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Simultaneous determination of pesticides and 5-hydroxymethylfurfural in honey by the modified QuEChERS method and liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

Nowadays, bee products are being produced in an environment polluted by different sources of contamination, such as pesticides, which can be transported by honey bees to the hive and incorporated into honey. In addition, the increasing consumption of honey has demanded efficient quality control for this product. In this study, the determination of the pesticides fipronil, imidacloprid, thiamethoxam, dimethoate, carbendazin, tebuconazole, amitraz, τ-fluvalinate and 5-hydroxymethylfurfural (HMF) which are used as indicators of honey quality, was carried out simultaneously. For the sample preparation, the optimized QuEChERS method was used and the determinations were done by LC-APCI-MS/MS. The LOQs of the method ranged between 0.005 and 1.0 mg kg^{-1}. For the recovery calculations and method evaluation a working curve was drawn. All the analytical curves showed *r* values higher than 0.99. The recoveries ranged between 70% and 112%, with RSD lower than 20% for all compounds. The matrix effect was evaluated, and most of the compounds showed signal enrichment. The applicability of the method for honey from different flowers was verified, and the method showed robustness and recoveries in the range 70–120% established for all compounds in samples belonging to different blossoms. HMF was detected in all samples, with concentrations ranging between 4.6 and 51.7 mg kg^{-1}; it was below the maximum concentration allowed by the legislation.

1. Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants. It is composed mainly of monosaccharides and oligosaccharides, totaling 77%, with glucose and fructose having average contents of 30% and 38%, respectively [1]. In many types of honeys, over 300 substances belonging to several chemical groups have been identified, such as phenolic acids, flavonoids and amino acids [2–4]. Honey composition is highly influenced by the types of flowers used by the bees as well as regional and climatic conditions [5].

Bee products are produced in an environment polluted by different sources of contamination since these contaminants can reach the raw materials of bee products (nectar, pollen, plant exudates) by air, water, plants and soil and then be transported into the bee hive by the bees [6]. Residues of pesticides have been found in apiarian products [7]; thus it is convenient to develop methods to evaluate their presence.

Pesticides protect agricultural crops, but overuse and incorrect use can pose risks to human health and the environment [8,9]. Even if small amounts of pesticide residues remain in the food supply, they constitute a potential risk for the human health because of their sub-acute and chronic toxicity [10,11].

Insecticides are a class of pesticides that affect the nervous system of target insects and also represent high toxicity to honey bees. The insecticides fipronil, imidacloprid and thiamethoxam have even been suspected of causing a significant decrease in honey bee colonies, a fact that has been observed in many countries since the past decade [12]. European and Canadian beekeepers have also reported serious losses of honey bees that they attribute to the use of the insecticides imidacloprid and fipronil in agricultural practices [13].

Dimethoate is an acaricide whose use is indicated for some crops such as citrus, tomatoes and apples, for example. It belongs to the organophosphate class, and has a more acute toxic effect on humans and other mammals than the organochlorine pesticides [14]. The fungicides carbendazin and tebuconazole are widely

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used in various crops in the southern region of Rio Grande do Sul state (Brazil), such as citrus, onion, rice and peaches, which are also important sources of pollen and nectar for bees [15].

The acaricides amitraz and τ-fluvalinate are used directly in hives, for the control of *Varroa jacobsoni. Varroosis* is a bee disease caused by *V. jacobsoni* which endangers beekeeping all over the world. In order to prevent economic losses, beekeepers treat their colonies with acaricides, such as amitraz and τ-fluvalinate. The latter remains stable in honey for 9 months [16].

Another important issue related to honey quality is the determination of transformation products. The compound created during the decomposition of monosaccharides (mainly fructose) is 5-hydroxymethylfurfural (HMF). It is usually absent in fresh honey but its concentration tends to increase during honey conditioning and storage and in excessive heat. It can also show adulteration with commercially available invert syrup [17–19]. The Codex Alimentarius set the maximum allowable concentration of HMF to 40 mg kg^{-1} for honey from non-tropical regions and 80 mg kg^{-1} for honey from tropical regions in 2001 [20].

Due to the complexity of the matrix, efficient sample preparation and trace-level detection and identification are important to obtain reliable results, especially in the analysis of trace compounds in food. Efficient sample preparation depends on the matrix, as well as on the properties and the analyte concentration [21].

The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) was developed in 2003 for the determination of multiclass pesticide residues in fruits and vegetables [22]. The QuEChERS method is particularly popular for the determination of polar, middle polar and non-polar pesticide residues in various food matrices because it is simple, inexpensive, amenable to high throughput, and quite efficient with a minimum number of steps [23]. A modified QuEChERS method was applied to honey samples for the extraction of chloramphenicol in 2006 [24], extraction of pesticides in 2007 [25] and determination of neonicotinoid pesticides in honey and bee products in 2010 [26].

In this study, a simple, rapid and effective method based on QuEChERS and LC-APCI-MS/MS was proposed for the simultaneous determination of eight different chemical classes of pesticides and HMF in honey. The optimized method was applied to determine all the analytes in different honey samples simultaneously, in order to evaluate the applicability of the method to real samples.

