Optimization of Method for Pesticide Detection in Honey by Using Liquid and Gas Chromatography Coupled with Mass Spectrometric Detection

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Abstract: This study aimed to optimize and validate a multi-residue method for identifying and quantifying pesticides in honey by using both gas and liquid chromatographic separation followed by mass spectrometric detection. The proposed method was validated to detect 168 compounds, 127 of them by LC-MS/MS (liquid chromatography tandem mass spectrometric detection) and 41 by GC-MS/MS (gas chromatography tandem mass spectrometric detection). The limit of detection (LOD) and limit of quantification (LOQ) values for the analytes determined by LC-MS/MS were 0.0001–0.0004 mg/kg and 0.0002–0.0008 mg/kg, respectively. For GC-MS/MS analyses, the LOD and LOQ values were 0.001–0.004 mg/kg and 0.002–0.008 mg/kg. In total, 33 samples of commercial honey produced by apiaries in six Brazilian states were analyzed with the validated method. Residual amounts of 15 analytes were detected in 31 samples (93.9%). The method described in the present study was able to detect an extensive and broad range of pesticides with very high sensitivity.

Keywords: residues in food; pesticides; LC-MS/MS; GC-MS/MS; QuEChERS; honey

1. Introduction

Honey is consumed by humans worldwide because of its characteristic sweet flavor and as a medicinal food. It is produced by honeybees, mainly from nectar collected from flowers. However, honey may be contaminated with pesticides used on crops foraged by bees. Contamination may occur through direct contact of the bee body to the pesticide or by bee consumption of the contaminated nectar, pollen, and guttation fluid (an exudate eliminated through the tips or edges of leaves of some plants) [1–3]. Furthermore, some pesticides are used to treat beehives against diseases [4].

The consumption of residual pesticides in contaminated foods has been linked to several toxic effects in humans, such as carcinogenesis, immunological disorders, and neurological disturbances [5]. Maximum residue levels (MRLs) have been established for pesticides in honey to ensure consumers’ safety [6–9]. It is mandatory to avoid the commercialization of honey containing residual pesticides at levels above the MRLs. To determine residual pesticide levels, precise and sensitive analytical methods must be able to detect an extensive and broad range of compounds.

Several analytical methods have been developed for detecting single compounds to a few dozen pesticides in honey. In these methods, detection and quantification are performed using techniques such as liquid chromatography (LC) with diode array [10], ultraviolet [11,12],
fluorescence [13], and electrochemical [11] detectors, gas chromatography (GC) with electron capture [14], flame ionization [15], nitrogen–phosphorus [16], flame photometric [17], thermionic-specific [18], and atomic emission [19] detectors, and excitation–emission matrix fluorescence data [20].

The performance of chromatographic analysis depends on adequate sample extraction and cleanup procedures. Matrix compounds are concentrated at the extraction procedure, whereas interfering substances are removed by the cleanup procedure [21]. An innovative technique developed for sample extraction and cleanup procedures is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method [22]. Compared to earlier procedures, this method reduces the volume of solvents, and offers practical performance. Modifications of the QuEChERS method have been used for the detection of pesticides in different matrices such as meat [23], fish [24], milk [25], and honey [3,26–31].

Simultaneous detection of the residual levels of several pesticides in honey is mandatory in several countries to inspect this food before commercialization. Multi-residue analysis of at least one hundred pesticides in honey has been achieved using LC and GC coupled to mass spectrometric (MS) or tandem mass spectrometric (MS/MS) detection [1,3,26–32].

This study aimed to develop and validate a multi-residue method for identifying and quantifying pesticides in honey by using both gas and liquid chromatographic separation followed by mass spectrometric detection.

2. Materials and Methods

2.1. Chemicals and Reagents

Acetonitrile, ethyl acetate (both high performance liquid chromatography [HPLC] grade), and formic acid (for analysis) were supplied by Merck (Darmstadt, Germany). Methanol (HPLC grade) was obtained from Honeywell (Charlotte, NC, USA). Ammonium formate (>99%) was purchased from Vetec (Rio de Janeiro, Brazil). A DisQuE™ CEN sample preparation kit in pouch format (each pouch containing 4.0 g of anhydrous magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate; all > 99%) was supplied by Waters (Milford, CT, USA). An ExtraBond® QuEChERS Dispersive kit EN (each tube containing 900 mg of anhydrous magnesium sulfate and 150 mg of primary and secondary amine (PSA); both > 99%) was obtained from Scharlab (Barcelona, Spain). D-Sorbitol (≥98%) and gluconolactone (>99%) were purchased from Sigma–Aldrich (Darmstadt, Germany). Ultrapure water was generated with a Millipore Milli-Q system (Milford, CT, USA). All reference standards were of high purity grade (>98.0%) and were obtained from Dr. Ehrenstorfer (Augsburg, Germany) or AccuStandard (New Haven, CT, USA). Individual stock solutions were prepared at an approximate concentration of 1000 ng/µL in acetonitrile or acetone and stored in a freezer at −20 °C. Working solutions were prepared through appropriate dilutions of the stock solutions.

2.2. Samples

Blank samples of honey were obtained from apiaries managed under an organic system, and repeated analyses confirmed the absence of residual pesticides. These blank samples were fortified with target analytes for the validation of the analytical method. Furthermore, 33 samples of commercial honey produced by apiaries in six Brazilian states (Distrito Federal, Goiás, Minas Gerais, Rio Grande do Norte, Rio Grande do Sul, and São Paulo) were analyzed using the validated method.

2.3. Sample Preparation

The modified QuEChERS method for extraction and cleanup was optimized from previously described procedures [22,28,32,33]. Each honey sample (5.0 g) was placed into a 50 mL polypropylene tube and spiked with appropriate amounts of pesticides in working solutions. Next, 10.0 mL of ultrapure water was added, and the mixture was agitated at 1750 rpm for 2 min. Exactly 10.0 mL of a solution of acetonitrile and ethyl acetate (70:30, v/v) was added, and each tube was agitated again
at 1750 rpm for 2 min. Then, 4.0 g of anhydrous magnesium sulfate, 1.0 g of sodium chloride, 1 g of trisodium citrate dehydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate were added, and the tubes were agitated at 1750 rpm for another 2 min and centrifuged at 4000 rpm for 5 min. The whole organic layer was transferred to a 15 mL polypropylene tube, and the mixture was kept at −40 °C for at least 2 h. The supernatant (6.0 mL) was mixed with 900 mg of anhydrous magnesium sulfate and 150 mg of PSA, and the mixture was agitated at 1750 rpm for 1 min and centrifuged at 3600 rpm for 5 min. The extract (4.0 mL) was transferred to two 13 × 100 mm glass tubes, with 2.0 mL in each tube. The solution was dried in an evaporator with a water bath maintained at 45 °C and nitrogen pressure of 15 psi.

The procedural internal standard (P-IS) [34] for the LC analysis was Propoxur, and the P-IS for GC analysis was 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE). After weighing the honey sample, 10 μL of the P-IS solution containing 4.0 ng/μL of Propoxur and 4.0 ng/μL of DDE 4,4 was added. Propoxur and 4,4′-DDE were then validated following the validation method described in Section 2.3.

For LC analysis, the dried residue was reconstituted with 200 μL of methanol:water (1:1), with both solvents containing 5 mM ammonium formate and 0.01% formic acid. After 30 min, the tube was vortexed for 1 min, and the solution was transferred to a vial containing a conical insert of 250 μL.

For GC analysis, the dried residue was reconstituted with 200 μL of acetonitrile:ethyl acetate (7:3) and 6 μL of analyte protectant solution, composed of 10 mg/mL gluconolactone and 5 mg/mL D-sorbitol in acetonitrile:water (7:3). The tube was then immediately vortexed for 0.5 min, and the solution was transferred to a vial containing a conical insert of 250 μL.

2.4. Liquid Chromatography

LC-MS/MS (liquid chromatography tandem mass spectrometric detection) analysis was performed using an Agilent 6495 Triple Quadrupole LC/MS system. Chromatographic separations were carried out on a Zorbax SB-C18 Rapid Resolution HT column (4.6 × 150 mm, 1.8 μm) at a 40 °C column temperature. The mobile phases were water containing 5 mM ammonium formate and 0.01% formic acid (phase A) and methanol containing 5 mM ammonium formate and 0.01% formic acid (phase B), with gradient elution at a flow rate of 0.6 mL/min. The gradient elution program was as follows: 0 min, 90% B; 2.0 min, 50% B; 20 min, 100% B. The total chromatographic run time was 25 min. The injection volume was 5 μL.

For mass spectrometric analysis, an electrospray ionization (ESI) source was used in both negative (ESI-) and positive (ESI+) modes. Source parameters were set as follows: gas temperature 120 °C, gas flow 15 L/min, nebulizer 45 psi, sheath gas flow 12 L/min, sheath gas temperature 300 °C, capillary voltage 3500 V (+ and −), nozzle voltage 300 V (+)/500 V (−), iFunnel RF high pressure 150 V (+)/90 V (−), and iFunnel RF low pressure 60 V (+ and −). The retention times, delta retention times, polarities, ion transitions, and collision energies are presented in Table 1. Two transitions were chosen for almost all pesticides, but an extra confirmatory transition was included for four pesticides to avoid false-positives at trace pesticide levels. The analysis was run according to all requirements for identifying analytes by MS/MS established by European Union SANTE/12682/2019 [34].
Table 1. Chromatographic parameters and MS/MS (tandem mass spectrometric) detection for compounds analyzed by LC-MS/MS (liquid chromatography tandem mass spectrometric detection).

