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Determination of pesticide residues in honey: a preliminary study from two of Africa's largest honey producers

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Abstract

Background: The presence of pollutants in honey can influence honey bee colony performance and devalue its use for human consumption. Using liquid chromatography tandem mass spectrometry (LC-MS/MS), various clean-up methods were evaluated for efficient determination of multiclass pesticide contaminants in honey. The selected clean-up method was optimized and validated and then applied to perform a preliminary study of commercial honey samples from Africa.

Results: The most efficient method was primary-secondary amine (PSA) sorbent which was significantly different from the others (P <0.05; average recovery ~94 %) and was applied to analyze 96 pesticide residues in 28 retail honey samples from Kenya and Ethiopia. From our preliminary data, a total of 17 pesticide residues were detected at ~10-fold below maximum residue limit (MRL) established for food products except for malathion which was detected at almost 2-fold above its acceptable MRL.

Conclusions: A highly efficient approach for determining pesticide residues in honey with good recoveries was developed. All residue contaminants were detected at levels well below their acceptable MRLs except malathion suggesting that the retail honey analyzed is safe for human consumption. Although PSA clean-up method was selected as the most efficient for cleaning honey samples, omitting the clean-up step was the most economical approach with potential applicability in the food industry.

Keywords: Pesticide residues, Honey bees, Liquid chromatography-tandem mass spectrometry (LC-MS/MS), Honey, Method development

Background

The recent sudden decline of honey bee colonies is of global concern not only because of pollination services they provide in food production process, but also due to honey production among other benefits. While there are multiple variables, including poor nutrition, pests, diseases, and loss of natural bee habitat, negatively affecting bee health, it is becoming increasingly clear that the widespread use of pesticides on agricultural crops is a major factor (Vanengelsdorp and Meixner 2010; Gill et al. 2012; Brodschneider and Crailsheim 2010). As such, to preserve honey bee health which is inextricably integrated with human health and to preserve the quality of bee by-products especially honey, requires regular monitoring using rigorous analytical methods to confirm product quality (Muli et al. 2014; Kujawski and Namiesnik 2008).

Honey is composed of over 300 compounds, mostly carbohydrates (>75 %) and water (~18 %), with minor components comprising of proteins, amino acids, vitamins, antioxidants, minerals, essential oils, sterols, pigments, phospholipids, and organic acids (Bogdanov et al. 2008; Kujawski and Namiesnik 2008). Whereas these diverse ranges of compounds make it a nutrient rich food commodity, they also make it a highly complex analytical matrix especially when analysing the presence of trace compounds such as toxins, pesticide residues and other environmental pollutants (Kujawski and Namiesnik 2008). The presence of pesticide residues and other contaminants in honey can have adverse health effects on bees and humans, decrease the quality of honey

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and devalue its beneficial properties (Bogdanov et al. 2008). Typically, pesticide residues in honey occurs when bees in search for food, visit crops that have been treated with various agro-chemicals and/or when beekeepers use chemicals to control bee pests or diseases (Bogdanov 2006). So far, several researchers have reported various residues of pesticides in honey at varying concentrations (De Pinho, et al. 2010; Irani 2009; Barganska et al. 2013; Blasco et al. 2011; Garcia-Chao et al. 2010; Herrera et al. 2005; Rissato et al. 2007; Weist et al. 2011; Fontana et al. 2010; Kujawski and Namiesnik 2011; Wang et al. 2010; Campillo et al. 2006; Choudhary and Sharma 2008; Martel et al. 2007; Erdog˘rul 2007; Blasco et al. 2003) confirming the need to constantly monitor the presence of pesticide residues in honey to assess any potential health risk and to ensure that its quality, whether as food or as a therapeutic, is not compromised. However, to date, only few studies have been carried out to monitor pesticide residues in honey produced from Africa (Eissa et al. 2014). A recent study conducted in Kenya in 2010 detected four pesticides from beeswax and bee bread at very low concentrations (Muli et al. 2014). However, the cumulative levels and presence of pesticides in hive products over time can pose health problems for both honeybees and humans. Therefore there is the need to develop highly sensitive and selective analytical techniques that have the ability to analyze multiple pesticides simultaneously in hive products.

Since honey is a complex analytical matrix, it is often necessary to clean-up the sample prior to instrumental analysis (Kujawski and Namiesnik 2008). This facilitates removal of matrix co-extractives that could result in enhancement or suppression of the signal of the targeted analytes during analysis (Ferrer et al. 2011; Kittlaus et al. 2011; Kruve et al. 2008). Conversely, this clean-up step is usually the most expensive, time consuming and laborious sample preparation step with the highest probability of introducing errors on recovery and method repeatability. Conventional extraction/clean-up methods such as liquid-liquid (LLE) or solid-phase extractions (SPE), require large volumes of organic solvents and usually target pesticides from a single chemical class (Fontana et al. 2010; Fernández and Simal 1991; Wang et al. 2010; Martel et al. 2007). Recently, extensive research has been geared towards finding more economical and environmental friendly methods that can yield good recoveries for a diverse range of pesticides. For instance, a recent study compared four different methods for extracting 12 organophosphates and carbamates from honey and concluded that the choice of the method depends on the targeted analytes (Blasco, et al. 2011). In another example (Kujawski et al. 2014), two methods; solid supported liquid-liquid extraction(SLE) and a modified Quick, Easy, Cheap, Effective and Safe (QuEChERS) method for multiresidue analysis were compared using extraction efficiencies for determination of 30 LC-amenable pesticides in honey at their MRLs. These authors concluded that in terms of recovery (ranged from 34 to 96 %) the methods had no significant difference but in terms of costs and time, the modified QuEChERS was better (Kujawski et al. 2014). In this study, an ultra-high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was employed to analyze multiclass chemical contaminants in African honey at parts per billion (ppb) levels. Four different clean-up methods including PSA plus graphitized carbon (GCB), PSA plus C18, PSA alone, and a no clean-up approach were investigated using 96 LC-amenable pesticides to determine their applicability in a multiclass residue analysis in honey by comparing their recoveries. The method was validated and applied to conduct a preliminary study of pesticide residues in commercial honey samples obtained from Kenya and Ethiopia which are among the major producers of honey in Africa. Previous data on honey production in Africa indicates that Ethiopia is the largest producer with an estimate of 41,233 tons of honey followed by Tanzania at 28,678 tons and Kenya at 25,000 tons in 2004- 2006 (FAOSTAT). To the best of our knowledge, this is the first in-depth multiclass pesticide residue analysis of commercial honey from Africa. These results provide some insights in the safety of honey from Africa and some baseline information for future studies on other components of the hive matrix in relation to honey bee colony losses.

Methods

Chemicals and reagents

All pesticide standards were of high purity (>94 %) and were obtained from Sigma-Aldrich (Chemie GmbH, Germany) and Dr Ehrenstorfer (Augsburg, Germany) and were stored according to manufacturer's recommendations until use. Pesticide stock solutions were prepared in acetonitrile at 1 μg/mL and stored in amber screw-capped glass vials at −20 °C.

LC-MS/MS instrumentation

An Agilent 1290 ultra high performance liquid chromatography (UHPLC) series coupled to a 6490 model triple quadrupole mass spectrometer (Agilent technologies) with an ifunnel JetStream electrospray source operating in the positive ionization mode was applied using dynamic multi-reaction monitoring (DMRM) software features. The electrospray ionization settings were gas temperature, 120 °C; gas flow, 15 L/min; nebuliser gas, 30 psi; sheath gas temperature, 375 °C; sheath gas flow, 12 L/min; capillary voltage, 3500 V; nozzle voltage, 300 V. The ifunnel parameters were high pressure RF 150 V and low pressure RF 60 V. Nitrogen was used both as a nebuliser and as the collision gas. Mass Hunter Data Acquisition; Qualitative and Quantitative analysis

software (Agilent Technologies, Palo Alto, CA, v.B.06 and v.B.07) were used for method development, data acquisition and data processing for all the analyses.

The chromatographic separation was performed on a Rapid Resolution reverse phase column-C18 1.8 μm, 2.1 × 150 mm column (Agilent Technologies). The mobile phases comprised of 100 % water in 5 mM ammonium formate containing 0.1 % formic acid for solvent A and acetonitrile in 5 mM ammonium formate containing 0.1 % formic acid for solvent B. A gradient elution at a flow rate of 0.4 mL/min was used.

