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Determination of Pesticide Residues in Honey using the GC×GC-TOFMS Technique

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Abstract

In this study, a QuEChERS method and gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) was developed for rapid extraction and simultaneous determination of 12 pesticide residues in honey. The GC×GC-TOFMS method was validated according to the SANCO guidance in terms of linearity, selectivity, reproducibility and recovery. Regarding the results, recovery rates ranged between 70-120% with relative standard deviations <20% in most cases. The method Limits of Quantification (LOQ) ranged between 6-26 ng/g. According to the estimated LOQ values the analytical procedure can be applied to analysis of real honey samples.

Keywords: Environmental monitoring; GC×GC-TOF-MS; Honey; Pesticide residues

Introduction

Honey produced by honey bees from pollen, plant nectars, and/or honeydew is composed of over 300 chemical substances which belong to different chemical compound groups. These are mainly carbohydrates, water, polysaccharides, fatty acids, proteins, minerals, dyes, fragrances, enzymes, hormones and vitamins in amounts depending on the plant from which the honey was made [1].

Honey bees can bring many pollutants deposited on plants into the hive. Therefore, plant protection products used in agriculture can not only cause mass poisoning of bees, but may also be transferred to bee products, especially honey affecting its quality, properties and posing a particular threat to human health [2,3]. Pesticides are a significant group of xenobiotics affecting the biota. Regulation 396/20 of the European Parliament and of the Council established values of the Maximum Residue Levels (MRLs) of pesticides in products of plant and animal origin Regulation (EC), 2005. Since September 1st 2008, the European Commission set new MRLs of some pesticides in honey, which are within the range of 10 and 50 ng/g [4]. Honey can be used as an indicator of environmental pollution with radioactive elements [5], heavy metals [6] or pesticides [7].

Sample preparation and isolation/enrichment of the target compounds are very important analysis steps because the pollutants are present in honey at very low concentration levels [8]. There are many extraction techniques, which are designed to disallow the determination of very low pesticide residues in honey. Solid Phase Extraction (SPE) [9,10] and Liquid-Liquid Extraction (LLE) [11,12] are the most common extraction and purification techniques used in the determination of pesticide residue in honey. Other extraction techniques, such as Supercritical Fluid Extraction (SFE) [13], Matrix Solid Phase Dispersion (MSPD) [14], Solid Phase Micro Extraction (SPME) [15,16] and Stir Bar Sorptive Extraction (SBSE) [13,17] have been developed to reduce the quantities of reagents and time spent on sample preparation.

In recent years, the QuEChERS (quick, easy, cheap, effective, rugged and safe) method developed in 2003 [18] has become the most frequently employed technique for determination of pesticide residues in food (especially in fruits and vegetables). The QuEChERS method has many advantages. It is less time-consuming, does not require high financial costs and it has wide applicability. In this method, pesticides are extracted with acetonitrile; water is removed by salting out. In most stage the acetonitrile extract is cleaned up by mixing with an SPE sorbent rather than passing it through an SPE column [19].

In this study, the QuEChERS method was applied to honey samples for the extraction of antibiotics in honey and bee products [20] and also for determination of neonicotinoid and other pesticides [21,22]. So far gas or Liquid Chromatography coupled with a tandem Mass Spectrometer (LC-MS/MS and GC-MS/MS) has been mainly used for determining pesticide residues in honey or other bee products [23,24].

We have described the first evaluation and adaptation of the QuEChERS approach in combination with two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) for determining pesticides in honey. The method provides good analytical results for the targeted pesticides in the method validation according to the SANCO guidance [25].

