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Gas chromatography-tandem mass spectrometry multiresidual method for determination of pesticide residues in honey

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Abstract: In our laboratory we introduced and validated a new analytical method for determination of environmental pesticide residues in honey. The extraction was conducted using acetone, petroleum ether and dichlorometane. The determination was conducted using gas chromatography coupled with tandem mass spectrometry. Practical usage of method was analyses of 31 samples of Slovenian honey. 33 active substances (pesticides) were sought. The insecticide cypermethrin was the only active substance found in three samples. The active substances sought were not found in 90.3 % of the samples analysed. The risk assessment showed that no unacceptable risk is expected for consumers. The results were compared with those from the literature. We revealed that honey from Slovenia contained a lower portion of positive samples per active substance sought as in Italy, comparable as in Estonia and Spain, comparable to higher as in Poland and higher as in Egypt.

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

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

Izvleček: V našem laboratoriju smo uvedli in validirali novo analizno metodo za določanje ostankov fitofarmacevtskih sredstev iz okolja v medu. Ekstrakcijo smo izvedli z acetonom, petroletrom in diklorometanom, določitev pa s plinsko kromatografijo sklopljeno s tandemsko masno spektrometrijo. Praktična uporaba metode je bila analiza 31 vzorcev slovenskega medu. Določali smo 33 aktivnih spojin (pesticidov). Edina najdena aktivna snov je bil insekticid cipermetrin v treh vzorcih. Iskanih aktivnih snovi nismo določili v 90,3 % analiziranih vzorcev. Ocena tveganja je pokazala, da ni pričakovati nesprejemljivega tveganja za potrošnika. Rezultate smo primerjali z literaturnimi podatki. Odkrili smo, da je slovenski med vseboval manjši delež pozitivnih vzorcev na aktivno snov kot v Italiji, primerljiv kot v Estoniji in Španiji, primerljiv do večji kot na Poljskem in večji kot v Egiptu.

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

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1 INTRODUCTION

Honey is produced from nectar collected by bees, which gets broken down into simple sugars stored inside the honeycomb. Therefore, honey is mainly composed of carbohydrates (approx. 80 %): glucose, fructose, sucrose and maltose, and water (approx. 20 %). It also contains minor compounds such as vitamins, minerals, amino acids, proteins and aroma compounds (Geană at al., 2020, Kahraman et al., 2010). Nutritional properties and therapeutic applications of honey are reason for its frequent use.

Honey bees can fly within a radius of 4.8 km in all directions from their apiary (Eckert, 1933). On their way they can come into contact with pesticide residues when they collect nectar and pollen on plants treated with plant protection products (PPPs) (Colin et al., 2004) and/or on the ground, in water, in the air, on melliferous in-field weeds and off-field plants where PPPs were carried by the drift after treatment (Bonmatin at al., 2015, Krupke et al., 2012, SANTE, 2023, Ward et al., 2022). Bees carry pesticide residues into the hive, from where they eventually end up in honey (Zhou et al., 2018).

Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) entered into force on 1 January 2020. With the introduction of this guideline, during PPPs authorisation of uses on plants with melliferous capacity, experiments are required to determine residues in honey. Therefore, monitoring of PPP residues in honey is recommended.

For extraction procedures of analytical methods for determination of PPP residues in honey nowadays mainly use modified Quick Easy Cheap Effective Rugged and Safe method also called QuEChERS method, where acetonitrile is used (Gawel et al., 2019, Karise et al., 2017, Shendy et al., 2016). In some laboratories extraction is performed with ethyl acetate (Panseri et al., 2014) or the mixture of ethyl acetate and cyclohexane (Brugnerotto et al., 2023). In our laboratory a mixture of acetone, dichloromethane and petroleum ether was used, to achieve the extraction of very polar (for instance, flonicamid) to non-polar (for instance, cyhalothrin-lambda) pesticides at the same time (Baša Česnik et al., 2019). Besides, when extracting materials containing high amount of sugar with acetone, no double layered extract is obtained like with acetonitrile (Luke et al., 1975).