| Name            | RT (min) | DRT (min) | Polarity | Transitions                        | Collision Energy |
|-----------------|----------|-----------|----------|------------------------------------|------------------|
| 2,4-D           | 9.60     | 1.5       | ESI−     | QI 3 218.9 > 161.                   | 11               |
| Acephate        | 4.56     | 1.5       | ESI+     | 1st CI 4 218.9 > 124.9             | 35               |
|                 |          |           |          | 2nd CI 220.9 > 162.9               |                  |
|                 |          |           |          | QI 184.0 > 143.0                   | 5                |
| Acetamiprid     | 6.56     | 1.0       | ESI+     | 1st CI 223.1 > 126.0               | 15               |
|                 |          |           |          | 2nd CI 223.1 > 56.0                |                  |
| Aldicarb        | 8.05     | 1.5       | ESI+     | 1st CI 208.1 > 116.2               | 10               |
|                 |          |           |          | 1st CI 208.1 > 89.1                | 24               |
| Aldicarb-Sulfone| 4.99     | 1.5       | ESI+     | 1st CI 223.1 > 148.0               | 5                |
|                 |          |           |          | 1st CI 223.1 > 76.0                | 5                |
| Aldicarb-Sulfoxide | 4.88   | 1.5       | ESI+     | QI 207.1 > 131.9                   | 0                |
| Allethrin       | 19.36    | 1.5       | ESI+     | 1st CI 207.1 > 89.1                | 8                |
| Ametryn         | 13.15    | 1.5       | ESI+     | QI 228.1 > 186.1                   | 20               |
|                 |          |           |          | 1st CI 228.1 > 116.1               |                  |
|                 |          |           |          | 2nd CI 228.1 > 96.0                | 25               |
| Aminocarb       | 7.32     | 1.5       | ESI+     | QI 209.1 > 152.2                   | 12               |
|                 |          |           |          | 1st CI 209.1 > 137.2               | 20               |
| Atrazine        | 11.34    | 1.5       | ESI+     | QI 216.1 > 174.1                   | 16               |
|                 |          |           |          | 1st CI 216.1 > 68.0                | 40               |
| Avermectin B1a  | 21.71    | 1.5       | ESI+     | QI 890.5 > 567.4                   | 8                |
|                 |          |           |          | 1st CI 890.5 > 305.1               | 16               |
| Azinphos-Ethyl  | 14.74    | 1.5       | ESI+     | QI 300.0 > 231.1                   | 16               |
|                 |          |           |          | 1st CI 300.0 > 159.0               |                  |
| Azinphos-Methyl | 12.15    | 1.5       | ESI+     | QI 318.0 > 261.0                   | 0                |
|                 |          |           |          | 1st CI 318.0 > 132.1               | 8                |
|                 |          |           |          | QI 404.1 > 372.1                   | 12               |
| Azoxytrobin     | 12.90    | 1.5       | ESI+     | 1st CI 404.1 > 344.1               | 28               |
|                 |          |           |          | 2nd CI 404.1 > 329.1               |                  |
| Benalaxyl       | 16.86    | 1.5       | ESI+     | QI 326.2 > 294.1                   | 4                |
|                 |          |           |          | 1st CI 326.2 > 148.1               | 27               |
| Bitertanol      | 16.98    | 1.5       | ESI+     | QI 338.2 > 99.1                    | 10               |
|                 |          |           |          | 1st CI 338.2 > 70.0                | 4                |
| Boscalid        | 13.34    | 1.5       | ESI+     | QI 343.0 > 307.1                   | 16               |
|                 |          |           |          | 1st CI 343.0 > 271.2               | 32               |
| Bromacil        | 9.29     | 1.5       | ESI+     | QI 261.0 > 205.0                   | 20               |
|                 |          |           |          | 1st CI 261.0 > 187.9               |                  |
| Bromuconazole   | 15.50    | 3.0       | ESI+     | QI 378.0 > 159.0                   | 32               |
|                 |          |           |          | 1st CI 378.0 > 70.0                | 35               |
| Buprofezin      | 19.13    | 1.5       | ESI+     | QI 306.2 > 201.1                   | 5                |
|                 |          |           |          | 1st CI 306.2 > 116.1               | 10               |
| Cadusafos       | 17.96    | 1.5       | ESI+     | QI 271.1 > 130.9                   | 20               |
|                 |          |           |          | 1st CI 271.1 > 97.0                | 40               |
| Carbaryl        | 9.80     | 1.5       | ESI+     | QI 202.1 > 145.1                   | 4                |
|                 |          |           |          | 1st CI 202.1 > 127.1               | 28               |
| Carbendazim     | 7.07     | 1.5       | ESI+     | QI 192.1 > 160.1                   | 16               |
|                 |          |           |          | 1st CI 192.1 > 132.1               | 32               |
| Carbofuran      | 9.35     | 1.5       | ESI+     | QI 222.1 > 165.1                   | 20               |
|                 |          |           |          | 1st CI 222.1 > 123.1               | 30               |
| 3-Hydroxycarbofuran | 6.35 | 1.5       | ESI+     | QI 238.1 > 220.1                   | 0                |
|                 |          |           |          | 1st CI 238.1 > 163.1               | 8                |
| Name                        | RT \(^1\) (min) | DRT \(^2\) (min) | Polarity | Transitions                      | Collision Energy |
|-----------------------------|-----------------|------------------|----------|---------------------------------|------------------|
| Carboxin                    | 9.88            | 1.5              | ESI+     | QI 236.1 > 143.1                | 12               |
|                            |                 |                  |          | 1st CI 236.1 > 93.1             | 36               |
| Chlorfenvinphos            | 17.20           | 1.5              | ESI+     | QI 358.9 > 155.0                | 8                |
|                            |                 |                  |          | 1st CI 358.9 > 99.2             | 28               |
| Chlorfluazuron              | 20.00           | 1.5              | ESI+     | QI 539.9 > 383.0                | 44               |
|                            |                 |                  |          | 1st CI 539.9 > 158.0            | 36               |
| Chlorpyrifos               | 20.20           | 1.5              | ESI+     | QI 349.9 > 198.0                | 20               |
|                            |                 |                  |          | 1st CI 349.9 > 97.0             | 20               |
| Chlorpyrifos-Methyl-Oxon   | 15.89           | 1.5              | ESI+     | QI 334.0 > 306.0                | 8                |
|                            |                 |                  |          | 1st CI 334.0 > 278.0            | 8                |
| Clofentezine               | 16.99           | 1.5              | ESI+     | QI 303.0 > 138.0                | 12               |
|                            |                 |                  |          | 1st CI 303.0 > 102.0            | 40               |
| Clomazone                  | 12.70           | 1.5              | ESI+     | QI 242.1 > 127.0                | 20               |
|                            |                 |                  |          | 1st CI 240.1 > 89.1             | 56               |
| Clothianidin               | 6.08            | 1.5              | ESI+     | QI 250.0 > 169.0                | 8                |
|                            |                 |                  |          | 1st CI 250.0 > 131.9            | 8                |
| Cyanazine                  | 8.43            | 1.5              | ESI+     | QI 241.1 > 214.1                | 18               |
|                            |                 |                  |          | 1st CI 241.1 > 104.0            | 44               |
| Cyanofenphos               | 16.60           | 1.0              | ESI+     | QI 304.1 > 276.0                | 12               |
|                            |                 |                  |          | 1st CI 304.1 > 157.0            | 24               |
| Cyazofamid                 | 15.43           | 1.5              | ESI+     | QI 325.0 > 261.0                | 4                |
|                            |                 |                  |          | 1st CI 325.0 > 108.0            | 8                |
| Cymoxanil                  | 6.97            | 1.5              | ESI+     | QI 199.1 > 128.0                | 4                |
|                            |                 |                  |          | 1st CI 199.1 > 110.9            | 12               |
| Cyproconazole              | 14.80           | 2.0              | ESI+     | QI 292.1 > 125.0                | 32               |
|                            |                 |                  |          | 1st CI 292.1 > 70.0             | 16               |
| Cyprodinil                 | 17.10           | 1.5              | ESI+     | QI 226.1 > 108.0                | 30               |
|                            |                 |                  |          | 1st CI 226.1 > 93.0             | 40               |
| Cyromazine                 | 4.48            | 1.5              | ESI+     | QI 167.1 > 125.0                | 16               |
|                            |                 |                  |          | 1st CI 167.1 > 85.0             | 16               |
| Diafenthiuron              | 20.82           | 1.5              | ESI+     | QI 385.2 > 329.2                | 16               |
|                            |                 |                  |          | 1st CI 385.2 > 276.2            | 32               |
| Diazinon                   | 17.10           | 1.5              | ESI+     | QI 305.1 > 169.1                | 32               |
|                            |                 |                  |          | 1st CI 305.1 > 97.0             | 40               |
| Dichlorvos                 | 9.11            | 1.5              | ESI+     | QI 221.0 > 109.0                | 12               |
|                            |                 |                  |          | 1st CI 221.0 > 79.0             | 24               |
| Dicrotophos                | 5.83            | 1.5              | ESI+     | QI 238.0 > 127.0                | 12               |
|                            |                 |                  |          | 1st CI 238.0 > 112.1            | 8                |
|                            |                 |                  |          | QI 406.1 > 337.0                | 18               |
| Difenoconazole             | 17.80           | 1.5              | ESI+     | QI 30.1 > 251.0                 | 28               |
|                            |                 |                  |          | 1st CI 406.1 > 251.0            | 8                |
| Diflubenzuron              | 14.96           | 1.5              | ESI+     | QI 311.0 > 158.0                | 8                |
|                            |                 |                  |          | 1st CI 311.0 > 141.0            | 32               |
| Dimethoate                 | 6.53            | 1.5              | ESI+     | QI 230.0 > 198.8                | 0                |
|                            |                 |                  |          | 1st CI 230.0 > 125.0            | 16               |
| Dimethomorph               | 13.80           | 3.0              | ESI+     | QI 388.1 > 301.1                | 20               |
|                            |                 |                  |          | 1st CI 388.1 > 165.1            | 32               |
| Diniconazole               | 18.00           | 1.5              | ESI+     | QI 326.1 > 159.0                | 28               |
|                            |                 |                  |          | 1st CI 326.1 > 70.0             | 28               |
| Disulfoton                 | 17.69           | 1.5              | ESI+     | QI 275.0 > 89.0                 | 12               |
|                            |                 |                  |          | 1st CI 275.0 > 61.0             | 44               |
| Disulfoton-Sulfone         | 10.72           | 1.5              | ESI+     | QI 307.0 > 125.0                | 10               |
|                            |                 |                  |          | 1st CI 307.0 > 97.0             | 30               |
| Disulfoton-Sulfoxide       | 10.78           | 1.5              | ESI+     | QI 291.0 > 185.0                | 10               |
|                            |                 |                  |          | 1st CI 291.0 > 157.0            | 20               |
| Name           | RT (min) | DRT (min) | Polarity | Transitions                  | Collision Energy |
|----------------|----------|-----------|----------|------------------------------|------------------|
| Diuron         | 11.38    | 1.5       | ESI+     | QI 2350, > 72.0              | 20               |
|                |          |           |          | 1st CI 233.0 > 160.0         |                  |
|                |          |           |          | 2nd CI 233.03 > 72.1         |                  |
| Emamectin B1a | 20.89    | 2.0       | ESI+     | QI 886.5 > 158.0             | 44               |
|                |          |           |          | 1st CI 886.5 > 82.1          |                  |
| Emamectin B1b | 20.34    | 2.0       | ESI+     | QI 872.5 > 158.3             | 40               |
|                |          |           |          | 1st CI 872.5 > 82.3          |                  |
| Epoxiconazole  | 15.21    | 1.5       | ESI+     | QI 330.1 > 121.0             | 68               |
|                |          |           |          | 1st CI 330.1 > 101.2         |                  |
| Ethion         | 19.65    | 1.5       | ESI+     | QI 385.0 > 199.1             | 4                |
|                |          |           |          | 1st CI 385.0 > 142.8         |                  |
| Etofenprox     | 22.82    | 1.5       | ESI+     | QI 394.2 > 359.0             | 5                |
|                |          |           |          | 1st CI 394.2 > 177.0         |                  |
| Ethoprophos    | 15.72    | 1.5       | ESI+     | QI 243.1 > 130.9             | 15               |
|                |          |           |          | 1st CI 243.1 > 97.0          |                  |
| Etrimfos       | 16.80    | 1.5       | ESI+     | QI 293.1 > 265.0             | 26               |
|                |          |           |          | 1st CI 293.1 > 125.0         |                  |
| Famoxadone     | 16.93    | 1.5       | ESI+     | QI 392.1 > 330.9             | 4                |
|                |          |           |          | 1st CI 392.1 > 238.0         |                  |
| Fenamiphos     | 15.92    | 1.5       | ESI+     | QI 304.1 > 234.0             | 12               |
|                |          |           |          | 1st CI 304.1 > 217.1         |                  |
| Fenbuconazole  | 15.11    | 1.5       | ESI+     | QI 337.1 > 125.1             | 40               |
|                |          |           |          | 1st CI 337.1 > 70.0          |                  |
| Fenpyroximate  | 20.91    | 1.5       | ESI+     | QI 422.2 > 366.2             | 12               |
|                |          |           |          | 1st CI 422.2 > 135.0         |                  |
| Fenthion       | 16.86    | 1.5       | ESI+     | QI 279.0 > 247.1             | 8                |
|                |          |           |          | 1st CI 279.0 > 169.1         |                  |
| Fipronil       | 15.50    | 1.5       | ESI+     | QI 437.0 > 368.0             | 18               |
|                |          |           |          | 1st CI 437.0 > 255.0         |                  |
| Flazasulfuron  | 11.24    | 1.5       | ESI+     | QI 408.1 > 182.1             | 28               |
|                |          |           |          | 1st CI 408.1 > 83.0          |                  |
| Fluazifop-Butyl| 18.80    | 1.5       | ESI+     | QI 384.1 > 328.1             | 12               |
|                |          |           |          | 1st CI 384.1 > 282.2         |                  |
| Flufenoxuron   | 19.88    | 1.5       | ESI+     | QI 489.1 > 158.0             | 20               |
|                |          |           |          | 1st CI 489.1 > 140.9         |                  |
| Fluquinconazole| 15.11    | 1.5       | ESI+     | QI 376.0 > 349.0             | 20               |
|                |          |           |          | 1st CI 376.0 > 307.1         |                  |
| Flutriafol     | 11.17    | 1.5       | ESI+     | QI 302.1 > 122.9             | 28               |
|                |          |           |          | 1st CI 302.1 > 70.1          |                  |
| Furathiocarb   | 19.30    | 1.5       | ESI+     | QI 383.2 > 251.9             | 8                |
|                |          |           |          | 1st CI 383.2 > 195.0         |                  |
| Heptenophos    | 11.79    | 1.5       | ESI+     | QI 251.0 > 127.0             | 15               |
|                |          |           |          | 1st CI 251.0 > 125.0         |                  |
| Hexaconazole   | 17.40    | 1.5       | ESI+     | QI 314.1 > 159.0             | 30               |
|                |          |           |          | 1st CI 314.1 > 70.1          |                  |
| Hexythiazox    | 19.90    | 1.5       | ESI+     | QI 353.1 > 227.9             | 8                |
|                |          |           |          | 1st CI 353.1 > 168.1         |                  |
| Imazalil       | 14.30    | 3.0       | ESI+     | QI 297.1 > 201.0             | 15               |
|                |          |           |          | 1st CI 297.1 > 159.0         |                  |
| Imazapyr       | 5.48     | 3.0       | ESI+     | QI 262.1 > 217.1             | 20               |
|                |          |           |          | 1st CI 262.1 > 131.0         |                  |
| Imazethapyr    | 7.42     | 1.5       | ESI+     | QI 290.1 > 245.1             | 24               |
|                |          |           |          | 1st CI 290.1 > 177.0         |                  |
| Imibenconazole | 19.60    | 1.5       | ESI+     | QI 411.0 > 171.0             | 20               |
|                |          |           |          | 1st CI 411.0 > 125.0         |                  |
| Name                  | RT (min) | DRT (min) | Polarity | Transitions               | Collision Energy |
|-----------------------|----------|-----------|----------|---------------------------|------------------|
| Imidacloprid          | 5.97     | 1.5       | ESI+     | QI 258.0 > 210.9          | 12               |
|                       |          |           |          | 1st CI 256.0 > 208.9      | 12               |
|                       |          |           |          | 2nd CI 256 > 175          | 12               |
|                       |          |           |          | QI 528.1 > 203.0          | 45               |
|                       |          |           |          | 1st CI 528.1 > 150.0      | 20               |
|                       |          |           |          | QI 330.0 > 287.9          | 10               |
|                       |          |           |          | 1st CI 330.0 > 244.9      | 14               |
|                       |          |           |          | QI 321.2 > 202.9          | 5                |
|                       |          |           |          | 1st CI 321.2 > 119        | 16               |
| Indoxacarb            | 17.88    | 1.5       | ESI+     | QI 314.1 > 267.0          | 0                |
|                       |          |           |          | 1st CI 314.1 > 222.1      | 10               |
|                       |          |           |          | QI 249.0 > 160.1          | 20               |
|                       |          |           |          | 1st CI 249.0 > 133.0      | 36               |
|                       |          |           |          | QI 510.9 > 158.0          | 20               |
|                       |          |           |          | 1st CI 510.9 > 141.0      | 57               |
|                       |          |           |          | QI 315.0 > 127.1          | 20               |
|                       |          |           |          | 1st CI 315.0 > 99.2       | 4                |
|                       |          |           |          | QI 331.0 > 126.9          | 5                |
|                       |          |           |          | 1st CI 331.0 > 99.0       | 10               |
|                       |          |           |          | QI 280.2 > 220.1          | 10               |
|                       |          |           |          | 1st CI 280.2 > 160.1      | 20               |
|                       |          |           |          | QI 320.1 > 125.0          | 48               |
|                       |          |           |          | 1st CI 320.1 > 70.1       | 24               |
|                       |          |           |          | QI 142.0 > 125.0          | 10               |
|                       |          |           |          | 1st CI 142.0 > 94.0       | 10               |
|                       |          |           |          | QI 302.9 > 145.0          | 0                |
|                       |          |           |          | 1st CI 302.9 > 85.1       | 15               |
|                       |          |           |          | QI 226.1 > 169.0          | 4                |
|                       |          |           |          | 1st CI 226.1 > 121.1      | 12               |
|                       |          |           |          | QI 163.1 > 106.0          | 4                |
|                       |          |           |          | 1st CI 163.1 > 88.0       | 0                |
|                       |          |           |          | QI 369.2 > 313.1          | 0                |
|                       |          |           |          | 1st CI 369.2 > 149.0      | 10               |
|                       |          |           |          | QI 284.1 > 252.1          | 8                |
|                       |          |           |          | 1st CI 284.1 > 176.1      | 24               |
|                       |          |           |          | QI 215.1 > 187.1          | 15               |
|                       |          |           |          | 1st CI 215.1 > 84.0       | 30               |
|                       |          |           |          | QI 225.0 > 193.1          | 0                |
|                       |          |           |          | 1st CI 225.0 > 127.0      | 12               |
|                       |          |           |          | QI 224.1 > 193.0          | 0                |
|                       |          |           |          | 1st CI 224.1 > 127.0      | 10               |
|                       |          |           |          | QI 289.1 > 125.1          | 32               |
|                       |          |           |          | 1st CI 289.1 > 70.1       | 16               |
|                       |          |           |          | QI 380.7 > 127.0          | 8                |
|                       |          |           |          | 1st CI 380.7 > 109.0      | 24               |
|                       |          |           |          | QI 214.0 > 125.0          | 16               |
|                       |          |           |          | 1st CI 214.0 > 109.0      | 24               |
|                       |          |           |          | QI 237.1 > 90.0           | 10               |
|                       |          |           |          | 1st CI 237.1 > 72.0       | 12               |
|                       |          |           |          | QI 294.1 > 125.2          | 40               |
|                       |          |           |          | 1st CI 294.1 > 70.1       | 20               |
|                       |          |           |          | QI 276.1 > 220.0          | 10               |
|                       |          |           |          | 1st CI 276.1 > 94.0       | 40               |
|                       |          |           |          | QI 248.0 > 201.9          | 20               |
|                       |          |           |          | 1st CI 248.0 > 90.0       | 25               |
Table 1. Cont.