2. Materials and methods

2.1. Chemicals and materials

Fipronil (97.6%), imidacloprid (99.9%), thiamethoxam (99.7%), dimethoate (99.4%), carbendazin (99.0%), tebuconazole (99.6%), amitraz (98.9%), τ-fluvalinate (93.8%) and 5-hydroxymethylfurfural (99.0%) were purchased from Sigma-Aldrich, Brazil. Acetonitrile and methanol, both of HPLC grade, were bought from JT Baker (Mallinckrodt, NJ, USA). The water was purified on an Ultrapure Water System (Millipore, Bedford, MA, USA). Magnesium sulfate (99.8%) and sodium acetate (99.5%) were obtained from JT Baker. Glacial acetic acid, formic acid (98%) and ammonium hydroxide were obtained from Merck. The primary secondary amine (PSA-Bondesil) was purchased from Varian, USA.

2.2. Standard solutions

The individual stock standard solution was prepared in acetonitrile and stored at −18 °C in a freezer. Working standard solutions were prepared by appropriately diluting the stock solution using acetonitrile.

Due to the different sensitivities of the compounds in the mass spectrometer detector, a working solution containing the mixture of nine analytes in different concentrations was prepared: 100 mg L^{-1} for HMF, 50 mg L^{-1} for thiamethoxam, 10 mg L^{-1} for imidacloprid, 5 mg L^{-1} for carbendazim, dimethoate and τ-fluvalinate, 1 mg L^{-1} for fipronil and tebuconazole and 0.5 mg L^{-1} for amitraz. Successive dilutions of this working solution were prepared daily.

2.3. Samples

Honey samples from eucalyptus (denominated blank samples) free from any traces of pesticides and obtained from organic beekeepers were used for method optimization and validation.

The samples used for the method applied (from different flowers and from different regions in the south of Brazil) were purchased in local supermarkets. The samples were stored at room temperature in a fresh and dark place before analysis.

2.4. Sample preparation: modified QuEChERS method

For the extraction procedure, 10.0 g honey was weighed in a 50.0 mL polypropylene tube and 10 mL of water with 150 μL of NH_4OH was added. The mixture was vortexed with a Certomat MV homogenizer (B. Braun Int., Germany) for 1 min. Afterwards, 10 mL acetonitrile was added and the sample was homogenized. After adding 4.0 g anhydrous magnesium sulfate, the mixture was vortexed again for 1 min, and then it was centrifuged for 10 min at 8000 rpm. An aliquot of 10 μL of the upper acetonitrile layer was injected into the LC system for analysis. All experiments were performed in triplicate.

2.5. Instrument and chromatographic conditions

Liquid chromatography with mass spectrometric detection was performed in a Waters Alliance 2695 Separations Module (Waters, Milford, MA, USA) fitted with an autosampler, a membrane degasser and a quaternary pump. Mass spectrometry was performed in a Micromass Quattro Micro API (Waters, Milford, MA, USA) with an APCI interface. The LC column was an XTerra of 3.5 μm particle size (50 × 3 mm i.d.; Waters, Milford, MA, USA). The drying gas, as well as the nebulizing gas, was nitrogen generated by pressurized air in a NG-7 nitrogen generator (Aquilo, Etten-Leur, NL). The nebulizing gas flow was 50 L h^{-1} and the desolvation gas flow was 550 L h^{-1}.

For the operation in the MS/MS mode, the collision gas was Argon 99.99% (White Martins, Brazil) with a pressure of 3.5×10^{-3} mbar in the collision cell. The optimized values were corona current 0.5 μA; source temperature 100 °C; desolvation temperature, 500 °C; and multiplier, 650 V. Detection by MS/MS was performed using an APCI interface operating in positive and negative ionizations.

For each compound, optimum collision energies with the aim of getting two characteristic multiple reaction monitoring (MRM) transitions with the best signal intensity were selected. After the optimization of the collision cell energy of the triple quadrupole, two different precursor ion-product ion transitions were selected for each analyte, one for quantification and one for confirmation. Table 1 shows the optimized MRM transitions of the pesticides with the respective retention times (t_R). Analytical instrument control, data acquisition and treatment were performed by the software MassLynx (Micromass, Manchester, UK), version 4.1.

The mobile phase components were methanol (A) and ultrapure water with 0.1% formic acid (B), in a gradient mode as follows: 0–5 min, 30–100% A; 5–9 min, 100% A; 9–10 min, 100–70% A; and 10–15 min, 70% A. The flow rates were 0–5 min, 0.2–0.4 mL min^{-1}; 5–9 min, 0.4 mL min^{-1}; 9–10 min 0.4–0.2 mL min^{-1}; and 10–15 min, 0.2 mL min^{-1}.

Table 1
MRM settings for the studied compounds.

