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Development of a new microextraction method based on elevated temperature dispersive liquid–liquid microextraction for determination of triazole pesticides residues in honey by gas chromatography-nitrogen phosphorus detection

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ABSTRACT

In the present study, a rapid, highly efficient, and reliable sample preparation method named "elevated temperature dispersive liquid–liquid microextraction" followed by gas chromatography-nitrogen-phosphorus detection was developed for the extraction, preconcentration, and determination of five triazole pesticides (penconazole, hexaconazole, diniconazole, tebuconazole, and difenoconazole) in honey samples. In this method the temperature of high-volume aqueous phase was adjusted at an elevated temperature and then a disperser solvent containing an extraction solvent was rapidly injected into the aqueous phase. After cooling to room temperature, the phase separation was accelerated by centrifugation. Various parameters affecting the extraction efficiency such as type and volume of the extraction and disperser solvents, temperature, salt addition, and pH were evaluated. Under the optimum extraction conditions, the method resulted in low limits of detection and quantification within the range 0.05–0.21 ng g^{-1} in honey (15–70 ng L^{-1} in solution) and 0.15–1.1 ng g^{-1} in honey (45–210 ng L^{-1} in solution), respectively. Enrichment factors and extraction recoveries were in the ranges of 1943–1994 and 97–100%, respectively. The method precision was evaluated at 1.5 ng g^{-1} of each analyte, and the relative standard deviations were found to be less than 4% for intra-day ($n = 6$) and less than 6% for inter-days. The method was successfully applied to the analysis of honey samples and difenoconazole was determined at ng g^{-1} levels.

1. Introduction

Honey is a wholesome natural product consumed worldwide. The nutritional and quality aspects of honey are important since they are among the significant attributes that affect consumer acceptance. Of even more significance is chemical safety of honey as it affects human health. So there is an increasing interest in monitoring honey for the presence of pesticides and other harmful chemical compounds. According to European Union (EU) regulations, honey must be free of chemical contamination, particularly those due to the presence of pesticides.

Maximum residue limits (MRLs) for some triazole pesticides in royal, jelly, pollen, and honeycomb are under statutory regulations by the EU Council (diniconazole, 0.01 mg kg^{-1}, Reg. (EU) No. 899/2012; tebuconazole, 0.05 mg kg^{-1}, Reg. (EU) No. 500/2013; and difenoconazole, 0.05 mg kg^{-1}, Reg. (EU) No. 834/2013) [1]. Considering the fact that beehives are frequently pastured on plants and agricultural crops contaminated by pesticides, there is a need for accurate and reliable determination of pesticide residues in honey products.

Triazole fungicides are among the flourishing new generations of pesticides applied to fruits, vegetables, and grain crops [2]. Besides their antifungal activity, they are also of concern as a group of compounds that disturb endocrine activity in human beings. Due to their lipophilic nature, these compounds can be bio-accumulated in various tissues of living organisms and they can be transported between various compartments of ecosystems and contaminate food chains.

Sample preparation plays a key role in the analysis of pesticide residues in complex matrices such as those found in honey samples [3]. The main objective of this challenging critical step

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is to transfer the analytes into a phase in which they are pre-purified, concentrated, and compatible with the analytical system [4,5]. Traditionally, the extraction and enrichment of analytes from the sample matrix are often accomplished by procedures such as liquid–liquid extraction (LLE) [6,7] and solid-phase extraction (SPE) [8,9]. However, these traditional pretreatment methods suffer from disadvantages such as demanding intensive labor, being time-consuming, resulting in unsatisfactory enrichment factors (EFs), and consuming large quantities of toxic solvent(s), which compel analysts to limit their application. Recent research on sample pretreatment and preparation methods have being oriented toward the development of efficient, economical, and miniaturized methods. As a result of this, solid-phase microextraction (SPME) [10,11], and liquid-phase microextraction (LPME) [12,13] were developed. SPME, a technique introduced in 1990 by Pawliszyn [14,15], was based on equilibration of analyte(s) between the sample matrix and a fused silica fiber coated with an adsorbent [16–21]. However, most of the commercial extractive fibers used in SPME were relatively expensive and fragile and occurrence of sample carry-over further complicated the problem with them [22]. LPME methods such as single-drop microextraction (SDME) and hollow-fiber supported LPME (HF-HPME) were developed as solvent-minimized sample pretreatment techniques that were inexpensive and caused minimal exposure to toxic organic solvents [23–26].