Optimization of LC-MS/MS parameters

Pesticide standard solutions, individually or as mixes, were used for method development and instrument parameters optimization. To ensure that the maximum sensitivity for identification and quantification of the targeted pesticides is obtained, careful optimization of all MS parameters was performed by infusing the standard solutions directly into the MS followed by infusion through the column to establish their respective retention times (RT). The parameters optimised included collision energy (CE), gas temperature; gas flow, sheath gas temperature and flow, high and low pressure radio-frequency. Table 1 demonstrates the parameters developed and optimised for the 96 pesticide residues targeted in this study.

Data analysis

Targeted analytes were identified by monitoring two transition ions where possible, for each analyte as recommended by SANCO guidelines for LC-MS/MS analysis (SANCO/12571/2013). The most dominant transition ion was used for quantification whereas the second most intense ion as a qualifier for confirmation purposes. Calibration standard solutions were prepared at seven calibration levels covering a concentration range of 0.1 to 100 parts per billion (ppb), including the zero point. The resulting calibration curve was used to determine the instrument's limit of reporting (LOR) and limits of detection (LOD). These were set as calibration standard concentrations producing signal to noise ratio of 3 and 10 respectively. The LOR was set as the minimum concentration that could be quantified with acceptable accuracy and precision. The LC-MS/MS system's linearity was evaluated by assessing the signal responses of the calibration standards.

Sample preparation

Prior analysis of a honey sample, obtained from the local organic farmer from Kenya, was performed to ensure that it did not contain any of the studied compounds. This sample was selected as a blank during method development for spiking, preparing matrix matched calibration curves and recovery purposes. Samples were prepared following the QuEChERS method (Anastassiades et al. 2003) with some modifications. Briefly, 5 g of this sample was weighed into a 50 ml falcon tube and 10 ml of water were added and the mixture homogenized. Acetonitrile (10 ml) plus a mixture of salts (4 g magnesium sulphate, 1 g sodium chloride, 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate) were added and the samples were vortexed for 1 min and centrifuged at 4200 rpm for 5 min. Aliquots of the supernatant were transferred to separate eppendorf tubes and subjected to either no clean-up or to various QuEChERS clean-up methods. A portion of 1 mL of the final solution was then transferred to an auto-sampler vial for LC-MS/MS analysis.

Extraction efficiency

A series of spiked samples were used to assess extraction efficiency of the method. These samples were prepared as follows: blank honey samples fortified at 10 times LOQ (10 ng/g) were dissolved in appropriate amounts of water and homogenized. Extractions of the spiked residues were performed following QuEChERS methods. Honey samples were spiked with a mixture of pesticide residues possessing different physic-chemical properties. After extraction, aliquots of the extract were subjected to three QuEChERS clean-up methods (PSA plus GCB or PSA plus C18 or PSA alone). Figure 1 represents a schematic diagram illustrating the workflow that was employed during method development. Extraction efficiencies of these clean-up methods were compared to extraction efficiencies of no clean-up methods to evaluate which of those methods will be best suited for our analysis. Instead, these samples were subjected to high centrifugation (12,000 rpm held at 4 °C) for 10 min and filtered through 0.22 μm PTFE filters on a Samplicity system (Merck Millipore, Germany). Each test was replicated three times.

Matrix effects

The effect of matrix co-extractives was performed by assessing ion suppression or enhancement effects of signals from chromatograms of matrix matched standard solutions compared to spiked extracts at the same concentration levels as per DG SANCO guidelines for LC-MS/MS analysis (SANCO/12571/2013). These were prepared using the extract of blank matrix (honey) covering a target analyte concentration range of 0.1 to 100 ng/g. Detection and quantification limits of the method were determined as described previously.

Validation of the analytical procedure

Analytes to be validated were spiked into the blank honey sample at LOR (1 ng/g) and at the lowest MRL level (0.01 mg/kg or 10 ng/g). Analysis was performed as

Table 1 Instrumental parameters of the MS/MS detector and retention times (RT) of the 96 pesticides standard mixture used for method development

Compound name	RT (min)	Parent ion (*m/z*)	[a]Trans1	CE1(V)	[a]Trans2	CE2(V)
Omethoate	2.72	214	125	20	109	25
Acetamiprid	2.84	223	126	20	90	35
Acephate	2.84	184	143	5	125	15
Propamocarb	3.19	189	144	5	102	15
Oxamyl	3.58	237	90	0	72	15
Methomyl	3.84	163	106	5	88	0
Thiamethoxam	3.95	292	211	5	181	20
Monocrotophos	3.95	224	193	0	127	10
Aldicarb	3.98	208	116	0	89	10
Imidacloprid	4.42	256	209	10	175	15
Thiabendazol	4.45	202	175	25	131	35
Cymiazole	4.70	219	171	25	144	35
Dimethoate	4.82	230	199	0	125	20
Thiacloprid	5.13	253	126	20	90	40
Propagite	5.25	368	231	5	175	10
Aldicarb fragment	5.43	116	89	4	70	4
Pirimicarb	5.90	239	182	10	72	20
Dichlorvos	6.13	221	109	12	79	24
Carbofuran	6.36	222	165	5	123	20
Nicosulfuron	6.40	411	213	12	182	16
Metsulfuron-methyl	6.51	382	199	20	167	15
Metribuzin	6.54	215	187	15	84	20
Malathion	6.64	331	126	5	99	10
Carbaryl	6.93	202	145	0	127	25
Fosthiazole	7.16	284	228	5	104	20
Thiodicarb	7.16	355	108	10	88	10
Amidosulfuron	7.22	370	261	10	218	20
DEET	7.75	192	119	16	91	32
Molinate	7.75	188	126	25	98	12
Tribenuron-methyl	7.87	396	155	5		
Metalaxyl	7.89	280	220	10	160	20
Flutriafol	8.01	302	70	15	123	30
Diuron	8.02	233	72	20	72	20
Isoxafluote	8.08	360	251	20	220	35
Methidathion	8.46	303	145	0	85	15
Flazasulfuron	8.73	408	182	15		
Fenobucarb	8.79	208	152	5	95	10
Azoxystrobin	9.01	404	372	10	344	25
Linuron	9.19	249	182	10	160	15
Fludioxonil	9.30	247	169	32	126	32
Promecarb	9.64	208	151	0		
Bosclid	9.67	343	271	28	307	12
Triadimefon	10.01	294	197	10	69	20

Table 1 Instrumental parameters of the MS/MS detector and retention times (RT) of the 96 pesticides standard mixture used for method development *(Continued)*

Bromuconazole	10.02	378	159	35	70	20
Bifenazate	10.09	301	170	15		
Cyproconazole	10.16	292	70	15	125	35
Fluquinconazole	10.27	376	349	16	307	24
Iprovalicarb	10.27	321	203	0	119	20
Triadimenol	10.36	296	70	5	99	10
Flufenacet	10.38	364	194	5	152	15
Bupirimate	10.42	317	166	20	108	25
Tetraconazole	10.45	372	159	30	70	20
Ethoprophos	10.48	243	131	15	97	30
Epoxyconazol	10.65	330	121	20	101	45
Cyazofamid	10.68	325	261	5	108	10
Cyprodinil	10.81	226	93	40	77	45
Fenbuconazole	10.85	337	125	35	70	15
Metolachlor	10.94	284	252	10	176	20
Fenamiphos	10.95	304	217	20	202	35
Flusilazole	10.97	316	247	15	165	25
Picoxystrobin	11.05	368	205	0	145	20
Tebufenozid	11.10	353	297	0	133	15
Diflubenzuron	11.17	311	158	10	141	35
Rotenone	11.24	395	213	20	192	20
Fipronil	11.25	435	330	12	250	28
Kresoxim-methyl	11.53	314	267	0	222	10
Tebuconazole	11.53	308	125	40	70	20
Procymidon	11.64	284	67	12	256	28
Benalaxyl	11.71	326	294	5	148	15
Diazinon	11.71	305	169	20	153	20
Coumaphos	11.76	363	307	16	227	28
Prochloraz	11.76	376	308	5	266	10
Chlorfenvinphos	11.77	359	170	40	155	8
Hexaconazole	11.93	314	159	30	70	15
Pyraclostrobin	12.04	388	194	5	163	20
Clofentezin	12.06	303	138	10	102	40
Pirimiphos-methyl	12.21	306	164	20	108	30
Spinosyn A	12.23	732	142	30	98	45
Metconazole	12.30	320	125	40		
Bitertenol	12.38	338	269	0	70	0
Chlorpyrifos-methyl	12.41	322	290	10	125	25
Trifloxystrobin	12.78	409	186	10	145	45
Spinosyn D	12.88	747	142	35	98	55
Ipconazole	12.97	334	125	45	70	25
Indoxacarb	12.99	528	203	45	150	20
Novaluron	13.32	493	158	20	141	45
Buprofezin	13.45	306	201	5	116	10

