Efficiency of QuEChERS approach for determining 52 pesticide residues in honey and honey bees

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\textbf{GRAPHICAL ABSTRACT}

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A comparison between QuEChERS and other pesticide extraction procedures for honey and honey bee matrices is discussed. Honey bee matrix was extracted by solvent based procedure whereas solid phase extraction was the protocol for the honey matrix. The citrate buffered QuEChERS method was used for both matrices. The methods were evaluated regarding cost (equipment and reagents), time, accuracy, precision, sensitivity and versatility. The results proved that the QuEChERS protocol was the most efficient method for the extraction of the selected pesticides in both matrices.

- QuEChERS is the most economical and less time-consuming procedure.
- SPE and solvent-based extraction procedures show equivalent recoveries to QuEChERS.
- QuEChERS can be used to extract pesticide residues from both matrices.

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Method details

QuEChERS approach for the extraction of pesticide residues in honey and honey bee matrices [1–3].

1) Weigh 5 g of honey or honey bees into 50 mL centrifuge tubes and add 7.5 mL of water, 10 mL of acetonitrile, 6 g of MgSO4 and 1 g of NaCl. Homogenize the mixture immediately and then, centrifuge for 5 min at 300 rpm.
2) Put 2 mL of the supernatant into another 15 mL centrifuge tube containing 50 mg C18, 50 mg PSA, and 150 mg MgSO4. Vortex the mix and centrifuge it for 5 min at 3000 rpm.
3) Finally, filter the supernatant using a PTFE 13 mm \( \times \) 0.22 \text{µm} into the autosampler vials for LC–MS/MS analysis.

Solvent approach for the extraction of pesticide residues in honey bee matrix [4].

1) Weigh 5 g of honey bees and pound thoroughly in a glass mortar. When homogenized place in a 250 mL flask and mix it vigorously for 10 min with 20 mL of acetone.
2) Filter the mixture in a Kitassato flask through a Buchner funnel of 13 cm with a paper filter packed with a layer of Celite 545 (5–10 mm) and wash the filter cake with 20 mL of acetone.
3) Prepare 100 mL, with 1% weight/volume (w/v) ammonium chloride and 2% volume/volume (v/v) orthophosphoric acid (85%) and add it to the filtrate. Allow it to stand for 30 min with occasional stirring and then filter with Celite 545.
4) After filtration, dilute the sample with 200 mL of 2% aqueous sodium chloride (w/v) and extract twice with 100 mL of dichloromethane.
5) Pass the resultant organic phase through a filter containing anhydrous sodium sulfate and evaporate it to dryness in a rotary evaporator at 35 °C.
6) Dissolve the extract obtained from the honey bee samples in acetone, up to 2 mL, for GC analysis. For LC–MS determination, evaporate to dryness a 1-mL aliquot of the previous extract using a gentle stream of nitrogen and then dissolve it in the same volume of methanol.

Solid phase extraction (SPE) approach for the extraction of pesticide residues in honey matrix [5].

1) Weigh honey (1.5 g) and mix it with 30 mL of hot water (<80 °C). Agitate by a stir bar for 10 min.
2) Pre-condition an Oasis HLB cartridge [poly (divinylbenzene-co-N-pyrrolidone)] with 5 mL of methanol and 5 mL of Milli-Q water.
3) Pass the mix through the cartridge at a flow rate of 10 mL min\(^{-1}\).
4) Rinse the cartridge with 5 mL of Milli-Q water.
5) Dry the cartridge under vacuum for 15 min.
6) Elute the retained pesticides by passing 10 mL of methanol–dichloromethane (3:7).
7) Evaporate the eluate to 0.5 mL using a gentle steam of nitrogen.
8) Then, transfer it into 1-mL volumetric flask with methanol, obtaining a final extract in 100% methanol.
Liquid chromatography–mass spectrometry

Inject 5 μL of the extract in the LC–MS/MS according to the conditions already reported [1] and detailed below.

Ionization and fragmentation settings were optimized by direct injection of pesticide standard solutions. MS/MS was performed in the SRM mode using ESI in positive mode. For each compound, two characteristic product ions of the protonated molecule [M+H]+ were monitored, the first and most abundant one was used for quantification, while the second one was used as a qualifier. Collision energy and cone voltage were optimized for each pesticide (Table 1). Nitrogen was used as collision, nebulising and desolvation gas. The ESI conditions were: capillary voltage 4000 V, nebulizer 15 psi, source temperature 300 °C and gas flow 10 L min⁻¹. In order to maximize sensitivity, dynamic MRM was used, with MS₁ and MS₂ at unit resolution and cell acceleration voltage of 7 eV for all the compounds.

