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Determining pesticide contamination in honey by LC-ESI-MS/MS – Comparison of pesticide recoveries of two liquid–liquid extraction based approaches

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ABSTRACT

Honey, a valuable food product, may be contaminated by xenobiotics during its production and/or harvest. The determination of trace levels of contaminants in a complex matrix like honey still presents a challenge to analytical chemists. The aim of this work was to assess and compare the extraction efficiencies of 30 pesticide residues (acaricides, insecticides, herbicides, fungicides), belonging to over 15 different chemical classes. Two common extraction approaches were applied – the increasingly popular QuEChERS method and extraction on a diatomaceous earth support. Both are used for pesticide determinations in fruits and vegetables, and with some adjustments they can be used for honey samples. In order to assess whether the differences in recoveries between the two investigated methods were statistically significant, the *F*-Snedecor and *T*-test were employed. The recoveries ranged from 34 to 96%, and in the case of 4 pesticides the differences in the values were statistically significant. Both methods showed good linearity ($R^2 > 0.991$), and the extraction efficiencies enabled method quantification limits well below EU-recommended Maximum Residue Levels to be achieved for the investigated pesticides.

1. Introduction

Produced by bees from flower nectar and honeydew, honey has antiseptic properties, stimulates the immunological system and is a source of many essential elements (Kędzia & Hołderna-Kędzia, 1998). However, on their foraging expeditions bees can also carry to the hive contaminants deposited in the environment, e.g. pesticides, heavy metals. Moreover, honey may be contaminated with antibiotics and/or pesticides as a result of inappropriate beekeeping practices, for example, when these substances are overdosed in beehive treatments.

Honey consists mainly of monosaccharides (ca. 70%), oligosaccharides (ca. 7%), water (ca. 18–20%) as well as other compounds from different chemical classes (essential elements, organic acids, proteins and amino acids, enzymes, flavonoids, anthocyanins, vitamins, sterols, phospholipids, essential oils and pigments), a total of approximately 300 compounds. Honey is thus a complex matrix and still presents a challenge to analysts aiming to determine contaminations at trace levels (Kujawski & Namieśnik, 2008). This implies the need for effective clean-up treatment before the analysis. Typical, "classic" clean-up/extraction procedures, such as liquid–liquid extraction (LLE) or solid phase extraction (SPE), require the use of significant amounts of organic solvents and usually enable the extraction of analytes belonging to only one chemical class (Balayianni & Balayiannis, 2008; Blasco, Lino, Picó, Pena, Font & Silveira, 2004; Herrera et al., 2005; Mukherjee, 2009).

Environmental concerns have encouraged researchers to develop more environmentally friendly approaches, resulting in the reduced use of organic solvents, or even the application of solventless techniques for pesticide residue analysis in honey.

Headspace solid-phase microextraction (HS-SPME) is a "green" technique, solventless and relatively fast (Arthur & Pawliszyn, 1990). However, its main drawbacks are possible sample carry-over and/or cross-contamination, as well as competitive sorption of analytes on the fibre, which in turn is fragile and of significant cost. In the case of HS-SPME, it is mainly the competitive sorption of analytes and the amount of volatile compounds naturally occurring in honey that render questionable its application in determining trace levels of contaminants in honey. Nonetheless, Direct Immersion solid-phase microextraction (DI-SPME) may be used with

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diluted honey solutions for routine screening analyses of pesticides (Volante et al., 2001).

In comparison with DI-SPME, under the same conditions (i.e. extraction followed by back-extraction into methanol and LC–MS analysis), stir bar sorptive extraction proves to be a better technique for the extraction of organophosphorus insecticides from honey, in terms of concentration capability, accuracy and sensitivity (Blasco, Fernández, Picó, & Font, 2004).

In recent years, several sample preparation approaches using reduced solvent volumes have been reported, such as the method employing LLE and low temperature purification, followed by Florisil® clean-up, for the determination of chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin (de Pinho, Neves, de Queiroz, & Silvério, 2010), or the method using pressurized solvent extraction (PLE) for the determination of organochlorine pesticide (OCP) residue levels (Wang, Kliks, Jun, & Li, 2010). Another example is coacervative microextraction ultrasound-assisted back-extraction (CME-UABE) coupled with GC–MS for the determination of organophosphorus pesticides (OPPs). This extraction technique uses non-ionic surfactants and the fact that under specific conditions they form micelles. Since the volume of extraction solvent is much smaller than the volume of aqueous sample (ca. 100-fold or more), it can also be regarded as preconcentration step. Back-extraction of the analytes into hexane is required prior to GC–MS analysis (Fontana, Camargo, & Altamirano, 2010). A different study reports on the development and validation of the method based on Florisil® SPE with LC–MS/MS detection and quantification of seven systemic insecticides (imidacloprid, thiamethoxam, fipronil and fipronil metabolites) in raw honey and pollen samples (García-Chao et al., 2010).

