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DETERMINATION OF SELECTED PESTICIDES IN HONEY BY LC-MS-IT: EFFECTS OF SAMPLE PREPARATION

Abstract: *Honey is well known in human diet and it is considered to be one of the healthiest foods with high nutritional value. Due to the growing presence of anthropogenic contaminants, with extremely long half-life and prevalence in all environments, some of them may be present in honey. Honey samples were spiked with six pesticides which are commonly used at the time of plant's blooming, in order to develop an efficient method for preparing honey samples for LC-MS-IT analysis. Samples of honey, spiked with soultion containing mixture of six pesticides (pirimicarb, atrazine, prometrine, malation, cyprodinil and famoxadone), were treated applying SPE with Florisil and C_{18} cartriges, as well as liquid-liquid extraction method. Afterwards, samples were injected in the HPLC with ESI ionisation source, and IT (ion trap) mass analyzer. Methanol solution of pesticides in the same concentrations as in spiked samples, were used as blank. In spiked honey samples, after treatment with Florisil prometrine and famoxadon were detected; with C_{18}, atrazine and prometrine; after LLE famoxedon and pirimicarb. In the specific case, as acceptable sample preparation method SPE with Florisil cartridge may be proposed, since it enables reliable identification of the most examined pesticides.*

Key words: honey, pesticides, liquid chromatography, mass spectrometry, solid phase extraction

INTRODUCTION

Honey is one of the most important healthy natural products, with excellent nutritional properties. The sources for producing honey by honey bees are nectar of plants, as well as honey dew. Some of the components (carbohydrates, water, traces of organic acids, enzymes, amino acids, pigments, pollen and wax) are due to maturation of the honey, some are added by the bees and some of them are derived from the plants. Raw honey contains extraneous matter, such as pollen, traces of wax, variable amounts of sugar-tolerant yeasts, and probably crystals of dextrose hydrate. The mineral content varies from about 0.04% in pale honeys to 0.2% in some dark honey samples. There are many other minor constituents of honey, including very low concentrations of vitamins and plant acids.

Since it is a product consumed worldwide, it is evident that human health should be taken into consideration in regards to its consumption. To ensure the safety and quality control of honey, it is necessary to perform the analysis of chemical contaminants in honey in order to assure that this natural product does not contain toxic residues in quantities that might imply a risk for consumers. In that way, sensitive, selective, fast, and reliable analytical methods for food analysis are continuously under development. The most method of analysis of hazardous compounds at trace level in complex matrices includes a procedure of sample preparation in order to remove matrix interferents [1,2].

The sample treatment techniques frequently applied as preparation step in analysis of chemical contaminants from honey are liquid–liquid extraction (LLE) [3,4], solid-phase extraction (SPE) [5,6,7], solid-phase microextraction (SPME) [8], matrix solid-phase dispersion (MSPD) [9], supercritical fluid extraction (SFE) [10], ultrasound-assisted extraction (UAE) [11] or pressurized liquid extraction (PLE) [12].

Pesticides play a very important role in agriculture, because of their action against the variety of pest that destroy crops, even though small amounts of pesticide residues remain in the food supply, constituting a potential risk for the human health, because of their sub-acute and chronic toxicity [13].

Pirimicarb (Figure 1) is a selective insecticide belonging to the group of carbamates. It is used specifically to target aphids and it is applied as a foliar spray to infested plant material. The mode of action of pirimicarb is acetyl cholinesterase inhibition, and in that way disrupting the neural pathways of the insect. Pirimicarb is approved for application on a huge variety of crops.

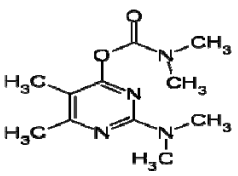

Figure 1. *Pirimicarb*

Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine, (Figure 2) is a widely used herbicide, though its use became controversial, because of widespread contamination. Although it has been banned by Serbian low, it is still one of the most widely used herbicides.

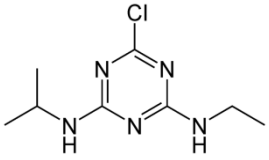

Figure 2. *Atrazine*

Malathion (Figure 3) is an organophosphate, acting as an acetylcholinesterase inhibitor, used against insects. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. Malathion itself is of low toxicity, but its it's absorption or ingestion into the human body readily results in metabolite malaoxon which is substantially more toxic.

