Analysis of insecticide residues in honey by liquid chromatography tandem mass spectrometry using QuEChERS optimized by the Plackett Burman design

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ABSTRACT
A liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analytical method using a modified QuEChERS sample preparation for the analysis of insecticide residues in honey was developed and validated. The use of the Plackett Burman design in the sample preparation step proved to be effective in optimizing recovery and reducing the matrix effect. For quantification purposes, extract-matched analytical curves were constructed showing linearity ($R^2$) higher than 0.99. The precision (RSD%) of the method was lower than 20% and the accuracy was between 74% and 104%. Lower limits of quantification than the maximum residue limits established by the European Commission and the Brazilian legislative framework were obtained. Insecticide residues were found in 37.3% of 51 real honey samples analyzed, with imidacloprid, clothianidin and dimethoate being the most frequently detected insecticides. The co-occurrence of insecticide residues in samples was frequent. Monitoring insecticide residues in honey is needed to avoid consumer exposure at unacceptable levels.

1. Introduction
Honey is complex food and has great diversity in terms of its chemical composition (more than 200 known substances). It consists mainly of carbohydrates (fructose, glucose and sucrose), water and other substances such as proteins, organic acids, vitamins, minerals, pigments and solid particles (Bogdanov, 2006). The use of honey has grown and has been adopted into consumption habits due to its high nutritional value, palatable flavor, and medicinal properties. Several authors have reported the presence in the honey of pesticide residues from different classes, mainly neonicotinoid insecticides, organophosphates and pyrethroids (Al Naggar et al., 2015; Codling et al., 2016; Orso et al., 2015).

The presence of insecticide residues induces adverse effects in bees (Jeschke & Nauen, 2005). Therefore, to protect human and environmental health, the European Union has set maximum residue limits (MRLs) for the presence of insecticides in honey (European Data Base, 2019). In Brazil, the Ministry of Agriculture, Livestock and Supply (MAPA) established reference limits for the residues of some pesticides in honey for inspection purposes (MAPA, 2019), and the National Health Surveillance Agency (ANVISA) of the Ministry of Health (ANVISA, 2019) established MRLs for amitraz and coumaphos.

The QuEChERS sample preparation approach has been the most used method for the simultaneous extraction and extract cleanup for the analysis of insecticide residues in honey (Codling et al., 2016; P. A. Tette et al., 2016a), and the Plackett Burman design is an alternative for optimization of the sample preparation step with a small number of experiments to assess the effects of the chosen variables...
under study and establish their levels of influence (Rodrigues & lemma, 2014).

Methods for quantification of pesticide residues in honey report the use of gas chromatography with detectors such as electron capture (GC-ECD) (Orso et al., 2014), nitrogen-phosphorus (GC-NPD) (Farajzadeh et al., 2014) and mass spectrometry (GC-MS) (Bargińska et al., 2014). Also, liquid chromatography (LC) with ultraviolet detection (LC-UV) (Jovanov et al., 2015) has been reported. In particular, LC coupled with tandem mass spectrometry (LC-MS/MS) has been used in multiresidue methods and shows promising performance in the quantitative analysis of several classes of pesticides with different physicochemical characteristics in matrices such as honey (P. A. S. Tette et al., 2016b).

Thus, this work reports a method based on LC-MS/MS with a QuEChERS sample preparation approach optimized through the Placket Burman design given the physical-chemical difference between pesticides and the complexity of the matrix. The method was validated and applied to real samples for the analysis of insecticide (imidacloprid [IMI], clothianidin [CLO], dimethoate [DIM], chlorpyrifos [CPF], cypermethrin [CYP], and permethrin [PER]) residues in honey. Samples from different origins (Brazil, Mexico, Paraguay, and some European countries) were analyzed to evaluate the robustness and reliability of the method.