Although these methods give satisfactory results and are characterized by high quality metrological parameters, they are limited to only single groups of pesticides.

For the multi-class/multi-residue analysis of pesticides the most convenient detector would be a mass spectrometer (MS/MS, MS-TOF), coupled with either GC or LC, depending mostly on the type of pesticides of interest (volatile, semi-volatile and thermally stable ones can be determined by GC, whereas non-volatile and/or thermally unstable ones should be determined by LC). With regard to quantitative analysis by LC–MS/MS, one should bear in mind that analysis of honey samples is considerably influenced by strong matrix effects, altering the ionization efficiency of target compounds. This, however, can be easily compensated for by the dilution of extracts and by applying matrix-matched calibration standards.

A method for the determination of multiclass pesticide residues in honey samples employing Single Drop Microextraction (SDME) and GC-ECD for quantification and GC–MS for identification was reported (Tsiropoulos & Amvrazi, 2011). The method is characterized by good recovery values (70.8–120%) and method quantification limits in range of 0.03–10.6 ng/g. The advantage of applying SDME is that the method requires minimal volumes of organic solvents.

Recently four different approaches (namely: QuEChERS, SPE, PLE and SPME) were compared in terms of best applicability to extract 12 organophosphorus and carbamate insecticides from honey samples (Blasco, Vazquez-Roig, Onghena, Masia, & Picó, 2011). The authors concluded that any of the four methods is suitable for the recovery of all 12 target compounds (depending on the analyte and method applied, recoveries varied from 28–90% for SPME to 82–104% for PLE, at detection levels).

In this work two extraction approaches were examined, both originally developed for fruit and vegetable samples. One (hereafter denoted as SLE – Solid supported Liquid–Liquid Extraction), involves the introduction of an aqueous sample with a certain amount of NaCl to the column and the extraction of analytes from thin layer of liquid, adsorbed on a diatomaceous earth support, with an organic solvent (e.g. dichloromethane or ethylene acetate) (Klein & Alder, 2003). This approach, adjusted for honey samples, has been described by us elsewhere (Kujawski & Namieśnik, 2011). The other methodology – the QuEChERS approach (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003) – was originally developed for fruit and vegetables containing considerable amounts of water (>75%). This is based on acetonitrile extraction from an aqueous sample solution containing NaCl, followed by solvent demixing with the addition of anhydrous $MgSO_4$, the cleanup of an aliquot of the acetonitrile fraction by dispersive solid phase extraction, and the analysis of the supernatant.

The approaches were adjusted to the honey matrix and the extraction efficiencies of 30 pesticides of choice (amenable by LC–MS/MS) were compared. These pesticides, among others, have Maximum Residue Limits (MRLs) in honey established by the EU in Regulation (EC) No 396/2005, and they belong to different chemical classes. The aim of this study was to assess the applicability of both approaches to the multiclass determination of pesticide residues in honey by LC–MS/MS, at action levels (MRLs) and below them, as the aforementioned Regulation states for many MRLs that these values are lower limits of analytical determination.

2. Materials and methods

2.1. Pesticide standards, organic solvents and reagents

Pesticide standard solutions of acrinathrin, azoxystrobin, buprofezin, chloridazon, cymoxanil, cyprodinil, diflubenzuron, dimethomorph, etofenprox, fenazaquin, fenbuconazole, fenoxaprop-P, fenpropidin, fenpyroximate, fluazifop-P-butyl, flusilazole, flutolanil, metazachlor, methiocarb, phosalone, phoxim, propaquizafop, propham, prosulfocarb, pyridaben, pyriproxyfen, quizalofop-P-ethyl, simazine and tebufenpyrad at 100 ng μL^{-1} in acetone were supplied by Ultra Scientific (North Kingston, RI, USA), and a standard solution of emamectin benzoate, also at 100 ng μL^{-1} in acetone, was supplied by Sigma–Aldrich (Schnelldorf, Germany). The individual stock standard solutions were stored at −20 °C. The working standard solution mixture was prepared in acetone on the day of analysis. The matrix-matched calibration curve was prepared by spiking honey extracts (obtained by each method) at five concentration levels.