| Name               | RT (min) | DRT (min) | Polarity | Transitions                      | Collision Energy |
|--------------------|----------|-----------|----------|----------------------------------|------------------|
| Parathion          | 16.40    | 1.5       | ESI+     | QI 292.0 > 236.1                 | 8                |
|                    |          |           |          | 1st CI 292.0 > 94.1              | 40               |
| Penconazole        | 16.70    | 1.5       | ESI+     | QI 284.1 > 159                   | 30               |
|                    |          |           |          | 1st CI 284.1 > 70.1              | 15               |
| Pencycuron         | 18.00    | 1.5       | ESI+     | QI 329.1 > 125.1                 | 24               |
|                    |          |           |          | 1st CI 329.1 > 89.1              | 60               |
| Pendimethalin      | 20.20    | 1.5       | ESI+     | QI 282.1 > 212.1                 | 4                |
|                    |          |           |          | 1st CI 282.1 > 194.1             | 16               |
| Phenthoate         | 16.20    | 1.5       | ESI+     | QI 321.0 > 163.1                 | 8                |
|                    |          |           |          | 1st CI 321.0 > 79.1              | 44               |
| Phorate            | 17.50    | 1.5       | ESI+     | QI 261.0 > 199.0                 | 2                |
|                    |          |           |          | 1st CI 261.0 > 75.1              | 5                |
| Phosmet            | 12.80    | 1.5       | ESI+     | QI 317.9 > 160.0                 | 8                |
|                    |          |           |          | 1st CI 317.9 > 133.0             | 36               |
| Phosphamidon       | 8.30     | 1.5       | ESI+     | QI 300.0 > 174.1                 | 8                |
|                    |          |           |          | 1st CI 300.0 > 127.1             | 16               |
|                    |          |           |          | 2nd CI 241 > 222.8               | 10               |
| Picloram           | 4.71     | 1.5       | ESI+     | QI 368.1 > 205.2                 | 4                |
|                    |          |           |          | 1st CI 368.1 > 145.0             | 20               |
| Picoxystrobin      | 16.03    | 1.5       | ESI+     | QI 239.1 > 182.1                 | 12               |
|                    |          |           |          | 1st CI 239.1 > 72.1              | 20               |
| Pirimicarb         | 11.08    | 1.5       | ESI+     | QI 334.1 > 198.1                 | 22               |
|                    |          |           |          | 1st CI 334.1 > 182.1             | 24               |
| Pirimiphos-Ethyl   | 19.39    | 1.5       | ESI+     | QI 306.2 > 164.1                 | 20               |
|                    |          |           |          | 1st CI 306.2 > 108.1             | 30               |
| Pirimiphos-Methyl  | 18.00    | 1.5       | ESI+     | QI 376.0 > 308.0                 | 4                |
|                    |          |           |          | 1st CI 376.0 > 265.9             | 12               |
|                    |          |           |          | 2nd CI 241 > 222.8               | 10               |
| Prochloraz         | 18.03    | 1.5       | ESI+     | QI 374.9 > 347.0                 | 12               |
|                    |          |           |          | 1st CI 374.9 > 304.9             | 19               |
|                    |          |           |          | QI 189.2 > 144.0                 | 8                |
| Profenofos         | 18.83    | 1.5       | ESI+     | QI 368.1 > 231.2                 | 0                |
|                    |          |           |          | 1st CI 368.1 > 175.2             | 8                |
| Propamocarb        | 4.72     | 1.5       | ESI+     | QI 342.1 > 159.0                 | 32               |
|                    |          |           |          | 1st CI 342.1 > 69.1              | 16               |
| Propargite         | 20.26    | 1.5       | ESI+     | QI 310.11 > 168.1                | 5                |
|                    |          |           |          | 1st CI 310.11 > 111.1            | 8                |
| Propiconazole      | 17.50    | 1.5       | ESI+     | QI 388.11 > 193.8                | 8                |
| Propoxur           | 9.33     | 1.5       | ESI+     | QI 374.1 > 222.1                 | 16               |
|                    |          |           |          | 1st CI 374.1 > 194.1             | 32               |
| Pyraclostrobin     | 17.50    | 1.5       | ESI+     | QI 365.1 > 309.1                 | 4                |
|                    |          |           |          | 1st CI 365.1 > 147.2             | 20               |
| Pyrazophos         | 17.26    | 1.5       | ESI+     | QI 341.0 > 205.1                 | 10               |
|                    |          |           |          | 1st CI 341.0 > 189.0             | 20               |
| Pyridaben          | 21.90    | 1.5       | ESI+     | QI 300.1 > 106.9                 | 20               |
|                    |          |           |          | 1st CI 300.1 > 82.0              | 25               |
| Pyridaphenthion    | 14.36    | 1.5       | ESI+     | QI 322.2 > 227.2                 | 12               |
|                    |          |           |          | 2nd CI 322.2 > 96                | 12               |
| Pyrimethanil       | 13.88    | 1.5       | ESI+     | QI 299.0 > 163.0                 | 20               |
|                    |          |           |          | 1st CI 299.0 > 147.0             | 20               |
| Pyriproxyfen       | 19.90    | 1.5       | ESI+     | QI 373.0 > 271.2                 | 24               |
|                    |          |           |          | 1st CI 373.0 > 255.1             | 36               |
| Quinalphos         | 16.48    | 1.5       | ESI+     | QI 373.0 > 271.2                 | 24               |
|                    |          |           |          | 1st CI 373.0 > 255.1             | 36               |
| Quizalofop-Ethyl   | 19.10    | 1.5       | ESI+     | QI 373.0 > 271.2                 | 24               |
|                    |          |           |          | 1st CI 373.0 > 255.1             | 36               |
Table 1. Cont.