Determination of pesticide residues is nowadays usually performed using gas chromatography coupled with mass spectrometry (GC-MS) (Brugnerotto et al., 2023, Karise et al., 2017, Mukiibi et al., 2021), gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) (Gawel et al., 2019, Lazarus et al., 2021, Panseri et al., 2014, Shendy et al., 2016, Sun et al., 2022) and/or liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Gawel et al., 2019, Karise et al., 2017, Liu et al., 2022). The most sensitive is tandem mass spectrometry, which was also used by our laboratory.

Numerous authors have analysed pesticide residues in honey with GC-MS/MS. Gawel et al. (2019) analysed 53 active substances in honey from Poland. Panseri et al. (2014) tested honey samples from Italy for 28 active substances. Shendy et al. (2016) introduced a method for determining 200 active substances in honey samples from Egypt. Wang et al. (2022) used a method for determining 203 active substances in China honey. In our study up to 24 of active substances sought in literature studies were introduced. 97.0 % of active substances selected in this paper are authorised for use in Slovenia. The rest were authorised in previous years. Of those selected, 57.6 % were fungicides, 21.2 % were acaricides and/or insecticides and 21.2 % were herbicides.

Our paper is presenting a new GC-MS/MS multiresidual method for determination of 33 active substances (pesticides) in honey. The old extraction procedure using acetone, dichlorometane and petroleum ether was used, but new active substances were introduced and validated with the new, more sensitive instrument. Method was used in practice. 31 honey samples, collected from Slovenian beekeepers, were analysed. Results were compared with literature data and consumer risk assessment was calculated.

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

The certified pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). For extraction procedure acetone - p.a. grade, dichlorometane – p.a. grade and petroleum ether – p.a. grade, were obtained from J.T.Baker (Deventer, Netherlands). Also acetone HPLC-grade, which was used for preparation of standards, was obtained from 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 of individual active substances were

prepared in acetone. Concentration of each active substance was 625 μg ml^{-1}. From 33 stock solutions, three mixed solutions of all 33 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

Extraction procedure was conducted with acetone, petroleum ether and dichlorometane. We used the same extraction procedure as the one for determination of chlorfenvinphos, coumaphos and thymol, described by Baša Česnik at al. (2019). The only difference was that the final dry extract was dissolved in acetone HPLC-grade.

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 presented in Table 1. For each active substance two to four transitions were scaned. For calibration matrix match standards were used.

2.4 VALIDATION OF METHODS

2.4.1 LOQ and linearity

The linearity was tested with matrix match standards. F test was used to check linearity and determine linearity range. Each calibration curve had three to seven concentration levels with two repetitions at each level.

Estimation of LOQs was conducted using matrix match standards. S/N ratio had to be at least 10.

2.4.2 Precision

Blank honey was purchased in store. It was analysed on presence of pesticide residues sought. After proving that it does not contain pesticides of our choice, it was spiked in two parallel samples at LOQ within the period of 10 days. For the determination of precision (ISO 5725), i.e. repeatability and reproducibility, the standard deviation of the repeatability of the level and the standard deviation of reproducibility of the level were both calculated from results obtained.

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 PPP residues should be 50 %, as proposed in SANTE/11312/2021. The method is fit for purpose when during validation it is proven that measurement uncertainty is ≤ 50 %.

2.4.4 Accuracy

The accuracy was verified by checking the recoveries. We used recoveries obtained during test for precision. 20 results for each active substance (pesticide) were averaged and RSD 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 %.

The guidelines for single-laboratory validation (Alder et al. 2000) require mean recoveries at level > 0.001 mg kg^{-1} and ≤ 0.01 mg kg^{-1} from 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. Chronic consumer exposure was expressed in % of the Acceptable Daily Intake (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. Acute consumer exposure

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

Active substance	Activity type[a]	MRM transitions (Q1, Q2, Q3)[b]	Dwell (ms)	CE (V)[c]
8-hydroxyquinoline	F	145->117.1, 145->89, 117->90	77.5	10, 40, 10
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
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
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	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
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
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
trifloxystrobin	F	222->162.1, 222->130, 131->116	11.1	10, 10, 20

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

was expressed in % of the Acute Reference Dose (ARfD). The acceptable limit for short-term exposure is 100 % of the ARfD.