Table 1 Instrumental parameters of the MS/MS detector and retention times (RT) of the 96 pesticides standard mixture used for method development *(Continued)*

Profenofos	13.48	375	347	5	305	15
Ethion	13.93	385	199	4	143	20
Temephos	14.02	467	419	20	125	44
Chlorpyrifos	14.08	350	200	15	198	15
Pyriproxyfen	14.17	322	185	20	96	10
Lufenuron	14.19	511	158	20	141	45
Hexythiazox	14.46	353	228	10	168	25
Fenazaquin	15.35	307	161	10	57	25
Pyridaben	15.44	365	309	10	147	25
Bifenthrin	16.47	440	181	5	166	20
Etofenprox	16.57	394	177	10	107	45

[a]Transition ions used to quantify and qualify the targeted analytes

described previously. The recoveries and precision of the extraction method were determined as the average of five replicates. The method linearity was evaluated by assessing the signal responses of the targeted analytes from matrix-matched calibration solutions prepared by spiking blank extracts at seven concentration levels, from 0.1 to 100 ng/g, including the zero point or the blank. The method precision was expressed as percent relative standard deviation (%RSD) of the intra-day and inter-day analyses ($n = 5$). Blank matrices along with reagent blank were run during validation to ensure minimal risk of interferences, guarantee specificity of the method and to check for potential solvent contamination.

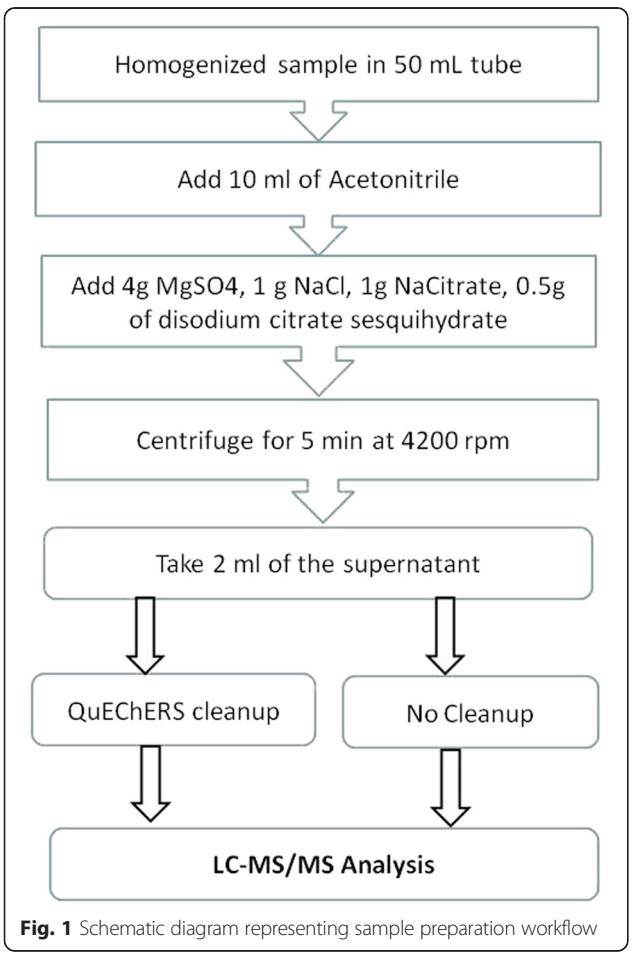

Fig. 1 Schematic diagram representing sample preparation workflow

Application to real samples

The developed method was applied to conduct a preliminary study on chemical contaminants present in commercial honey in Africa. Ethiopia and Kenya were selected for this study as they are among the major producers of honey in Africa. From each country, 14 commercial honey samples were collected from local markets/farmers. These samples consisted of five honey samples from stingless (*Apis meliponina*) and nine honey bee (*Apis mellifera*) samples from various regions in each country. A total of 28 samples were analyzed at the African Reference Laboratory for Bee Health, International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Nairobi, Kenya at two different seasons (November 2014 and July 2015). All samples were stored in their original packaging under the recommended conditions prior to use and were prepared as previously described. The same calibration curve described above was run at the end of the sample series to check the stability of the detector after data acquisition of the unknown samples.

Statistical analysis

Data were analyzed using R version 3.1.1 (R Core Team 2014). For each pesticide or compound, the four clean-up methods were compared using one-way Analysis of Variance (ANOVA) and the means separated using the Student-Newman-Kuels (SNK) test. All tests were performed at 5 % significance level. Means with the same letter across are not significantly different.

Results and discussion

LC-MS/MS analysis

In this study, the methods investigated were selected based on the known matrix interferences expected from honey. Since sugars constitute the greatest proportion of honey (>75 %), three of the four methods investigated included PSA, as it removes sugars, along other interferences. Samples were spiked with a mixture of 96 pesticide standards at the default MRL value (0.01 mg/kg) since it provided great recoveries with the best reproducibility across multiple analytes during method development. Figure 2 shows representative chromatograms of honey extract processed using the four clean-up methods. Although the chromatographic profiles appeared similar for the four clean-up methods, the lowest recoveries were obtained from pesticides subjected to PSA combined with GCB clean-up with recoveries ranging from 5 to 117 % (Table 2). The use of GCB was important in removing pigment in honey; however, it also resulted in significant analyte losses during sample clean-up which could potentially lead to false negative results. Out of the 96 pesticides evaluated, 51 pesticides had the lowest recoveries from this method compared to the other methods (Table 2). Additionally, more than 45 % of the pesticides subjected to this method did not meet the minimum recommended criteria (>70 %) as indicated in the Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed (SANCO/12571/2013). On the other hand, for most pesticides, the best recoveries were obtained when PSA was used as a clean-up method. When compared to PSA plus C18 clean-up method, there were significant (P <0.05) differences in more than 10 % of the pesticides evaluated. Results from this study also indicate that out of the 96 pesticides studied, only three pesticides, nicosulfuron (43 %), procymidon (58 %) and propamocarb (58 %), had recoveries that were below the acceptable limit when PSA was used alone. There was no significant (P <0.05) difference in recoveries for procymidon cleaned using C18 plus PSA (78 %) and PSA alone (58 %). Therefore, to improve recoveries for nicosulfuron and propamocarb, other alternatives must be considered. For instance, for nicosulfuron, based on the data provided in Table 2, the clean-up step can be omitted to yield 100 % recovery. This suggests that in the absence of clean-up resources, satisfactory information on levels of residue contamination in honey can still be achieved with minimal sample manipulations as found in other studies (Kujawski et al. 2014). Although omitting the clean-up step offers time savings in sample processing and is more economical, further precaution must be taken to avoid any potential clogging of the LC-MS system or eventual contamination of the MS ionization source. Based on the findings highlighted in Table 2, the use of PSA was selected as the best method for our analysis but was complemented with the no clean-up method to maximize on recoveries of all targeted pesticides.