Pestanal® grade acetonitrile and LiChrosolv® grade *n*-hexane were purchased from Merck (Darmstadt, Germany). Dichloromethane (Pestanal® grade), methanol and 0.1% aqueous formic acid (both LC–MS grade) were supplied by Fluka (Buchs, Switzerland). NaCl p.a. was purchased from Stanlab (Lublin, Poland), disposable QuEChERS extraction kits were supplied by Agilent Technologies (Warsaw, Poland) and disposable ChemElut 5 mL cartridges were supplied by Varian Inc. (Darmstadt, Germany). Purified water was generated by a Millipore Milli-Q Ultra Pure Water System (Bedford, MA, USA). PTFE syringe filters (3 mm diameter, 0.45 μm pore size) were obtained from Macherey-Nigel (Düren, Germany).

Sample of forest raspberry honey free from contaminations was used throughout the validation process as a matrix blank and was provided by Bee Product Quality Testing Laboratory, Research Institute of Horticulture, Apiculture Division, Puławy, Poland.

2.2. Sample preparation procedures

Sample preparation by SLE was carried out as described elsewhere (Kujawski & Namieśnik, 2011) with one change – the agitation time was reduced to 20 min, as it was sufficient for complete dissolution of honey sample. A flow chart of the modified QuEChERS procedure is presented in Fig. 1.

2.3. LC–MS/MS analysis

The analyses were performed on an Agilent Infinity 1290 UHPLC system with a Kinetex C18 column (50 × 2.1 mm i.d., 1.7 μm particle size, Phenomenex, Torrance, CA, USA) coupled to an Agilent QqQ 6460 tandem mass spectrometer working in selected reaction monitoring mode (EMV: +300 V; dwell time: 5 ms). Two transitions for each analyte were monitored. The first, more intensive one, was used for quantification and the second for confirmation purposes. Mobile phase A consisted of 90:10 (v/v) H_2O:MeOH with 10 mmol L^{-1} ammonium acetate, and mobile phase B of 10:90 (v/v) H_2O:MeOH with 10 mmol L^{-1} ammonium acetate.

Gradient elution at a flow rate of 0.4 mL min^{-1} was applied as follows: 0–0.5 min 20% B, to 60% B in 2 min, hold 60% B for 2 min, to 90% B in 4.5 min, hold 90% B for 6 min. The total analysis time was 14 min, plus 2 min equilibration before each run.

A series of matrix-matched calibration solutions was prepared, covering a target analyte concentration range of 0.5 ng g^{-1} honey to 4xMRL; linear regression was assessed in terms of R^2.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated at matrix-matched calibration standard concentrations producing signal-to-noise ratios (S/N) of 3 and 10 respectively. The method detection limit (MDL) and method quantification limit (MQL) were estimated as the concentrations of standards in spiked samples producing S/N of 3 and 10 (respectively) after the whole procedure.

2.4. Extraction efficiency studies

In order to compare the two extraction approaches, a series of spiked samples used for recovery studies was prepared as follows: homogenized blank honey samples were spiked at 2 levels (respective MRLs and 75% MRLs of investigated pesticides) in pentuplicates, dissolved in appropriate amounts of water, homogenized and left for 1 h.

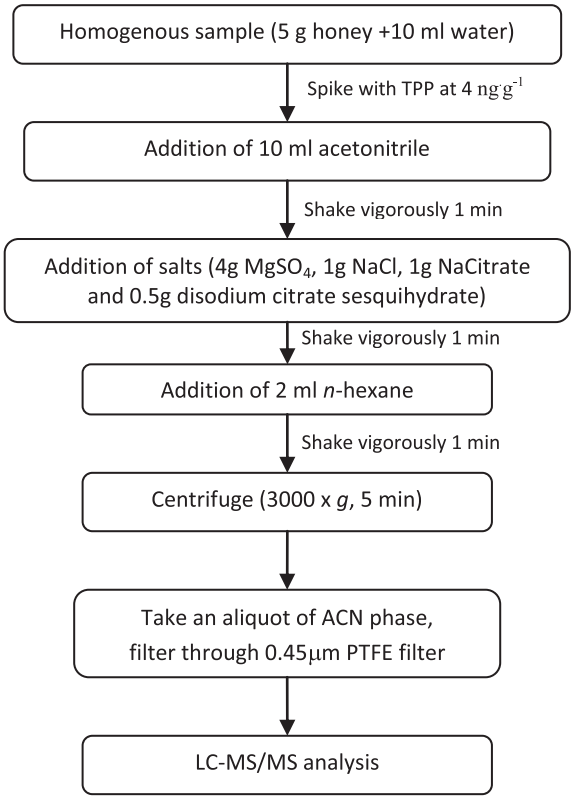


Fig. 1. Flow chart of the modified QuEChERS approach (TPP – triphenyl phosphate; ACN – acetonitrile).

After that time the operations were carried out in accordance with the flow chart and the recoveries for each of 30 pesticides calculated.