2.6 SAMPLING

31 honey samples were collected from Slovenian beekeepers from 11 statistical regions in Slovenia in 2023. The sampling distribution is presented in Table 2.

3 RESULTS AND DISCUSSION

3.1 VALIDATION OF METHOD

3.1.1 LOQ and linearity

The linear model is valid for all active substances presented in Table 3. Linearity was proven in the range of 0.005 mg kg^{-1} to 0.02 mg kg^{-1} for pendimethalin, in the range of 0.005 mg kg^{-1} to 0.04 mg kg^{-1} for 8-hydroxyquinoline and prosulfocarb, in the range of 0.005 mg kg^{-1} to 0.05 mg kg^{-1} for flonicamid and in the range of 0.005 mg kg^{-1} to 0.03 mg kg^{-1} for all other active substances. R^2 ranged from 0.987 to 1.000. Results are presented in Table 3.

3.1.2 Accuracy

The recoveries at LOQs for the active substances scanned with GC-MS/MS are in the range of 92.8 % to 98.9 %, with RSDs of 6.0 % to 11.3 %. The results are presented in Table 3.

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

3.1.3 Uncertainty of repeatability and uncertainty of reproducibility

The uncertainty of repeatability and uncertainty of reproducibility were determined at concentrations equal to the LOQs. Uncertainty of repeatability ranged from 0.0004 mg kg^{-1} to 0.0009 mg kg^{-1}, which is 7.6 % to 18.3 % of LOQ. Uncertainty of reproducibility ranged from 0.0007 mg kg^{-1} to 0.0013 mg kg^{-1}, which is 13.3 % to 25.2 % of LOQ. The results are presented in Table 3.

Table 2: Sampling distribution according to statistical regions of Slovenian honey samples collected in 2023

	No of samples		
Region	Pouring in 2022	Pouring in 2023	sum
Goriška	5	0	5
Jugovzhodna Slovenija	1	1	2
Koroška	1	3	4
Obalno Kraška	1	0	1
Osrednja Slovenija	3	2	5
Podravska	5	1	6
Pomurska	1	1	2
Posavska	0	1	1
Primorsko-Notranjska	1	0	1
Savinjska	3	0	3
Zasavska	1	0	1
sum	22	9	31

3.2 SURVEY OF PESTICIDE RESIDUES IN HONEY SAMPLES

Of the 31 honey samples analysed, only 3 contained one active substance: cypermethrin in concentrations 0.006 (honey poured in 2022, Osrednja Slovenija), 0.015 (honey poured in 2023, Koroška) and 0.048 mg kg^{-1} (honey poured in 2023, Koroška). This means that in 90.3 % of all samples analysed, were free of pesticides sought. In Slovenia, cypermethrin is authorised as insecticide for seed treatment of cereals (formulation ES, Emulsion for seed treatment), and for use on soil at planting of melliferous crops like oilseed rape, pumpkin and aubergines and on non-melliferous crops like onion, garlic, head cabbage, horseradish, chinese cabbage, carrot, potatoes, kale, tomatoes, parsnips, parsley, beetroots, radishes, sugar beet, shallots, tobacco, celery and grass (formulation GR, Granule). Cypermethrin is a non-systemic and cannot be translocated in plants. But granules of PPPs contain 10 % dust (SANTE, 2023). Dust from treated seeds and/or granules of PPPs can be deposited on melliferous in-field weeds and off-field plants like clover or dandelion (Bonmatin at al., 2015, SANTE, 2023). The consequence is that residues of all active substances used in the field near the hive can be present in honey up to 0.05 mg kg^{-1}, which is MRL for cypermethrin in honey. Value of 0.05 mg kg^{-1} is calculated as a default value for all active sub-