Analytes eluted in 17 min followed by a short high-organic rinse to maintain the column and also in avoiding matrix carryover into the next sample. Elution of the remaining matrix material during subsequent analysis can cause unexpected matrix effects resulting in significant ionization inefficiencies. Matrix effects may either result to signal enhancement leading to recoveries >100 % or signal suppression resulting in poor recoveries. Aside from polar pesticides, other pesticides were well distributed across the elution window facilitating proper scan rate for scheduled

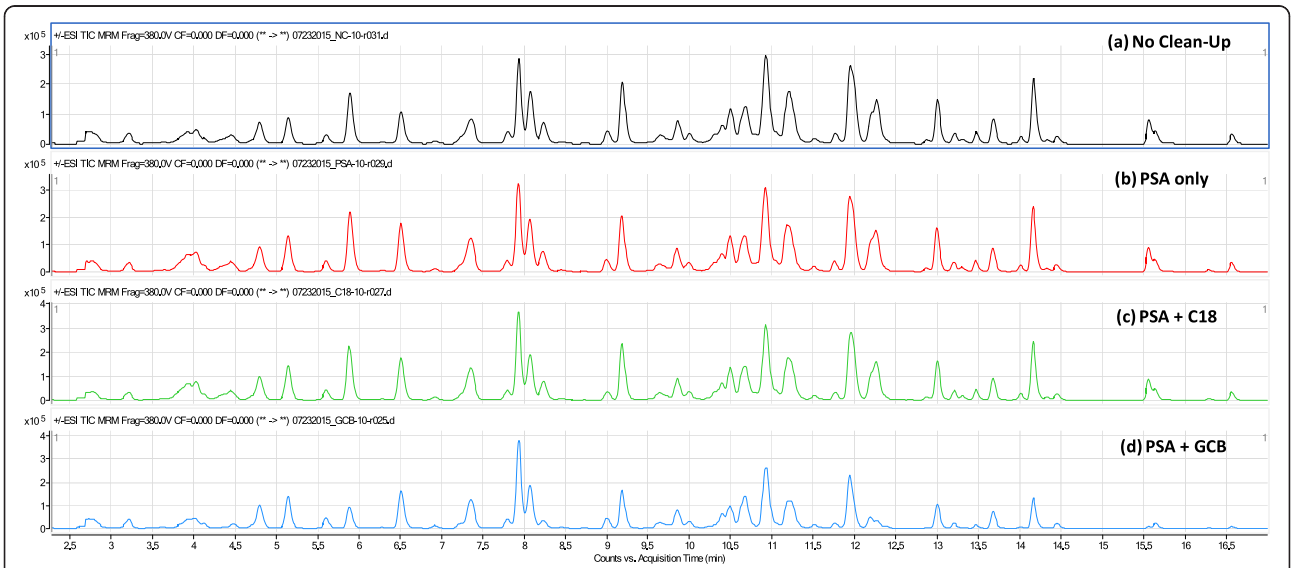

Fig. 2 Example of total ion chromatograms (TIC) of 96 pesticides extracted from spiked honey sample at 10 ng/g level and cleaned up using (**a**) No clean-up (**b**) PSA only (**c**) PSA+C18 (**d**) PSA+GCB

Table 2 Percentage recoveries (±SD) of 96 pesticides subjected to either QuEChERS clean-up methods or no clean-up

	% recovery at 10LOR (10 ng/g) ± SD			
Compound name	GCB+PSA	C18+PSA	PSA	No clean-up
Acephate	72.8 ± 0.8$^{(b)}$	85.1 ± 0.6$^{(a)}$	76.1 ± 0.8$^{(ab)}$	52.5 ± 0.7$^{(c)}$
Acetamiprid	98.1 ± 0.6$^{(a)}$	99.8 ± 0.03$^{(a)}$	99.6 ± 0.3$^{(a)}$	74.8 ± 0.0$^{(b)}$
Aldicarb fragment	104.5 ± 0.4$^{(a)}$	100.5 ± 0.3$^{(b)}$	97.9 ± 0.2$^{(b)}$	70.6 ± 0.1$^{(c)}$
Amidosulfuron	87.0 ± 0.8$^{(b)}$	74.3 ± 0.4$^{(c)}$	89.5 ± 0.1$^{(b)}$	94.3 ± 0.2$^{(a)}$
Azoxystrobin	77.0 ± 0.7$^{(b)}$	108.8 ± 0.8$^{(a)}$	106.5 ± 0.8$^{(a)}$	101.8 ± 0.5$^{(a)}$
Benalaxyl	88.9 ± 0.6$^{(a)}$	97.3 ± 1.0$^{(a)}$	97.5 ± 0.4$^{(a)}$	97.7 ± 0.6$^{(a)}$
Bifenazate	23.7 ± 0.8$^{(b)}$	117.5 ± 0.3$^{(a)}$	111.6 ± 1.2$^{(a)}$	121.6 ± 0.4$^{(a)}$
Bifenthrin	45.7 ± 1.1$^{(b)}$	92.5 ± 0.9$^{(a)}$	79.8 ± 0.8$^{(a)}$	90.2 ± 0.02$^{(a)}$
Bitertanol	88.9 ± 0.6$^{(b)}$	105.6 ± 0.4$^{(a)}$	99.4 ± 0.01$^{(a)}$	100.6 ± 0.7$^{(a,b)}$
Bosclid (Nicobifen)	39.6 ± 1.0$^{(b)}$	113.1 ± 1.3$^{(a)}$	106.3 ± 0.4$^{(a)}$	115.3 ± 0.7$^{(a)}$
Bromuconazole	85.2 ± 1.6$^{(b)}$	96.9 ± 0.1$^{(ab)}$	103.0 ± 0.4$^{(a)}$	92.3 ± 0.4$^{(ab)}$
Bupirimate	61.3 ± 0.6$^{(b)}$	104.6 ± 0.1$^{(a)}$	102.5 ± 0.6$^{(a)}$	110.7 ± 1.4$^{(a)}$
Buprofezin	84.6 ± 0.5$^{(c)}$	104.0 ± 0.4$^{(ab)}$	106.9 ± 0.4$^{(a)}$	102.9 ± 0.8$^{(b)}$
Carbaryl	98.2 ± 1.2$^{(a)}$	110.9 ± 0.9$^{(a)}$	102.5 ± 0.2$^{(a)}$	72.6 ± 0.1$^{(b)}$
Carbofuran	108.3 ± 0.8$^{(b)}$	120.4 ± 0.9$^{(a)}$	119.9 ± 0.1$^{(ab)}$	64.1 ± 0.6$^{(c)}$
Chlorfenvinphos	78.1 ± 0.7$^{(c)}$	93.6 ± 0.1$^{(b)}$	103.6 ± 0.2$^{(a)}$	94.9 ± 0.7$^{(b)}$
Chlorpyrifos	21.4 ± 0.6$^{(b)}$	93.4 ± 0.6$^{(a)}$	94.2 ± 0.3$^{(a)}$	87.6 ± 1.0$^{(a)}$
Chlorpyrifos-methyl	26.4 ± 0.8$^{(c)}$	105.3 ± 0.5$^{(a)}$	99.5 ± 0.1$^{(a)}$	95.5 ± 0.4$^{(b)}$
Clofentezin	6.2 ± 0.7$^{(b)}$	97.3 ± 0.3$^{(a)}$	98.5 ± 0.3$^{(a)}$	91.1 ± 0.8$^{(a)}$
Coumaphos	5.4 ± 0.4$^{(b)}$	102.6 ± 1.3$^{(a)}$	105.2 ± 0.5$^{(a)}$	109.2 ± 0.5$^{(a)}$
Cyazofamid	79.1 ± 0.2$^{(c)}$	102.2 ± 0.1$^{(a)}$	100.6 ± 0.2$^{(a)}$	92.3 ± 0.2$^{(b)}$
Cymiazol	56.0 ± 0.9$^{(c)}$	92.2 ± 0.4$^{(a)}$	89.5 ± 0.1$^{(a)}$	75.2 ± 0.5$^{(a)}$
Cyproconazole	100.3 ± 0.2$^{(b)}$	87.8 ± 1.0$^{(b)}$	106.8 ± 0.2$^{(a)}$	92.3 ± 0.5$^{(b)}$
Cyprodinil	10.5 ± 0.6$^{(c)}$	90.8 ± 0.2$^{(b)}$	104.5 ± 0.4$^{(a)}$	105.0 ± 1.0$^{(ab)}$
DEET	109.1 ± 0.4$^{(a)}$	100.4 ± 1.2$^{(a)}$	96.0 ± 0.0$^{(a)}$	83.5 ± 0.5$^{(b)}$
Diazinon	81.4 ± 0.01$^{(b)}$	98.7 ± 0.3$^{(a)}$	99.0 ± 0.3$^{(a)}$	99.4 ± 1.1$^{(a)}$
Dichlorvos	107.0 ± 0.8$^{(a)}$	97.3 ± 0.6$^{(b)}$	99.4 ± 0.3$^{(b)}$	85.7 ± 0.2$^{(c)}$
Diflubenzuron	18.9 ± 4.8$^{(b)}$	101.9 ± 0.3$^{(a)}$	106.3 ± 1$^{(a)}$	104.8 ± 0.6$^{(a)}$
Dimethoate	99.2 ± 1.0$^{(a)}$	101.7 ± 0.2$^{(a)}$	94.3 ± 0.3$^{(a)}$	62.8 ± 0.1$^{(b)}$
Diuron	33.0 ± 0.8$^{(c)}$	100.7 ± 0.6$^{(a)}$	108.1 ± 0.2$^{(a)}$	92.1 ± 0.4$^{(b)}$
Epoxyconazol	38.8 ± 2.6$^{(b)}$	91.2 ± 0.2$^{(a)}$	96.4 ± 1.1$^{(a)}$	89.9 ± 0.6$^{(a)}$
Ethion	78.7 ± 0.2$^{(b)}$	98.3 ± 0.1$^{(a)}$	103.0 ± 0.5$^{(a)}$	95.1 ± 0.1$^{(a)}$
Ethoprophos	87.7 ± 0.9$^{(a)}$	94.1 ± 0.4$^{(a)}$	98.3 ± 1.0$^{(a)}$	90.8 ± 0.7$^{(a)}$
Etofenprox	24.2 ± 0.5$^{(b)}$	98.5 ± 0.4$^{(a)}$	99.5 ± 0.1$^{(a)}$	92.2 ± 0.0$^{(a)}$
Fenamiphos	56.4 ± 1.0$^{(b)}$	107.8 ± 0.2$^{(a)}$	111.6 ± 0.3$^{(a)}$	107.8 ± 0.5$^{(a)}$
Fenazaquin	9.9 ± 1.7$^{(d)}$	93.5 ± 0.5$^{(b)}$	98.4 ± 0.1$^{(a)}$	89.4 ± 0.1$^{(c)}$
Fenbuconazole	40.5 ± 1.2$^{(b)}$	107.8 ± 0.3$^{(a)}$	109.1 ± 0.9$^{(a)}$	107.0 ± 0.3$^{(a)}$
Fenobucarb	94.7 ± 2.0$^{(b)}$	80.6 ± 0.4$^{(c)}$	101.6 ± 0.1$^{(a)}$	90.4 ± 0.2$^{(b)}$
Fipronil	107.9 ± 1.0$^{(a)}$	111.4 ± 0.1$^{(a)}$	108.3 ± 0.3$^{(a)}$	111.1 ± 0.4$^{(a)}$
Flazasulfuron	81.1 ± 1.5$^{(b)}$	46.7 ± 0.5$^{(d)}$	70.3 ± 0.3$^{(c)}$	96.7 ± 0.4$^{(a)}$
Fludioxonil	34.1 ± 2.9$^{(b}$	105.9 ± 0.5$^{(a)}$	104.7 ± 0.1$^{(a)}$	110.8 ± 0.5$^{(a)}$
Flufenacet	102.8 ± 1.9$^{(a)}$	117.5 ± 0.9$^{(a)}$	103.8 ± 0.7$^{(a)}$	100.9 ± 0.5$^{(a)}$
Fluquinconazole	43.6 ± 2.0$^{(b)}$	92.7 ± 1.1$^{(a)}$	99.3 ± 0.9$^{(a)}$	90.5 ± 0.9$^{(a)}$