2. Materials and methods

2.1. Reagents and analytical standards

All analytical insecticide standards (imidacloprid, clothianidin, chlorpyrifos, permethrin, dimethoate, cypermethrin and the internal standard (IS) imidacloprid d-4) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and had purities greater than 99%. Acetonitrile and methanol of HPLC grade were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetone, ammonium formate, formic acid, anhydrous magnesium sulfate and sodium chloride supplied by Sigma-Aldrich (St. Louis, MO, USA) were of analytical grade. Primary and secondary amine (PSA), silica-bonded C18 and graphitized carbon black (GCB) sorbents were supplied by Supelco (Bellefonte, PA, USA). PVDF (polyvinylidene fluoride) syringe filters (0.22 μm) were purchased from Analytica (São Paulo, SP, Brazil). Ultrapure deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Standard solutions

Individual stock standard solutions of all analytes (including the internal standard imidacloprid d-4) were prepared in acetonitrile at a concentration of 200 μg mL−1, except for clothianidin stock solution, which was prepared in acetone. From the stock solutions, intermediate solutions were prepared in acetonitrile at two concentrations (10 and 100 μg mL−1). For the preparation of analytical curves [in solvent (acetonitrile) and extract (extract-matched)], proper volumes of the intermediate standard solutions were added to acetonitrile and extracts to prepare 7-point analytical curves of each insecticide standard (equivalent to 10, 25, 50, 75, 100, 150 and 250 μg kg−1 in the blank honey matrix).

2.3. Instrumentation and chromatographic conditions

Analyses were carried out using an Agilent UHPLC 1290 system coupled with a 6460 triple quadrupole tandem mass spectrometer (Agilent Technologies, CA, USA). Chromatographic separation was achieved on an Agilent Zorbax Eclipse plus RRHD C18 column (2.1 mm x 50 mm, 1.8 μm) (Agilent Technologies, CA, USA). The mobile phase consisted of (A) 20 mmol L−1 ammonium formate at pH 3.0 and (B) methanol containing 0.1% formic acid. A gradient elution program was used, starting with 15% B increasing linearly until reaching 95% over 2.2 min, remaining constant for 2.5 min and then returning to 15%, with a total run time of 5.0 min. A post-run time of 3.0 min was necessary to re-equilibrate the column at the initial conditions. The flow rate was 0.6 mL min−1, the column temperature was 45°C, and the injection volume was 2 μL.

The MS/MS system was equipped with an electrospray ionization (ESI) source and operated in positive ionization mode. The ionization source conditions were optimized employing the Source Optimizer tool and were as follows: gas temperature, 300°C; gas flow, 10 L min−1; nebulizer, 25 psi; sheath gas flow 10 L min−1; sheath gas temperature, 300°C; capillary, 4.5 kV; and nozzle, 0 kV. These parameters were established by directly injecting individual standard solutions (1 μg mL−1) prepared in acetonitrile into the ESI source. The Optimizer tool was also used to provide the most abundant product ions (quantifier and qualifier ions) and the corresponding collision energies (CE) in selected reaction monitoring (SRM) mode (Table 1). The identification criteria were based on the Guidance document SANTE/12682/2019 for LC-MS/MS analysis with acquisition of two transitions ions (quantifier and qualifier ions), retention time (tolerance of ±0.2 min) and ion ratio compliance with a tolerance of ±30% between the ions (SANTE, 2019).

2.4. Samples

Blank samples of honey employed for optimization and validation purposes were obtained from Apiário Cambará, Rio Grande do Sul, Brazil.

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**Table 1.** Selected reaction monitoring (SRM) settings for the targeted insecticides using electrospray ionization (ESI) source in positive ionization mode.

| Insecticides   | Chemical Class | tR(min) | Precursor ion (m/z) | Quantifier ion (CE1) | Qualifier ion (CE2) | Fragmentor (V) |
|----------------|----------------|---------|---------------------|----------------------|---------------------|----------------|
| Clothianidin   | Neonicotinoid  | 1.7     | 249.0 [M + H]⁺      | 250.0 > 169.0 (9)    | 250.0 > 131.9 (13)  | 64             |
| Imidacloprid   | Neonicotinoid  | 1.8     | 255.0 [M + H]⁺      | 256.1 > 209.0 (13)   | 256.1 > 175.0 (17)  | 64             |
| Dimethoate     | Organophosphate| 3.9     | 229.0 [M + H]⁺      | 230.0 > 124.8 (17)   | 230.0 > 198.8 (5)   | 64             |
| Chlorpyrifos   | Organophosphate| 3.9     | 348.9 [M + H]⁺      | 349.9 > 197.9 (21)   | 349.9 > 124.9 (9)   | 113            |
| Cypermethrin   | Pyrethroid     | 4.1     | 415.1 [M + NH₄]⁺    | 433.1 > 191.0 (10)   | 433.1 > 127.0 (40)  | 76             |
| Permethrin     | Pyrethroid     | 4.3     | 390.8 [M + NH₄]⁺    | 408.1 > 183.0 (17)   | 408.1 > 355.1 (5)   | 64             |

Dwell time = 20 ms; tR = retention time; CE1 = collision energy 1; CE2 = collision energy 2.
A total of 51 honey samples were purchased at the retail market or supplied by producers and honey cooperatives in Brazil. Also, samples from other Latin American countries (Paraguay (n = 1) and Mexico (n = 1)) and Europe (France (n = 1), Spain (n = 1), Germany (n = 1), Portugal (n = 1), Cyprus (n = 2) and Italy (n = 3)) were analyzed. All samples were collected between 2017 and 2019, stored at 10°C and analyzed within their listed shelf life.