Table 3: Validation parameters for honey

Active substance	Linearity range (mg kg^{-1})	R^2	LOQ (mg kg^{-1})	Recovery (%)	RSD[a] (%)	U_r[b] (mg kg^{-1})	U_r[c] (%)	U_R[d] (mg kg^{-1})	U_R[e] (%)
8-hydroxyquinoline	0.005-0.04	0.995	0.005	95.5	8.2	0.0007	13.8	0.0009	17.9
benthiavalicarb-isopropyl	0.005-0.03	0.999	0.005	97.3	7.4	0.0004	7.6	0.0008	16.6
boscalid	0.005-0.03	0.997	0.005	95.1	7.4	0.0006	11.4	0.0008	16.2
clomazone	0.005-0.03	0.999	0.005	96.3	7.3	0.0005	10.9	0.0008	16.2
cypermethrin	0.005-0.03	0.997	0.005	93.3	11.0	0.0009	18.3	0.0012	23.4
cyprodinil	0.005-0.03	0.999	0.005	95.0	6.1	0.0005	10.8	0.0007	13.3
deltamethrin	0.005-0.03	0.997	0.005	92.8	9.8	0.0008	16.5	0.0010	20.8
fenhexamid	0.005-0.03	0.999	0.005	96.4	11.2	0.0005	9.8	0.0012	24.9
flonicamid	0.005-0.05	0.987	0.005	98.3	7.0	0.0006	11.9	0.0008	15.7
fluazifop-p-butyl	0.005-0.03	0.999	0.005	96.9	8.6	0.0008	15.6	0.0009	18.9
fludioxonil	0.005-0.03	0.998	0.005	95.7	8.0	0.0007	13.3	0.0009	17.5
flufenacet	0.005-0.03	0.999	0.005	96.5	7.6	0.0006	12.5	0.0008	16.8
fluopicolide	0.005-0.03	0.998	0.005	97.0	7.6	0.0007	13.2	0.0008	16.9
fluopyram	0.005-0.03	0.999	0.005	97.3	6.4	0.0004	8.5	0.0007	14.2
flutolanil	0.005-0.03	0.999	0.005	95.6	8.2	0.0007	14.9	0.0009	17.9
iprovalicarb	0.005-0.03	0.999	0.005	96.1	8.1	0.0008	15.9	0.0009	17.8
kresoxim-methyl	0.005-0.03	0.999	0.005	97.0	7.4	0.0006	11.5	0.0008	16.4
lambda-cyhalothrin	0.005-0.03	0.999	0.005	98.7	7.8	0.0009	18.0	0.0009	18.0
metazachlor	0.005-0.03	0.999	0.005	96.2	6.8	0.0005	9.7	0.0007	15.0
myclobutanil	0.005-0.03	0.998	0.005	97.1	7.0	0.0005	10.8	0.0008	15.7
napropamide	0.005-0.03	0.999	0.005	95.9	6.0	0.0007	14.0	0.0007	14.0
penconazole	0.005-0.03	1.000	0.005	96.8	8.0	0.0006	11.3	0.0009	17.8
pendimethalin	0.005-0.02	1.000	0.005	93.7	7.3	0.0007	13.8	0.0008	15.6
pirimicarb	0.005-0.03	0.997	0.005	96.7	8.0	0.0007	13.7	0.0009	17.6
proquinazid	0.005-0.03	0.999	0.005	96.4	7.0	0.0005	10.5	0.0008	15.6
prosulfocarb	0.005-0.04	1.000	0.005	93.7	8.1	0.0008	15.7	0.0009	17.2
pyraclostrobin	0.005-0.03	0.993	0.005	96.9	11.3	0.0006	12.7	0.0013	25.2
pyrimethanil	0.005-0.03	1.000	0.005	95.1	7.7	0.0007	13.8	0.0008	16.8
tebuconazole	0.005-0.03	0.999	0.005	96.7	8.5	0.0007	14.1	0.0009	18.9
tebufenpyrad	0.005-0.03	0.998	0.005	95.9	7.2	0.0004	8.7	0.0008	15.9
tefluthrin	0.005-0.03	0.999	0.005	95.9	6.8	0.0005	9.9	0.0008	15.0
tetraconazole	0.005-0.03	0.999	0.005	94.1	8.7	0.0006	11.4	0.0009	18.9
trifloxystrobin	0.005-0.03	0.998	0.005	97.7	10.2	0.0008	15.5	0.0011	22.9