| Name           | RT \(^1\) (min) | DRT \(^2\) (min) | Polarity | Transitions              | Collision Energy |
|----------------|-----------------|------------------|----------|--------------------------|------------------|
| Simazine       | 9.45            | 1.5              | ESI+     | QI 202.1 > 132.0         | 22               |
| Spinosyn A     | 19.87           | 2.0              | ESI+     | QI 732.5 > 142.1         | 28               |
| Spinosyn D     | 20.78           | 2.0              | ESI+     | QI 746.5 > 142.1         | 35               |
| Spirodiclofen  | 21.25           | 1.5              | ESI+     | QI 411.1 > 313           | 8                |
| Spiromesifen   | 20.67           | 1.5              | ESI+     | QI 371.2 > 273.1         | 12               |
| Sulfentrazone  | 9.38            | 1.5              | ESI+     | QI 404.0 > 306.9         | 28               |
| Tebuconazole   | 16.80           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Tebufenozide   | 16.08           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Teflubenzuron  | 19.00           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Temephos       | 18.90           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Terbufos       | 19.60           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Tetraconazole  | 15.09           | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Thiabendazole  | 8.22            | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Thiacloprid    | 7.10            | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Thiabendazole  | 8.22            | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Thiacloprid    | 7.10            | 1.5              | ESI+     | QI 381.0 > 158.0         | 12               |
| Thiamethoxam   | 5.42            | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Thiobencarb    | 18.03           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Thiodicarb     | 10.59           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Thiophanate-Methyl | 8.65  | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Tolyfluquinid  | 15.99           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Triadimefon    | 14.80           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Triadimenol    | 14.70           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Triazophos     | 14.30           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Trichlorfon    | 6.55            | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Trifloxystrobin| 18.35           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Triflumizole   | 18.50           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Vamidothion    | 6.39            | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |
| Zoxamide       | 16.96           | 1.5              | ESI+     | QI 292.0 > 211.0         | 8                |

\(^1\) RT: retention time. \(^2\) DRT: delta retention time. \(^3\) QI: quantification ions. \(^4\) CI: confirmation ions.
2.5. Gas Chromatography

GC-MS/MS (gas chromatography tandem mass spectrometric detection) analysis was performed using an Agilent 7000C Triple Quadrupole GC/MS system with a multimode inlet. The temperature of the injector was maintained at 150 °C (0.1 min), ramped up to 300 °C at 600 °C/min (20 min hold), and then ramped down to 200 °C at 20 °C/min until the end of the analysis. The injection volume was 2 µL. The pulsed splitless injection was at 50 psi for 0.5 min with a split flow of 50 mL/min for 0.6 min. The gas saver was set to 20 L/min and started after 5 min. The carrier gas was helium, and the inlet pressure was 5.59 psi (constant pressure mode) during the run and 2.0 psi during the backflush. From the inlet, two Agilent HP-5ms Ultra Inert (5%-phenyl)-methylpolysiloxane columns (0.25 mm, 0.25 µm) were coupled to each other through a purged ultimate union for post-run backflushing; the first column was 30 m, and the second column was 2 m. The total chromatographic run time was 29.5 min, and backflushing started after 25.5 min with 8.92 psi. The column oven temperature was maintained at 60 °C for 1.0 min, ramped up to 180 °C at 30 °C/min, and then ramped up to 300 °C at 5 °C/min.

For the mass spectrometric analysis, an electron ionization source was used with an ionization voltage of 70 eV, ion source temperature of 290 °C, and interface temperature of 280 °C. The retention times, delta retention times, polarities, ion transitions, and collision energies are presented in Table 2. Two transitions were chosen for almost all pesticides, but an extra confirmatory transition was included for seven pesticides to avoid false-positives at trace pesticide levels. The analysis was run according to all requirements for identifying analytes by MS/MS established by European Union SANTE/12682/2019 [34].

| Name                     | RT  (min) | DRT  (min) | Quantification Transition | Collision Energy |
|--------------------------|-----------|------------|---------------------------|------------------|
| Alachlor                 | 11.33     | 1          | QI 3 188.1 > 160.1        | 10               |
|                          |           |            | 1st CI 1 188.1 > 130.1    |                  |
|                          |           |            | QI 263.0 > 193.0          | 40               |
|                          |           |            | 1st CI 2 298.0 > 263.0    | 8                |
|                          |           |            | QI 182.0 > 167.0          | 12               |
|                          |           |            | 1st CI 1 181.0 > 165.0    | 25               |
| Aldrin                   | 12.62     | 1          | QI 330.9 > 315.9          | 16               |
|                          |           |            | 1st CI 2 329.0 > 314.0    | 16               |
| Bifenthrin               | 19.78     | 2          | QI 341.0 > 185.0          | 5                |
|                          |           |            | 1st CI 1 341.0 > 183.0    | 20               |
|                          |           |            | 1st CI 2 153.0 > 96.9     | 10               |
| Carbophenothion          | 17.71     | 1          | 1st CI 153.0 > 79.0       | 30               |
|                          |           |            | 2nd CI 157.0 > 75.1       | 40               |
|                          |           |            | QI 162.9 > 127.0          | 5                |
| Cyfluthrin               | 24.39     | 2          | 1st CI 226.9 > 77.1       | 30               |
|                          |           |            | QI 162.9 > 127.0          | 5                |
| Cypermethrin             | 25.01     | 2          | 1st CI 181.1 > 127.1      | 35               |
| Clordane Gama (Trans)    | 14.42     | 2          | QI 272.0 > 237.0          | 16               |
|                          |           |            | 1st CI 375.0 > 266.0      | 25               |
| Chlorfenapyr             | 16        | 1          | QI 247.0 > 227.0          | 15               |
|                          |           |            | 1st CI 247.0 > 200.0      | 25               |
|                          |           |            | 1st CI 2 247.0 > 197.0    | 5                |
| Chlorothalonil           | 10.17     | 1          | QI 265.9 > 230.9          | 20               |
|                          |           |            | 1st CI 2 263.8 > 229.0    | 20               |
| Chlorpyrifos-Methyl      | 11.14     | 2          | QI 288.0 > 93.0           | 26               |
|                          |           |            | 1st CI 2 288.0 > 273.0    | 15               |
|                          |           |            | 2nd CI 2 286.0 > 271.0    | 16               |
| Name                | RT 1 (min) | DRT 2 (min) | Quantification Transition | Collision Energy |
|---------------------|------------|-------------|---------------------------|------------------|
| Chlorthiophos       | 16.93      | 1           | QI 297.0 > 269.0          | 14               |
| 2,4′-DDD            | 15.7       | 1           | QI 237.0 > 165.0          | 20               |
| 2,4′-DDE            | 14.47      | 2           | QI 246.0 > 176.0          | 20               |
| 4,4′-DDE            | 15.92      | 1           | QI 246.0 > 211.0          | 20               |
| 2,4′-DDT            | 16.84      | 1           | QI 237.0 > 165.0          | 20               |
| 4,4′-DDT            | 18         | 1           | QI 237.0 > 165.0          | 20               |
| Deltamethrin        | 27.94      | 1           | QI 253.0 > 174.0          | 15               |
| Dicofol             | 18.46      | 2           | QI 249.9 > 139.1          | 10               |
| Dieldrin            | 15.69      | 1           | QI 263.0 > 191.0          | 35               |
| Endosulfan Alpha    | 14.84      | 2           | QI 238.8 > 204.0          | 15               |
| Endosulfan Beta     | 16.35      | 1           | QI 241.0 > 206.0          | 15               |
| Endosulfan Sulfate  | 17.94      | 1           | QI 240.8 > 190.0          | 15               |
| Endrin              | 16.06      | 1           | QI 263.0 > 193.0          | 35               |
| Esfenvalerate       | 26.91      | 2           | QI 225.0 > 119.0          | 10               |
| Fenpropathrin       | 20.1       | 1           | QI 265.0 > 210.0          | 15               |
| Fenarimol           | 21.94      | 1           | QI 159.0 > 111.0          | 26               |
| Fenitrothion        | 11.94      | 1           | QI 247.0 > 260.0          | 15               |
| Phosalone           | 20.91      | 1           | QI 182.0 > 111.0          | 15               |
| HCH Alpha           | 9.1        | 2           | QI 189.0 > 145.0          | 12               |
| HCH Beta            | 9.57       | 2           | QI 180.9 > 145.0          | 12               |
| HCH Delta           | 10.38      | 1           | QI 180.9 > 145.0          | 12               |
| HCH Gamma           | 9.81       | 2           | QI 180.9 > 145.0          | 12               |
| Heptachlor          | 11.63      | 1           | QI 271.9 > 236.8          | 25               |
| Heptacloro Exo Epoxid | 13.71    | 1           | QI 353.0 > 263.0          | 15               |
| Hexachlorobenzene (HCB) | 9.23     | 1           | QI 283.9 > 213.9          | 35               |
| Lambda Cyhalothrin  | 21.65      | 1           | QI 181.1 > 152.1          | 30               |
| Methoxychlor        | 20         | 2           | QI 227.0 > 141.1          | 40               |
Table 2. Cont.

| Name            | RT 1 (min) | DRT 2 (min) | Quantification Transition | Collision Energy |
|-----------------|------------|-------------|----------------------------|------------------|
| Mirex           | 21.68      | 1           | QI 271.9 > 235.0           | 25               |
|                 |            |             | 1st CI 272.0 > 237.0       |                  |
|                 |            |             | QI 174.8 > 111.1           | 10               |
|                 |            |             | 2nd CI 302.0 > 175.0       | 4                |
| Ovex (Clorfenson) | 15.11      | 1           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       | 32               |
|                 |            |             | QI 263.0 > 109.1           | 15               |
| Oxyfluorfen     | 15.64      | 1           | QI 263.0 > 79.1            | 30               |
|                 |            |             | 1st CI 263.0 > 79.1        |                  |
| Parathion-Methyl| 11.28      | 2           | QI 183.1 > 153.1           | 15               |
|                 |            |             | 1st CI 183.0 > 115.2       | 25               |
| Permethrin      | 23.38      | 2           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |
| Procymidone     | 13.97      | 2           | QI 263.0 > 79.1            | 30               |
|                 |            |             | 1st CI 263.0 > 79.1        |                  |
| Prothiofos      | 15.21      | 1           | QI 252.0 > 146.0           |                  |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |
| Quintozene      | 9.73       | 1           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |
| Tetradifon      | 20.71      | 1           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |
| Trifluralin     | 8.48       | 1           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |
| Vinclozolin     | 11.22      | 2           | QI 252.0 > 146.0           | 32               |
|                 |            |             | 1st CI 252.0 > 170.0       |                  |

1 RT: retention time. 2 DRT: delta retention time. 3 QI: quantification ions. 4 CI: confirmation ions.

2.6. Method Validation

Validation was performed following the European Union SANTE/12682/2019 [34] and Codex Alimentarius CXG90-2017 [35] guidelines. The following analytical performance parameters were assessed: linearity, selectivity, trueness, precision (repeatability and within-lab reproducibility), limit of detection (LOD), and limit of quantification (LOQ). A total of 209 different analytes were tested, 159 of them by LC-MS/MS and 50 by GC-MS/MS.

Matrix-matched calibration (MMC) was used to minimize the matrix effect. For the preparation of analytical MMC curves, blank honey extracts were spiked with appropriate amounts of standard solutions at the six final concentrations. Three independent solutions were prepared for each level of the curve (n = 18), and the samples were injected randomly. The difference between the calculated concentration and the theoretical concentration must be less than or equal to 20% for the curve’s best fit. The selectivity was determined by identifying the pesticide in the presence of the matrix and other analytes. If interfering peaks were detected at the same retention time as some pesticides, the interfering agents’ areas had to be less than or equal to 30% of the analyte LOQs.

