



## Determination of Pesticide Residues in Honey using GC-MS Technique

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**Abstract:** Ten samples of honey (*Mountain Honey, Mountain flower honey, Flower honey and Chestnut honey*) were analysed for 26 organochlorine, carbamate and organophosphorus pesticides residues. An analytical procedure was based on QuEChERS extraction with acetonitrile followed by gas chromatography-mass spectrometry. The calibration curves constructed were linear over the range from 0.01-0.25 mL<sup>-1</sup>. The correlation coefficient were  $\geq 0.995$  for all pesticides standards. The mean recoveries for extractions were 70-125% for lower concentration range (0.02 mg/kg) and 62-135% for the higher concentration range (0.10 mg/kg) for pesticides analysed. Six different pesticides, prothion and carbofuran (carbamate pesticides), methyl parathion, dichlorvos, sulfotep and malathion (organophosphate pesticides) were detected in the analysed honey samples.

## INTRODUCTION

Pesticides are widely used in producing food. Their residues may remain in small amounts in or on fruits, vegetables, grains, and other foods (Echobichon, 2001). The way in which pesticides are used varies from country to country. There is evidence that pesticides or their residues have a negative impact on the environment and human and animal health since they are exposed to them through their diets. To ensure the safety of food, most governments regulate the maximum level of each permitted pesticide residue (MRLs). These values also vary according to the intake of different types of food in different countries. In 2008 the European Commission set new MRLs of some pesticides in honey, which are within the range of 10 and 50 ng/g (Regulation EC, 2005). Therefore, determination of these contaminants in honey is essential, since the use of pesticides has increased significantly in recent decades because of the growing demand for food production.

Besides common extraction techniques for the extraction of pesticides from different samples, other more recent approaches such as QuEChERS, solid-phase extraction (SPE), solid-phase microextraction (SPME), supercritical fluid extraction (SFE), microwave-assisted extraction

(MAE). These techniques have results in new possibilities in sample treatment and a lot of advantages such as reduction of extraction time and volume of solvents, which is also ecologically acceptable (Lambropoulou and Albanis, 2007; Nadaf, Yadav and Kumari, 2015; Pang, Fan, Liu *et al.*, 2006).

The most widely used technique for the determination of pesticides is gas chromatography (GC), due to its high separation power and availability of selective detectors such as electron capture detector (ECD), mass spectrometry detector (MS) and nitrogen phosphorus, (NPD). Also, liquid chromatography (LC) is good alternative technique to GC because of its application to polar, non-volatile and thermolabile pesticides (Tette, Roche, Gloria *et al.*, 2016; Bargańska, Ślebioda, and Namieśnik, 2014; Bargańska, Olkowska, Dymerski, *et al.*, 2014; Rial-Otero, Gaspar, Moura *et al.*, 2007).

The aim of this study was to analyse 26 pesticides residue in 10 samples of honey, mostly origin from Bosnia, using QuEChERS extraction method and GC-MS for their detection.

## EXPERIMENTAL

**Chemicals and Reagents:** The Certified Reference Materials (CRMs) standard solutions of all pesticides (aldrine, ethyl-aziphos, methyl-bromophos, carbofuran, chlorfenvinphos, chlorpyrifos, diazinon, dichlorvos, dimethoate,  $\alpha$ -endosulfan, ethion, fenthion, lindane, malathion, methazachlor, methoprotryne, metholachlor, methyl-parathion, pendimethalin, procymidone, prothion, simazin, sulfotep, terbufos, terbutrin, triadimefon) were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). The stock standard solutions were prepared in the concentration of 200 mg/L and were stored at  $-20^{\circ}\text{C}$ . The calibration standards and working standards were prepared by dilution with acetonitrile on the day of analysis. Acetonitrile, acetone and *n*-hexane (pro analysis) were obtained from J.T.Baker (U.S.A) HPLC grade. Water was purified with a Milli-Q water system (Millipore Corporation, Billerica, MA, USA). The QuEChERS kits with salt packets containing 4 g of anhydrous magnesium sulphate, 1 g of sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g, trisodium citrate hemihydrate) as well as tubes with 900 mg anhydrous magnesium sulphate and 150 mg primary-secondary amine (PSA) for dispersive solid phase extraction (dSPE) were purchased from Restek (USA).