3. Results and discussion

3.1. Comparison of the two extraction approaches

In SLE approach SPE columns filled with inert solid phase (diatomaceous earth) are used. This solution enables realising the extraction

Table 1
Instrumental parameters of the MS/MS detector and retention times of target analytes after optimisation.

Compound	Retention time [min]	Precursor ion [m/z]	Product ion [m/z]	Fragmentor [V]	Collision energy [V]
Acrinathrin	9.01	559	208	100	8
		559	181	100	28
Azoxystrobin	6.22	404	372	100	8
		404	344	100	24
Buprofezin	9.17	306	201	100	4
		306	116	100	12
Chloridazon	1.78	222	104	150	20
		222	92	150	24
Cymoxanil	9.34	199	128	50	4
		199	83	50	20
Cyprodinil	7.99	226	93	150	36
		226	77	150	48
Diflubenzuron	8.26	311	158	100	8
		311	141	100	32
Dimethomorph	6.37	388	301	100	16
		388	165	100	32
Emamectin benzoate	10.86	886	158	200	40
		886	82	200	96
Etofenprox	12.83	394	177	100	8
		394	107	100	40
Fenazaquin	11.04	307	161	100	12
		307	147	100	16
Fenbuconazole	7.80	337	125	100	28
		337	70	100	16
Fenoxaprop-P	6.81	332	260	100	4
		332	152	100	12
Fenpropidin	6.55	274	147	150	28
		274	117	150	60
Fenpyroximate	11.36	422	366	100	12
		422	135	100	28
Fluazifop-P-butyl	9.94	384	282	150	16
		384	328	150	12
Flusilazole	7.76	316	247	100	12
		316	165	100	28
Flutolanil	6.89	324	262	100	12
		324	242	100	24
Metazachlor	4.23	278	134	50	16
		278	210	50	4
Methiocarb	5.79	243	169	50	8
		243	121	50	20
Phosalone	8.79	368	182	100	8
		368	111	100	44
Phoxim	8.90	299	129	50	4
		299	77	50	32
Propaquizafop	10.13	444	100	100	16
		444	299	100	20
Propham	4.24	180	138	50	4
		180	120	50	12
Prosulfocarb	8.73	252	128	100	4
		252	91	100	20
Pyridaben	10.88	365	147	100	20
		365	309	100	8
Pyriproxyfen	10.17	322	96	100	12
		322	185	100	20
Quizalofop-P-ethyl	9.74	373	299	146	16
		373	299	146	16
Simazine	3.08	202	132	100	16
		202	124	100	16
Tebufenpyrad	9.37	334	117	150	40
		334	145	150	24

in relatively short time, without the need for shaking, and in consequence avoiding creation of emulsion. Addition of sodium chloride (0.5 g mL^{-1} solution) to the aqueous sample improves recovery. After loading the column, the solution is adsorbed on the porous support. The extraction equilibrium is achieved within several minutes and subsequently the analytes can be eluted with ethyl acetate (up to 8% dissolves in water), methylene dichloride or other water-immiscible organic solvent. Analytes are extracted from adsorbed water film to the passing organic phase. This way the contact area between sample and the extraction solvent is significantly higher than in typical extraction approaches. The eluate can be then evaporated completely to change the solvent to the one most suitable for LC analysis, evaporated partially to concentrate the extract or analysed directly (for example by GC). In the described procedure aqueous/acetonitrile (1/1, v/v) sample was prepared and methylene dichloride was used as the elution solvent, the eluate was evaporated to dryness and reconstituted with methanol/water (70/30, v/v).