2.5. Optimization of the sample preparation step

Sample preparation was based on the QuEChERS approach (Anastassiades et al., 2003), and optimized through the Plackett-Burman (PB) design (Rodrigues & Lemma, 2014). Eight sample preparation variables were studied at two levels, low (-1) and high (+1): the amount of salts used in the extraction step (NaCl and MgSO₄), extraction time, amount of sorbents (C18, GCB, PSA) used in the dispersive solid-phase extraction (d-SPE) and extraction time (Table S1, supplemental material). The effect of each variable on insecticide recoveries was estimated using Statistica 7.0 software, adopting a significance level of 10% (p ≤ .1).

2.6. Sample preparation

Sample preparation was carried out using the in-house optimized QuEChERS. Ten grams of a homogenized honey sample was weighed into a 50 mL PTFE centrifuge tube and 10 mL of water was added, followed by vortexing for 5 min. Then, 10 mL of acetonitrile was added, and the samples were vortexed again for 5 min. To induce phase separation and force the insecticides into the acetonitrile phase, 4 g of MgSO₄ and 1.5 g of NaCl were added into the tubes and the mixture was vortexed for 5 min followed by centrifugation at 3,000 g for 5 min. A 5 mL aliquot of the supernatant was transferred to another PTFE centrifuge tube containing 750 mg of MgSO₄, 250 mg of PSA and 125 mg of C18 and was vortexed for 2 min and centrifuged at 3,000 g for 5 min. The cleaned extracts were filtered through a Millex HV filter unit (0.22 μm pore size, Millipore) directly into an LC vial and then injected into the UHPLC-MS/MS system.

2.7. In-house validation

The performance characteristics of the analytical method were established according to the European Commission (EU) guidance document for pesticide residue analysis in food and feed (SANTE, 2019). The parameters evaluated were selectivity, matrix effect, linearity, precision, accuracy, limit of quantification (LOQ) and robustness.

The selectivity was determined based on the LC-MS/MS analysis of analyte-free samples against spiked samples to provide the ability of the method to discriminate the targeted insecticides from interfering matrix compounds in SRM mode. Linearity was assessed through 7-point (10–250 μg kg⁻¹) analytical curves prepared in solvent (acetonitrile) and blank honey matrix extract (extract-matched). The matrix effects (ME) were estimated according to Sapozhnikova and Lehotay (2013). Accuracy and precision (intraday and interday) were assessed through recovery of additions of known amounts of the analytical insecticide standards to the blank honey matrix at two concentration levels, namely, a low level (the LOQ obtained for each target insecticide) and high level (100 μg kg⁻¹). Accuracy, expressed as recovery (%), was obtained by comparison between the real and measured concentrations from five individual replicates at each concentration level. Precision, expressed as relative standard deviation (RSD%), was evaluated under repeatability conditions (intraday precision) from five individual replicates at each spiked concentration level analyzed on the same day and under within-laboratory reproducibility (interday precision), which was evaluated from a total of ten individual replicates at each spiked concentration level analyzed on two different days.

The LOQ was stated as the lowest analyte concentration in the matrix that could be quantified with acceptable accuracy (between 70% and 120%) and precision (RSD values ≤20%) with a signal-to-noise ratio of 10:1 (SANTE, 2019).

The robustness was evaluated by applying a 2³ factorial design, considering small variations inherent in the laboratory routine, such as analytical column batch (# B13211 and # B12238), oven temperature (40°C and 50°C) and flow rate of the mobile phase (0.5 mL min⁻¹ and 0.7 mL min⁻¹). All data were analyzed using Statistica 7.0 software and adopting a significance level of 5% (p ≤ .05). The applicability of the method was verified through the analysis of real samples from different origin.