Table 1
Dynamic MRM conditions used for LC–MS/MS determination of pesticide residues.

| Target Pesticide | t₀ᵃ (min) | Δ tᵇ | Precursor Ion | SRM₁: Frag<sup>d</sup> (V) | CE<sup>e</sup> (V) | SMR₂: Frag<sup>d</sup> (V) | CE<sup>e</sup> (V) | SMR₂/SRM₁ (%) | (%) RSD |
|------------------|-----------|------|---------------|----------------|----------------|----------------|----------------|---------------|--------|
| Acetamiprid      | 2.67      | 3.21 | 223           | 126 111 22     |                 | 56 111 14     |                | 37.4 (12)     |
| Acetochlor       | 10.07     | 2    | 270           | 224 120 10     |                 | 148 120 10    |                | 46.8 (22)     |
| Alachlor         | 10.07     | 2    | 270           | 238 80 15      |                 | 162 80 10     |                | 50.4 (13)     |
| Atrazine         | 6.52      | 2.63 | 216           | 132 120 15     |                 | 174 120 20    |                | 17.3 (14)     |
| Atrazine-desethyl| 2.54      | 2.5  | 188           | 146 120 15     |                 | 104 121 24    |                | 29.1 (15)     |
| Atrazine-desisopropyl | 1.75 | 2.08 | 174           | 96 120 15      |                 | 132 120 15    |                | 78.6 (13)     |
| Azinphos-ethyl   | 10.16     | 1.71 | 346           | 97 80 20       |                 | 137 80 32     |                | 83.5 (12)     |
| Azinphos-methyl  | 8.17      | 1.24 | 318           | 125 80 8       |                 | 132 80 12     |                | 85.4 (11)     |
| Buprofezin       | 14.5      | 1.1  | 306           | 201 120 10     |                 | 116 120 15    |                | 64.6 (13)     |
| Carbazindazim    | 4.54      | 4.74 | 192           | 160 95 17      |                 | 132 95 25     |                | 11.4 (14)     |
| Carbofuran       | 4.37      | 2.91 | 222           | 123 120 10     |                 | 165 70 15     |                | 98.0 (9.3)    |
| Carbofuran-3-hydroxy | 1.85 | 2.48 | 255           | 163 70 5       |                 | 220 70 15     |                | 90.8 (9)      |
| Chlorfenvinphos  | 11.74     | 1.61 | 359           | 155 120 10     |                 | 127 120 15    |                | 63.8 (11)     |
| Chlorpyrifos     | 15.33     | 2.23 | 350           | 350 92 13      |                 | 198 97 13     |                | 78.6 (14)     |
| Coumaphos        | 14.05     | 2.15 | 363           | 335 134 10     |                 | 307 134 10    |                | 24.8 (10)     |
| Diazon           | 11.77     | 1.89 | 305           | 169 128 17     |                 | 153 128 21    |                | 66.3 (12)     |
| Diflubenzuron    | 14.68     | 2    | 315           | 259 120 10     |                 | 287 120 5     |                | 44 (11)       |
| Dimethoate       | 2.06      | 2.59 | 230           | 199 80 10      |                 | 171 80 5      |                | 45.3 (12)     |
| Diuron           | 7.5       | 1.25 | 233           | 72 120 20      |                 | 160 120 20    |                | 3.2 (13)      |
| DMF              | 5.14      | 4.5  | 150           | 132 111 10     |                 | 107 111 15    |                | 41.6 (16)     |
| Ethion           | 14.88     | 1.23 | 385           | 199 80 5       |                 | 171 80 15     |                | 35.3 (11)     |
| Fenitrothion     | 10.03     | 1.18 | 278           | 125 140 15     |                 | 109 121 12    |                | 95.5 (12)     |
| Fenthion         | 11.51     | 1.83 | 279           | 247 114 5      |                 | 169 114 13    |                | 76.6 (10)     |
| Fipronil         | 13.33     | 2.85 | 437           | 368 150 15     |                 | 290 150 25    |                | 21.8 (11)     |
| Flumethrin       | 18.53     | 1.85 | 527           | 267 50 10      |                 | 239 50 10     |                | 48.3 (18)     |
| Flufenalinate    | 18.11     | 1.81 | 503           | 208 50 10      |                 | 181 50 26     |                | 73.4 (10)     |
| Hexythiazole     | 15.11     | 1.15 | 353           | 228 120 20     |                 | 168 120 10    |                | 67.4 (9)      |
| Imazalil         | 11.4      | 1.71 | 297           | 159 120 20     |                 | 201 120 15    |                | 56 (14)       |
| Imidacloprid     | 1.61      | 1.96 | 256           | 209 80 10      |                 | 175 80 10     |                | 75 (11)       |
| Isoproturon      | 6.83      | 2.37 | 207           | 72 120 20      |                 | 165 120 10    |                | 16.8 (12)     |
| Malathion        | 9.36      | 1.96 | 331           | 99 80 10       |                 | 127 80 5      |                | 98.5 (4)      |
| Methiocarb       | 8.64      | 1.93 | 226           | 121 80 5       |                 | 169 80 10     |                | 66.6 (11)     |
| Metholachlor     | 10.49     | 2.04 | 284           | 252 120 15     |                 | 176 120 10    |                | 10 (14)       |
| Molinate         | 9.41      | 1.98 | 188           | 126 80 20      |                 | 55 80 10      |                | 61.7 (11)     |
| Omethoate        | 1.06      | 2.67 | 214           | 125 80 5       |                 | 183 80 20     |                | 72.3 (12)     |
| Parathion-ethyl  | 11.11     | 1.91 | 292           | 236 88 4       |                 | 264 88 8      |                | 45.5 (13)     |
| Parathion-methyl | 8.17      | 1.5  | 264           | 125 120 20     |                 | 232 110 5     |                | 34.5 (13)     |
| Prochloraz       | 12.08     | 1.91 | 376           | 308 80 10      |                 | 266 80 10     |                | 14.3 (9)      |
| Propanil         | 8.6       | 2.01 | 218           | 162 120 20     |                 | 127 120 15    |                | 92.4 (11)     |
Quality assurance/quality control (QA/QC)