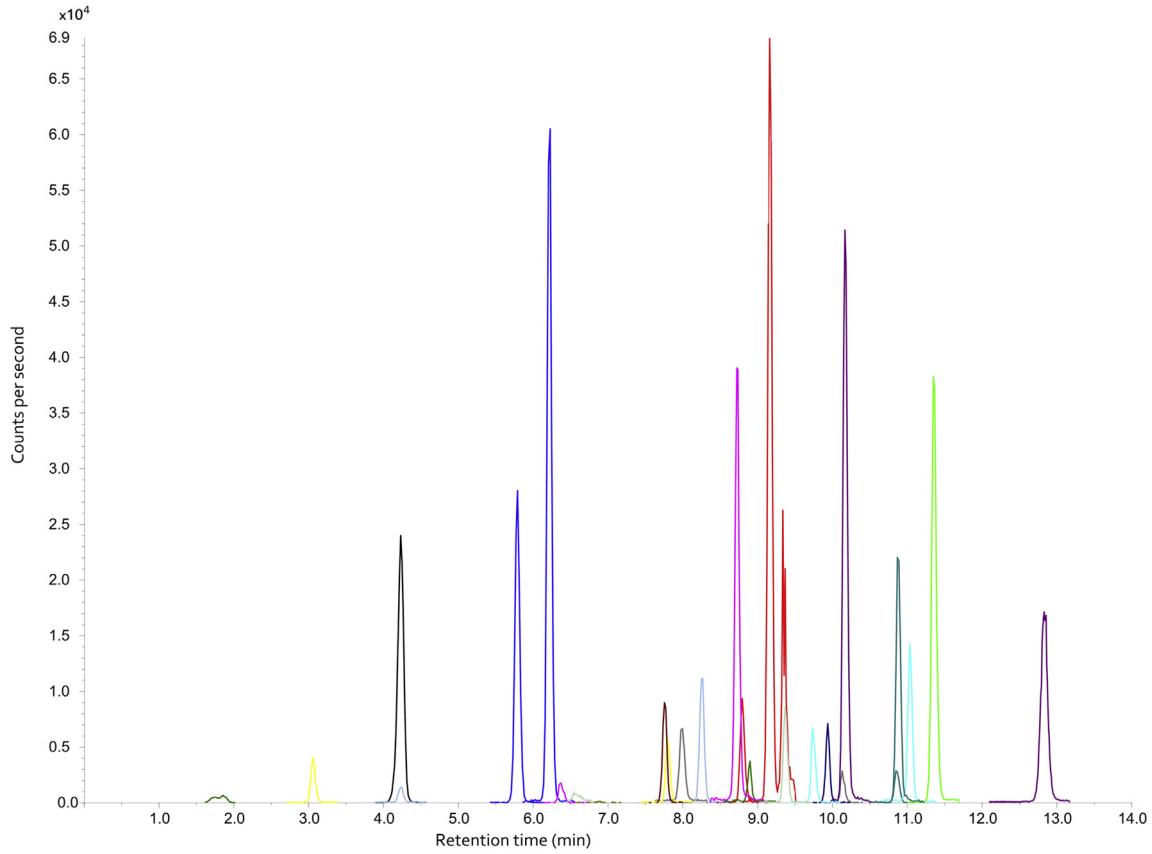
In the QuEChERS approach the sample should have more than 75% of water, so initial dissolution of honey sample was required. Acetonitrile is used as the water-miscible extraction solvent, enabling infinite contact area between phases. Phase separation is achieved by the addition of dehydrated $MgSO_4$, and the heat produced by water binding process promotes extraction to acetonitrile. It also causes waxes present in the sample to change their structure from highly porous to the one of low porosity, facilitating desorption of the analytes from the matrix. Addition of NaCl also increases the extraction efficiency. Additional extract clean-up by dispersive SPE allowed achieving lower noise and lower MDLs and MQLs. An aliquot of the acetonitrile extract was diluted with water (to obtain 1/1, v/v ratio) to get closer to the elution strength of initial LC gradient.

In general, the extract can also be analysed directly, evaporated partially, to concentrate the sample, or completely, to change the solvent to a more suitable one, if there is a need. However, concentration of honey extract, apart from increasing the analyte concentration (and hopefully signal), can also increase the matrix effects and cause ion suppression in the ionisation source and in consequence produce poorer signals with higher noise.

Modified QuEChERS approach seems to have an advantage over the SLE method in terms of time consumption, as the procedure does not require evaporation step. Also less toxic extraction solvent is used (acetonitrile vs. methylene dichloride), and its consumption is also in favour of the former (10 mL vs. 15 mL). Approximate sample preparation times are 20 min for QuEChERS and ca. 60 min for SLE, but in the latter, the steps consists of mainly waiting – for equilibration on the support and then for the complete evaporation under gentle stream of nitrogen.

3.2. LC–MS/MS

Most suitable MS/MS parameters for the target analytes were determined in flow injection mode, and after that, the optimisation of gradient elution conditions was realised using matrix-matched calibration standard. Using matrix-matched calibration compensates for changes in ionisation efficiency of analytes caused by matrix constituents. Determined most suitable MS/MS parameters and retention times for all analytes are given in Table 1.



Identity (RT in min) of peaks in elution order: Chloridazon (1.78), Simazine (3.08), Metazachlor (4.23), Propham (4.24), Methiocarb (5.79), Azoxystrobin (6.22), Dimethomorph (6.37), Fenpropidin (6.55), Fenoxaprop-P (6.81), Flutolanil (6.89), Flusilazole (7.76), Fenbuconazole (7.80), Cyprodinil (7.99), Diflubenzuron (8.26), Prosulfocarb (8.73), Phosalone (8.79), Phoxim (8.90), Acrinathrin (9.01), Buprofezin (9.17), Cymoxanil (9.34), Tebufenpyrad (9.37), Quizalofop-P-ethyl (9.74), Fluazifop-P-butyl (9.94), Propaquizafop (10.13), Pyriproxyfen (10.17), Emmamectin benzoate (10.86), Pyridaben (10.88), Fenazaquin (11.04), Fenpyroximate (11.36), Etofenprox (12.83)

Fig. 2. Representative Selected Reaction Monitoring (SRM) chromatogram of matrix-matched (extraction using modified QuEChERS approach) calibration sample at 10 ng g^{-1} level.