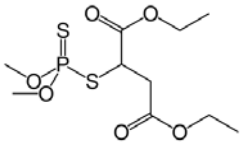

Figure 3. *Malathion*

Pesticide cyprodinil *4-cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine* (Figure 4) belongs to the group of fungicides, designated for the control of a broad spectrum of plant diseases, but also via water pollution possible threat to the environment.

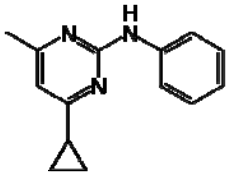

Figure 4. *Cyprodinil*

Famoxadone, (*RS*)-5-Methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-1,3-oxazolidine-2,4-dione (Figure 5) is a fungicide aimed to protect agricultural products (fruits at first place), and in that way it can be ingested into the human organism and cause various disorders.

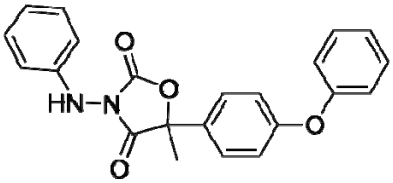

Figure 5. *Famoxadone*

Prometryn (Figure 6) (N2-isopropyl-N4-methyl-6-methylthio-1,3,5-triazine-2,4-diamine) (Figure 1f) is selective triazine used as a selective pre- and post-emergence herbicide in growing celery, parsley, fennel, dill, broccoli, uncultivated areas, cotton, used to control broadleaf and grassy weeds through photosynthetic electron transport inhibition easily absorbed by leaves and roots.

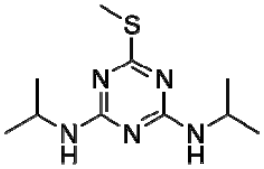

Figure 6. *Prometryn*

For determination of pesticides in complex matrices, such as honey, a method is needed that eliminates the influence of matrix compounds and most frequently it can be utilized the LC-MS capability of an ion trap system. Prior to the analysis, sample preparation is required and different SPE and LLE extraction techniques are recommended [14,15,16].

The aim of the present study was to examine if the honey sample preparation technique may possibly influence the determination of pesticide mixture, applying ion trap LC-MS method analysis.

EXPERIMENTAL

All reagents, applied in the experiment, were p.a. grade, products of Merck, Germany. Deionized water, with specific conductivity less than 0.05 μS/cm, was used for all solutions preparation and for final vessels washing.

SPE of honey samples

Solid phase extraction by Florisil cartridge

To 1g of honey 2 mL of MeOH were added. Preconditioning of the cartridge was started by 5 mL of n-hexan:dichlormethan (1:1, v/v) mixture, followed by rinsing with 15 mL of the preconditioning solution [10].

To the samples of honey of 1 g, volumes of 5 μL and 10 μL of mixed pesticide solution were added, to make final concentrations of 2 ppb. The solutions, obtained in that way were evaporated to dryness, and solid residues were dissolved in 1 mL of MeOH, and then filtered through the 2 μm filters. The volumes of 20 μL were chromatographed and detected by ESI-MS-IT spectrometer.

Solid phase extraction by C_{18} cartridge

To 1 g of honey 2 mL of H_2O were added.

Preconditioning of the cartridge was firstly performed by adding 10 mL MeOH, and then 10 mL H_2O. Rinsing was done by 10 mL of etylacetate, followed by 4 mL of MeOH and finished by 1 mL of dichlormethan. To the two other samples of honey, 5 μL and 10 μL of mixed pesticide solution were added, to make final concentrations of 2 ppb [17].

The solutions obtained were evapporated to dryness, then dissolved in 1 mL of MeOH and filtered through 20 μm filter. Volume of 20 μL of resulted solution was subjected to the HPLC-ESI-MS-IT analysis.

Liquid-liquid extraction (LLE) of honey samples

To the honey of 5 g, 32.5 mL acetonythryl and 10 mL water were added and shaked for 30 minutes. The mixture was transfered to the separating funnel, organic leyer was separated, evaporated to dryness and dissolved in 1 mL of MeOH [18].

To te rest of honey samples, prepared in the same way, prior to LLE, volumes of 5 μL and 10 μL of mixed pesticide solution were added, to make final concentrations of 2 ppb.