The trueness and precision (repeatability and within-lab reproducibility) were determined from the recovery assay results of blank samples spiked with all of the analytes at two distinct levels (LOQ and 10× LOQ) for GC-MS/MS and three distinct levels (LOQ, 2× LOQ, and 10× LOQ) for LC-MS/MS. Repeatability was evaluated using data from replicate samples (n = 6) analyzed on the same day for each level. The within-lab reproducibility was evaluated using replicate data (n = 12) from two different days and two analysts for each level. Repeatability and within-lab reproducibility are expressed by the relative standard deviation (RSD in %), whereas average recovery values express trueness. The expanded measurement uncertainty (U) was estimated by the top-down approach. All results are reported in Tables 3 and 4. Average recovery ranging from 70% to 120% was considered adequate. Precision deviations of up to 20% were considered acceptable [34].
Table 3. Linearity, recovery (in %), repeatability relative standard deviation (RSD; in %), expanded measurement uncertainty (U; in %), limit of detection (LOD; in mg/kg), and limit of quantification (LOQ; in mg/kg) for each analyte of the LC-MS/MS method for analysis of pesticides in honey.

| Compound                          | Type of Adjust | Ponderation | LR $^1$ (µg/kg) | Average Recovery | RSD | U   | LOD  | LOQ  |
|-----------------------------------|----------------|-------------|-----------------|-----------------|-----|-----|------|------|
| 3-Hydroxycarbofuran               | Linear         | 1/x         | 1–10            | 110             | 91  | 92  | 6    | 6    | 8    | 6    | 6    | 8    | 0.00010 | 0.00020 |
| Acetate                           | Linear         | 1/x         | 1–10            | 109             | 91  | 81  | 4    | 10   | 5    | 8    | 10   | 10   | 0.00010 | 0.00020 |
| Acetamiprid                       | Linear         | 1/x         | 1–10            | 101             | 96  | 101 | 10   | 7    | 4    | 20   | 19   | 8    | 0.00010 | 0.00020 |
| Aldicarb                          | Linear         | 1/x         | 1–10            | 95              | 98  | 86  | 8    | 11   | 12   | 12   | 15   | 12   | 0.00010 | 0.00020 |
| Aldicarb-sulfone                  | Linear         | 1/x         | 1–10            | 118             | 104 | 90  | 5    | 11   | 6    | 5    | 11   | 6    | 0.00010 | 0.00020 |
| Aldicarb-sulfoxide                | Linear         | 1–10        | 118              | 111             | 100 | 7   | 11   | 9    | 8    | 14   | 17   | 0.00010 | 0.00020 |
| Allethrin                         | Linear         | 1–10        | 92              | 80              | 83  | 10  | 18   | 20   | 10   | 18   | 20   | 0.00010 | 0.00020 |
| Aminocarb                         | Linear         | 1/x         | 1–10            | 97              | 100 | 90  | 13   | 9    | 4    | 14   | 9    | 12   | 0.00010 | 0.00020 |
| Atrazine                          | Linear         | 1–10        | 107             | 104             | 103 | 4   | 5    | 4    | 14   | 10   | 6    | 0.00010 | 0.00020 |
| Azinphos-ethyl                    | Linear         | 1–10        | 108             | 107             | 108 | 12  | 10   | 4    | 12   | 11   | 10   | 0.00010 | 0.00020 |
| Azinphos-methyl                   | Linear         | 1–10        | 104             | 106             | 96  | 8   | 7    | 7    | 8    | 8    | 9    | 0.00010 | 0.00020 |
| Azoxystrobin                      | Linear         | 1/x         | 1–10            | 119             | 98  | 88  | 9    | 3    | 4    | 9    | 14   | 14   | 0.00010 | 0.00020 |
| Benzalaxyl                        | Linear         | 1–10        | 119             | 119             | 97  | 97  | 6    | 7    | 8    | 10   | 7    | 8    | 0.00010 | 0.00020 |
| Bitertanol                        | Linear         | 1–10        | 111             | 103             | 94  | 4   | 8    | 5    | 7    | 8    | 5    | 0.00010 | 0.00020 |
| Boscalid                          | Linear         | 1–10        | 119             | 97              | 88  | 10  | 4    | 6    | 12   | 20   | 14   | 0.00010 | 0.00020 |
| Bromacil                          | Linear         | 1/x         | 1–10            | 100             | 95  | 92  | 6    | 6    | 4    | 16   | 19   | 12   | 0.00010 | 0.00020 |
| Bromuconazole                     | Linear         | 2–20        | 118             | 108             | 101 | 4   | 5    | 4    | 7    | 5    | 4    | 0.000020 | 0.000040 |
| Buprofezin                        | Linear         | 1/x         | 1–10            | 102             | 103 | 88  | 18   | 17   | 14   | 19   | 17   | 14   | 0.00010 | 0.00020 |
| Cadusafos                         | Linear         | 1–10        | 110             | 102             | 92  | 5   | 7    | 11   | 9    | 7    | 11   | 0.00010 | 0.00020 |
| Carbaryl                          | Linear         | 1–10        | 116             | 95              | 95  | 10  | 7    | 4    | 10   | 12   | 11   | 0.00010 | 0.00020 |
| Carbendazim                       | Linear         | 1/x         | 1–10            | 114             | 108 | 114 | 6    | 8    | 3    | 7    | 12   | 12   | 0.00010 | 0.00020 |
| Carbofuran                        | Linear         | 1/x         | 1–10            | 106             | 99  | 101 | 4    | 2    | 3    | 9    | 18   | 15   | 0.00010 | 0.00020 |
| Chlorfenpyrphos                   | Linear         | 1/x         | 1–10            | 111             | 110 | 108 | 4    | 6    | 5    | 10   | 6    | 5    | 0.00010 | 0.00020 |
| Chlorpyriños                      | Linear         | 1–10        | 91              | 79              | 74  | 14  | 18   | 16   | 16   | 18   | 16   | 0.00010 | 0.00020 |
| Chlorpyriños-methyl-oxon          | Linear         | 1–10        | 113             | 108             | 100 | 4   | 6    | 9    | 6    | 6    | 9    | 0.00010 | 0.00020 |
| Clorfenpyrphos                    | Linear         | 1–10        | 117             | 105             | 92  | 6   | 9    | 9    | 9    | 12   | 9    | 0.00010 | 0.00020 |
| Clomazone                         | Linear         | 1/x         | 1–10            | 105             | 88  | 99  | 8    | 5    | 5    | 12   | 19   | 15   | 0.00010 | 0.00020 |
| Clothianindin                     | Linear         | 1–10        | 119             | 105             | 100 | 5   | 6    | 6    | 10   | 8    | 6    | 0.00010 | 0.00020 |
| Cyanazine                         | Linear         | 1–10        | 118             | 95              | 90  | 9   | 4    | 7    | 9    | 19   | 19   | 0.00010 | 0.00020 |
| Compound               | Type of Adjust | Ponderation | LR $^1$ (µg/kg) | Average Recovery | RSD | U | LOD (mg/kg) | LOQ (mg/kg) |
|------------------------|----------------|-------------|-----------------|------------------|-----|---|------------|-------------|
| Cyanofenphos           | Linear         | 1/x         | 1–10            | 105              | 102 | 96 | 9          | 15          | 11          | 10          | 15          | 11          | 0.00010     | 0.00020     |
| Cyazofamid             | Linear         | 1/10        | 2–20            | 105              | 110 | 115 | 3          | 4           | 2           | 15          | 7           | 4           | 0.00020     | 0.00040     |
| Cyproconazole          | Linear         | 1/x2        | 1–10            | 100              | 106 | 95  | 7          | 18          | 11          | 13          | 18          | 11          | 0.00010     | 0.00020     |
| Cyprodinil             | Linear         | 1/10        | 1–10            | 104              | 92  | 93  | 5          | 5           | 4           | 13          | 19          | 16          | 0.00010     | 0.00020     |
| Diclofop              | Linear         | 1/x         | 1–10            | 113              | 105 | 100 | 5          | 7           | 8           | 5           | 8           | 9           | 0.00010     | 0.00020     |
| Difenoconazole         | Linear         | 1–10        | 1–10            | 113              | 106 | 101 | 3          | 9           | 10          | 5           | 9           | 10          | 0.00010     | 0.00020     |
| Diflubenzuron          | Linear         | 1–10        | 1–10            | 100              | 100 | 99  | 8          | 8           | 4           | 10          | 12          | 4           | 0.00010     | 0.00020     |
| Dimethoate            | Linear         | 1–10        | 1–10            | 110              | 109 | 102 | 5          | 3           | 5           | 6           | 8           | 5           | 0.00010     | 0.00020     |
| Dimethomorph          | Linear         | 1–10        | 1–10            | 97               | 101 | 97  | 7          | 8           | 10          | 10          | 12          | 11          | 0.00010     | 0.00020     |
| Diniconazole          | Linear         | 1–10        | 1–10            | 110              | 109 | 106 | 3          | 4           | 5           | 10          | 8           | 6           | 0.00010     | 0.00020     |
| Disulfoton-sulfone     | Linear         | 1–10        | 1–10            | 112              | 104 | 110 | 4          | 10          | 3           | 13          | 11          | 4           | 0.00010     | 0.00020     |
| Dimethoate            | Linear         | 1–10        | 1–10            | 113              | 112 | 111 | 8          | 9           | 6           | 13          | 10          | 6           | 0.00010     | 0.00020     |
| Ethicon                | Linear         | 1–10        | 1–10            | 109              | 110 | 102 | 8          | 5           | 5           | 8           | 6           | 8           | 0.00010     | 0.00020     |
| Ethoprophos           | Linear         | 1–10        | 1–10            | 106              | 103 | 100 | 7          | 5           | 7           | 8           | 6           | 9           | 0.00010     | 0.00020     |
| Etrifos               | Linear         | 1–10        | 1–10            | 106              | 100 | 97  | 7          | 5           | 7           | 8           | 8           | 5           | 0.00010     | 0.00020     |
| Fambidoxone           | Linear         | 1–10        | 1–10            | 106              | 98  | 88  | 6          | 11          | 12          | 16          | 11          | 12          | 0.00010     | 0.00020     |
| Fenbuconazole         | Linear         | 1–10        | 1–10            | 113              | 112 | 111 | 8          | 9           | 6           | 13          | 10          | 6           | 0.00010     | 0.00020     |
| Fenpyroximate         | Linear         | 1–10        | 1–10            | 110              | 110 | 102 | 8          | 5           | 5           | 8           | 6           | 8           | 0.00010     | 0.00020     |
| Fenthion              | Linear         | 1–10        | 1–10            | 106              | 94  | 90  | 5          | 10          | 8           | 19          | 12          | 11          | 0.00010     | 0.00020     |
| Fipronil              | Linear         | 1–10        | 1–10            | 106              | 101 | 99  | 5          | 11          | 8           | 6           | 11          | 8           | 0.00020     | 0.00040     |
| Fluazifop-P-butyl     | Linear         | 1–10        | 1–10            | 106              | 87  | 77  | 8          | 12          | 16          | 9           | 12          | 16          | 0.00010     | 0.00020     |
| Fluquinconazole       | Linear         | 1–10        | 1–10            | 106              | 108 | 106 | 7          | 6           | 6           | 10          | 8           | 6           | 0.00010     | 0.00020     |
| Furathiocarb          | Linear         | 1–10        | 1–10            | 107              | 96  | 88  | 4          | 6           | 10          | 6           | 6           | 10          | 0.00010     | 0.00020     |
| Heptenophos           | Linear         | 1–10        | 1–10            | 104              | 97  | 94  | 7          | 7           | 8           | 8           | 9           | 12          | 0.00010     | 0.00020     |
| Hexaconazole          | Linear         | 1–10        | 1–10            | 105              | 108 | 100 | 6          | 9           | 6           | 14          | 11          | 9           | 0.00010     | 0.00020     |
| Hexythiazox           | Linear         | 1–10        | 1–10            | 97               | 87  | 77  | 5          | 16          | 15          | 20          | 16          | 15          | 0.00010     | 0.00020     |
| Imazalil              | Linear         | 1–10        | 1–10            | 106              | 102 | 103 | 9          | 4           | 6           | 17          | 16          | 8           | 0.00010     | 0.00020     |
| Imibenconazole        | Linear         | 1–10        | 1–10            | 89.5             | 85.8 | 79.9 | 4.8        | 9.9          | 13.6        | 20.0        | 12.9        | 13.6        | 0.00010     | 0.00020     |
| Imidacloprid          | Linear         | 1–10        | 1–10            | 99               | 91   | 92  | 10         | 10          | 4           | 10          | 18          | 18          | 0.00010     | 0.00020     |
| Indoxacarb            | Linear         | 1–10        | 1–10            | 105              | 95   | 87  | 5          | 11          | 11          | 6           | 11          | 11          | 0.00010     | 0.00020     |
Table 3. Cont.