The use of an MS/MS detector enables the simultaneous detection and distinction of co-eluting analytes and the influence of matrix effect on chromatograms obtained by analysis of spiked extracts is moderate and comparable for both procedures. Matrix-matched calibration curves produced linear responses ($R^2 > 0.991$) over the range studied, i.e. LOQ-(4xMRL) ng g^{-1}. Optimized analysis parameters permit the definitive determination of analytes at levels below MRLs. Representative chromatogram of matrix-matched calibration solution containing all 30 pesticides (at 10 ng g^{-1} level) is presented in Fig. 2. Retention times correspond to the data in Table 1.

3.3. Recovery studies

The EC Regulation 396/2005 with annexes states for many MRLs that these values are lower limits of analytical determination, thus the extraction efficiencies were determined at MRL and 75% MRL levels. In order to compare the two extraction approaches, first the *F*-Snedecor test ($\alpha = 0.05$) was performed to determine whether the RSDs differed significantly; in the case of statistically insignificant differences, the *T*-test ($\alpha = 0.05$) was performed, otherwise the Cochran–Cox test was employed. The recoveries determined along with repeatability and the results of the statistical analysis are presented in Table 2.

In two cases, namely for flusilazole at 37.5 ng g^{-1} and fenazaquin at 10 ng g^{-1}, the *F*-Snedecor test results indicated that the variances of the extraction methods (their precisions) differed significantly. In this case a Cochran–Cox test ($\alpha = 0.05$) was performed to compare two results with significantly different variances and it was concluded that the recoveries for analytes in question do not differ statistically significantly between the methods.

The recoveries obtained for emamectin benzoate (at 7.5 ng g^{-1}), fluazifop-P-butyl and methiocarb (both at 37.5 ng g^{-1}) differed statistically significantly (*T*-test), depending on the extraction approach chosen. At the higher concentration level, only the recovery of cymoxanil (50 ng g^{-1}) differed significantly (*T*-test). At lower analyte concentrations the differences in the efficiencies of the extraction approaches for some pesticides may become significant. Differences in recoveries at different levels for same procedure and analyte are within 16% span (except for acrinathrin by SLE, where 22% difference may be observed) and are quite common when using MS/MS. These variations may occur due to the uncertainty budget of each result obtained (including variations in analyte-to-IS area ratios caused by run-to-run signal variations, even for replicate analyses of the same solution), however they both overlap with the range of 2xRSD, what is acceptable in such a case and for multiresidue methods developed and used for routine analyses.

The extraction yield for cymoxanil is poor in both cases, but such a situation may occur in the case of multiresidue methods, and a low recovery is acceptable, provided the repeatability is good.

The method detection and quantification limits for both approaches are given in Table 3. The values presented are for the "worst case scenario", i.e. for the highest level required to satisfy the S/N criteria. As can be seen, both methodologies allow for the unequivocal determination of analytes at levels below established MRLs.

4. Conclusions

The two reported methods used for the extraction of 30 multiclass pesticides from spiked honey samples are variants of liquid–liquid extraction requiring reduced amounts of organic solvents

Table 2
Extraction efficiencies of 30 pesticides spiked at respective 0.75 Maximum Residue Limits (MRLs) and MRLs after application of the methodologies investigated, along with repeatability (in terms of RSD, $n = 5$) and results of F-Snedecor test ($n_1 = n_2 = 5$, d.f. = 4, $\alpha = 0.05$) and *T*-test ($n_1 = n_2 = 5$, d.f. = 8, $\alpha = 0.05$).