3. Results and discussion

3.1. Chromatographic conditions

The studied compounds belong to three different chemical classes of insecticides, including neonicotinoids, pyrethroids and organophosphates, with partition coefficients (log P) ranging from 0.57 (IMI) to 6.6 (CYP). Therefore, to achieve suitable simultaneous analyses of the insecticides in terms of selectivity and peak shape, different compositions and proportions of the mobile phase, such as water-methanol, water-acetonitrile or water-acetonitrile-methanol, as well as an aqueous ammonium formate solution at different concentrations (5, 10 or 20 mmol L⁻¹) and pH (3.0 or 6.0) were tested using a reverse-phase C18 column.

The ionization of pyrethroids is favored with methanol, and this insecticide class ionized more efficiently with ammonium formate at a concentration of 20 mmol L⁻¹, increasing ammonium adduct ion formation [M + NH₄]⁺. A pH of 3.0 provided enhanced ionization of the neonicotinoids and the acidification of methanol with 0.1% formic acid provided analytical signals with optimal shape with ESI in positive ionization mode, as indicated by Lehotay and Mastovska (2005).

Neonicotinoids are extremely polar compounds (log P 0.57 and 0.7 for IMI and CLO, respectively) and show poor interaction with the stationary phase used; thus, the elution gradient was optimized to improve the separation efficiency and avoid the elution of these compounds in the column dead volume. In addition, the permethrin isomers could be visualized in the chromatogram, and both were considered for quantification purposes. Thus, the chosen mobile phase was a 20 mmol L⁻¹ aqueous ammonium formate solution at pH 3.0 (A) and methanol with 0.1% formic acid (B) with gradient elution. Figure 1 shows SRM chromatograms of a standard solution in the honey extract at a level of 100 μg kg⁻¹.
3.2. Optimization of the sample preparation step

Honey is a complex matrix that requires a sample preparation with efficient cleanup step to eliminate possible LC-MS/MS interferences (Bogdanov, 2006; Rissato et al., 2007). The QuEChERS procedure has been widely used for honey analyses and several modifications to the original method have been reported due to the physicochemical characteristics of the compounds investigated (Al Naggar et al., 2015; Codling et al., 2016; P. A. Tette et al., 2016a). In the present study, the target insecticides cover a wide range of polarities with log P values between 0.57 (IMI) and 6.6 (CYP). Thus, a Plackett-Burman screening design was used to evaluate sample preparation variables that could affect the insecticide extraction as well as to minimize the amount of chemicals employed in the sample preparation step.

The experimental design was conducted in according to Rodrigues and lemma (2014) and resulted in recoveries from 36% to 138% (Table S1, supplemental material). The effect (%) was calculated from the recoveries obtained (Table 2).

It was verified that the amount of NaCl and MgSO₄ salts used to induce phase separation as well as the amount of PSA and C18 sorbents used in the cleanup step significantly influenced (p ≤ .1) the insecticide recoveries.

The variation in the amount of MgSO₄ from 2 g to 6 g resulted in a significantly negative effect (p ≤ .1) on the recovery of neonicotinoid (imidacloprid and clothianidin) and organophosphate (dimethoate and chlorpyrifos) insecticides, whereas an increase in the amount of NaCl from 0.5 to 1.5 g resulted in a significantly positive effect (p ≤ .1) on the recovery of pyrethroids (permethrin and cypermethrin). Therefore, 4 g of MgSO₄ (central point condition) and a high amount of NaCl (1.5 g) were chosen as optimal conditions for the extraction step. In addition, variations in the time of vortex agitation from 5 to 25 min resulted in no significant effects (p > .1) on the recoveries; thus, the time was maintained at 5 min.

Regarding the d-SPE step, variations in the amount of C18 (from 125 to 375 mg), GCB (from 0 to 75 mg) and PSA (from 125 to 375 mg) sorbents were evaluated. C18 has been used in d-SPE to remove lipids, and GCB is extremely efficient in removing pigments such as chlorophyll and carotenoids, but it has a significant effect on the recovery of some structurally planar pesticides. PSA is an anion-exchange sorbent used mainly to remove polar coextracted matrix components, including sugars, organic acids and some pigments, such as anthocyanidins (Lehotay & Mastovska, 2005). The increase in the amounts of C18 and GCB resulted in a significantly negative effect (p ≤ .1) on permethrin recovery; thus, the minimal amount of both sorbents was adopted for the cleanup step.