| Compound                  | Type of Adjust | Ponderation | LR $^1$ (µg/kg) | Linearity | Average Recovery | RSD | U   | LOD  | LOQ  |
|---------------------------|----------------|-------------|-----------------|-----------|------------------|-----|-----|------|------|
| Iprodione                 | Linear         | 1/x2        | 1–10            | 94        | 101              | 95  | 14  | 10   | 15   | 14  | 11  | 16  | 0.00010 | 0.00020 |
| Iprovalicarb              | Linear         | 1–10        | 103             | 106       | 105              | 4   | 7   | 4    | 16   | 14  | 7   | 10  | 0.00010 | 0.00020 |
| Kresoxim-methyl           | Linear         | 1–10        | 112             | 104       | 96               | 6   | 6   | 4    | 6    | 9   | 8   | 10  | 0.00010 | 0.00020 |
| Linuron                   | Linear         | 1–10        | 117             | 91        | 90               | 18  | 11  | 3    | 18   | 19  | 10  | 10  | 0.00010 | 0.00020 |
| Malaoxon                  | Linear         | 1–10        | 118             | 117       | 100              | 19  | 10  | 7    | 19   | 11  | 8   | 10  | 0.00010 | 0.00020 |
| Malathion                 | Linear         | 1–10        | 102             | 104       | 109              | 4   | 3   | 4    | 20   | 19  | 16  | 10  | 0.00010 | 0.00020 |
| Metalaxyl                 | Linear         | 1–10        | 107             | 105       | 107              | 2   | 4   | 4    | 14   | 14  | 11  | 11  | 0.00010 | 0.00020 |
| Metaldehyde               | Linear         | 1–10        | 111             | 106       | 94               | 4   | 7   | 9    | 5    | 7   | 9   | 11  | 0.00010 | 0.00020 |
| Methidathion              | Linear         | 1–10        | 108             | 100       | 96               | 9   | 4   | 5    | 10   | 12  | 11  | 9   | 0.00010 | 0.00020 |
| Methiocarb                | Linear         | 1–10        | 120             | 100       | 91               | 7   | 6   | 3    | 10   | 20  | 19  | 10  | 1.00010 | 0.00020 |
| Methomyl                  | Linear         | 1–10        | 92              | 85        | 88               | 10  | 10  | 6    | 19   | 20  | 20  | 10  | 0.00010 | 0.00020 |
| Methoxyfenozide           | Linear         | 1–10        | 110             | 108       | 99               | 3   | 6   | 7    | 6    | 6   | 7   | 5   | 0.00010 | 0.00020 |
| Metolachlor               | Linear         | 1–10        | 105             | 105       | 100              | 5   | 4   | 9    | 20   | 12  | 9   | 9   | 0.00010 | 0.00020 |
| Metribuzin                | Linear         | 1–10        | 96              | 97        | 98               | 15  | 9   | 5    | 20   | 10  | 12  | 6   | 0.00010 | 0.00020 |
| Monocrotophos             | Linear         | 1–10        | 108             | 90        | 82               | 13  | 15  | 6    | 15   | 16  | 17  | 17  | 0.00010 | 0.00020 |
| Methylbutanil             | Linear         | 1–10        | 110             | 108       | 103              | 5   | 3   | 5    | 11   | 5   | 5   | 5   | 0.00010 | 0.00020 |
| Omeothoate                | Linear         | 1–10        | 95              | 84        | 78               | 7   | 5   | 3    | 8    | 10  | 6   | 10  | 0.00010 | 0.00020 |
| Oxamyl                    | Linear         | 1–10        | 99              | 101       | 100              | 10  | 10  | 3    | 19   | 17  | 10  | 10  | 0.00010 | 0.00020 |
| Paclobutrazol             | Linear         | 1–10        | 107             | 98        | 97               | 6   | 3   | 3    | 10   | 9   | 10  | 9   | 0.00010 | 0.00020 |
| Paraoxon                  | Linear         | 1–10        | 111             | 119       | 117              | 10  | 8   | 5    | 10   | 15  | 15  | 15  | 0.00010 | 0.00020 |
| Parathion                 | Linear         | 1–10        | 94              | 99        | 93               | 16  | 11  | 14   | 16   | 11  | 14  | 14  | 0.00004 | 0.000080 |
| Penconazole               | Linear         | 1–10        | 107             | 103       | 103              | 8   | 6   | 4    | 10   | 8   | 6   | 8   | 0.00010 | 0.00020 |
| Pencycuron                | Linear         | 1–10        | 92              | 86        | 89               | 7   | 12  | 15   | 12   | 12  | 15  | 12  | 0.00010 | 0.00020 |
| Pendimethalin             | Linear         | 1–10        | 98              | 82        | 80               | 12  | 15  | 13   | 14   | 13  | 13  | 13  | 0.00010 | 0.00020 |
| Phenoxythoate             | Linear         | 1–10        | 109             | 101       | 93               | 5   | 10  | 12   | 7    | 10  | 15  | 10  | 0.00010 | 0.00020 |
| Phosmet                   | Linear         | 1–10        | 107             | 104       | 108              | 5   | 4   | 4    | 14   | 16  | 10  | 10  | 0.00010 | 0.00020 |
| Phosphamidon              | Linear         | 1–10        | 118             | 108       | 100              | 4   | 3   | 4    | 7    | 6   | 5   | 5   | 0.00010 | 0.00020 |
| Piracyxystrobin           | Linear         | 1–10        | 110             | 107       | 110              | 6   | 5   | 7    | 15   | 9   | 7   | 10  | 0.00010 | 0.00020 |
| Pirimicarb                | Linear         | 1–10        | 115             | 110       | 111              | 3   | 2   | 4    | 9    | 4   | 4   | 4   | 0.00010 | 0.00020 |
| Pirimiphos-ethyl          | Linear         | 1–10        | 102             | 90        | 89               | 9   | 8   | 13   | 16   | 8   | 13  | 13  | 0.00010 | 0.00020 |
| Pirimiphos-methyl         | Linear         | 1–10        | 104             | 101       | 95               | 7   | 10  | 11   | 10   | 14  | 13  | 13  | 0.00010 | 0.00020 |
| Procichloraz              | Linear         | 1–10        | 108             | 106       | 106              | 3   | 7   | 6    | 12   | 10  | 11  | 10  | 0.00010 | 0.00020 |
| Profenofos                | Linear         | 1–20        | 103             | 98        | 95               | 5   | 10  | 10   | 10   | 10  | 12  | 12  | 0.00020 | 0.00040 |
| Compound               | Type of Adjust | Ponderation | LR $^1$ (µg/kg) | Pt $^2$ 1 | Pt 2 | Pt 6 | Pt 1 | Pt 2 | Pt 6 | Pt 1 | Pt 2 | Pt 6 | U   | LOD | LOQ |
|------------------------|----------------|-------------|-----------------|-----------|------|------|------|------|------|------|------|------|-----|-----|-----|
| Propargite             | Linear         | 1/10        | 104 88 73       | 12 18 19 | 12 18 | 19   | 0.00010 | 0.00020 |
| Propoxur               | Linear         | 1/10        | 108 98 95       | 10 4 5   | 15 20 | 19   | 0.00010 | 0.00020 |
| Pyraclostrobin         | Linear         | 1/10        | 110 99 96       | 7 10 11 10 | 10 12 | 13   | 0.00010 | 0.00020 |
| Pyrazophos             | Linear         | 1/10        | 111 104 96      | 4 7 7   | 6 7   | 7    | 0.00010 | 0.00020 |
| Pyridaphenthion        | Linear         | 1/10        | 117 111 102     | 5 6 4   | 6 6   | 4    | 0.00010 | 0.00020 |
| Pyriproxyfen           | Quadratic      | 1/10        | 98 92 93        | 18 15 17 | 20 15 | 17   | 0.00010 | 0.00020 |
| Quinalphos             | Linear         | 1/10        | 110 105 98      | 5 7 8   | 6 7   | 10   | 0.00010 | 0.00020 |
| Quizalofop-P-ethyl     | Linear         | 1/10        | 96 88 82        | 8 10 15 | 8 10 17 | 0.00010 | 0.00020 |
| Simazine               | Linear         | 1/10        | 104 103 96      | 5 5 5   | 15 18 | 14   | 0.00010 | 0.00020 |
| Spinosyn A             | Linear         | 1/10        | 105 106 101     | 4 5 6   | 5 5   | 6    | 0.00010 | 0.00020 |
| Spinosyn D             | Linear         | 1/10        | 106 101 97      | 13 17 14 | 17 17 | 16   | 0.00010 | 0.00020 |
| Spirodiclofen          | Linear         | 1/10        | 99 84 84        | 11 19 16 | 11 19 | 20   | 0.00010 | 0.00020 |
| Spiromesifen           | Linear         | 1/10        | 86 81 73        | 10 12 17 | 10 12 | 17   | 0.00010 | 0.00020 |
| Tebuconazole           | Linear         | 1/10        | 96 102 100      | 7 4 8   | 12 9  | 8    | 0.00010 | 0.00020 |
| Tebufenozide           | Linear         | 1/10        | 106 100 84      | 11 10 16 | 11 10 | 16   | 0.00010 | 0.00020 |
| Teflubezuron           | Linear         | 1/10        | 104 90 81       | 9 13 18 | 10 13 | 18   | 0.00020 | 0.00040 |
| Terbufos               | Linear         | 1/20        | 86 74 71        | 13 12 19 | 11 13 | 11   | 0.00020 | 0.00040 |
| Tetraconazole          | Linear         | 1/10        | 102 105 105     | 5 6 6   | 9 11  | 10   | 0.00010 | 0.00020 |
| Thiabendazole          | Linear         | 1/10        | 111 114 95      | 17 18 5 | 19 18 | 5    | 0.00010 | 0.00020 |
| Thiacloprid            | Linear         | 1/10        | 117 103 98      | 6 5 3   | 6 6   | 3    | 0.00010 | 0.00020 |
| Thiamethoxam           | Linear         | 1/10        | 106 94 91       | 8 7 6   | 8 9   | 8    | 0.00010 | 0.00020 |
| Thiobencarb            | Linear         | 1/10        | 106 94 91       | 11 7 8 | 13 9  | 9    | 0.00010 | 0.00020 |
| Thiodicarb             | Linear         | 1/10        | 111 98 97       | 10 3 4 10 | 14 9   | 0.00010 | 0.00020 |
| Tolyfluquinid          | Linear         | 1/10        | 100 95 96       | 8 5 7   | 12 8  | 8    | 0.00010 | 0.00020 |
| Triadimefon            | Linear         | 1/10        | 103 106 104     | 6 8 5   | 14 13 | 11   | 0.00010 | 0.00020 |
| Triadimenol            | Linear         | 1/10        | 116 116 106     | 4 3 5   | 6 9   | 11   | 0.00010 | 0.00020 |
| Triazophos             | Linear         | 1/10        | 113 104 109     | 4 5 3   | 14 13 | 11   | 0.00010 | 0.00020 |
| Trichlorfon            | Linear         | 1/20        | 107 101 97      | 9 7 5   | 9 9   | 9    | 0.00010 | 0.00020 |
| Trifoxystrobin         | Linear         | 1/10        | 105 96 93       | 5 11 12 | 9 11  | 12   | 0.00010 | 0.00020 |
| Triflumizole           | Linear         | 1/10        | 93 94 92        | 9 7 7   | 13 10 | 7    | 0.00010 | 0.00020 |
| Vamidothion            | Linear         | 1/10        | 105 92 90       | 6 7 4   | 12 15 | 6    | 0.00010 | 0.00020 |
| Zoxamide               | Linear         | 1/10        | 104 92 88       | 6 9 8   | 9 10  | 10   | 0.00010 | 0.00020 |