Analytes	MRM transitions (m/z)		Collision energy (eV)	Cone voltage (V)	t_R (min)	Ion ratio (%)
Carbendazin	APCI	192 > 160[a]	28	28	1.29	46.95
	+	192 > 132[b]	29	28		
HMF	APCI	127 > 109[a]	5	12	1.63	9.67
	+	127 > 81[b]	5	12		
Thiamethoxam	APCI	292 > 211[a]	30	23	2.04	56.18
	+	292 > 122[b]	31	25		
Imidacloprid	APCI	256 > 209[a]	14	30	3.12	26.52
	+	256 > 175[b]	27	11		
Dimethoate	APCI	230 > 125[a]	20	16	3.87	98.04
	+	230 > 199[b]	10	20		
Fipronil	APCI	435 > 330[a]	15	30	6.42	5.40
	-	435 > 250[b]	26	25		
Tebuconazole	APCI	308 > 70[a]	20	40	6.58	9.77
	+	308 > 125[b]	22	28		
Amitraz	APCI	294 > 163[a]	20	40	7.18	9.02
	+	294 > 122[b]	20	40		
τ-Fluvalinate	APCI	406 > 251[a]	11	20	7.43	14.00
	+	406 > 337[b]	11	20		

Dwell time: 0.2 s positive mode; 0.3 s negative mode.
[a] Quantification ions.
[b] Confirmatory ions.

2.6. Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) of the method for each analyte were obtained considering 3 and 10 times the ratio of signal to baseline (noise), respectively. The signal/noise ratio was calculated by the "software" of the instrument. The limits of the method were obtained by the injection of analytical solutions in different concentrations prepared by dilution of the standard solution in the matrix blank extract which were obtained by the modified QuEChERS method and confirmed experimentally. The LOQ is defined as the lowest validated spike level which meets the method performance acceptability criteria (mean recoveries were in the range 70–120%, with an RSD $\leq$20%).

2.7. Analytical curves and linearity

The analytical curves in acetonitrile and blank matrix extract were constructed to evaluate and compensate for the matrix effect (ME) and to assess the linearity according to the procedures for method validation suggested by SANCO [27].

Due to the different sensitivities of the compounds, the analytical curves responded in different ranges of concentration. Three sets of samples were prepared and for each one, analytical curves were constructed according to Varga et al. [28];

Set 1: consisted of neat calibration standards,
Set 2: the samples were first extracted and spiked after extraction with the analytes in the same solvent at the same concentration level as in set 1. Any difference of the peak areas of the analytes in comparison with those observed in set 1 would indicate a ME,
Set 3: analytes were spiked into the samples before extraction. The differences would reflect a combined effect of a sample matrix and potential differences in the recovery (R%) of analytes. By comparing the absolute peak areas obtained in sets 1–3, the process efficiency (PE) can be determined.

The curves were obtained by plotting the peak area versus the concentration of analytes in working solutions, from the limit of quantification of each compound up to 10 times the concentration of the LOQ, with five levels.

2.8. Recoveries (%)

Accuracy was evaluated by recovery studies at five different spiked levels. Firstly, the area values from the fortified samples were replaced in the equation of the working curve to find the concentration in the extract. To calculate the recoveries, the following equation was used: recovery (%)=$[(C1-C2)/C3]\times 100$, where $C1$=concentration of the analyte in the final extract, $C2$=concentration of the analyte in the blank sample, and $C3$=concentration of the analyte added to the sample.

2.9. Matrix effect

ME occurs when molecules co-eluting with the compounds of interest alter the ionization efficiency of the interface. The exact mechanism of the ME is unknown, but it probably originates from the competition between an analyte and the co-eluting undetected matrix components [29].

A comparison between curves of sets 1 and 2 was made for the evaluation of the ME: ME=slope X2−slope X1/slope X1 × 100, where X2 corresponds to curves prepared in set 2 and X1 corresponds to curves prepared in set 1. Sets of samples are described in Section 2.7.

When the values found for the ME are between −20% and +20%, it is considered low; if they are between −50% and −20% or between +20% and +50%, it is considered medium; and if these values are below −50% or above +50% the ME is considered high [30].

2.10. Process efficiency

PE summarizes the efficiency of sample preparation (R%) and analyte ionization (ME) [28]. Therefore, process efficiency is a suitable quantity to assess the overall performance of the analysis method. PE (%) was calculated by comparing the analytical curves of sets 1 and 3.

PE (%) values near 100% generally indicate that recoveries (R%) are near 100% and there is no influence of ME. Ion enhancement increasing the PE and ion suppression can cause lower PE, in spite of high recovery. Significant differences between R% and PE (%) mean that pre-extraction addition results must be compared to post-extraction addition results (sets 3 and 2, respectively) in order to determine recovery, because it means that the ME is high [31].

3. Results and discussion

3.1. Modified QuEChERS method

The original QuEChERS method involves an extraction of 10.0 g sample with acetonitrile, followed by liquid–liquid partitioning formed by the addition of anhydrous $MgSO_4$, and a cleanup step by dispersive solid-phase extraction with PSA (primary–secondary amine) [22]. In a recent study carried out by our group, the QuEChERS method was optimized and applied to the extraction of fipronil in honey samples [32]. Some features such as extractor solvent and salt addition were studied and optimized; they were kept in this study. Other parameters, such as the cleanup step and the pH adjustment of honey samples, were analyzed due to different chemical properties of the compounds under study. Recovery studies were carried out using honey samples with no detectable pesticide residues (blank). During optimization, the

samples were spiked with a mixture of analyte standard, in all linear ranges of analytical curves for the study of the cleanup step and in an intermediate concentration of linear range for the pH adjustment. The extracts were analyzed by three replicates and each one was injected three times into the LC-APCI-MS/MS system.