An increase in the amount of PSA resulted in a significantly positive effect (p ≤ .1) on the recoveries of imidacloprid, clothianidin and dimethoate, all compounds with relatively low log P values (0.57, 0.7 and 0.78, respectively). On the other hand, negative effects were observed for compounds with relatively high log P values, such as permethrin (6.5) and cypermethrin (6.6). Thus, the amount of PSA was fixed at the central point (250 mg).

In addition to cleanup sorbents, the amount of MgSO₄ used in the d-SPE step and the time of vortex agitation were also evaluated. Variation in the amount of MgSO₄ from 250 to 750 mg resulted in positive effects on the recovery of all compounds; thus, the maximum amount studied was adopted on the d-SPE step. Since the increment in the agitation time from 2 to 8 min produced negative effects on the recovery of cypermethrin and clothianidin, this variable was fixed at the minimum level studied.

Therefore, based on the Plackett-Burman screening design, the optimal sample preparation conditions were 4 g of MgSO₄ and 1.5 g of NaCl in the liquid-liquid partitioning step, under 5 min of vortex agitation, followed by 750 mg of MgSO₄, 250 mg of PSA and 125 mg of C18 in the d-SPE step and under 2 min of vortex agitation. Under these conditions, clean honey extracts were obtained, and the insecticide recoveries ranged from 77% to 88%, which met the criteria established by SANTE 12682/2019 (SANTE, 2019) (Figure S1, supplemental material).

3.3. LC–MS/MS method validation

No analytical signals were observed at the insecticide retention times when comparing the SRM chromatograms...
Table 2. Main effects of evaluated parameters on the recovery (%) of insecticides estimated according the Plackett-Burman design.

| Extraction step | CYP | PER | CPY | CLO | DIM | IMI |
|-----------------|-----|-----|-----|-----|-----|-----|
| NaCl (g)        |     |     |     |     |     |     |
| Effect (%)      | 44.33 | 13 | −0.33 | 2.33 | 1.33 | −0.83 |
| t(6)            | 3.72  | 8.98 | −0.20 | 1.07 | 0.85 | −0.52 |
| p-value         | 0.01  | 0.0001 | 0.85 | 0.33 | 0.43 | 0.62 |
| MgSO₄ (g)       |     |     |     |     |     |     |
| Effect (%)      | 12.67 | 0.33 | −4.33 | −18.33 | −6.67 | −9.50 |
| t(6)            | 1.06  | 0.23 | −2.62 | −8.37 | −4.23 | −5.92 |
| p-value         | 0.33  | 0.08 | 0.03 | 0.0002 | 0.006 | 0.001 |
| Vortex (min)    |     |     |     |     |     |     |
| Effect (%)      | −14  | −2.33 | 0 | 2.33 | −1.33 | −1.83 |
| t(6)            | −1.18 | −1.61 | 0 | 1.07 | −0.85 | −1.14 |
| p-value         | 0.28  | 0.16 | 1 | 0.33 | 0.43 | 0.30 |

Table 2. Principales efectos de los parámetros evaluados sobre la recuperación (%) de insecticidas, estimados según el diseño de Plackett-Burman.

| Extracción | CYP | PER | CPY | CLO | DIM | IMI |
|------------|-----|-----|-----|-----|-----|-----|
| NaCl (g)   |     |     |     |     |     |     |
| Porcentaje | 44.33 | 13 | −0.33 | 2.33 | 1.33 | −0.83 |
| Error      | 3.72  | 8.98 | −0.20 | 1.07 | 0.85 | −0.52 |
| MgSO₄ (g)  |     |     |     |     |     |     |
| Porcentaje | 12.67 | 0.33 | −4.33 | −18.33 | −6.67 | −9.50 |
| Error      | 1.06  | 0.23 | −2.62 | −8.37 | −4.23 | −5.92 |
| Vortex (min)|     |     |     |     |     |     |
| Porcentaje | −14  | −2.33 | 0 | 2.33 | −1.33 | −1.83 |
| Error      | −1.18 | −1.61 | 0 | 1.07 | −0.85 | −1.14 |

Table 3. Maximum residue limits (MRL) and validation data of the analytical method for the determination of insecticide residues in honey.