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

Table 4: Literature data for active substances analysed by our laboratory, but not found in Slovenian honey samples

Active substance	Max content (mg kg $^{-1}$)	Ratio of positive samples (%)	Country of origin	Reference
boscalid	not reported	27.8	Italy	Panseri et al., 2014
boscalid	0.005	5	Poland	Gawel et al., 2019
cyhalothrin	0.0073	6.0	Egypt	Malhat et al., 2015
tebuconazole	0.012	10	Poland	Gawel et al., 2019
tebuconazole	0.005	9.1	Estonia	Karise et al., 2017
tebuconazole	0.004	9.1	Spain	Juan-Borrás et al., 2016
tetraconazole	0.005	3	Poland	Gawel et al., 2019
trifloxystrobin	not reported	20.8	Italy	Panseri et al., 2014

stances and presumes that the lowest ARfD is 1.5 x 10^{-4} mg (kg bw)$^{-1}$ d^{-1} (for active substance carbofuran) and the highest portion of consumed honey is 3.58 g (kg bw)$^{-1}$ (children consumption) (SANTE/11956/2016, rev. 9), meaning that residue of 0.05 mg kg^{-1} does not present acute risk for consumer. When residues are < 0.05 mg kg^{-1} it is not suspected that violation of PPPs happened. We do not have data about exact location of hives where Slovenian honey with cypermethrin residues was produced. Cypermethrin was probably found in Slovenian honey as a consequence of its use in vicinity of agricultural fields with melliferous off-field plants. We assume that in-field weeds were not present at application of PPPs and cereal seeds, containing cypermethrin, on soil. Farmers probably removed in-field weeds before sowing/planting. Therefore it is recommended that before PPPs are used, off-field plants near hives are mowed, to prevent presence of pesticide residues in honey.

A consumer risk assessment was performed using the EFSA PRIMo model rev. 3.1, which includes 36 national diets from EU countries. Slovenia did not create its own model, therefore EU model was used. The same model is also used during authorisation of PPPs in Slovenia and EU. For chronic exposure ADI of 0.005 mg (kg bw)$^{-1}$ d^{-1} and Supervised Trial Median Residue (STMR) of 0.015 mg kg^{-1} were used. The calculations of chronic exposure showed that the highest was observed in the German diet for children. It represented 0.03 % of ADI. For acute exposure ARfD of 0.005 mg (kg bw)$^{-1}$ d^{-1} and the Highest Residue (HR) of 0.048 mg kg^{-1} were used. The calculations of acute exposure showed that the highest was observed for children. It represented 3 % of ARfD. Based on these calculations, the conclusion was that the analysed honey samples do not represent unacceptable risk for consumers.

Our results were compared with the results from other scientific papers. Cypermethrin was not found in literature by our knowledge. Panseri et al. (2014), Malhat et al. (2015) and Juan-Borrás et al. (2016) did not measure presence of cypermethrin in Italy, Egypt and Spain. Cypermethrin was measured only by Gawel et al. (2019), but was not found in honey samples from Poland. The reason is probably that PPPs containing cypermethrin were not used in vicinity of locations of Polish hives. Other active substances (pesticides) analysed in our laboratory, namely boscalid, lambda-cyhalothrin, tebuconazole, tetraconazole and trifloxystrobin, were not found in Slovenian honey, but were found in samples analysed in Egypt, Estonia, Italy, Poland and Spain. Literature data for these active substances are presented in Table 4.