Materials and Methods

Chemicals and reagents

The solution of hexachlorobenzene (HCB) in acetonitryle, 1000 mg/mL, analytical grade, used as an internal standard was purchased from Sigma-Aldrich (Schnelldorf, Germany). Certified Reference Materials (solutions in acetonitrile, 100 mg/mL) of bifenthrin, diazinon, pyriproxyfen were purchased from LGC Standards (Łomianki, Poland). The CRM solutions (in acetonitrile, 100 mg/mL) of alachlor (in methanol, 100 mg/mL), vinclozoline and quinalphos were obtained from Ultra Scientific (North Kingston, RI, USA), and the CRM solutions of haloxyfrop-R-methyl (in acetonitrile, 10 mg/mL) was obtained from Dr. Ehrenstorfer GmBH (Germany). The CRM solutions of 4,4'-DDE (in methanol, 500 µg/mL), 4,4'-DDD and endosulfan

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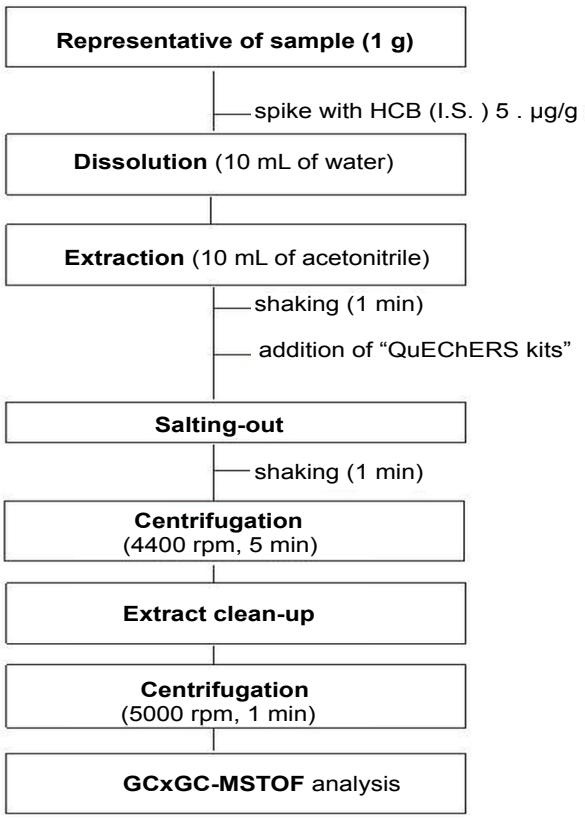

Figure 1: Analytical procedure work-up flow chart.

(analytical standard, 250 mg), dieldrin (in acetonitrile, 100 mg/mL) were obtained from Sigma-Aldrich (Schnelldorf, Germany). The stock standard solutions were stored at –18°C. The calibration standards and working standards were prepared by dilution with acetonitrile on the day of analysis.

Acetonitrile and methanol (LC-MS Chromosolv® 99.9%) were obtained from Fluka (Sigma-Aldrich, Germany). Water was purified with a Milli-Q water system (Millipore Corporation, USA). The QuEChERS kits (part no. 5682-5650) with salt packets containing 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g of sodium citrate and 0.5 g sodium hydrogencitrate sesquihydrate, and two-milliliter centrifuge tubes with 150 mg anhydrous magnesium sulfate and 25 mg primary-secondary amine (PSA) for dispersive solid phase extraction (dSPE, part no. 5982-5021) were purchased from Agilent Technologies (USA).

Instrumentation

GC×GC–TOFMS instrument from LECO, Co. (USA) consisted of Agilent 7890A gas chromatograph with cooled injection system of Gerstel (CIS4), 7683 Series autosampler and time-of-flight mass spectrometer Pegasus IV LECO Co. The column set consisted of a 30 m × 0.25 mm × 1 µm primary column (1D) with Equity 1 stationary phase (Supelco, Germany) and a 1.0 m × 0.25 mm × 0.25 µm secondary column (2D) with SG-Wax stationary phase (SGE, Austin, TX, USA).

Inside the GC oven, a dual-stage jet modulator and the secondary oven were utilized. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by a gaseous nitrogen, previously cooled by liquid nitrogen. Instrumental parameters of the GC×GC–TOF-MS method were as mentioned below.