**Sample Collection.** Ten honeybee samples were purchased in 2011 and 2012. Eight samples were from Bosnia, one from FYR of Macedonia, and one from Germany. All samples were stored at room temperature in the dark until analysis.

Four types of honey were analyzed. Three samples of *Mountain honey* (season 2011) from Bosnia (Olovo, Vareš and Sokolac), three samples *Mountain flower honey* (season 2012), two of them from Bosnia (Olovo, Kupres) and one obtained from FYR of Macedonia (2012). Three *Flower honey samples* (2011) from Bosnia (Gradačac), Germany, and one collected 2012 from Bosnia (Ljubuški). A *Chestnut honey* (2012) sample from Bosnia was collected from Velika Kladuša area (2012).

**Sample Preparation.** The honey samples (5 g) were thoroughly homogenized with 10 mL ultrapure water and approximately 5 g of the homogenate was transferred into polypropylene centrifuge tube (50 mL), ultrapure water (10 mL) was added and hand-shaken for 5 minutes. Thereafter 10 mL of acetonitrile (ACN) was added and mixed for 2 minutes, and the QuEChERS salt kit was added. The samples were immediately hand shaken and for 2 minutes and subsequently centrifuged at 3500 rpm for 5 minutes. Thereafter, 6 mL of supernatant was transferred in a 15 mL dSPE polypropylene tube. The tube was hand-shaken for 30 seconds and subsequently centrifuged at 3500 rpm or 5 minutes. Finally, 0.5 mL or 1  $\mu\text{L}$  of supernatant was taken into glass auto sampler vial.

**Instrumentation.** GC analysis was performed on a GC-MS Agilent (GC 7890A i MS 5975) with column backflush capability. The injector temperature was  $280^{\circ}\text{C}$ . The samples was injected in the splitless mode, and the splitless was opened after 2 minutes. Injection

volume was 2  $\mu\text{L}$ . A capillary column HP-5MS (5%-phenyl, 95%-dimethylpolysiloxane), 30 m x 0.25 mm x 0.25 $\mu\text{m}$ , was used. Gas carrier was He with constant flow of 1.90 mL/min. The oven temperature was as follows: initial temperature of  $60^{\circ}\text{C}$ , held for 2 min, increased to  $25^{\circ}\text{C}/\text{min}$  up to  $150^{\circ}\text{C}$ , at  $3^{\circ}\text{C}/\text{min}$  up to  $200^{\circ}\text{C}$  held for 1 min, and then increased to  $290^{\circ}\text{C}$  at  $8^{\circ}\text{C}/\text{min}$  and held for 43 min.

The MS ionization potential was 70 eV, and the temperatures were as follows: interface  $280^{\circ}\text{C}$ , MS source  $230^{\circ}\text{C}$  (ion source), MS Quad  $150^{\circ}\text{C}$ , electron multiplier 1200 V, in both SCAN and SIM modes  $250^{\circ}$ , transfer line  $200^{\circ}\text{C}$ , and analyzer  $230^{\circ}\text{C}$ . Analysis was performed in SIM mode monitoring specific ions of each analyte as it is shown in Table 1.