Table 2 Percentage recoveries (±SD) of 96 pesticides subjected to either QuEChERS clean-up methods or no clean-up *(Continued)*

Flusilazole	97.8 ± 0.8$^{(b)}$	117.4 ± 0.7$^{(a)}$	108.3 ± 0.8$^{(ab)}$	98.6 ± 0.5$^{(ab)}$
Flutriafol	94.3 ± 0.2$^{(b)}$	97.9 ± 0.4$^{(ab)}$	101.3 ± 0.1$^{(a)}$	96.8 ± 0.5$^{(ab)}$
Fosthiazate	101.8 ± 0.2$^{(a)}$	107.1 ± 0.9$^{(a)}$	103.6 ± 0.1$^{(a)}$	70.1 ± 0.1$^{(b)}$
Hexaconazole	90.0 ± 1.0$^{(b)}$	99.3 ± 1.1$^{(b)}$	110.5 ± 0.5$^{(a)}$	97.9 ± 0.1$^{(b)}$
Hexythiazox	77.2 ± 0.6$^{(c)}$	99.8 ± 0.1$^{(a)}$	99.6 ± 0.2$^{(a)}$	94.1 ± 0.4$^{(b)}$
Imidacloprid	80.3 ± 0.2$^{(a)}$	88.3 ± 0.2$^{(a)}$	87.6 ± 0.3$^{(a)}$	66.4 ± 0.3$^{(b)}$
Indoxacarb	56.4 ± 1.6$^{(b)}$	103.0 ± 0.5$^{(a)}$	102.1 ± 0.1$^{(a)}$	96.1 ± 0.3$^{(a)}$
Ipconazole	57.7 ± 0.9$^{(b)}$	103.7 ± 0.2$^{(a)}$	102.7 ± 0.1$^{(a)}$	98.9 ± 0.5$^{(a)}$
Iprovalicarb	58.6 ± 6.5$^{(a)}$	95.6 ± 0.1$^{(a)}$	99.1 ± 1.3$^{(a)}$	74.5 ± 1.2$^{(a)}$
Isoxaflutole	99.0 ± 0.3$^{(a)}$	98.9 ± 0.5$^{(a)}$	105.6 ± 0.4$^{(a)}$	120.8 ± 2.0$^{(a)}$
Kresoxim-methyl	74.7 ± 0.2$^{(a)}$	96.0 ± 1.1$^{(a)}$	93.1 ± 0.3$^{(a)}$	92.3 ± 0.8$^{(a)}$
Linuron	39.7 ± 3.4$^{(b)}$	103.3 ± 0.03$^{(a)}$	107.9 ± 0.5$^{(a)}$	97.5 ± 0.1$^{(a)}$
Lufenuron	5.9 ± 3.2$^{(d)}$	105.1 ± 0.4$^{(a)}$	98.6 ± 0.2$^{(b)}$	95.4 ± 0.2$^{(c)}$
Malathion	102.9 ± 0.2$^{(a)}$	113.6 ± 0.2$^{(a)}$	109.4 ± 0.3$^{(a)}$	98.1 ± 0.1$^{(a)}$
Metalaxyl	100.3 ± 0.1$^{(a)}$	102.8 ± 0.4$^{(a)}$	108.1 ± 0.2$^{(a)}$	99.3 ± 0.5$^{(a)}$
Metconazole	56.8 ± 1.7$^{(b)}$	101.8 ± 0.8$^{(a)}$	109.9 ± 0.1$^{(a)}$	101.2 ± 0.2$^{(a)}$
Methidathion	76.4 ± 0.7$^{(b)}$	98.2 ± 0.4$^{(a)}$	99.8 ± 0.5$^{(a)}$	77.7 ± 0.2$^{(b)}$
Methomyl	63.9 ± 7.9$^{(a)}$	111.1 ± 0.6$^{(a)}$	105.6 ± 0.3$^{(a)}$	86.0 ± 0.4$^{(a)}$
Metolachlor	88.6 ± 0.3$^{(a)}$	100.2 ± 0.1$^{(a)}$	97.7 ± 0.3$^{(a)}$	98.4 ± 0.9$^{(a)}$
Metribuzin	106.3 ± 0.1$^{(a)}$	103.9 ± 0.5$^{(a)}$	106.0 ± 0.5$^{(a)}$	48.7 ± 0.3$^{(b)}$
Metsulfuron-methyl	72.7 ± 1.7$^{(b)}$	46.6 ± 0.7$^{(c)}$	72.8 ± 0.5$^{(b)}$	122.1 ± 0.4$^{(a)}$
Monocrotophos	86.4 ± 0.1$^{(a)}$	98.4 ± 0.2$^{(a)}$	86.3 ± 0.3$^{(a)}$	14.7 ± 7.1$^{(b)}$
Nicosulfuron	43.9 ± 2.5$^{(b)}$	19.0 ± 1.4$^{(c)}$	42.6 ± 0.3$^{(b)}$	100.6 ± 2.1$^{(a)}$
Novaluron	16.5 ± 2.5$^{(c)}$	104.4 ± 0.2$^{(a)}$	107.4 ± 0.3$^{(a)}$	92.1 ± 0.6$^{(b)}$
Omethoat	88.0 ± 0.2$^{(b)}$	90.6 ± 0.2$^{(a)}$	86.4 ± 0.3$^{(b)}$	83.5 ± 0.1$^{(b)}$
Oxamyl	90.3 ± 0.1$^{(a)}$	94.7 ± 0.0$^{(a)}$	94.4 ± 0.1$^{(a)}$	67.8 ± 0.2$^{(b)}$
Picoxystrobin	76.7 ± 0.1$^{(b)}$	94.3 ± 0.5$^{(a)}$	92.6 ± 1.1$^{(a)}$	79.1 ± 0.3$^{(b)}$
Pirimicarb	37.1 ± 2.2$^{(c)}$	103.6 ± 1.1$^{(a)}$	102.3 ± 0.5$^{(a)}$	81.2 ± 0.5$^{(b)}$
Pirimiphos-methyl	44.6 ± 1.1$^{(b)}$	99.2 ± 1.1$^{(a)}$	99.1 ± 0.2$^{(a)}$	91.2 ± 0.2$^{(a)}$
Prochloraz	34.1 ± 3.7$^{(b)}$	106.5 ± 0.4$^{(a)}$	111.4 ± 0.4$^{(a)}$	103.6 ± 0.1$^{(a)}$
Procymidon	54.1 ± 1.0$^{(a)}$	78.8 ± 0.7$^{(a)}$	58.3 ± 1.7$^{(a)}$	66.5 ± 0.7$^{(a)}$
Profenofos	31.7 ± 2.6$^{(b)}$	95.9 ± 0.7$^{(a)}$	97.2 ± 0.2$^{(a)}$	89.1 ± 0.4$^{(a)}$
Promecarb	106.6 ± 0.4$^{(a)}$	107.8 ± 0.1$^{(a)}$	102.6 ± 0.2$^{(a)}$	95.1 ± 0.1$^{(a)}$
Propamocarb	75.9 ± 0.0$^{(a)}$	49.3 ± 0.7$^{(c)}$	57.9 ± 0.2$^{(b)}$	73.4 ± 0.1$^{(a)}$
Propargit	70.1 ± 1.4$^{(ab)}$	98.7 ± 0.4$^{(a)}$	99.4 ± 0.7$^{(a)}$	64.2 ± 0.2$^{(ab)}$
Pyraclostrobin	5.2 ± 0.9$^{(c)}$	111.8 ± 0.0$^{(a)}$	106.7 ± 0.2$^{(a)}$	100.3 ± 0.3$^{(b)}$
Pyridaben	53.1 ± 2.0$^{(b)}$	100.0 ± 0.8$^{(a)}$	102.6 ± 0.3$^{(a)}$	89.9 ± 1.3$^{(a)}$
Pyriproxyfen	36.9 ± 2.6$^{(b)}$	98.1 ± 0.5$^{(a)}$	97.9 ± 0.3$^{(a)}$	92.3 ± 0.1$^{(a)}$
Rotenone	46.7 ± 2.7$^{(b)}$	101.2 ± 0.5$^{(a)}$	100.1 ± 0.5$^{(a)}$	96.5 ± 0.1$^{(a)}$
Spinosyn A	12.5 ± 2.6$^{(d)}$	98.3 ± 0.3$^{(b)}$	109.2 ± 0.7$^{(a)}$	87.0 ± 0.5$^{(c)}$
Spinosyn D	9.3 ± 3.2$^{(c)}$	92.0 ± 0.1$^{(b)}$	101.6 ± 0.4$^{(a)}$	90.5 ± 0.3$^{(b)}$
Tebuconazole	59.7 ± 1.6$^{(b)}$	99.5 ± 0.7$^{(a)}$	114.4 ± 0.6$^{(a)}$	111.4 ± 0.4$^{(a)}$
Tebufenozid	100.9 ± 0.5$^{(b)}$	112.0 ± 0.4$^{(a)}$	120.4 ± 1.5$^{(a)}$	98.6 ± 0.4$^{(b)}$
Temephos	25.4 ± 3.0$^{(c)}$	105.9 ± 0.4$^{(a)}$	100.7 ± 0.2$^{(ab)}$	98.0 ± 0.6$^{(b)}$
Tetraconazole	92.5 ± 0.6$^{(c)}$	108.2 ± 1.0$^{(a)}$	102.4 ± 0.5$^{(ab)}$	93.4 ± 0.6$^{(bc)}$

