0.01	0.01
tolylfluanid	0.01	0.010	0.001	0.001	0.2	0.19	0.01	0.01
triadimefon	0.02	0.020	0.001	0.001	0.2	0.19	0.01	0.01
triadimenol	0.02	0.0195	0.002	0.002	0.2	0.19	0.01	0.01
triazophos	0.01	0.0097	0.0008	0.0009	0.2	0.19	0.01	0.01
trifloxystrobin	0.03	0.029	0.002	0.003	0.2	0.19	0.01	0.01
vinclozolin	0.01	0.0099	0.0006	0.0006	0.2	0.19	0.01	0.01

3.4 Uncertainty of repeatability and uncertainty of reproducibility

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

$Ur = t_{95;9} \times s_r$; $UR = t_{95;9} \times s_R$

The results are presented in Table 7. The measurement uncertainty for PPP residues is set in the Official Gazette of the Republic of Slovenia (Republic of Slovenia, 2007). Its value is 50 %. With validation, analysts must prove that their measurement uncertainty is below or equal to the official measurement uncertainty.

3.5 Sample analysis

The method was checked in practice. 19 strawberry samples were analysed for the presence of all 53 validated active substances. 10 samples, which represent 52.6 % of all the analysed samples contained no residues. 5 active substances, all fungicides, were found: chlorothalonil, cyprodinil, fludioxonil, metalaxyl+metalaxyl-M and pyrimethanil. Other active substances were below the LOQ. The most frequently measured was cyprodinil, which was found in 8 samples, representing 42.1 % of all the analysed samples. The reason is probably that this substance is included in the PPP Switch 62.5 WG, which is the mixture of fungicides cyprodinil and fludioxonil used for strawberries and sold in Slovenia. 9 samples, which represent 47.4 % of all the analysed samples contained PPP residues in the range 0.01 – 0.44 mg/kg. Multiple residues (2 or more active substances) were found in 5 samples, representing 26.3 % of all the analysed samples. None of the substances exceeded the valid MRL. Therefore, the conclusion was drawn that farmers were using PPPs according to good agriculture practice described on the labels of the PPPs. Also, these strawberries presented no risk to consumers. The results are presented in Table 8.

Comparing our results with the literature we observed that PPP residues in strawberries in Slovenia are mainly comparable to observations of other authors. Jardim et al. (2012) found pesticide residues in Brazilia in 76.3 % of strawberry samples; 71.6 % of them had multiple residues and 13.5 % of them were exceeding the MRL. In Slovenia, the amount of positive samples was about 29 % lower, the amount of multiple residues was about 45 % lower and no MRL exceedances were observed. On the other hand Poulsen et al. (2017) reported that in Denmark, 37 % of the analysed samples contained multiple residues, which is approximately 11 % higher than in Slovenia.

In strawberry samples in Poland, Szpyrka et al. (2015) found cypermethrin, deltamethrin and trifloxystrobin among the active substances that we both analysed. On the other hand, again in strawberry samples in Poland, Sójka et al. (2015) found the fungicides cyprodinil (mean content 0.16 mg kg^{-1}), fludioxonil (mean content 0.115 mg kg^{-1}) and pyrimethanil (mean content 0.056 mg kg^{-1}), as well as the insecticide chlorpyrifos (mean content 0.012 mg kg^{-1}) among the active substances that we both analysed. The mean contents of cyprodinil and fludioxonil were comparable to ours, while the content of pyrimethanil was slightly lower. Chlorpyriphos was not found in our research. In protected strawberries Allen et al. (2015) found cyprodinil (mean content 0.062 mg kg^{-1}) and iprodione (mean content 0.055 mg kg^{-1}) among the active substances that we both analysed. The cyprodinil mean content was in the range of contents that we measured, while iprodione was not found in our research.

Table 7: Uncertainty of repeatability (U_r) and reproducibility (U_R) of the method

active substance	spiking level (mg kg^{-1})	U_r (mg kg^{-1})	U_r (%)	U_R (mg kg^{-1})	U_R (%)	spiking level (mg kg^{-1})	U_r (mg kg^{-1})	U_r (%)	U_R (mg kg^{-1})	U_R (%)
aldrin	0.005	0.0006	12.0	0.0006	12.0	0.2	0.02	10.0	0.02	10.0
azinphos-methyl	0.01	0.003	30.0	0.003	30.0	0.2	0.04	20.0	0.05	25.0
azoxystrobin	0.04	0.01	25.0	0.01	25.0	0.2	0.04	20.0	0.04	20.0
bifenthrin	0.01	0.002	20.0	0.002	20.0	0.2	0.02	10.0	0.03	15.0
bromopropylate	0.01	0.002	20.0	0.002	20.0	0.2	0.02	10.0	0.03	15.0
bupirimate	0.02	0.003	15.0	0.003	15.0	0.2	0.03	15.0	0.03	15.0
captan	0.1	0.04	40.0	0.04	40.0	0.2	0.04	20.0	0.1	50.0
chlorothalonil	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
chlorpropham	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0
chlorpyriphos	0.01	0.001	10.0	0.002	20.0	0.2	0.02	10.0	0.02	10.0
chlorpyriphos-methyl	0.02	0.003	15.0	0.003	15.0	0.2	0.02	10.0	0.03	15.0
cyhalotrin-lambda	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
cypermethrin	0.03	0.007	23.3	0.007	23.3	0.2	0.03	15.0	0.03	15.0
cyprodinil	0.01	0.001	10.0	0.002	20.0	0.2	0.02	10.0	0.03	15.0
DDT	0.05	0.007	14.0	0.008	16.0	1.0	0.12	12.0	0.14	14.0
deltamethrin	0.03	0.006	20.0	0.006	20.0	0.2	0.04	20.0	0.04	20.0
diazinon	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0
dichlofluanid	0.01	0.001	10.0	0.002	20.0	0.2	0.03	15.0	0.02	10.0
dimethoate	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
diphenylamine	0.01	0.0009	9.0	0.0011	11.0	0.2	0.02	10.0	0.02	10.0
endrin	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.03	15.0
fenitrothion	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
fenthion	0.005	0.0007	14.0	0.0008	16.0	0.2	0.02	10.0	0.02	10.0
fludioxonil	0.01	0.002	20.0	0.003	30.0	0.2	0.03	15.0	0.04	20.0
folpet	0.02	0.009	45.0	0.009	45.0	0.2	0.03	15.0	0.03	15.0
HCH-alpha	0.005	0.0005	10.0	0.0005	10.0	0.2	0.02	10.0	0.02	10.0
heptachlor	0.005	0.0006	12.0	0.0006	12.0	0.2	0.02	10.0	0.02	10.0