**Table 1.** SIM Conditions of Pesticides Detected by GC-MS

Pesticide	SIM ions (m/z)
Aldrine	262.80; 264.80; 260.80; 291.00
Ethyl Aziphos	128.60; 132.00; 159.80; 206.60
Methyl-bromophos	124.80; 328.80; 331.00; 333.00
Carbofuran	123.00; 131.00; 149.00; 164.00
Chlorfenvinphos	266.80; 268.80; 323.00; 325.00
Chlorpyrifos	196.80; 198.80; 257.80; 314.00
Diazinon	179.00; 199.00; 275.80; 304.00
Dichlorvos	109.00; 185.00; 187.00; 219.60
Dimethoate	92.80; 124.80; 142.60
$\alpha$ -Endosulfan	238.80; 264.80; 268.80; 276.80
Ethion	124.80; 153.00; 231.00; 384.00
Fenthion	153.00; 168.80; 124.80; 278.00
Lindane	180.80; 182.80; 216.80; 218.80
Malathion	157.80; 124.80; 126.80; 173.00
Methazachlor	132.00; 133.00; 134.00; 209.00
Methoprotryne	213.00; 226.00; 240.20; 256.00
Metholachlor	162.00; 238.00; 240.00
Methyl parathion	109.00; 125.00; 263.00
Pendimethalin	162.00; 190.80; 219.80; 252.00
Procymidone	254.80; 286.80; 283.00; 285.00
Prothion	93.00; 120.00; 137.00; 179.00
Simazin	137.80; 173.00; 186.00; 201.00
Sulfotep	202.00; 245.00; 265.80; 322.00
Terbufos	153.00; 232.80; 231.00; 287.80
Terbutrin	241.20; 170.00; 185.00; 226.00
Triadimefon	128.00; 180.80; 208.00; 210.00

The most intense ion was used for quantification and the second and third ion for confirmation. Identification criteria was based on (a) the chromatographic retention data, and (b) the relative peak heights of the three characteristic masses in the sample peak that must be within  $\pm 20\%$  of the relative intensity of these masses in the mass spectrum of the standard analyzed in the GC-MS system.

## RESULTS AND DISCUSSION

Ten samples of honey were analysed for 26 organochlorine, carbamate and organochlorous pesticides residues. The calibration curves constructed were linear over the range from 0.01-0.25 mL<sup>-1</sup>. The correlation coefficient were  $\geq 0.995$  for all pesticides standards. The mean recoveries for extractions were 70-125% for lower concentration range (0.02 mg/kg) and 62-135% for the higher concentration range (0.10 mg/kg) for analysed pesticides.

The limits of detection (LODs) for the pesticides detected in the investigated samples were: 0.010±0.003 (propham), 0.010±0.005 (methyl parathion), 0.010±0.005 (carbofuran), 0.010±0.008 (dichlorvos), 0.010±0.004 (sulfotep) and 0.020±0.002 mg/kg for (melathion).

Results of analyzed honey samples are shown in Table 2.

**Table 2.** Pesticide residues detected in honeybee samples

The variety of honey	The geographical origin and the season of collection	Detected Pesticides
Mountain honey	(Olovo, BiH 2011)	propham, methyl parathion
Mountain honey	(Vareš, BiH 2011)	nd
Mountain honey	(Sokolac, BiH 2011)	nd
Mountain flower honey	(FYR of Macedonia 2012)	propham malathion
Mountain flower honey	(Olovo, BiH 2012)	nd
Mountain flower honey	(Kupres, BiH 2012)	nd
Flower honey	(Gradačac, BiH 2011)	propham
Flower honey	(Germany 2011)	carbofuran
Flower honey	(Ljubuški, BiH 2012)	dichlorvos propham sulfotep
Chestnut honey	(Velika Kladuša, BiH 2012)	carbofuran propham
nd-not detected		

The Table 2 shows that six different pesticides, propham and carbofuran (carbamate pesticides) and methyl parathion, dichlorvos, sulfotep and malathion (organophosphate pesticides) were detected.