Table 2 Percentage recoveries (±SD) of 96 pesticides subjected to either QuEChERS clean-up methods or no clean-up *(Continued)*

Thiabendazol	17.2 ± 1.6$^{(d)}$	82.2 ± 0.4$^{(a)}$	77.3 ± 0.1$^{(b)}$	57.9 ± 0.2$^{(c)}$
Thiacloprid	85.3 ± 1.2$^{(a)}$	99.5 ± 0.6$^{(a)}$	94.7 ± 0.0$^{(a)}$	61.0 ± 0.3$^{(b)}$
Thiamethoxam	95.6 ± 0.6$^{(a)}$	101.2 ± 0.1$^{(a)}$	99.7 ± 0.2$^{(a)}$	59.7 ± 0.1$^{(b)}$
Thiodicarb	43.3 ± 2.4$^{(c)}$	101.4 ± 0.6$^{(a)}$	103.5 ± 0.5$^{(a)}$	76.8 ± 0.3$^{(b)}$
Triadimenol	92.8 ± 0.1$^{(b)}$	108.6 ± 1.0$^{(a)}$	111.3 ± 0.03$^{(a)}$	97.9 ± 0.6$^{(a)}$
Triadimefon	117.7 ± 0.3$^{(a)}$	107.4 ± 0.8$^{(a)}$	115.7 ± 0.1$^{(a)}$	110.6 ± 0.7$^{(a)}$
Tribenuron-methyl	64.2 ± 1.7$^{(b)}$	73.3 ± 0.1$^{(a)}$	81.5 ± 0.1$^{(a)}$	63.4 ± 0.4$^{(b)}$
Trifloxystrobin	60.1 ± 1.7$^{(b)}$	101.2 ± 0.2$^{(a)}$	103.8 ± 0.1$^{(a)}$	93.7 ± 0.1$^{(a)}$

*For each pesticide, mean recoveries with the same letter are not significantly different

MRM methods of targeted analytes as shown in Fig. 3. This figure illustrates an example of MRM chromatogram of the 96 pesticides targeted in this study that were extracted from spiked honey after PSA clean up. From this chromatogram, each colored peak represent a unique pesticide identified based on the MRM transition ions. A detailed summary indicating the identity of each peak shown in Fig. 3 and their corresponding retention times along with their molecular masses are provided in Table 1.

Validation of the selected method

The developed method was validated following the guidelines provided in the Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed (SANCO/12571/2013). To meet these guidelines, the method was validated in terms of recovery, linearity, LOQ, matrix effects, intra-day and inter-day precision. The mean recovery values used in this study were within the range of 70–120 %, with an associated repeatability, RSD <20 %, for all compounds within the scope of the method. Matrix-matched calibration standards were used to calculate recoveries as this helped in compensating for any matrix effects arising from matrix interferences or co-extractives that can change the ionization efficiency of an analyte causing signal suppression or enhancement leading to poor recoveries. This could have an adverse effect on the quality of the data and can erroneously result in false positive or negative results. It is therefore imperative for any LC-MS/MS method to give acceptable quantitative results; matrix effects must be considered (Ferrer et al. 2011; Kittlaus et al. 2011).

Table 3 shows the list of pesticides validated and demonstrates the summarized recovery results along with the linearity of the validated analytes. This table illustrates recoveries obtained at LOR using PSA and no clean-up approach. Percent recovery values for these analytes were calculated using matrix-matched calibration curves. The LOR for the method was determined as the lowest spike level of the validation meeting these method performance acceptability criteria. Although the LOD and LOR varied depending on the pesticides in question, most compounds could be detected at 0.1 and quantified below 1 ng/g. Overall, the LOD and the LOR was set at 0.5 and 1 ng/g, respectively. From this study, approximately 10 % of the studied compounds had poor recoveries from either method but there was tremendous improvement on recoveries when both methods were combined. In this case, all pesticides, except for two (fluquinconazole –68 % and propamocarb - 63 %) had good recoveries which were well within the recommended limits provided in SANCO/12575/2013 document. It is worth noting that pesticides with good recoveries had good reproducibility (RSD <20 %) whereas those with poor recoveries were characterized by poor reproducibility. As a result, during