active substance	spiking level (mg kg^{-1})	U_r (mg kg^{-1})	U_r (%)	U_R (mg kg^{-1})	U_R (%)	spiking level (mg kg^{-1})	U_r (mg kg^{-1})	U_r (%)	U_R (mg kg^{-1})	U_R (%)
heptenophos	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.03	15.0
iprodione	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
kresoxim-methyl	0.02	0.003	15.0	0.003	15.0	0.2	0.03	15.0	0.03	15.0
lindane	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0
mecarbam	0.04	0.007	17.5	0.007	17.5	0.2	0.02	10.0	0.03	15.0
metalaxyl+metalaxyl-M	0.01	0.0009	9.0	0.0010	10.0	0.2	0.02	10.0	0.03	15.0
methidathion	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
myclobutanil	0.05	0.009	18.0	0.009	18.0	0.2	0.02	10.0	0.03	15.0
parathion	0.03	0.005	16.7	0.005	16.7	0.2	0.03	15.0	0.03	15.0
penconazole	0.01	0.001	10.0	0.002	20.0	0.2	0.02	10.0	0.03	15.0
permethrin	0.02	0.004	20.0	0.005	25.0	0.2	0.03	15.0	0.03	15.0
phosalone	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
pirimicarb	0.01	0.001	10.0	0.001	10.0	0.2	0.03	15.0	0.03	15.0
pirimiphos-methyl	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0
propyzamide	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.03	15.0
pyridaphenthion	0.01	0.002	20.0	0.003	30.0	0.2	0.03	15.0	0.03	15.0
pyrimethanil	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.03	15.0
quinalphos	0.01	0.002	20.0	0.002	20.0	0.2	0.02	10.0	0.02	10.0
spiroxamine	0.03	0.003	10.0	0.004	13.3	0.2	0.02	10.0	0.02	10.0
tolclofos-methyl	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0
tolylfluanid	0.01	0.002	20.0	0.002	20.0	0.2	0.02	10.0	0.03	15.0
triadimefon	0.02	0.003	15.0	0.003	15.0	0.2	0.02	10.0	0.03	15.0
triadimenol	0.02	0.003	15.0	0.004	20.0	0.2	0.02	10.0	0.03	15.0
triazophos	0.01	0.002	20.0	0.002	20.0	0.2	0.03	15.0	0.03	15.0
trifloxystrobin	0.03	0.005	16.7	0.006	20.0	0.2	0.03	15.0	0.03	15.0
vinclozolin	0.01	0.001	10.0	0.001	10.0	0.2	0.02	10.0	0.02	10.0

Table 8: Contents of active substances found in 19 strawberry samples

	chlorothalonil (mg kg^{-1})	cyprodinil (mg kg^{-1})	fludioxonil (mg kg^{-1})	metalaxyl+metalaxyl-M (mg kg^{-1})	pyrimethanil (mg kg^{-1})
MRL (mg kg^{-1})	4.0	5.0	4.0	0.6	5.0
sample no.					
1	-	-	-	-	-
2	-	-	-	-	-
3	-	0.04	-	-	-
4	-	0.04	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	0.01	-	-	-	-
9	-	-	-	-	-
10	-	0.02	-	-	-
11	0.10	0.02	-	-	-
12	-	-	-	-	-
13	-	0.24	0.17	-	0.13
14	0.06	0.02	-	-	-
15	-	-	-	-	-
16	-	0.24	-	0.02	-
17	0.02	0.08	-	-	0.44
18	-	-	-	-	-
19	-	-	-	-	-

- means <LOQ
MRL is maximum residue level

4 CONCLUSIONS

According to the validation, the method is suitable for the determination of at least 53 active compounds and their residues in strawberries. The method could be expanded to more active substances. The system is linear with an R^2 higher or equal than 0.977. The LOQs range from 0.005 mg kg^{-1} for aldrin to 0.1 mg kg^{-1} for captan. Recoveries range from 96.6 % (chlorothalonil) to 105.4 % (triadimenol) at a spiking level equal to the LOQ. Uncertainty of reproducibility ranges from 10 % for vinclozolin to 50 % for captan. The method is fit for purpose and is accredited according to the SIST EN ISO/IEC 17025 standard by the Slovenian accreditation body SA.

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