3.1.1. Cleanup step

To remove the matrix interference, a cleanup step is usually performed. In this study, PSA was employed in dispersive solid-phase extraction for cleanup, in order to investigate the ME.

The sorbent was chosen due to its ability to retain matrix components, such as polar organic acids, sugars and fatty acids, as well as allow the analytes of interest into the acetonitrile phase [23].

For the study of the cleanup step, the procedure described in Section 2.4 was performed (without the addition of 150 μL of NH_4OH). One mL of the sample extract is added to a vial containing 25 mg of PSA, and the mixture is mixed in a vortex. The sorbent is then separated by centrifugation and 10 μL of the final extract is taken for analysis.

The evaluation of the ME was carried out by comparing the slopes of analytical curves obtained by standards in acetonitrile and curves obtained by the QuEChERS method with and without a cleanup step. As can be seen in Fig. 1, among the substances that have a high ME, it only decreased for HMF, amitraz and imidacloprid. Thiamethoxam, tebuconazole and dimethoate showed high ME in both situations. In other analytes that have medium and low ME, no significant changes were observed. Thus, the use of a cleanup step was not necessary. Consequently, time was decreased and a lower amount of reagents and materials was needed. To compensate for the occurrence of ME, analytical curves prepared in a blank matrix extract were used.

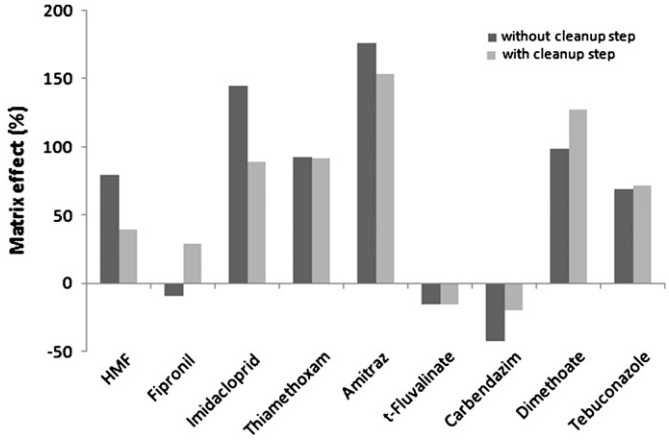

Fig. 1. Influence of the cleanup step in the ME. Negative values indicate signal suppression and positive ones indicate signal enrichment.

3.1.2. pH adjustment of honey samples

The influence of pH on recoveries was checked in acidic (pH 2.9) and alkaline (pH 9.7) media and on the pH of the sample (pH 4.6). To maintain constant pH in the sample, acetate buffer was added. Therefore, acetonitrile was acidified with 100 μL of glacial acetic acid and 1.0 g of acetate sodium with magnesium sulfate was added. For the acidic medium, the experiments were performed by acidifying the aqueous sample with 300 μL of glacial acetic acid, and for the experiments in alkaline medium, 150 μL of ammonium hydroxide was added to the aqueous solution containing the sample.

As can be seen in Fig. 2, for all analytes and pH values, the recoveries were above 70%, with the exception of amitraz. Amitraz is a toxic acaricide that undergoes rapid hydrolysis in acidic medium. Korta et al. analyzed honey samples by LC and showed that amitraz in honey undergoes complete degradation in 10 days; the main products resulting from this degradation are 2,4-dimethyl–aniline (DMA) and 2,4-dimethylphenylformamide (DMF) [16]. In another study [33] different half-life times for amitraz were presented in a pH range of 1.3 to 10.7. With increasing pH, the constant of degradation of amitraz decreases, and hence its half-life increases [33]. Since it was possible to obtain recoveries above 70% for amitraz only in an alkaline medium, the optimized method uses ammonium hydroxide to adjust the pH.

3.2. Method validation

3.2.1. Limits of detection and quantification

Table 2 shows the LOD and LOQ obtained by the method developed by LC-APCI-MS/MS and the MRLs established by the Commission of the European Union [34]. As can be seen, the LOQs of the method were lower than the MRLs for most analytes.

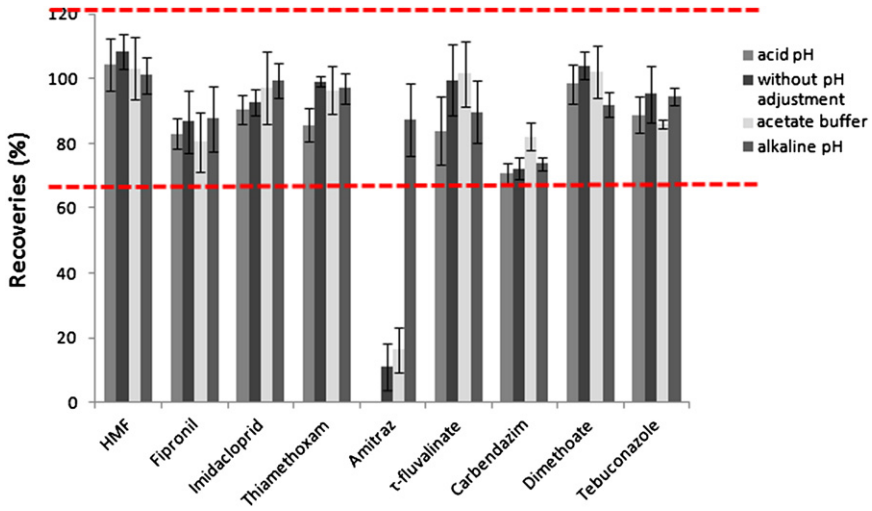

Fig. 2. Influence of sample pH on recoveries. Error bars indicate RSD% values.