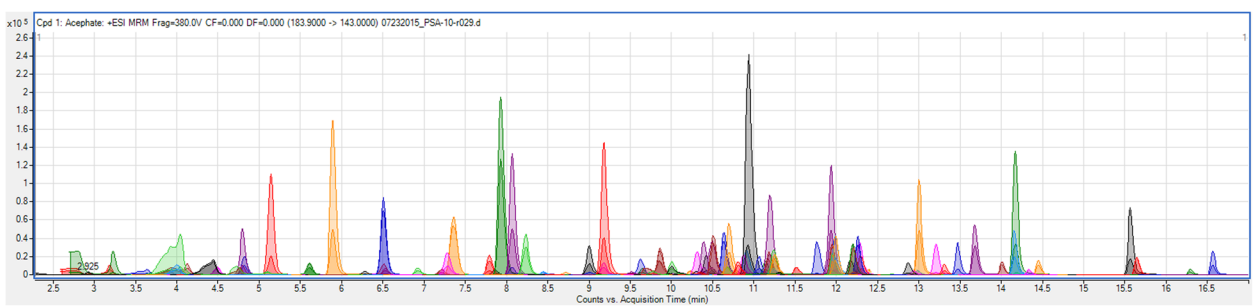

Fig. 3 Representative example of MRM chromatogram of 96 pesticides extracted from a spiked honey sample at 10 ng/g level and cleaned up using PSA only

Table 3 Extraction efficiencies of validated pesticides spiked at LOR, precision in terms of RSD ($n = 5$) and coefficients of determination for the investigated pesticides

Compound name	% recovery at LOR (1 ng/g)				
	No clean-up		PSA		
	% recovery	% RSD, $n = 5$	% recovery	% RSD, $n = 5$	R^2
Acephate	70.1	2.6	97.4	4.7	0.9989
Acetamiprid	82.6	3.2	115.0	3.9	0.9982
Aldicarb fragment	73.0	0.9	102.8	1.5	0.9476
Amidosulfuron	87.1	1.2	54.2	24.3	0.9990
Azoxystrobin	84.5	4.5	90.8	4.9	0.9986
Benalaxyl	87.6	3.3	87.5	1.5	0.9989
Bifenazate	89.6	2.4	96.3	4.3	0.9919
Bifenthrin	91.3	6.5	99.0	5.0	0.9996
Bitertanol	85.7	0.4	89.9	0.1	0.9982
Bosclid (Nicobifen)	81.7	0.7	88.2	1.5	0.9977
Bromuconazole	117.3	11.5	102.3	9.3	0.9991
Bupirimate	87.6	1.7	95.6	4.1	0.9998
Buprofezin	81.9	9.0	86.1	4.3	0.9985
Carbaryl	83.8	1.8	118.3	1.3	0.9980
Carbofuran	67.8	3.1	115.8	5.1	0.9987
Chlorfenvinphos	95.0	5.7	103.0	1.1	1.0000
Chlorpyriphos	93.1	6.2	99.3	1.8	0.9996
Chlorpyriphos-methyl	82.9	16.9	92.7	8.6	0.9990
Clofentezin	93.8	1.8	94.0	1.9	0.9996
Coumaphos	70.3	4.7	75.9	3.3	0.9935
Cyazofamid	108.4	9.4	113.6	1.6	0.9992
Cymiazol	86.6	0.5	109.6	5.9	0.9987
Cyproconazole	84.8	0.9	89.2	4.1	0.9976
Cyprodinil	93.7	1.0	96.7	1.6	0.9995
DEET	68.5	3.3	96.1	4.0	0.9999
Diazinon	87.6	3.6	100.4	0.5	0.9999
Dichlorvos	72.8	15.4	101.4	10.9	0.9998
Diflubenzuron	95.6	0.4	99.5	3.9	0.9999
Dimethoate	67.5	2.9	108.7	0.3	0.9997
Diuron	55.9	0.7	98.4	4.7	0.9979
Epoxyconazol	100.4	8.4	100.7	0.4	1.0000
Ethion	74.9	7.1	82.8	8.9	0.9986
Ethoprophos	91.6	3.7	103.8	11.8	0.9998
Etofenprox	95.2	0.8	101.3	2.8	1.0000
Fenamiphos	100.2	8.9	103.8	2.0	0.9991
Fenazaquin	95.1	2.3	99.5	5.2	0.9999
Fenbuconazole	94.9	1.9	101.3	0.4	1.0000
Fenobucarb	83.0	5.1	96.7	0.3	0.9999
Fipronil	99.7	2.6	98.3	13.7	0.9982
Flazasulfuron	87.1	3.7	25.7	62.6	0.9995
Fludioxonil	75.6	5.3	62.2	1.8	0.9981
Flufenacet	82.2	14.1	79.1	17.0	0.9926
Fluquinconazole	68.3	6.9	68.3	1.7	0.9981
Flusilazole	79.5	4.7	90.9	6.1	0.9991
Flutriafol	93.5	4.9	93.9	1.8	0.9999
Fosthiazate	73.2	4.1	109.4	1.6	0.9998
Hexaconazole	94.6	0.3	98.8	0.9	1.0000
Hexythiazox	89.7	2.7	98.2	0.8	0.9997
Imidacloprid	69.3	6.0	101.4	3.4	0.9996
Indoxacarb	93.2	1.1	96.8	1.9	0.9998
Ipconazole	89.8	2.4	95.7	4.9	0.9996
Iprovalicarb	90.7	13.6	100.6	9.9	0.9988
Isoxaflutole	91.4	0.5	76.0	5.7	0.9951
Kresoxim-methyl	108.8	10.7	113.5	13.2	0.9962
Linuron	76.3	7.1	74.0	3.0	0.9958
Lufenuron	91.9	15.8	90.2	9.0	0.9990
Malathion	78.8	8.3	86.3	1.0	0.9972
Metalaxyl	83.1	1.3	91.4	0.7	0.9995
Metconazole	78.2	11.0	81.0	8.2	0.9984
Methidathion	72.7	8.2	89.5	2.6	0.9993
Methomyl	96.1	0.4	114.6	3.5	0.9996
Metolachlor	111.6	1.1	115.3	3.7	0.9984
Metribuzin	66.3	14.3	115.4	2.8	0.9976
Metsulfuron-methyl	121.6	0.5	53.5	28.1	0.9994
Monocrotophos	77.9	0.4	112.5	2.7	0.9968
Nicosulfuron	90.1	2.1	10.5	39.9	0.9994
Novaluron	94.9	0.2	95.5	0.6	0.9999
Omethoate	78.3	5.9	81.3	15.8	0.9995
Oxamyl	72.9	15.3	112.4	2.5	0.9995
Picoxystrobin	93.1	2.6	101.4	9.4	0.9992
Pirimicarb	87.0	2.5	113.1	0.4	0.9992
Pirimiphos-methyl	99.3	1.0	112.2	4.4	0.9999
Prochloraz	79.9	0.4	80.1	1.2	0.9978
Procymidon	79.9	6.0	111.7	10.9	0.9956
Profenofos	98.5	3.7	109.2	11.2	0.9994
Promecarb	85.1	1.3	87.7	0.2	0.9993
Propamocarb	62.8	0.0	29.7	83.9	0.9996
Propargit	25.2	52.7	76.5	19.8	0.9983
Pyraclostrobin	80.0	10.4	88.1	5.6	0.9972
Pyridaben	84.3	1.5	87.2	1.2	0.9998
Pyriproxyfen	92.7	4.4	100.4	1.0	1.0000
Rotenone	97.7	1.9	113.0	1.7	0.9985

Table 3 Extraction efficiencies of validated pesticides spiked at LOR, precision in terms of RSD ($n = 5$) and coefficients of determination for the investigated pesticides *(Continued)*

Spinosyn A	92.0	0.8	95.9	0.8	0.9999
Spinosyn D	85.1	0.1	92.3	2.0	0.9997
Tebuconazole	92.1	4.0	97.1	0.1	0.9993
Tebufenozid	75.3	0.5	87.7	9.5	0.9958
Temephos	94.4	1.6	95.5	2.6	0.9999
Tetraconazole	83.1	6.1	108.6	3.1	0.9997
Thiabendazol	91.3	2.7	110.9	1.9	0.9946
Thiacloprid	67.7	0.4	108.2	1.8	0.9995
Thiamethoxam	55.4	5.5	100.0	1.6	1.0000
Thiodicarb	78.3	1.0	102.4	3.5	0.9996
Triadimenol	73.7	8.5	69.8	13.5	0.9903
Triadimefon	85.6	8.9	83.8	2.2	0.9948
Tribenuron-methyl	71.8	4.9	65.7	13.4	0.9997
Trifloxystrobin	94.7	3.1	100.6	0.6	0.9999

recovery studies, blank matrix was fortified at 10 times the LOR since it gave the best reproducibility for all studied compounds. The method linearity was evaluated by assessing the signal responses of the targeted analytes from matrix-matched calibration solutions prepared in blank extracts at seven concentration levels. The developed method was proven satisfactory with linear chromatographic response for the tested pesticides, ranging from 0.1 to 100 ng g^{-1}. Majority of the correlation coefficients (R^2) was higher or equal to 0.995, see Table 3.