$^1$ LR: linearity range. $^2$ Pt: point.
Table 4. Linearity, recovery (in %), repeatability relative standard deviation (RSD; in %), expanded measurement uncertainty (U; in %), limit of detection (LOD; in mg/kg), and limit of quantification (LO Q; in mg/kg) for each analyte of the GC-MS/MS method for analysis of pesticides in honey.

| Compound                  | Type of Adjust | Ponderation | FT (µg/kg) | Average Recovery | RSD  | U    | LOD  | LOQ  |
|---------------------------|----------------|-------------|------------|------------------|------|------|------|------|
|                           |                |             |            | Pt 1  | Pt 6 | Pt 1 | Pt 6 | Pt 6 |      |
| DDE 4,4                   | Linear         | 1/x         | 10–100     | 119   | 94   | 8    | 7    | 20   | 7    |
|                           |                |             |            |       |      |      |      |      | 0.001| 0.002|
| Alachlor                  | Linear         | 10–100      | 103        | 109   | 7    | 5    | 19   | 12   |      |
|                           |                |             |            |       |      |      |      |      | 0.001| 0.002|
| Aldrin                    | Linear         | 1/20–200    | 110        | 99    | 10   | 9    | 18   | 11   |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Azoxystrobin              | Linear         | 10–100      | 98         | 78    | 9    | 7    | 13   | 8    |      |
|                           |                |             |            |       |      |      |      |      | 0.001| 0.002|
| Bifenthrin                | Linear         | 20–200      | 117        | 91    | 3    | 4    | 11   | 4    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Bromophos-methyl          | Linear         | 20–200      | 119        | 100   | 6    | 7    | 15   | 6    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Bromopropylate            | Linear         | 1/20–200    | 113        | 91    | 6    | 6    | 14   | 7    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Carbophenothion           | Linear         | 1/20–200    | 115        | 93    | 5    | 5    | 17   | 5    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Cyfluthrin                | Linear         | 40–400      | 106        | 92    | 5    | 8    | 13   | 8    | 0.004|
|                           |                |             |            |       |      |      |      |      | 0.008|
| Cypermethrin              | Linear         | 20–200      | 102        | 89    | 7    | 8    | 16   | 8    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Chlorpyrifos-methyl       | Linear         | 1/20–200    | 112        | 99    | 7    | 5    | 18   | 10   |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| Chlorthiophos             | Linear         | 1/20–200    | 118        | 94    | 9    | 6    | 18   | 6    |      |
|                           |                |             |            |       |      |      |      |      | 0.002| 0.004|
| DDD 2,4                   | Linear         | 1/20–100    | 115        | 95    | 10   | 7    | 14   | 7    |      |
|                           |                |             |            |       |      |      |      |      | 0.001| 0.002|
| DDT 2,4                   | Linear         | 1/20–100    | 112        | 98    | 5    | 7    | 20   | 8    |      |
|                           |                |             |            |       |      |      |      | 0.001| 0.002|
| DDT 4,4                   | Linear         | 1/20–100    | 109        | 98    | 5    | 5    | 19   | 7    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Deltamethrin              | Linear         | 1/20–100    | 96         | 119   | 13   | 4    | 20   | 10   |      |
|                           |                |             |            |       |      |      |      | 0.001| 0.002|
| Dieldrin                  | Linear         | 1/20–100    | 113        | 96    | 10   | 7    | 20   | 8    | 0.002|
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Difenoconazole            | Linear         | 1/20–100    | 99         | 85    | 9    | 7    | 16   | 8    | 0.001|
|                           |                |             |            |       |      |      |      | 0.002| 0.002|
| Endosulfan alpha          | Linear         | 1/20–200    | 116        | 98    | 8    | 5    | 17   | 8    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Endosulfan beta           | Linear         | 1/20–200    | 111        | 95    | 5    | 5    | 18   | 7    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Endosulfan sulfate        | Linear         | 1/20–200    | 108        | 103   | 7    | 5    | 18   | 6    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Endrin                    | Linear         | 1/20–200    | 111        | 98    | 10   | 5    | 19   | 6    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Esfenvalerate             | Linear         | 1/10–200    | 105        | 105   | 8    | 6    | 19   | 9    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Fenpropofothrin           | Linear         | 1/80–200    | 109        | 92    | 5    | 5    | 16   | 5    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Fenarimol                 | Linear         | 1/80–200    | 105        | 85    | 6    | 7    | 12   | 10   |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Fipronil                  | Linear         | 1/80–200    | 109        | 104   | 11   | 4    | 19   | 15   |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| Fluquinconazole           | Linear         | 1/20–100    | 108        | 90    | 7    | 5    | 13   | 6    | 0.001|
|                           |                |             |            |       |      |      |      | 0.002| 0.002|
| Phosalone                 | Linear         | 1/20–200    | 102        | 92    | 8    | 7    | 18   | 7    |      |
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
| HCH alpha                 | Linear         | 1/20–200    | 107        | 84    | 8    | 11   | 13   | 11   | 0.002|
|                           |                |             |            |       |      |      |      | 0.002| 0.004|
Table 4. Cont.

| Compound                              | Linearity | Type of Adjust | Ponderation | FT (µg/kg) | Average Recovery | RSD | U        | LOD (mg/kg) | LOQ (mg/kg) |
|---------------------------------------|-----------|----------------|-------------|------------|------------------|-----|----------|-------------|-------------|
| Heptachlor                            | Linear    | 1/x            | 20–200      | 108 90     | 12 11            | 18 16 | 0.002   | 0.004       |
| Hexachlorobenzene (HCB)               | Linear    | 1/x            | 10–100      | 98 80      | 12 9             | 13 13 | 0.001   | 0.002       |
| Iprodione                             | Linear    | 1/x            | 20–200      | 108 89     | 13 8             | 15 8  | 0.002   | 0.004       |
| Lambda cyhalothrin                    | Linear    | 1/x            | 20–200      | 110 101    | 7 6              | 16 6  | 0.002   | 0.004       |
| Methoxychlor                          | Linear    | 1/x            | 20–200      | 108 97     | 5 6              | 19 6  | 0.002   | 0.004       |
| Mirex                                 | Linear    | 1/x            | 10–100      | 113 108    | 5 9              | 18 18 | 0.001   | 0.002       |
| Chlortefonson                          | Linear    | 1/x            | 20–200      | 109 93     | 9 6              | 17 8  | 0.002   | 0.004       |
| Oxyfluorfen                           | Linear    | 1/x2           | 20–200      | 108 113    | 5 6              | 19 15 | 0.002   | 0.004       |
| Pendimethalin                          | Linear    | 1/x            | 10–100      | 99 104     | 6 6              | 18 18 | 0.001   | 0.002       |
| Permethrin                            | Linear    | 1/x            | 20–200      | 99 86      | 11 5             | 18 6  | 0.002   | 0.004       |
| Pirimicarb                            | Linear    | 1/x            | 10–100      | 115 104    | 13 6             | 14 16 | 0.001   | 0.002       |
| Pirimiphos-ethyl                       | Linear    | 1/x            | 10–100      | 102 105    | 7 5              | 18 16 | 0.001   | 0.002       |
| Procymidone                           | Linear    | 1/x            | 20–200      | 103 96     | 6 7              | 10 10 | 0.002   | 0.004       |
| Profenofos                            | Linear    | 1/x            | 20–200      | 112 102    | 4 5              | 14 13 | 0.002   | 0.004       |
| Prothiofos                            | Linear    | 1/x            | 20–200      | 113 97     | 8 5              | 15 8  | 0.002   | 0.004       |
| Quintozene                            | Linear    | 1/x            | 10–100      | 106 87     | 9 4              | 14 14 | 0.001   | 0.002       |
| Tetradifon                            | Linear    | 1/x            | 40–400      | 107 90     | 4 7              | 15 8  | 0.004   | 0.008       |
| Trifluralin                           | Linear    | 1/x            | 20–200      | 112 94     | 9 11             | 15 14 | 0.002   | 0.004       |
| Vinclozolin                           | Linear    | 1/x            | 20–200      | 111 101    | 6 7              | 8 14  | 0.002   | 0.004       |
The LOQ was determined as the lowest concentration level of the calibration curve with acceptable accuracy. The LOD corresponded to 50% of the estimated value for the quantification limit, provided that the recoveries presented an area greater than or equal to 50% of the point in the matrix solution injected and that the signal/noise ratio was higher than or equal to 3.

3. Results and Discussion

3.1. Extraction Method

The extraction procedure is a crucial step for detecting pesticides, and it can be challenging for a complicated matrix such as honey. Extraction procedures that have been developed for honey samples include solvent extraction, supercritical fluid extraction, solid-phase extraction, matrix solid-phase dispersion, solid-phase microextraction, stir bar sorptive extraction [36], purge and trap, dispersive liquid–liquid microextraction, microextraction by packed sorbent, single-drop microextraction, magnetic solid-phase extraction [37], and solvent floatation [38]. In the present method, the QuEChERS method was optimized for the extraction and cleanup of honey samples from the original method [22] with modifications for honey [28,33] and bee pollen samples [32]. The original QuEChERS method consists of an extraction step with acetonitrile and separation using extraction salts, followed by a cleanup step with purification salts [22].

Different extraction and cleanup conditions were evaluated for this method. Honey samples were diluted in water prior to extraction. Acetonitrile:ethyl acetate (70:30, v/v) solution provided better extraction efficiency, similar to Souza Tette et al. [28]. On the other hand, Mitchell et al. [33] used acetonitrile:water (50:50, v/v) solution without the sample’s previous dilution. In the present study, the extracted solution was subjected to freeze-out before the dispersive solid phase extraction (d-SPE) cleanup, following the method developed for bee pollen by Vázquez et al. [32]. The extraction recoveries for most pesticides were improved by keeping the extract in the freezer at −40 °C for at least 2 h (Supplementary Materials Table S1). Furthermore, extracted solutions that were subjected to freeze-out were visually more translucent than solutions that were not subjected to freeze-out.

The cleanup procedure of the present study was performed with magnesium sulfate and PSA. The same purification salts were also used by Mitchell et al. [33], but at different amounts (150 mg magnesium sulfate and 100 mg PSA); in contrast, Souza Tette et al. [28] also included Florisil (50 mg) to magnesium sulfate (150 mg) and PSA (50 mg). The extract was concentrated ten times after cleanup to achieve lower LOD and LOQ values, similarly to an earlier study [33]. The effectiveness of the modifications to the QuEChERS method in the present study was confirmed by the wide range of pesticides successfully detected and the high sensitivity evidenced by the low LOD and LOQ values.