| Insecticides | Brazil MRL (µg kg⁻¹) | EU MRL (µg kg⁻¹) | LOQ (µg kg⁻¹) | Solvent | Extract ME (%) | Low | High | Precision |
|--------------|-----------------------|-----------------|---------------|---------|----------------|-----|------|-----------|
|              |                       |                 |               |         |                |     |      | Intra-day | Inter-day |
|              |                       |                 |               |         |                |     |      | (n = 5)   | (n = 10)  |
| Cypemethrin  | 50                    | 50              | 50.0          | 0.955   | 0.9906         | 3.2 | 85   | 82       | 16        | 13      | 9        |
| Permethrin   | 880                   | N.A.            | 10.0          | 0.9987  | 0.9953         | 0.84| 84   | 74       | 11        | 4       | 12       | 5        |
| Chlorpyrifos | 50                    | 50              | 10.0          | 0.9918  | 0.9950         | 9.3 | 91   | 74       | 14        | 2       | 15       | 5        |
| Clothianidin | 50                    | N.A.            | 10.0          | 0.9968  | 0.9950         | −0.17| 94   | 79       | 12        | 6       | 15       | 6        |
| Dimethoate   | 10                    | N.A.            | 10.0          | 0.9979  | 0.9984         | −4.9| 104  | 75       | 4         | 3       | 4        | 2        |
| Imidacloprid | 50                    | 50              | 10.0          | 0.9980  | 0.9987         | 8.9 | 100  | 83       | 4         | 2       | 3        | 5        |

N.A. = no available; ME = matrix effect; LOQ = limit of quantification; Low = LOQ, 50 µg kg⁻¹ for cypemethrin and 10 µg kg⁻¹ for the others; High = 100 µg kg⁻¹ for all insectes.
(25%) presented residues below the LOQ, whereas one sample (8.3%) exceeded the MRL (Figure 2). This noncompliant honey sample was from Spain and contained cypermethrin residues at 102 µg kg⁻¹ (Figure 3).

In all samples the most common insecticides detected (in at least five samples) were imidacloprid, clothianidin, dimethoate and cypermethrin. The frequency of detection for imidacloprid (27.5%, 13 Brazilian and 1 Spanish sample), clothianidin (11.8%, 4 Brazilian, 1 Italian and 1 Spanish samples), dimethoate (31.4%, 14 Brazilian, 1 French and 1 Spanish sample) and cypermethrin (9.8%, 2 Brazilian, 1 Italian, 1 Greek and 1 Spanish sample) were relatively high. Since 2013, imidacloprid and clothianidin have use restrictions in the European Union to protect pollinating insects (European Food Safety Authority [EFSA], 2018).

In monitoring studies conducted in Brazil, the presence of chlorpyrifos and dimethoate at levels below the MRL established by the EU and the reference limits adopted in Brazil (MAPA, 2019) was reported in honey samples from the State of Rio Grande do Sul (Orso et al., 2015). Tomasinii et al. (2012) did not found residues of imidacloprid nor thiametoxan in samples from the south of Brazil.

Regarding other monitoring studies, neonicotinoid insecticides such as imidacloprid, thiametoxan, thiabendazid and some metabolites have been reported in honey from Canada (Codling et al., 2016), Serbia (Jovanov et al., 2015), Spain (Sánchez-Hernández et al., 2016) and Poland (Gawel et al., 2019).

In the most recent annual report, the European Food Safety Authority (EFSA) reported the results of the EU-coordinated control program on food analyzed in 2018. Among the honey and other bee products analyzed (762 samples), 78.9% of samples did not show pesticide residues, whereas 1.2% of samples contained residues above the MRL. The substances that exceeded the MRL value established by the EU were the insecticide acetamiprid, the herbicide glyphosate, the fungicide boscalid and the antibiotic dimoxystrobin (EFSA, 2020).

Considering the results found in this study, it is suggested to improve the management on the use of pesticides in general, mainly insecticides, in agricultural practice to avoid inappropriate residue levels in honey, protecting the human and environmental health and maintaining trade for this food commodity.

4. Conclusion

The LC-MS/MS analytical method developed and validated according to European Commission SANTE/12682/2019 was reliable and appropriate for the intended purpose of quantification of insecticide residues in honey. The optimization of the sample preparation step (extraction and cleanup) using an experimental design (Plackett Burman approach) enable higher analytes recovery and reduces the matrix effect, allowing obtaining lower LOQ values. The analysis of real honey samples from different origins proved the method’s robustness and applicability. Imidacloprid, clothianidin, dimethoate and cypermethrin were the insecticide more detected in the samples, being that in one sample...
cypermethrin MRL was exceeded. These indicate there is a recurrent environmental contamination. Thus, it is recommended that regulatory agencies conduct surveillance programs to assess pesticide residues in honey to avoid unnecessary consumer exposure to these toxic compounds.

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