HMF, thiamethoxam and imidacloprid showed higher LOQ values in comparison with other analytes. This can be explained by the use of APCI as ionization source on the mass spectrometry detector. The APCI has been increasingly used for the analysis of non-polar molecules of high volatility, since the ionization occurs in the gas phase [35]. This method was developed for nine compounds of different classes; consequently, these analytes have different polarities and physico–chemical properties. Therefore, the LOQ values were different for them. HMF, thiamethoxam and imidacloprid are the most polar analytes; they also have the lowest vapor pressure, and the LOQ was higher for these compounds.

3.2.2. Analytical curves and linearity

The method showed good linearity with correlation coefficients (r) between 0.996 and 0.999 for analytical curves prepared for acetonitrile and between 0.994 and 0.999 for the analytical curves prepared for the blank matrix extract. The correlation coefficients 0.992–0.999 for analytical curves obtained from spiked samples, after extraction and analysis, showed good linearity of the whole analytical method, from sample preparation to the analysis in LC-APCI-MS/MS. These curves were also used for recovery calculations, precision and process efficiency of the method.

Table 2
LMR established by the EU Pesticides Database (Regulation (EC) No. 396/2005), LOQs and LODs of the proposed method.

Analytes	EU LMR (mg kg^{-1})	LOQ of proposed method (mg kg^{-1})	LOD of proposed method (mg kg^{-1})
Amitraz	0.2	0.005	0.0016
Carbendazin	1.0	0.05	0.016
Dimethoate	n.f	0.05	0.016
Fipronil	0.01[a]	0.01	0.0033
τ-fluvalinate	0.01	0.05	0.016
Imidacloprid	0.05	0.1	0.033
Tebuconazole	0.05	0.01	0.0033
Thiamethoxam	0.01[a]	0.5	0.16
HMF	80.0	1.0	0.33

n.f: not found.

[a] LMR of pesticide and its metabolites.

3.2.3. Accuracy and precision of the method

To examine the accuracy and the precision of the method, the curves prepared for set 3 were used for calculations of *R%* and RSD. Each level of a curve was prepared in triplicate and the extracts were injected into the chromatographic system in triplicate ($n=9$).

The results shown in Table 3 indicate that the method provides acceptable recoveries (70–112%). The precision was evaluated in terms of repeatability (RSD_r), which was lower than 20%, an evidence of good precision of the method, since values up to 20% are accepted. The chromatograms are shown in Fig. 3.

The interday precision was evaluated in LOQ and 5LOQ levels. The RSD was lower than 16.7% with recoveries of 70.4–119.8% (these data are not shown). All results comply with SANCO [27].

3.3. Evaluation of the matrix effect

The results of the evaluation of the matrix effect in the optimized QuEChERS condition can be observed in Fig. 4. High signal enrichment was observed for most of the analytes but only carbendazin presented signal suppression. Although τ-fluvalinate did not show any matrix effect, fipronil showed a medium one.

Different behaviors are reported in literature when electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) sources are used. Many authors observed signal

Table 3
Recoveries (*R%*) and repeatability (RSD_r).

Analytes	Spiked levels (mg kg^{-1})	R (%)	RSD_r (%)	Analytes	Spiked levels (mg kg^{-1})	R (%)	RSD_r (%)
Amitraz	0.005	86.7	19.8	Imidacloprid	0.1	111	16.6
	0.0125	92.1	12.4		0.25	97.1	7.3
	0.025	107	8.9		0.5	97.4	8.9
	0.0375	103	8.9		0.75	101	6.6
	0.05	97.2	6.2		1.0	100	8.3
Carbendazim	0.05	87.8	10.1	Tebuconazole	0.01	90.5	9.9
	0.125	98.2	6.1		0.025	100	6.1
	0.25	104	4.3		0.05	101	7.8
	0.375	98.1	5.3		0.075	103	12.5
	0.5	100	3.2		0.1	98.1	6.2
Dimethoate	0.05	104	12.5	Thiamethoxam	0.5	112	19.8
	0.125	99.2	7.2		1.25	99.4	13.8
	0.25	101	6.1		2.5	93.8	12.4
	0.375	98.2	5.2		3.75	103	8,9
	0.5	101	4.6		5.0	99.7	10.5
Fipronil	0.01	87.6	12.4	HMF	1.0	70.0	11.1
	0.025	95.7	7.0		2.5	75.0	10.7
	0.05	111	8.3		5.0	109	12.7
	0.075	95.8	7.2		7.5	97.1	16.6
	0.1	100	7.2		10.0	99.8	6.3
τ-fluvalinate	0.05	77.5	18.4				
	0.125	104	17.1				
	0.25	100	13.1				
	0.375	100	15.7				
	0.5	99.5	16.7				

Results obtained for $n=9$.