Analyte	Recovery (RSD) at 0.75 MRL					$F_{crit} = 6.39$	Recovery (RSD) at MRL					$F_{crit} = 6.39$
	0.75 MRL [ng g^{-1}]	QuEChERS ($n = 5$)		SLE ($n = 5$)		*F*-value	MRL [ng g^{-1}]	QuEChERS ($n = 5$)		SLE ($n = 5$)		*F*-value
Acrinathrin	37.5	86%	(24%)	96%	(20%)	1.14	50	72%	(17%)	75%	(16%)	1.05
Azoxystrobin	7.5	73%	(19%)	71%	(12%)	2.69	10	59%	(16%)	67%	(16%)	1.22
Buprofezin	37.5	66%	(13%)	73.7%	(9.8%)	1.47	50	56.1%	(7.4%)	58%	(12%)	2.55
Chloridazon	37.5	65.4%	(7.4%)	67%	(10%)	2.10	50	58.0%	(7.2%)	62.6%	(8.9%)	1.78
Cymoxanil	37.5	36%	(11%)	36%	(10%)	1.24	50	34%	(12%)	41%	(11%)	1.25a
Cyprodinil	37.5	74.5%	(8.4%)	84%	(11%)	2.00	50	63.6%	(9.0%)	69%	(10%)	1.30
Diflubenzuron	37.5	70.5%	(7.6%)	74.2%	(8.6%)	1.42	50	61%	(13%)	67.1%	(8.1%)	2.06
Dimethomorph	37.5	70%	(16%)	70.6%	(8.4%)	3.41	50	59%	(14%)	66%	(12%)	1.06
Emamectin benzoate	7.5	77%	(11%)	64%	(11%)	1.50[a]	10	65%	(14%)	70%	(11%)	1.43
Etophenprox	37.5	49%	(10%)	46.5%	(9.8%)	1.26	50	43.5%	(8.8%)	47%	(10%)	1.54
Fenazaquin	7.5	53%	(14%)	43%	(18%)	1.07	10	40.4%	(8.0%)	48%	(23%)	**11.38**[b]
Fenbuconazole	37.5	73%	(12%)	70.8%	(6.8%)	3.06	50	60%	(24%)	68.1%	(9.2%)	5.20
Fenoxaprop-P	37.5	73%	(11%)	63.2%	(8.2%)	2.39	50	65%	(10%)	70%	(10%)	1.14
Fenpropidin	15.0	74%	(12%)	67.0%	(5.8%)	5.45	20	62.6%	(6.4%)	68.6%	(9.0%)	2.38
Fenpyroximate	7.5	66%	(13%)	73.1%	(8.0%)	2.11	10	62%	(20%)	65%	(10%)	3.37
Fluazifop-P-butyl	37.5	68%	(10%)	53.2%	(7.6%)	2.74[a]	50	59.5%	(7.4%)	65.6%	(8.7%)	1.68
Flusilazole	37.5	69%	(14%)	61.8%	(4.8%)	**11.07**[b]	50	56%	(22%)	63%	(10%)	4.11
Flutolanil	15.0	72%	(15%)	60.4%	(7.6%)	5.56	20	64%	(15%)	68%	(11%)	1.66
Metazachlor	37.5	73.1%	(7.6%)	74.0%	(7.2%)	1.08	50	62.4%	(9.0%)	68.4%	(7.4%)	1.23
Methiocarb	37.5	84.8%	(8.0%)	67.0%	(8.4%)	1.45[a]	50	74.6%	(8.6%)	79.8%	(8.2%)	1.04
Phosalone	37.5	76.8%	(8.6%)	70%	(12%)	1.50	50	70%	(13%)	75%	(10%)	1.54
Phoxim	15.0	64%	(20%)	55%	(10%)	4.81	20	60%	(15%)	62%	(15%)	1.03
Propaquizafop	37.5	67%	(14%)	71.4%	(7.6%)	3.09	50	61%	(10%)	64%	(11%)	1.23
Propham	37.5	86%	(18%)	78%	(19%)	1.18	50	73%	(18%)	80%	(19%)	1.33
Prosulfocarb	37.5	58.1%	(6.2%)	55%	(10%)	2.34	50	49.6%	(9.4%)	54.9%	(8.1%)	1.10
Pyridaben	15.0	60%	(15%)	60%	(11%)	1.99	20	52%	(24%)	56%	(13%)	2.90
Pyriproxyfen	37.5	65%	(10%)	70%	(10%)	1.25	50	56.4%	(6.2%)	61%	(10%)	2.96
Quizalofop-P-ethyl	37.5	67%	(15%)	66.5%	(9.8%)	2.25	50	60%	(11%)	65%	(12%)	1.50
Simazine	37.5	69%	(21%)	70%	(21%)	1.02	50	57%	(23%)	54%	(20%)	1.45
Tebufenpyrad	37.5	66%	(15%)	55%	(14%)	1.84	50	57%	(12%)	62%	(15%)	1.62

d.f. – degrees of freedom.
[a] Denotes significantly different recoveries (*T*-test, $\alpha = 0.05$).
[b] Results of *F*-Snedecor test over critical value are in bold. In this case Cochran–Cox test was performed ($\alpha = 0.05$).

