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 396/2005 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 acetonitrile, 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...
Representative of sample (1 g)

- spike with HCB (I.S.) 5 µg/g

Dissolution (10 mL of water)

Extraction (10 mL of acetonitrile)

- shaking (1 min)
- addition of "QuEChERS kits"

Salting-out

- shaking (1 min)

Centrifugation (4400 rpm, 5 min)

Extract clean-up

Centrifugation (5000 rpm, 1 min)

GC×GC-MSTOF analysis

Figure 1: Analytical procedure work-up flow chart.
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.
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, these analytical techniques can be applied to identify various ranges of contaminants present in honey and other bee products.

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References


**Table 2: Method performance data.**

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>LOD [ng/g]</th>
<th>LOQ [ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alachlor</td>
<td>0.9998</td>
<td>10</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.9988</td>
<td>12</td>
</tr>
<tr>
<td>4,4′-DDD</td>
<td>0.9977</td>
<td>1.5</td>
</tr>
<tr>
<td>4,4′-DDD</td>
<td>0.9999</td>
<td>1.4</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.9999</td>
<td>10</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.9986</td>
<td>4.5</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.9988</td>
<td>2.5</td>
</tr>
<tr>
<td>Haloxyfrop-R-methyl</td>
<td>0.9989</td>
<td>10</td>
</tr>
<tr>
<td>Hexachlorobenzene (HCB)</td>
<td>0.9977</td>
<td>15</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>0.9934</td>
<td>15</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>0.9998</td>
<td>14</td>
</tr>
<tr>
<td>Vinclozoline</td>
<td>0.9999</td>
<td>16</td>
</tr>
</tbody>
</table>

**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 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].