In the three tested samples of *Mountain honey*, two pesticides, propham and parathion-methyl, were detected. Also in one of the 3 analyzed samples of *Mountain flower honey*, pesticides propham and malathion were detected. In all three samples of flower honey, pesticides were found. The sample collected from area Ljubuški, BiH, was contaminated by three different pesticides, dichlorvos, propham, sulfotep. Similarly, in sample of chesnut honey, carbofuran and propham were detected. In four analysed honey samples pesticides were not found. The detected concentration of propham, methyl parathion, carbofuran, dichlorvos and sulfotep were 0.01 mg/kg, and for melathion was 0.02 mg/kg. Those values were the same as LOD values of detected pesticides.

The most common pesticide detected in even five samples of honey is *propham*. It is used as plant growth regulator in agricultural application as a selective herbicide for annual grasses and broadleaf weeds on forage crops, lettuce, spinach, sugar beets, lentils and peas. Since in this honey areas origin those vegetables are grown in large quantities, use of pesticides has increased, and is possible the reason for the contamination of honey. Mujić, Alibabić, Jokić *et al.*, (2011) evaluated the health safety of honey (meadow, chestnut, acacia, amphorae, and honeydew) produced at 18 different locations in the region of Una-Sana Canton in the northwestern part of Bosnia and Herzegovina. They determined the pesticides, heavy metals, radioactive elements, and antibiotic residues in 46 honey samples. The content of pesticides were not found. The results of this work indicate that this area is not polluted and is suitable for the development of beekeeping. Kartalovic Jovanic, Jaksic *et al.*, (2015) from the Pannonian region in the Republic of Serbia investigated organochlorine residues in honey samples. It was found the presence of organochlorine pesticides in all samples of honey. But all of the detected concentrations of pesticides were below the maximum allowed value. Das and Kaya (2009) investigated 15 organophosphorus insecticides in 275 honey samples in 33 different cities of Turkey, using gas chromatography with electron capture detector. No insecticide residue was detected in the samples analyzed. This result is highly significant because of its impacts on public health and food safety. Bogdanov, Ryll and Roth (2003) investigated pesticide 36 organochlorine, 32 organophosphorus in 27 honey samples. The investigations discovered that detected amount of all analyzed pesticides were lower than detection limits, which varied between 0.005-0.050 mg/kg. Blasco, Fernandez, Pena *et al.*, (2003), analysed honey from Portugal and Spain. The residues of more than one pesticide were found in honeys from both countries.

In many cases, pollution of honey is caused by pesticide application in the surrounding area or by environmental contamination. Given the use of those pesticides, it is clear why the samples of honey from rural areas consisted more types of pesticides. Fortunately, the contents of these pesticides detected in this work do not exceed the MRL values.

## CONCLUSION

Based on results obtained in this work it can be concluded that the samples of honeybee analyzed are safe for human consumption, in particular mountain honeybees, with less possibility of contamination by pesticides used in farming. In general, in mountainous areas is less influence of anthropogenic factors.

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## Summary/Sažetak

U deset uzorka meda (planinski, planinski cvjetni, livadski med i med od kestena) je analizirano 26 organohlorinih, karbamatnih i organofosforinih ostataka pesticida. Analitička procedura je bazirana na QuEChERS ekstrakciji na acetonitrilom, nakon čega su uzorci analizirani uz pomoć gasne hromatografije-masene spektrometrije. Linearnost za sve ispitivane pesticide je postignuta u opsegu koncentracija od 0.01-0.250 mL<sup>-1</sup>. Koeficijenti korelacije su bili ≥ 0.995 za sve ispitivane standarde pesticida. Srednje *recovery* vrijednosti ekstrakcija su iznosile 70-125% za niže koncentracijsko područje (0.02 mg/kg) i 62-135% za više koncentracijsko područje (0.10 mg/kg) za analizirane analite. Različiti pesticidi su detektovani u analiziranim uzorcima meda, i to: profam i karbofuran (karbamatni pesticidi) i metil paration, dihlorvos, malation i sulfotep (organofosfatni pesticidi).