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Simultaneous determination of 200 pesticide residues in honey using gas chromatography–tandem mass spectrometry in conjunction with streamlined quantification approach

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ABSTRACT

A sensitive, accurate and reliable multi-class GC–MS/MS assay protocol for quantification and confirmation of 200 common agricultural pesticides in honey was developed and validated according to EU guidelines. A modified extraction procedure, based on QuEChERS method (quick, easy, cheap, effective, rugged and safe) was employed. Mass spectrophotometric conditions were individually optimized for each analyte to achieve maximum sensitivity and selectivity in MRM mode. The use of at least two reactions for each compound allowed simultaneous identification and quantification in a single run. The pesticides under investigation were separated in less than 31 min using the ultra-inert capillary column (DB-35MS). For all analytes, neat standard calibration curves in conjunction with correction for matrix effect were successfully employed. The detection limits of the assay ranged from 1.00 to 3.00 ng mL^{-1} for the studied pesticides. The developed assay was linear over concentration range of 10.00–500.00 ng mL^{-1}, with correlation coefficient of more than 0.996. At the LOQ, 81% of the studied pesticides were efficiently recovered in the range of 70.00–120.00%, with CV% less than 15.00% while 99.3% compounds had mean percentage recovery of 60.00–140.00%, with CV% less than 21.00% (N = 18, over three different days). The proposed assay was successfully applied for the analysis of the studied pesticide residues in one PT sample and 64 commercial honey samples collected over 1 year from different districts around Egypt. Results revealed that only one honey sample out of the 64 analyzed samples was contaminated with tau-Fluvalinate (10.00 µg kg^{-1}). This wide scope assay protocol is applicable for monitoring pesticide residues in honey by national regulatory authorities and accredited labs; that should help ensure safety of such widely used product.

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1. Introduction

Honey and bee products have the image of being natural, healthy and free of contaminants although in many places they are produced in polluted environment [1–5]. Owing to the extensive utilization and usual persistence in the environment, honey may get contaminated by pesticides [1,6–9]. It has been reported that pesticide residues can cause genetic mutations, cellular degradation in addition to several public health problems [10–12]. This may occur through direct contamination from beekeeping practices as well as indirect contamination from environmental sources [13–15]. Acaricides, fungicides, insecticides and many other toxic substances are used inside beehive colonies to control bee diseases especially *varroatosis* and ascospheriosis carrying the risk of direct contamination of honey and other hive products [1,10,16,17]. On the other hand, the indirect contamination from environment occurs because of the widespread use and extensive distribution of pesticides that helped introduce their residues into honey by bees that have been fed on contaminated blossom [1,4,7,11,18,19].

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Reportedly, more than 150 different pesticides have been detected in colony samples [20]. In general, the frequently detected pesticide residues are often from varroacides that have the ability to migrate and accumulate in beeswax, pollen, and bee bread [21,22]. Organophosphorus (OPPs) and carbamates have almost replaced organochlorine pesticides (OCPs). However, owing to the persistent nature of OCPs, they are still within the scope of recently developed analysis procedures [19,23]. According to EU regulations [24], honey is considered not suitable for human consumption if residues are beyond the maximum residue levels (MRL) that are usually in the range of 10.0–50.0 ng g^{-1} [11]. Based on the EU directive 96/23/EC (Annex I) [25] for imported honey from the developing countries, many pesticide residue groups have been identified as highly desirable to be monitored in honey samples. Thus, OCPs, OPPs, carbamates, pyrethroids and polychlorinated biphenyls (PCBs) should be monitored in honey samples.

Determination of pesticides in honey at trace levels is a challenging task due to its complex composition and particularly the presence of waxes and pigments. Conventional extraction protocols using organic solvents followed by subsequent cleanup procedures prior to GC determination have been a common practice [4,7,26–29]. The drawbacks of this traditional approach are limited scope, large amounts of toxic solvents, prolonged analysis time and the need for large volume glassware. QuEChERS (quick, easy, cheap, effective, rugged and safe) method on the other hand is based on liquid–liquid partitioning with acetonitrile followed by a cleanup step via dispersive solid phase extraction (d-SPE) using primary secondary amine (PSA) [30]. Coupling of QuEChERS protocol to GC–MS enabled multi-class, multi-residue analysis over short analysis time. Determination of pesticide residues in honeybees using GC–MS/MS has been previously reported [31]. To the best of our knowledge, very few studies were reported for the simultaneous determination of pesticide residues in honey samples using QuEChERS protocol coupled to tandem mass spectrometry. Paradise et al. [32] reported the simultaneous determination for 22 insecticides of three chemical families in honey using QuEChERS/GC–MS/MS. The percentage recovery, correlation coefficient and LOD/LOQ were 63.00–139.00% (CV% <25), 0.96–0.98 and 0.07–0.20 ng g^{-1}/0.20–0.50 ng g^{-1}; respectively. In another study, Wiest et al. [33] reported the determination of 80 environmental contaminants in honey using a modified QuEChERS coupled to LC–MS/MS and GC-ToF. The percentage recoveries and correlation coefficients using GC-ToF were 60.00–120.00 (CV% >25) and >0.990; respectively while LOD and LOQ were 1.10–47.50 ng g^{-1} and 10.80–128.00 ng g^{-1}, respectively. In both studies, a strong matrix effect was obtained and thus matrix matched calibration was essential [32,33].