In 2006, a microextraction technique termed dispersive liquid–liquid microextraction (DLLME) was developed by Rezaee et al. [27]. Similar to homogeneous liquid–liquid extraction and cloud-point extraction, it was based on a ternary component solvent system. In this method, an appropriate mixture of an extraction solvent and a dispersive solvent was rapidly injected by a syringe into aqueous sample which resulted in the formation of a cloudy solution. Then the analytes were rapidly extracted into the fine droplets of extraction solvent. After extraction, phase separation was performed by centrifugation and the analytes were enriched in the organic phase and determined by a chromatographic or spectrophotometric method. The advantages of the DLLME method were simplicity of operation, rapidity, low cost, and high extraction recoveries (ERs) and EFs [28–31]. However, the relatively high volumes (in the mL range) of polar solvent (*e.g.* methanol or acetonitrile) consumed as dispersive solvent lead to lower extraction efficiencies because of increased solubility of the analytes in the solution. In 2008, Zhou et al. [32] developed a novel ionic liquid (IL) LPME method termed temperature-controlled ionic liquid dispersive LPME. The method was based on the dispersion of IL into aqueous phase by changing the temperature. Many analytical methods have been applied to measure pesticides in honey samples, mainly including high-performance liquid chromatography (HPLC) with different detectors such as variable wavelength detector (VWD) [33], diode-array detector (DAD) [34], and tandem mass spectrometer (MS–MS) [35], and gas chromatography (GC) with detectors such as MS [36], flame ionization detector (FID) [37], and electron capture detector (ECD) [38].

The goal of this study was to develop a sensitive procedure for the trace determination of triazole pesticides in honey samples using elevated-temperature dispersive liquid–liquid microextraction (ET-DLLME) combined with GC-nitrogen-phosphorous detection (NPD) method. In this method, large volumes of aqueous phase, along with small volumes of extracting phase improved ERs. Temperature can have an important effect in DLLME method and help reaching higher EFs and ERs in spite of very large volume ratio of aqueous phase to organic phase, because higher temperatures can be a driving force for better dispersion of extraction solvent in the aqueous phase. The main disadvantage of the DLLME technique lies in its extractant solvent which is usually a halogenated solvent of highly toxic nature that is difficult to handle in the laboratory. Furthermore, 1,1,2,2-tetrachloroethane (1,1,2,2-TCE) has considerable hepatotoxicity and 1,2-dibromoethane (1,2-DBE) is classified by IRAC as Group 2A, suspected carcinogen to humans with evidence of carcinogenicity in animals [39]. To the best of our knowledge, this is the first report on application of ET-DLLME to the determination of triazole pesticides using large-volume aqueous sample. The proposed method was successfully applied to the quantification of residues of some selected triazole pesticides in honey samples of different floral origins.

2. Experimental

2.1. Chemicals and solutions

All triazole pesticides used (penconazole, hexaconazole, diniconazole, tebuconazole, and difenoconazole) with purity of >98% were kindly provided by GYAH Corporation (Karadj, Iran). The tested extraction solvents were supplied by the following sources: 1,2-DBE was from Merck (Darmstadt, Germany), 1,1,2,2-TCE, and 1,1,2,2-tetrabromoethane (1,1,2,2-TBE) were from Janssen Chimica (Beerse, Belgium). Dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and *n*-propanol tested as disperser solvents were from Merck. Analytical-reagent grade sodium chloride, hydrochloric acid, and sodium hydroxide were also obtained from Merck. De-ionized water (Ghazi Company, Tabriz, Iran) was used for the preparation of aqueous solutions.

A stock solution of pesticides (1000 mg L^{-1} of each pesticide) was prepared by dissolving an appropriate amount of each pesticide in acetone. Working solutions were prepared daily by appropriate dilutions of the stock solution with de-ionized water. Another standard solution of analytes was prepared in 1,2-DBE at a concentration of 100 mg L^{-1} (each pesticide). This solution was directly injected into the chromatographic system three times a day for quality control and areas of the obtained peaks were used in calculation of EFs and ERs.

2.2. Samples

Four honey samples of different floral origins were purchased from local vendors (East Azarbaijan Province, Iran). One further honey sample was obtained from beehives located in virgin mountainous lands which are far away from the agricultural areas (Kaleybar, East Azarbaijan Province, Iran). It seems plausible to assume such honey to be free of any pesticides. Some preliminary tests performed on the basis of our previous works confirmed plausibility of this assumption. So it was used as a pesticide-free sample in optimization of the proposed method. All samples were stored in their original containers at ambient temperature just like normal storage conditions in their everyday use. To prepare aqueous samples, 15.0 g honey was dissolved in de-ionized water and the obtained homogeneous solution was brought to 50 mL by water. This solution was left to equilibrate for at least 15 min prior to performing the proposed extraction method. This solution was directly subjected to the extraction procedure without filtration or any other pretreatment.

2.3. Apparatus

Chromatographic analyses were performed on a gas chromatograph (GC-1000, Dani, Italy) equipped with a splitless/split injector operated at 290 °C in splitless mode (sampling time 1 min) and an NPD. Helium (99.999%, Gulf Cryo, United Arabic Emirates) was used as the carrier gas (at a constant linear velocity of 30 cm s^{-1}) and make-up gas (25 mL min^{-1}). Chromatographic separations were achieved on a BPX-5 capillary column (5% phenyl methyl siloxane, 95% dimethyl siloxane, 30 m × 0.25 mm i.d., and film thickness