4 CONCLUSIONS

A method for determining pesticide residues originating from the environment in honey was introduced and validated by our laboratory. The limit of quantification was 0.005 mg kg^{-1} for all active substances. The calibration curves gave a linear response with R^2 0.987 to 1.000. The recoveries ranged from 92.8 % to 98.7 % with RSDs from 6.0 % to 11.3 %. The measurement uncertainty of repeatability ranged from 7.6 to 18.3 % and the measurement uncertainty of reproducibility from 13.3 to 25.2 %. The method was found to be fit for purpose for analysing 33 active substances and for determination of possible MRL exceedances.

In practice method was tested by analysing 31 honey samples gathered from Slovenian beekeepers, all from conventional production. A total of 33 active substances were sought, but only the insecticide cypermethrin was found in three of these samples, below valid MRL. In 90.3 % of the samples analysed, the active substances sought were not found. A risk assessment revealed that the analysed Slovenian honey samples are safe for consumers.

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6 REFERENCES

Alder L., Hill A., Holland P.T., Lantos J., Lee S.M., MacNeil 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. https://doi.org/10.1039/9781847551757-00179

Baša Česnik H., Kmecl V., Velikonja Bolta Š. (2019). Pesticide and veterinary drug residues in honey - validation of methods and a survey of organic and conventional honeys from Slovenia. *Food Additives & Contaminants. Part A*, *36*, 1358-1375. https://doi.org/10.1080/19440049.2019.1631492

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A., Noome, D. A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Pollution Research, 22*, 35–67. https://doi.org/10.1007/s11356-014-3332-7

Brugnerotto P., Costa A.C.O., Fuente-Ballesteros A., Ares A.M., Gonzaga L.V., Fett R., Bernal J. (2023). Determination of seven pesticide residues in Mimosa scabrella honeydew honey from Brazil by GC-MS. *Journal of Food Composition and Analysis, 122*, 105433-105439. https://doi.org/10.1016/j.jfca.2023.105433

Colin, M.E., Bonmatin, J.M., Moineau, I., Gaimon, C., Brun, S., Vermandere, J.P. (2004). A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Archives of Environmental Contamination and Toxicology, 47*, 387–395. https://doi.org/10.1007/s00244-004-3052-y

Eckert J.E. (1933). The flight range of the honeybee. *Journal of Agricultural Research, 47,* 257-285.

Gawel M., Kiljanek T., Niewiadowska A., Semeniuk S., Goliszek M., Burek O., Posyniak A. (2019). Determination of neonicotinoids and 199 other pesticide residues in honey by liquid and gas chromatography coupled with tandem mass spectrometry. *Food Chemistry, 282*, 36-47. https://doi.org/10.1016/j.foodchem.2019.01.003

Geană E.-I., Ciucure C.T., Costinel D., Ionete R.E. (2020). Evaluation of honey in terms of quality and authenticity based on the general physicochemical pattern, major sugar composition and δ13C signature. *Food Control, 109*, 106919-106928. https://doi.org/10.1016/j.foodcont.2019.106919

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. https://doi.org/10.1016/j.foodcont.2016.02.051

Juan-Borrás M., Domenech E., Escriche I. (2016). Mixture-risk-assessment of pesticide residues in retail polyfloral honey. *Food Control, 67*, 127-134. https://doi.org/10.1016/j.foodcont.2016.02.051

Kahraman T., Buyukunal S.K., Vural A., Altunatmaz S.S. (2010). Physico-chemical properties in honey from different regions of Turkey. *Food Chemistry*, *123*, 41-44. https://doi.org/10.1016/j.foodchem.2010.03.123

Karise R., Raimets R., Bartkevics V., Pugajeva I., Pihlik P., Keres I., Williams I.H., Viinalass H., Mänd M. (2017). Are pesticide residues in honey related to oilseed rape treatments? *Chemosphere*, *188*, 389-396. https://doi.org/10.1016/j.chemosphere.2017.09.013

Krupke C.H., Hunt G.J., Eitzer B.D., Andino G., Given K. (2012). Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One, *7*, e29268. https://doi.org/10.1371/journal.pone.0029268