In the current study, QuEChERS method was revisited, modified accordingly and implemented for the simultaneous determination of 200 pesticide residues, belonging to more than 50 functional and chemical classes (Table S1) and residues in honey samples using GC–MS/MS. A streamlined quantification approach employing neat standard calibration curves in conjunction with correction for matrix effect was used. The validation parameters have been evaluated for each of the studied compounds according to EU guidelines [34–36]. This wide scope assay protocol is applicable for monitoring pesticide residues in honey by national regulatory authorities and accredited labs. The applicability of the developed protocol for the routine monitoring of locally produced honey was investigated. This work is part of the national initiative for developing a monitoring program for pesticide residues in Egyptian honey that should help locally produced products to penetrate international markets.

2. Materials and methods

2.1. Chemicals, reagents and standard solutions

Pesticide reference standards were obtained from Dr. Ehrenstorfer GmbH (Germany), 99.00% purity. An overview of the physicochemical properties of the studied compounds are summarized in Table S1. Ethyl acetate, hexane, acetone, acetonitrile and toluene of residue analysis grade, were purchased from Sigma–Aldrich (USA). The QuEChERS kits (part no. 5682-5650) with salt packets containing 4.00 g anhydrous magnesium sulfate, 1.00 g sodium chloride, 1.00 g sodium citrate and 0.50 g sodium hydrogen citrate sesquihydrate, and 15 mL centrifuge tubes with 150.00 mg anhydrous magnesium sulfate and 25.00 mg PSA for d-SPE (part no. 5982-5021) were purchased from Agilent Technologies (USA). Stock solutions (1000 μg mL^{-1}) of each pesticide standard were prepared by dissolving 0.10 g of each pesticide in 100 mL toluene. Working mixture standard solution of the studied pesticides (2.50 μg mL^{-1}, each) was prepared by diluting suitable aliquot of the stock solutions with toluene, and used to fortify honey samples. A set of calibration standard solutions 10.00–500.00 ng mL^{-1} was prepared in hexane/acetone (9:1 v/v). Stock standards and working solutions were stored at -20 ± 2 °C and 4–8 °C away from direct light, respectively. Ultra-pure water was obtained using a MilliQ UF-Plus system (Millipore, Germany) with a resistivity of at least 18.2 MΩ cm at 25 °C and TOC below 5 ppb.

2.2. Instrumentation and analysis conditions

Analysis was carried out using an Agilent 7980A Gas Chromatography system equipped with tandem mass spectrometer 7000B Quadrupole (Agilent Technologies, USA). Mass Hunter software was employed for instrument control and data acquisition/processing (Agilent Technologies, USA). NIST 08 mass spectral library, ver. 2.0f (Agilent P/N G1033A) was used for confirmation of the studied compounds as well as identification of co-extractives. Chromatographic separations were accomplished using the DB-35MS Ultra-inert capillary column (20 m length × 0.18 mm id × 0.25 μm) that was obtained from Agilent Technologies (USA). The GC oven temperature was programmed to initially be held at 70 °C for 1.3 min then increased to 135 °C at 50 °C min^{-1} (held for 0 min), and raised to 200 °C at the rate of 6 °C min^{-1} (held for 0 min), then increased from 200 to 310 °C at 16 °C min^{-1} (held for 8.2 min). The injection volume was 1 μL and detection was achieved using EI source (−70 eV). Samples were injected in a splitless mode and ultra-high purity helium (>99.999%) was used as both the carrier gas at flow rate of 0.7 mL min^{-1} and quench gas at 2.25 mL min^{-1}, and nitrogen served as the collision gas at 1.5 mL min^{-1}. Injector temperature, transfer line temperature, ion source temperature and quadrupole temperature were 250 °C, 285 °C, 280 °C and 150 °C, respectively. The filament current (35 μA) was switched off during a solvent delay time of 4 min. Acquisition was performed in MRM mode in which one MRM was used for quantification (quantifier peak) and the others were used for confirmation (qualifier peaks). The MS/MS transitions and optimal operational conditions used for analysis are summarized in Table 1. The correct identification of the studied pesticides was based on the t_R and the ion ratio of the qualifiers to quantifiers, compared to that obtained via analysis of neat standard solutions. Regular maintenance was carried out, where the liner (uni-taper) was replaced daily to avoid liner priming. In addition, 2 cm from the front part of the column was trimmed to remove any accumulated non-volatile components after about 500 injections.

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