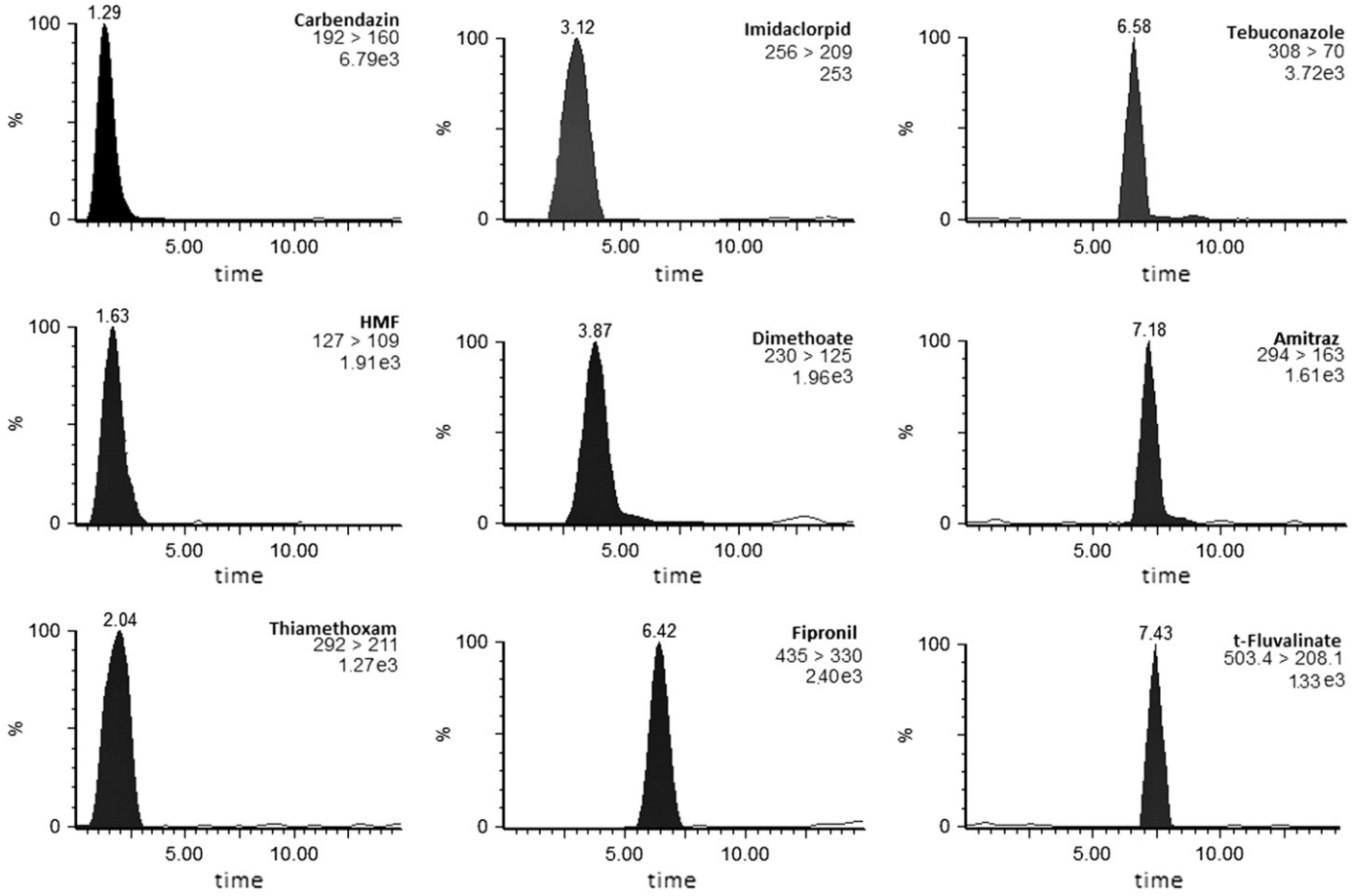

Fig. 3. MRM chromatogram of transitions monitored for quantification.

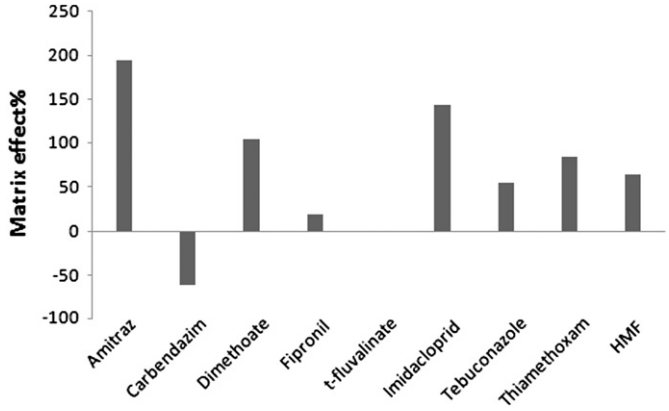

Fig. 4. Matrix effect observed for the 9 analytes, employing modified QuEChERS method and LC-APCI-MS/MS.

suppression both in ESI and APCI sources and most of them found that the effect is lower in APCI and a signal enhancement is mainly observed [36], as was observed in this study.