Lazarus M., Tariba Lovaković B., Orct T., Sekovanić A., Bilandžić N., Đokić M., Solomun KolanoviB., Varenina I., Jurič A., Denžić Lugomer M., Bubalo D. (2021). Difference in pesticides, trace metal(loid)s and drug residues between certified organic and conventional honeys from Croatia. *Chemosphere, 266*, 128954-128965. https://doi.org/10.1016/j.chemosphere.2020.128954

Liu Z., Wang J., Wang Z. , Xu H. , Di S. , Zhao H. , Qi P., Wang X. (2022). Development of magnetic solid phase extraction using magnetic amphiphilic polymer for sensitive analysis of multi-pesticides residue in honey. *Journal of Chromatography A*, *1664*, 462789-462797. https://doi.org/10.1016/j.chroma.2021.462789

Luke M.A., Froberg J.E., Masumoto H.T. (1975). Extraction and cleanup of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. *Journal of AOAC,58,* 1020–1026. https://doi.org/10.1093/jaoac/58.5.1020

Malhat F.M., Haggag N., Loutfy N.M., Osman M.A.M., Ahmed M.T. (2015). Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. *Chemosphere, 120*, 457-461. https://doi.org/10.1016/j.chemosphere.2014.08.032

Mukiibi S.B., Nyanzi S.A., Kwetegyeka J., Olisah C., Taiwo A.M., Mubiru E., Tebandeke E., Matovu H., Odongo S., Moses Abayi J.J., Chelangat Ngeno E., Sillanp M., Ssebugere P. (2021). Organochlorine pesticide residues in Uganda's honey as a bioindicator of environmental contamination and reproductive health implications to consumers. *Ecotoxicology and Environmental Safety, 214*, 112094-112106. https://doi.org/10.1016/j.ecoenv.2021.112094

Panseri S., Catalano A., Giorgi A., Arioli F., Procopio A., Britti D., Chiesa L.M. (2014). Occurrence of pesticide residues in Italian honey from different areas in relation to its potential

contamination sources. *Food Control, 38*, 150-156. https://doi.org/10.1016/j.foodcont.2013.10.024

SANTE/11956/2016 rev. 9. Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey. *SANTE/11956/2016 rev. 9.* DG SANTE, European Comission, 2020.

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.

SANTE (2023). *Guidance document for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey*. In formation. It circulated in EU for comments.

Shendy A.H., Al-Ghobashy M.A., Mohammed M.N., Gad Alla S.A., Lotfy H.M. (2016). Simultaneous determination of 200 pesticide residues in honey usinggas chromatography–tandem mass spectrometry in conjunction withstreamlined quantification approach. *Journal of Chromatography A, 1427*, 142-160. https://doi.org/10.1016/j.chroma.2015.11.068

Sun X., Fu Z., Jiang T., Ning F., Cheng Y., Fu T., Zhu M., Zhang H., Zhang M., Hu P. (2022). Application of β-cyclodextrin metal-organic framework/titanium dioxide hybrid nanocomposite as dispersive solid-phase extraction adsorbent to organochlorine pesticide residues in honey samples. *Journal of Chromatography A*, *1663*, 462750-462760. https://doi.org/10.1016/j.chroma.2021.462750

Wang F., Wang Y., Li Y., Zhang S., Shi P., Li-Byarlay H., Luo S. (2022). Pesticide residues in beebread and honey in *Apis cerana cerana* and their hazards to honey bees and human. *Ecotoxicology and Environmental Safety, 238*, 113574-113581. https://doi.org/10.1016/j.ecoenv.2022.113574

Ward L.T. Hladik M.L., Guzmana A., Winsemius S., Bautista A., Kremen C., Millsa N.J. (2022). Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. *Science of the Total Environment, 831*, 154697-154708. https://doi.org/10.1016/j.scitotenv.2022.154697

Zhou X., Taylor M.P., Davies P.J., Prasad S. (2018). Identifying sources of environmental contamination in European honey bees (*Apis mellifera*) using trace elements and lead isotopic compositions. *Environmental Science & Technology, 52,* 991-1001. https://doi.org/10.1021/acs.est.7b04084