GC×GC–TOFMS analysis

Injection mode: Programmed Temperature Vaporization (PTV); injection volume: 25 µL; modulation time: 4 s (hot pulse 0.8 s); modulation temperature offset: 30°C and cool time between stages: 1.20 s. The PTV temperature was programmed as follows: 105°C (0.2 min) →280°C (at 10°C/s) (2 min).

Gas chromatography: The temperature program of the first column (main GC oven) was as follows: 100°C (2 min), 100°C→120°C (at 40°C/min), 120°C→280°C (at 5°C/min) (13 min). The temperature of the second oven was programmed from 130°C (2 min), 130°C→150°C (at 40°C/min), 150°C→280°C (at 5°C/min) with a final hold time of 13 min.

Mass spectrometric detection: Acquisition rate: 125 Hz; acquisition delay: 520 s; mass range: 40-550 amu; ion source temperature: 250°C; transfer line temperature: 250°C; detector voltage: 1600V, Electron Impact ionization (EI); electron energy –70 eV.

Data analysis: Data processing was done automatically using the algorithm for peak deconvolution implemented in the Chroma TOF software (LECO Corp., version 4.44). Analytes were tentatively identified through MS library search using the NIST 2011 mass spectral library. Tentative identification was confirmed for some compounds by injecting authentic standards.

Sample preparation

The honey samples were thoroughly homogenized and approximately 1 g of the homogenate was transferred into a polypropylene centrifuge tube (50 mL) containing 10 mL of water, 10 mL of Acetonitrile (ACN) and 50 mL of the internal standard solution (HCB at 100 mg/mL). The content of the tube was shaken manually and the QuEChERS salt kit was added. The sample was immediately hand-shaken for 1 min and subsequently centrifuged at 4400 rpm for 3 min.

Thereafter, 1 mL of the acetonitrile fraction was transferred into a 2 mL dSPE polypropylene tube. The tube was hand-shaken for 1 min and centrifuged for 1 min at 5000 rpm. Finally, 0.5 mL of the supernatant was taken into a glass auto sampler vial. Figure 1 illustrates the scheme of the entire analytical protocol used during the present study.

Results and Discussion

Figure 2A shows a contour plot obtained by the analysis of the honey blank sample. By mass spectral comparison to a library, major co-extracts were identified and also confirmed by a comparison with authentic standards. Figure 2B shows the contour plot of the pesticides standard at a concentration of 20 ng/g. For the identification of individual pesticides in this figure 2, data presented in Table 1 were used data mentioned in Table 1.

Method performance

For evaluation of the method performance calibration sets were prepared at concentrations of 10, 20, 50, 100, 200, and 500 ng/mL, both in solvent, thereby enabling evaluation of linearity. Validation of the overall method was performed according to an EU guideline [25], which involved analysis of two control samples five samples spiked with 12 pesticides and contaminants at 20 ng/g and five samples spiked at 50 ng/g.

All standards and extracts samples were analyzed in one sequence, starting with the solvent standards, then the standards in matrix, and