Application of the method to real samples

As a natural product manufactured by bees, honey is considered to be free from any extraneous material. However, chemical residues have been reported in honey by several investigators. The presence of these residues in honey has prompted the need for setting up monitoring programs to determine the proper assessment of human exposure to pesticides (Choudhary and Sharma 2008). Unfortunately, there is no homogeneity on MRLs as different national regulations have established their own maximum concentrations of pesticide residues permitted in honey. In the absence of MRLs set for honey in the two African countries studied, the European Union set MRLs were employed and where no MRL existed, it was presumed at 10 which is the default MRL for pesticides with no specific value set as recommended in Regulation(EC)No 396/2005.

So far, there is little information that is currently available on chemical residues present in honey or hive products from most African countries (Muli et al. 2014; Eissa et al. 2014). Previous studies have shown that whereas in North America honey bees are exposed to at least 7 pesticides per food visit, this is not the case in Africa (Mullin et al. 2010). Results from a recent study carried out in Kenya detected less than four pesticides for the whole study duration at very minimal concentrations in honey bees and their hive products (Muli et al. 2014). In the current study, a preliminary analysis of pesticide residues in 28 honey samples obtained from local farmers' markets and supermarkets from various regions in Kenya and Ethiopia during the period of November 2014 to July 2015 revealed the presence of 17 pesticide residues out of the 96 pesticides investigated. The concentrations for each detected pesticide were compared with the set MRL values. Table 4 indicates the summarized results obtained from the two countries. Our preliminary results show that, with the exception of malathion, an organophosphate that has multiple uses in Africa, no other pesticide was detected at a level higher than the set MRL levels. For most pesticides, the levels obtained were about 10-fold lower than the set MRL levels, with concentration levels at <100 ng/g. However, the maximum concentration detected for malathion was 0.092 mg/kg, a level that far exceeds its acceptable MRL of 0.05 mg/kg. Although this compound is quickly metabolized from the body and is known to be non-persistent in the environment, exposure to the levels detected (0.092 mg/kg) in this study over a long period could result in adverse health effects to both humans and honey bees. Thus, further investigation is required to determine its cumulative effects and whether there are any potential synergistic effects when other contaminants are present. Malathion is also considered to be highly toxic to honey bees with LD_{50} of 0.16 μg/bee (Allison 2011). It is worth noting that data from the present study does not reflect seasonality of pesticide present in honey samples obtained from the two countries. This would require in-depth systematic studies using large samples obtained directly from specific beekeeping sites over different seasons in the two countries. Follow up studies are underway to investigate how seasonality affects residues present in honey from various African countries.

Conclusion

A highly efficient approach for determining pesticide residues in honey with good recoveries was developed. This approach involved using a modified QuEChERS method along with or without any clean-up. The viability of this approach was demonstrated by using 96 pesticides. About 98 % of these pesticides investigated had recoveries that are well within the acceptable limits of 70–120 %. The methods were linear (>0.995) over the range tested (0.1–100 ng/g) with LOR for

Table 4 Detected pesticide residues in honey obtained from Kenya and Ethiopia

	Identified pesticide residues																
SampleID	ACTM	AF	CF	CAR	CHP	Cy	DEET	DDVP	DM	BPMC	HEX	Mal	Met	Metri	Rot	TBN	THIA
Kenya																	
Taita	<LOQ	N/D	N/D	N/D	<LOQ	<LOQ	0.708	N/D	N/D	N/D	<LOQ	56.9	N/D	49.4	N/D	<LOQ	<LOQ
VapA	<LOQ	1.37	<LOQ	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	92.3	1.81	N/D	N/D	N/D	<LOQ
Cab	<LOQ	N/D	N/D	N/D	<LOQ	1.59	<LOQ	N/D	N/D	<LOQ	<LOQ	N/D	1.95	14.1	N/D	N/D	N/D
Nak	N/D	<LOQ	N/D	<LOQ	N/D	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Ken	<LOQ	<LOQ	N/D	N/D	N/D	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Mwi	N/D	N/D	N/D	1.26	N/D	N/D	1.01	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Kak	<L/OQ	<LOQ	N/D	N/D	N/D	<LOQ	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	<LOQ	N/D	N/D	N/D
ML	ND	N/D	N/D	<LOQ	N/D	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	34.0	N/D	N/D	N/D
HR	<LOQ	N/D	N/D	2.87	N/D	<LOQ	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	70.4	N/D	N/D	N/D
Gedi	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
K-B	N/D	N/D	N/D	<LOQ	N/D	N/D	1.37	2.58	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
K-M	N/D	N/D	N/D	N/D	N/D	N/D	<LOQ	<LOQ	<LOQ	N/D	N/D	N/D	N/D	<LOQ	N/D	N/D	N/D
K-N	<LOQ	N/D	N/D	<LOQ	N/D	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
VapB	<LOQ	<LOQ	N/D	N/D	<LOQ	N/D	1.09	N/D	N/D	N/D	1.66	76.7	5.29	N/D	N/D	<LOQ	<LOQ
Ethiopia																	
MB	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	9.52	N/D	N/D	N/D
Tol	<LOQ	N/D	N/D	N/D	<LOQ	<LOQ	<LOQ	N/D	N/D	<LOQ	N/D	60.5	4.77	11.2	N/D	<LOQ	N/D
Tig	<LOQ	<LOQ	N/D	N/D	<LOQ	<LOQ	<LOQ	N/D	N/D	N/D	<LOQ	15.3	N/D	N/D	N/D	N/D	N/D
SapV	<LOQ	<LOQ	<LOQ	N/D	<LOQ	<LOQ	<LOQ	N/D	N/D	<LOQ	<LOQ	45.1	1.25	14.2	N/D	<LOQ	<LOQ
E-1	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	2.60	N/D	N/D	N/D
E-2	N/D	N/D	N/D	N/D	N/D	N/D	N/D	1.16	<LOQ	N/D	N/D	N/D	N/D	44.2	N/D	N/D	N/D
E-3	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
E-4	ND	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	7.95	6.99	N/D	N/D
E-5	ND	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
E-M1	<LOQ	<LOQ	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
E-H	<LOQ	N/D	N/D	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Tol2	N/D	N/D	1.10	N/D	N/D	N/D	N/D	3.46	<LOQ	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Tig2	N/D	N/D	N/D	N/D	N/D	N/D	4.98	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Sap S2	<LOQ	N/D	<LOQ	N/D	<LOQ	10.5	<LOQ	N/D	N/D	<LOQ	<LOQ	22.3	N/D	21.0	N/D	N/D	N/D
MRL	50	10	10	50	*10	*10	*10	*10	*10	*10	*10	50	50	100	10	50	10

*Set at default MRL value; *N/D* not detected, *<LOQ* below the quantification limits
Identified pesticide residues: *ACTM* Acetamiprid, *AF* Aldicarb fragment, CF Carbofuran, *CHP* Chlopyrifos, *Cy* Cymiazole, *DDVP* Dichlorvos, *DM* Dimethoate, *BPMC* Fenobucarb, *HEX* Hexaconazole, *Mal* Malathion, *Met* Metalaxyl, *Metri* Metribuzin, *Rot* Rotenone, *TBN* Tebuconazole, *THIA* Thiomethoxam

most pesticides at 1 ng/g or ppb. The applicability of the developed methods to real samples was tested by performing a preliminary study of commercial honey from Africa. A total of 17 pesticide residues were detected at levels 10-fold lower than their set MRL values except malathion which was detected at almost 2-fold higher than its set MRL. Overall, these results suggest that honey from these regions maybe safe for both bees and human consumption but further investigation is required to determine the cumulative effect of these pesticides. In-depth follow up studies using this method are underway to verify this observation in honey samples collected from different agro-ecological regions from various African countries.

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Authors' contributions
JI designed the study and the experimental setting, performed the analytical work and wrote the manuscript. BT contributed in experimental design and critically revised the manuscript. SK edited and proofread the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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