3.4. Process efficiency

High PE can be observed for most analytes as shown in Table 4. This can be explained as due to the high recoveries and high ME that were observed. For the analytes with low or null ME, such as fipronil and τ-fluvalinate, the PE values were near 100%. For these analytes the whole method is efficient, resulting in good recoveries and low ME.

3.5. Application of the validated method to real samples

The validated method was applied to the analysis of three honey samples originating from native forest flowers (honey A), citrus flowers (honey B) and eucalyptus flowers (honey C).

The applicability of the method under investigation was evaluated using two levels of fortification of honey samples: LOQ and 5LOQ. The *R*%, RSD and ME were calculated for each honey sample. The recoveries ranged from 70.8% to 118.3% with RSD lower than 15.6% for all the samples.

In Table 5, the ME for the three honey samples is presented. The evaluation of ME shows that the floral origin of the honey plays an important role in the response of the LC-MS, requiring quantification based on the standard addition method [37], which was performed during the application of this method.

No pesticides were detected in the samples under analysis. However, HMF was found in all samples, at concentrations of 12.1, 11.8 and 51.7 mg kg^{-1}. These concentrations are in accordance with the legislation for honeys from tropical regions [20].

4. Conclusions

This study describes the optimization and validation of a new, rapid, easy, efficient and robust method based on QuEChERS and LC-APCI-MS/MS for the simultaneous determination of 5-hydroxymethylfurfural and eight pesticides in honey.

The modified QuEChERS method provides high-quality results, minimizes the number of analytical steps, uses few reagents in small quantities, and requires little glassware. The method showed good

Table 4
Process efficiency (PE) for each analyte in 5 levels of concentration.

Level	Amitraz			Carbendazin			Dimethoate		
	A	C	PE (%)	A	C	PE (%)	A	C	PE (%)
1	163.952	176.915	108	1.889.347	600.411	31.8	169.072	407.202	240
2	281.038	357.366	127	4.829.642	1.456.553	30.2	459.924	1.032.700	224
3	450.081	770.942	171	9.492.983	2.958.036	31.2	956.448	2.141.002	224
4	560.495	1.084.676	193	14.031.000	4.120.036	29.4	1.464.325	3.151.970	215
5	686.499	1.357.052	197	17.328.290	5.554.861	32.1	2.024.299	4.333.961	214
Level	Fipronil			τ-fluvalinate			Imidacloprid		
1	18.794	35.778	190	28.980	33.801	116	63.679	209.405	328
2	59.386	69.042	116	79.402	83.918	106	163.297	480.510	294
3	123.721	138.003	111	154.033	149.924	97.3	402.009	985.514	245
4	181.681	174.263	95.9	251.070	219.175	87.3	628.320	1.539.159	245
5	225.560	235.934	104	334.861	285.840	85.4	838.334	2.051.073	244
Level	Tebuconazole			Thiamethoxam			HMF		
1	590.912	1.140.833	193	116.578	212.552	182	465.388	1.034.064	222
2	1.566.707	2.875.828	184	296.572	582.522	196	1.631.453	3.338.685	204
3	3.291.830	5.649.234	172	673.221	1.178.616	175	3.166.993	5.734.987	181
4	5.270.803	8.564.450	162	1.122.549	1.999.860	178	4.655.240	6.947.690	149
5	7.078.774	10.816.228	153	1.492.980	2.608.390	175	6.304.337	8.747.669	139

A and C correspond to areas of chromatographic peaks obtained from the sets of samples 1 and 3, respectively.

Table 5
Matrix effect (%) for real samples.

Analytes	Honey A (%)	Honey B (%)	Honey C (%)
Amitraz	63.7	32.1	33.2
Carbendazin	−63.0	−71.6	−65.5
Dimethoate	23.7	63.4	6.50
Fipronil	21.4	88.5	35.8
τ- fluvalinate	6.11	11.2	19.6
Imidacloprid	39.7	130	102
Tebuconazole	53.0	64.5	21.1
Thiamethoxam	52.7	96.9	118
HMF	−5.43	72.1	112

performance, which was verified through the construction of the analytical curves. Acceptable recoveries and good precision were achieved. The study of the matrix effect demonstrated that it depends on the floral origin of honey samples and that quantification by the standard addition method in blank matrix extract is needed.

The experimental results indicated that the proposed method would be a valuable alternative for the simultaneous determination of HMF and eight pesticide residues in honey.