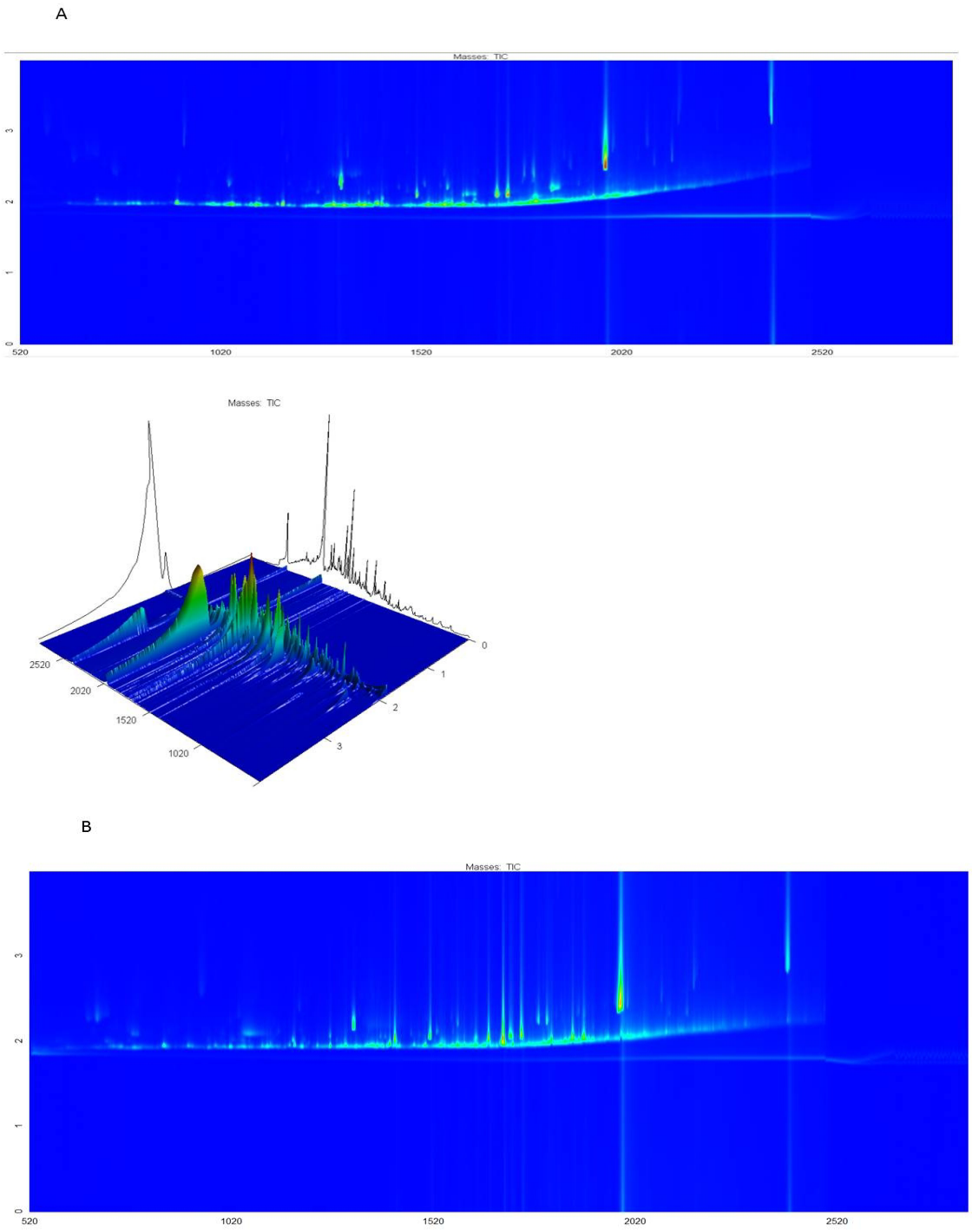

Figure 2: GC×GC chromatograms (TIC plots) of pesticides isolated by QuEChERS from (A) blank honey sample and (B) spiked honey sample at 20 ng/g.

finally the validation extracts. The validation extracts, in sets of four, were bracketed by additional calibration standards (HCB) in matrix at the level of 5 µg/mL (corresponding to 5 µg/g). The repetitive injections of the 50 ng/mL standards in matrix were used to assess the stability of the system performance.

The Limit of Detection (LOD) is the lowest concentration of analytes detectable by an analytical method and the Limit of Quantification (LOQ) is the lowest solute concentration that can be determined with acceptable precision and accuracy, under the stated experimental conditions. In this study, LOD and LOQ values were determined regarding the LOD as 3 times the baseline noise and the LOQ as the concentration that produced a relation signal to baseline noise of 10 [26].