3.2. Validation Assay

The proposed method was validated to detect 168 compounds, 127 of them by LC-MS/MS and 41 by GC-MS/MS. The matrix effect was minimized by using MMC. The method’s selectivity was determined by identifying the pesticide in the presence of the matrix and other analytes. All validated compounds showed average recoveries ranging from 70% to 120%. The mean repeatability relative standard deviation (RSD) for all samples in the LC-MS/MS method was 7.75%, ranging from 2% to 20%, and in the GC-MS/MS method the RSD was 7.24%, ranging from 3% to 15%. The expanded measurement uncertainty (U) for all samples in the LC-MS/MS method was 11.4%, ranging from 3% to 20%, and in the GC-MS/MS method was 13.1%, ranging from 4% to 20%. Average recoveries ranging from 70% to 120% and precision RSD of up to 20% were considered adequate [34]. The estimation of the uncertainty of an analytical method can be performed in different ways, including empirical, practical, or top-down approaches [39]. In the present study, the uncertainty was estimated using the top-down approach. In this way, the experimental design to estimate the RSD under conditions of partial reproducibility varied the day and the analysts to reproduce the variations.
Tables 3 and 4 show the linearity, recovery, RSD, expanded measurement uncertainty (U), LOD, and LOQ results for analytes determined using LC-MS/MS and GC-MS/MS, respectively. The LOD and LOQ values for 119 analytes determined by LC-MS/MS were 0.0001 mg/kg and 0.0002 mg/kg, respectively, whereas seven analytes showed LOD and LOQ values of 0.0002 mg/kg and 0.0004 mg/kg, and the values for one analyte were 0.0004 mg/kg and 0.0008 mg/kg. For GC-MS/MS analyses, the LOD and LOQ values were 0.001 mg/kg and 0.002 mg/kg for nine analytes, 0.002 mg/kg and 0.004 mg/kg for 30 analytes, and 0.004.0 mg/kg and 0.008 mg/kg for two analytes.

A total of 41 analytes could not be validated, 32 of which were analyzed by LC-MS/MS and 9 by GC-MS/MS (Supplementary Materials Tables S2 and S3). These compounds were detected, but the obtained values for linearity, recovery rate, RSD, and U were not following the European Union SANTE/12682/2019 [34] and Codex Alimentarius CXG90-2017 [35] guidelines.

Pacifico da Silva et al. [1] developed an analytical method with an LC-MS/MS system for the simultaneous detection of 152 pesticides in honey after extraction with ethyl acetate and cleanup using Florisil. The LOD and LOQ values for all the tested pesticides were 0.005 and 0.01 mg/kg, respectively [1]. Paoloni et al. [40] used Florisil for sample cleanup after extraction with n-Hexane for determining 13 pesticides in honey using GC-MS/MS. The LOQ for all tested pesticides was 0.01 mg/kg, and the LOD was not provided [40]. Česnik et al. [31] used a GC-MS method for detecting 75 pesticides and an LC-MS/MS method for detecting 60 pesticides in honey after extraction with a mixture of petroleum ether and dichloromethane. The LOQ ranged from 0.01 to 0.05 mg/kg with the GC-MS method and from 0.003 to 0.01 mg/kg with the LC-MS/MS method [31].

The QuEChERS method was applied for pesticide extraction in honey by other authors [26–30,41,42]. The LC-MS/MS method described by Souza Tette et al. [28] was validated to measure 116 pesticides in honey, but 11 compounds showed recoveries at 0.010 mg/kg out of the 70–120% range. The LOD was 0.005 mg/kg and the LOQ varied between 0.01 and 0.025 mg/kg [28]. The LC-ESI-MS/MS method of Kasiotis et al. [26] detected 115 pesticides, but some analytes showed recoveries below 70%. The LOD ranged from 0.00003 to 0.0233 mg/kg, and the LOQ ranged from 0.0001 to 0.078 mg/kg [26]. Another LC-MS/MS method for analyzing honey samples was described for 207 pesticides [30], with LOQ values ranging from 0.001 to 0.01 mg/kg. However, the LOD was not reported, and some pesticides showed recoveries out of the 70–120% range [30]. In another LC-MS/MS method [29], 132 tested compounds were measured in honey, obtaining recoveries ranging from 70% to 120% for 116 compounds. However, the LOD and LOQ were not provided in the manuscript nor supplementary material [29]. The GC-MS/MS method described by Zheng et al. [41] was validated to measure six pesticides in honey. The LOD ranged from 0.0004 to 0.002 mg/kg and the LOQ varied between 0.001 and 0.005 mg/kg [41]. Another GC-MS/MS method was developed by Shendy et al. [27] for the detection of 200 pesticides in honey. The LOD ranged from 0.001 to 0.003 mg/kg and the LOQ was 0.005 to 0.01 mg/kg, but the recoveries ranged from 51.13–126.55% [27]. Both LC-MS/MS and GC-MS/MS analysis of residual pesticides in honey was described by Bargąnska et al. [42]. This method was validated for 51 compounds, 18 of them determined by LC-MS/MS, 21 compounds by GC-MS/MS, and 12 compounds by both methods. The LOD ranged from 0.0028 to 0.09 mg/kg with the LC method and from 0.0023 to 0.027 mg/kg with the GC method [42]. Compared with these above articles, the method described in the present study was able to detect extensive and broad-spectrum pesticides (168) with very high sensitivity.

### 3.3. Real Samples

Of the 33 honey samples analyzed, 31 (93.9%) showed residual levels of pesticides (Table 5). Each sample contained up to 15 detected analytes. The most frequently detected compounds were carbendazim (20 samples), thiacetazole (20 samples), azoxystrobin (15 samples), chlorpyrifos (12 samples), and imidacloprid (12 samples). Carbendazim is a fungicide that is widely used in agriculture. Its toxic effects include liver damage, disruption of endocrine and hematological functions, and reproductive toxicity [43]. Thiacetazole is a fungicide and anthelmintic compound
with hepatotoxic and teratogenic effects, and it is probably a carcinogen [44]. Azoxystrobin is also a fungicide, and its toxicity includes lesions in the liver and kidneys [45]. Chlorpyrifos is an organophosphorus insecticide that is used as an insecticide and acaricide. It is considered moderately toxic and can cause disruption of neuronal, reproductive, immune, and endocrine systems, cancer, and chromosome damage [46]. Imidacloprid is a neonicotinoid insecticide that is highly toxic to honeybees [1,2], with neurotoxic, immunotoxic, teratogenic, and mutagenic effects in mammals [47]. The presence of pesticides in a considerable percentage of the analyzed samples is indicative of widespread environmental contamination by these compounds. However, the consumption of the analyzed honey may not be considered unsafe because the residual levels of all detected pesticides were below the MRLs established for Brazil [9] and the European Union [6–8].

Table 5. Detected pesticides (in mg/kg) in 33 samples of honey using the developed LC-MS/MS and GC-MS/MS method.

| Compound          | Positive Samples | Maximum Levels | LOD 1 | LOQ 2 | MRL 3 |
|-------------------|------------------|----------------|-------|-------|-------|
| Acephate          | 8                | 0.00779        | 0.0001| 0.0002| 0.020 |
| Acetamiprid       | 1                | <LQ            | 0.0001| 0.0002| 0.050 |
| Azoxystrobin      | 15               | 0.00019        | 0.0001| 0.0002| 0.050 |
| Bifenthrin        | 3                | <LQ            | 0.002 | 0.004 | 0.010 |
| Boscalid          | 1                | <LQ            | 0.0001| 0.0002| 0.050 |
| Carbaryl          | 2                | 0.00050        | 0.0001| 0.0002| 0.050 |
| Carbendazim       | 20               | 0.00350        | 0.0001| 0.0002| 0.050 |
| Clomazone         | 5                | <LQ            | 0.0001| 0.0002| -     |
| Chlorpyrifos      | 12               | 0.00034        | 0.0001| 0.0002| 0.010 |
| Clothianidin      | 2                | 0.00063        | 0.0001| 0.0002| -     |
| Diflubenzuron     | 3                | 0.00026        | 0.0001| 0.0002| 0.050 |
| Dimethoate        | 6                | 0.0194         | 0.0001| 0.0002| 0.010 |
| Diuron            | 5                | <LQ            | 0.0001| 0.0002| 0.050 |
| Imidacloprid      | 12               | 0.00618        | 0.0001| 0.0002| 0.050 |
| Metoxyphenazidine | 1                | <LQ            | 0.0001| 0.0002| 0.050 |
| Omethoate         | 2                | <LQ            | 0.0001| 0.0002| 0.010 |
| Pyraclostrobin    | 2                | <LQ            | 0.0001| 0.0002| 0.050 |
| Pyrimethanil      | 3                | 0.00040        | 0.0001| 0.0002| -     |
| Pyriproxyfen      | 3                | <LQ            | 0.0001| 0.0002| 0.050 |
| Tebuconazole      | 10               | 0.00045        | 0.0001| 0.0002| 0.050 |
| Thiamendazole     | 20               | 0.0130         | 0.0001| 0.0002| 0.010 |
| Thiamethoxam      | 9                 | 0.00209        | 0.0001| 0.0002| 0.050 |
| Triazophos        | 1                | <LQ            | 0.0001| 0.0002| 0.010 |
| Trifloxystrobin   | 5                | 0.00030        | 0.0001| 0.0002| 0.050 |

1 LOD: limit of detection (in mg/kg). 2 LOQ: limit of quantification (in mg/kg). 3 MRL: maximum residue level (in mg/kg) [9].

Few studies have been aimed at determining the presence of residual pesticides in honey in Brazil. Organophosphorous trichlorfon was detected in just one sample from one hundred commercial honey samples from five states of Brazil [28]. A total of 19 pesticides were found in 53 honey samples collected directly from colonies in the Rio Grande do Norte state, northeastern Brazil. Thirteen of these pesticides were detected in honey produced by honeybees pollinating melon crops (23 samples); however, only six were found in honey from honeybees foraging in the forest (20 samples), and four in honey produced by the stingless bee Melipona subnitida (10 samples) [1]. In another study, honey produced by M. subnitida from the Rio Grande do Norte state was tested for residual pesticides. Of the 35 analyzed samples, 25 showed residual pesticides, and the detected compounds were chlorpyrifos-methyl, monocrotoxophos, and trichlorfon [3]. These data support the requirement for testing honey for the presence of pesticides to avoid commercialization of batches containing residual levels above the MRLs.
4. Conclusions

The proposed method was successfully optimized and validated for multi-residue identification and quantification of pesticides in honey. It was able to detect an extensive and broad range of pesticides with remarkably high sensitivity and precision. The developed method was successfully applied to Brazilian commercial honey, showing the analyzed honey was considered safe for consumption.

Supplementary Materials: The following are available online at http://www.mdpi.com/2304-8158/9/10/1368/s1, Table S1. Modified QuEChERS method optimization. Extraction with acetonitrile, or a solution of acetonitrile and ethyl acetate (70:30, v/v), and inclusion of a freezing out step prior to clean up (900 mg of anhydrous magnesium sulfate and 150 mg of PSA). Results are presented as recovery (in %) for each analyte of the LC-MS/MS; Table S2. Non-approved analytes. Linearity, recovery (in %), repeatability relative standard deviation (RSD; in %), expanded measurement uncertainty (U; in %), limit of detection (LOD; in mg/kg), and limit of quantification (LOQ; in mg/kg) for each analyte of the LC-MS/MS method for analysis of pesticides in honey; Table S3: Non-approved analytes. Linearity, recovery (in %), repeatability relative standard deviation (RSD; in %), expanded measurement uncertainty (U; in %), limit of detection (LOD; in mg/kg), and limit of quantification (LOQ; in mg/kg) for each analyte of the GC-MS/MS method for analysis of pesticides in honey.

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