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References

[1] M.W. Kujawski, J. Namiesnik, Trends Anal. Chem. 27 (2008) 785–793.
[2] K. Pyrzynska, M. Biesaga, Trends Anal. Chem. 28 (2009) 893–902.
[3] L. Yao, Y. Jiang, B. D'arcy, R. Singanusong, N. Datta, N. Caffin, K. Raymont, J. Agric. Food Chem. 52 (2004) 210–214.
[4] I. Hermosín, R.M. Chicón, M.D. Cabezudo, Food Chem. 83 (2003) 263–268.
[5] S. Ajlouni, P. Sujirapinyokul, Food Chem. 119 (2010) 1000–1005.
[6] S. Bogdanov, Apidologie 37 (2006) 1–18.
[7] C. Pirard, J. Widart, B.K. Nguyen, C. Deleuze, L. Heudt, E. Haubruge, E. De Pauw, J.-F. Focant, J. Chromatogr. A. 1152 (2007) 116–123.
[8] S.S. Caldas, A. Demoliner, E.G. Primel, J. Braz. Chem. Soc. 20 (2009) 125–132.
[9] R. Zanella, E.G. Primel, F.F. Gonçalves, M.L. Martins, M.B. Adaime, E. Marchesan, S.L.O. Machado, J. Braz. Chem. Soc. 19 (2008) 987–995.
[10] I. Mukherjee, Bull. Environ. Contam. Toxicol. 83 (2009) 818–821.
[11] S.R. Rissato, M.S. Galhiane, F.R.N. Knoll, R.M.B. de Andrade, M.V. de Almeida, Quim. Nova 29 (2006) 950–955.
[12] M. García-Chao, M.J. Agruña, G.F. Calvete, V. Sakkas, M. Llompart, T. Dagnac, Anal. Chim. Acta 672 (2010) 107–113.
[13] P. Fidente, S. Seccia, F. Vanni, P. Morrica, J. Chromatogr. A 1094 (2005) 175–178.
[14] C.F.B. Coutinho, S.T. Tanimoto, A. Galli, G.S. Garbellini, M. Takayama, R.B. Amaral, L.H. Mazo, L.A. Avaca, S.A.S. Machado, Pestic.: Rev. Ecotoxicol. Meio Ambiente 15 (2005) 65–72.
[15] 〈www.extranet.agricultura.gov.br〉. (accessed April 2011).
[16] E. Korta, A. Bakkali, A. Berrueta, B. Gallo, F. Vicente, V. Kilchenmann, S. Bogdnanov, J. Agric. Food Chem. 49 (2001) 5835–5842.
[17] N. Spano, L. Casula, A. Panzanelli, M.I. Pilo, P.C. Piu, R. Scanu, A. Tapparo, G. Sanna, Talanta 68 (2006) 1390–1395.
[18] M. Zappalà, B. Fallico, E. Arena, A. Verzera, Food Control 16 (2005) 273–277.
[19] M. Driffield, D. Chan, R. Macarthur, S. Macdonald, P. Brereton, R. Wood, J. AOAC Int. 88 (2005) 121–127.
[20] Codex Alimentarius Commission, Revised codex standard for honey, Codex Standard, FAO and WHO, Rome, 2001 12-1981.
[21] S.A. Rodrigues, S.S. Caldas, E.G. Primel, Anal. Chim. Acta 678 (2010) 82–89.
[22] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412–431.
[23] U. Koesukwiwat, K. Sanguankaew, N. Leepipatpiboon, Anal. Chim. Acta 626 (2008) 10–20.
[24] C. Pan, H. Zhang, S. Chen, Y. Xu, S. Jiang, Acta Chromatogr. 17 (2006) 320–327.
[25] A.A. Barakat, H.M.A. Badawy, E. Salama, E. Attallah, G. Maatook, J. Food Agri. Environ. 5 (2007) 97–100.
[26] A. Kamel, J. Agric. Food Chem. 58 (2010) 5926–5931.
[27] SANCO, Comission of the European Communities, Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, Document No. SANCO/10684/2009, Uppsala, Sweden, 01/01/2010.
[28] R. Varga, I. Somogyvári, Z. Eke, K. Torkos, Talanta 83 (2011) 1447–1454.
[29] P.J. Taylor, Clin. Biochem. 38 (2005) 328–334.
[30] A. Economou, H. Botitsi, S. Antoniou, D. Tsipi, J. Chromatogr. A 1216 (2009) 5856–5867.
[31] A. Kruve, A. Kunnapas, K. Herodes, I. Leito., J. Chromatogr. A 1187 (2008) 58–66.
[32] D. Tomasini, M.R.F. Sampaio, L.V. Cardoso, S.S. Caldas, E.G. Primel, Anal. Methods 3 (2011) 1893–1900.
[33] E. Corta, A. Bakkali, A. Berrueta, B. Gallo, F. Vicente, Talanta 48 (1999) 189–199.
[34] EU Pesticides Database–Regulation EC No. 396/2005, Available on: 〈http://ec.europa.eu/sanco_pesticides/public/index.cfm〉. (accessed April 2011).
[35] M.C. Chiaradia, C.H. Collins, I.C.S.F. Jardim, Quim. Nova 31 (2008) 623–636.
[36] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, J. Chromatogr. A 1217 (2010) 3929–3937.
[37] D. Debayle, G. Dessalces, M.F. Grenier-Loustalot, Anal. Bioanal. Chem. 39 (2008) 1011–1020.