The equations of calibration curves in the solvent are included

Pesticides	Quantify mass *m/z*	Retention time in 1 dimension [s]	Retention time 2 dimension [s]
Alachlor	270 238 162	1520	2.208
Bifenthrin	181 165 115	2080	2.520
4,4′-DDE	246	1824	2.312
4,4′-DDD	235	1832	2.432
Diazinon	304 137	1388	2.112
Dieldrin	261 263 265	1836	2.336
Endosulfan	337 339 341	1784	2.296
Haloxyfrop-R-methyl	315 91	1736	2.176
Hexachlorobenzene (HCB)	284	1320	2.216
Pyriproxyfen	321 136	2156	3.352
Quinalphos	298 146	1700	2.312
Vinclozoline	285 287	1500	2.296

Table 1: Target compounds, qualifier ions and retention times.

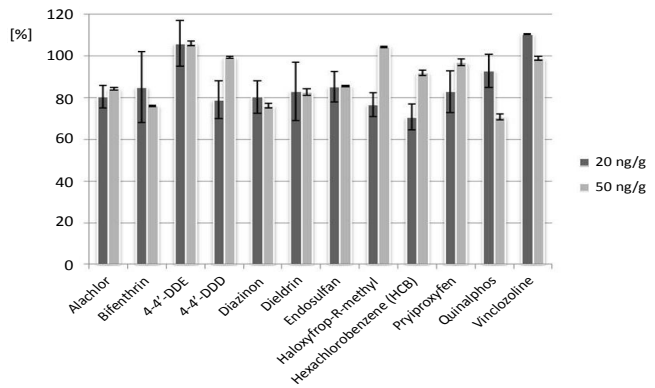

Figure 3: Pesticide recoveries (%) with error bars representing the residue standard deviation (n=5) obtained by QuEChERS in honey spiked at 20 ng/g and 50 ng/g.

in Table 2. Based on correlation coefficients of calibration curves, the proposed analytical procedure is characterized by linearity in the investigated range. The other method performance parameters (limit of detection, limit of quantification) also are included in Table 2. Figure 3 shows the mean recovery of analytes with residue standard deviation (RSD) for n=5. The obtained recoveries ranged between % (RSD 2.4-19.5). Recovery values are in accordance with the SANCO guidance [25].

Conclusions

This study describes a new, rapid, easy, efficient and robust analytical procedure based on the QuEChERS and GC×GC-TOFMS technique for the simultaneous determination of 12 pesticide residues in honey samples. The developed methodology is characterized by low values of limits of detection and quantification and relatively good recovery rates

Pesticides	Calibration curves solvent	Correlation coefficient r	LOD [ng/g]	LOQ [ng/g]
Alachlor	y=0.07154x+0.4588	0.9998	10	14
Bifenthrin	y=0.8312x+0.3235	0.9988	12	15
4,4′-DDE	y=0.7574x+0.3041	0.9997	1.5	7.5
4,4′-DDD	y=0.8148x−0.002	0.9999	1.4	9
Diazinon	y=0.7388x+0.2243	0.9999	10	19
Dieldrin	y=0.9555x+0.459	0.9996	4.5	16
Endosulfan	y=0.8112x+0.3134	0.9988	2.5	6
Haloxyfrop-R-methyl	y=0.7029x−0.3007	0.9989	10	17
Hexachlorobenzene (HCB)	y=0.9676x+0.4666	0.9997	15	26
Pyriproxyfen	y=0.6112x+0.4134	0.9996	15	19
Quinalphos	y=0.2120x+0.2132	0.9998	14	18
Vinclozoline	y=0.6212x+0.2114	0.9999	16	19

Table 2: Method performance data.

of analytes. According to the aforementioned aspects, it can be used for the determination of pesticide residues in real honey samples.

In summary, the obtained results show that high-resolution 2-D chromatography coupled with mass spectrometry can be used for identification and quantification of pesticide residues in the aforementioned samples Moreover, those analytical techniques can be applied to identify various ranges of contaminants present in honey and other bee products.

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