Table 3
Method detection and quantification limits along with coefficients of determination for the investigated procedures, with pesticide types and set Maximum Residue Limit (MRL) values.

Analyte	Pesticide type	QuEChERS			SLE			MRL [ng g^{-1}]
		R^2	MDL [ng g^{-1}]	MQL [ng g^{-1}]	R^2	MDL [ng g^{-1}]	MQL [ng g^{-1}]	
Acrinathrin	Insecticide, Acaricide	0.9970	7.37	23.65	0.9921	7.09	22.75	50
Azoxystrobin	Fungicide	0.9931	0.45	1.50	0.9928	0.41	1.34	10
Buprofezin	Insecticide, Acaricide	0.9988	1.11	3.74	0.9926	1.08	3.64	50
Chloridazon	Herbicide	0.9948	1.16	3.80	0.9918	1.07	3.51	50
Cymoxanil	Fungicide	0.9911	1.97	6.48	0.9922	1.87	6.15	50
Cyprodinil	Fungicide	0.9938	0.88	2.99	0.9924	0.81	2.76	50
Diflubenzuron	Insecticide	0.9949	0.77	2.63	0.9919	0.70	2.38	50
Dimethomorph	Fungicide	0.9916	1.18	3.87	0.9919	1.05	3.46	50
Emamectin benzoate	Insecticide, Acaricide	0.9985	0.45	1.48	0.9918	0.45	1.50	10
Etophenprox	Insecticide	0.9952	0.92	2.99	0.9999	0.86	2.80	50
Fenazaquin	Acaricide, Insecticide	0.9930	1.04	3.46	0.9941	0.97	3.24	10
Fenbuconazole	Fungicide	0.9980	0.66	2.18	0.9976	0.57	1.91	50
Fenoxaprop-P	Herbicide	0.9939	0.70	2.30	0.9989	0.73	2.37	50
Fenpropidin	Fungicide	0.9922	0.61	2.08	0.9971	0.57	1.94	20
Fenpyroximate	Acaricide	0.9947	0.65	2.10	0.9932	0.62	2.00	10
Fluazifop-P-butyl	Herbicide	0.9949	0.67	2.19	0.9944	0.75	2.45	50
Flusilazole	Fungicide	0.9933	0.61	1.98	0.992	0.55	1.78	50
Flutolanil	Fungicide	0.9923	0.33	1.10	0.9957	0.35	1.17	20
Metazachlor	Herbicide	0.9951	1.35	4.49	0.9941	1.23	4.09	50
Methiocarb	Insecticide, Molluscicide	0.9926	0.64	2.14	0.9959	0.72	2.39	50
Phosalone	Insecticide, Acaricide	0.9914	0.69	2.28	0.9942	0.69	2.29	50
Phoxim	Insecticide, Disinfectant	0.9971	0.69	2.34	0.9915	0.75	2.55	20
Propaquizafop	Herbicide	0.9935	0.68	2.31	0.9998	0.64	2.18	50
Propham	Herbicide, Plant growth regulator	0.9959	5.35	17.84	0.9942	5.02	16.75	50
Prosulfocarb	Herbicide	0.9949	0.60	1.97	0.9917	0.55	1.79	50
Pyridaben	Insecticide, Acaricide	0.9915	1.57	5.17	0.9979	1.46	4.81	20
Pyriproxyfen	Insecticide	0.9937	0.74	2.48	0.9997	0.68	2.28	50
Quizalofop-P-ethyl	Herbicide	0.9995	0.74	2.35	0.9963	0.68	2.17	50
Simazine	Herbicide	0.9966	0.69	2.29	0.9996	0.72	2.41	50
Tebufenpyrad	Acaricide	0.9970	0.89	2.98	0.9946	0.93	3.10	50

(the QuEChERS approach and the extraction on diatomaceous earth support) and final determination by LC-ESI-MS/MS. The extraction yields of both approaches were compared; for lower analyte concentrations, the differences in recoveries may become significant. The recoveries obtained for emamectin benzoate, fluazifop-P-butyl, methiocarb and cymoxanil differed significantly (*T*-test), depending on the extraction approach chosen. For the rest of analytes, comparable results were obtained by both approaches. Both methods exhibit good linearity ($R^2 > 0.991$), and the extraction approaches proved to be efficient enough to achieve the quantitative determination of pesticide residues in honey samples at levels below EU-recommended MRLs.

Modified QuEChERS approach seems to have an advantage over the SLE method, because of smaller volumes of less toxic extraction solvent used in the sample preparation step, and also in regard to time consumption, as the procedure does not require evaporation to dryness. However, as performance of the both methods in terms of repeatability and achievable MQLs is comparable, it is up to the analyst's preference which one to choose.

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