In order to compare QuEChERS to other routine procedures, methods were validated according to the European Union Guidelines [6]. Furthermore, the main elements of uncertainty as the amount of sample used for a determination, the recovery value of the analytical procedure and the repeatability of determinations for a true sample [7], were considered through the validation process (for detailed information of the validation parameters, see Supplementary material Table S1 and S2).

The sensitivity of the method was estimated by establishing the limits of quantification (LOQs) (Fig. 1). The LOQs were determined in pure solvent and in spiked honey and honey bees samples. LOQs were calculated as the lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. LOQs were from 0.2 to 10 ng g\(^{-1}\) and

![Fig. 1](image-url)

**Fig. 1.** Limits of quantitation (LOQs) of QuEChERS, SPE and solvent methods in honey and honey bee matrices.
from 0.03 to 10 ng g\(^{-1}\) for honey and honey bee matrices respectively. Solvent and SPE methods were slightly more sensitive than QuEChERS approach.

Matrix effects were evaluated by comparing the slope of the previous calibration curve and the slope of that prepared in the extract of honey or honey bee matrix with six concentration levels of

![Matrix effects of QuEChERS, SPE and solvent methods in honey and honey bee matrices.](image)

![Accuracy (Recoveries) and precision (RSDs) validation parameters of QuEChERS, SPE and solvent methods in honey and honey bee matrices.](image)
standard solutions (Fig. 2). Matrix effects were mostly suppressive in both matrices and ranged from −60 to 50 and from −60 to 35% in honey and honey bee matrices, respectively.

Mean recovery (as accuracy) and relative standard deviation (as precision) were evaluated by spiking the samples at the LOQ and 10 x LOQ, with a minimum of 5 replicates (Fig. 3). Recovery values of honey bee matrix were from 34 to 96%, whereas RSDs were in all cases <20%. Honey matrix showed recoveries that ranged from 30 to 96% and RDS were <20% except for 17 compounds that were from 21 to 42%. QuEChERS approach showed better results than solvent method in the honey bee matrix while SPE was slightly better both in accuracy and precision than QuEChERS extraction procedure for honey.

Additional information

The use of pesticides in agricultural cropping systems is often discussed as a factor influencing honey bee health [9, 1]. Furthermore, honey, which is considered a healthy natural product, can be contaminated during its production from both agricultural and beekeeping practices [8, 5]. The development of extraction procedures able to process samples in an economic way is crucial.

This paper presents some of the currently applied sample preparation methods for the separation and pre-concentration of pesticides in honey and honey bee samples. The composition of honey and honey bees is very different but both are complex matrices. In order to achieve an accurate and reliable analytical result, an efficient pre-concentration/separation step is usually required prior to determination, even when such a sensitive detection method as LC–MS/MS is used.

From an analytical point of view, honey can be considered as a highly concentrated sugar solution (mostly fructose). Then, after water dilution it can be extracted using protocols similar to those applied to water as SPE. The protocol described here requires a medium cost in reagent and equipment because the SPE sorbents involve a high cost. The extraction of a sample requires between 60 and 90 min, being evaporation the step that takes more time. The performance of the method provides the best sensitivity and lower matrix effects.

On the contrary, honey bees are rich in lipids and proteins, requiring most sophisticated and extensive sample preparation methods. Traditional methods as the solvent approach are long, tedious and require high amounts of expensive organic solvents [4]. Considering the use of reagents and equipment this method has high cost, requires between 150 and 180 min to process a sample and provides recoveries slightly lower for more polar pesticides.

The results pointed out that QuEChERS approach is used in many different matrices as hive products (beeswax, pollen, honey, honey bee) [9, 3, 10]. Honey and honey bee composition (Fig. 4) evidence the versatility of the QuEChERS method compared to other extraction procedures as those used in the present work. Appropriate results in terms of specificity, selectivity, accuracy and sensitivity, low cost and quickness make QuEChERS a suitable procedure for determining pesticides in less studied hive matrices as royal jelly and propolis. Furthermore, QuEChERS approach meets important components of green analytical chemistry [11] due to its small amounts of solvent needed compared to the traditional methods.

| Protein (%) | Fat (%) | Sugars (%) | Water (%) | Others (%) |
|-------------|---------|------------|-----------|------------|
| Honey       | 0.3     | 0          | 79.7      | 17.2       | 0.7        |
| Honey bee   | 14.5    | 7.9        | 6.3       | 68.4       | 2.9        |

Fig. 4. Honey and honey bee composition (%) [12, 13, 14].
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mex.2016.05.005.

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