LC-ESI-MS analysis

Analysis were performed on an Agilent 1100 pump LC system (Agilent Technologies, Santa Clara, CA, USA) coupled to a LCQ Decca (Termo Finnigan) mass spectrometer instrument equipped with an electrospray ionisation source. HPLC analyses were run on a Zorbax SB-C_{18} reverse phase column (2.1×50 mm, 1.8 μm). The mobile phases were constituted with solvent A: water containing 0.5% formic acid (v/v) and solvent B: acetonitrile/methanol (50/50,v/v) containing 0.5% formic acid (v/v). The injection volume was 10 μL of each of the four honey extracts. HPLC gradient and MS/MS acquisition started once the final honey extract no. 1 was injected. MS tuning was performed in positive electrospray ionization (ESI) by infusing individual solution of each analyte mixed with a HPLC flow made of solvents A and B. Nitrogen was used for the nebuliser at pressure of 50 psi. The interface heater was activated and the block source temperature was maintained at 251^{0}C with a capillary voltage set at 3 kV. Nitrogen was also used as collision gas at a medium pressure selection. MS/MS detection was realized using the selected reaction monitoring (SRM) acquisition mode.

RESULTS AND DISCUSSION

Since all studied pesticides belong to the chemically different families, for all and each of them optimal conditions for MS analysis were established, considering both ionization source and trap conditions. The obtained mass spectra are presented in the Figure 7.

Mixed standard solutions (containing all six pesticides), were subjected to the high pressure liquid chromatography under optimal conditions and total ion chromatograms were presented in the Figure 8 as a) case.

Total ion chromatogram of honey sample treated with SPE Florisil is presented in Figure 8b, while TIC of pesticide spiked honey sample, treated in the same way as previous is presented in Figure 8c.

Sample of honey, treated with C_{18} cartridge resulted with TIC in the Figure 9b, while pesticides spiked honey sample gave TIC in the Figure 9c.

After performing LLE, for honey sample and pesticide spiked honey sample, TIC were recorded (Figure 10b) and c) respectively.

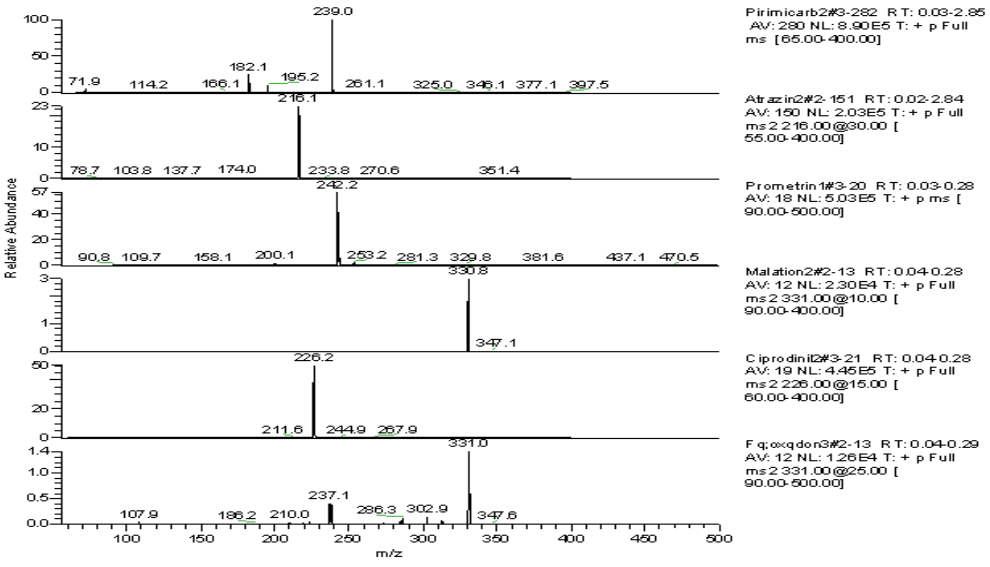

Figure 7. *Mass spectra of six pesticides:*

Pirimicarb (m/z 239, 195, 182); Atrazine (m/z 216, 174) ; Prometrine (m/z 242, 200, 158) ; Malation (m/z 331, 285, 127) ; Cyprodinil (m/z 226, 93,108) Famoxadone (m/z 397, 353, 261)

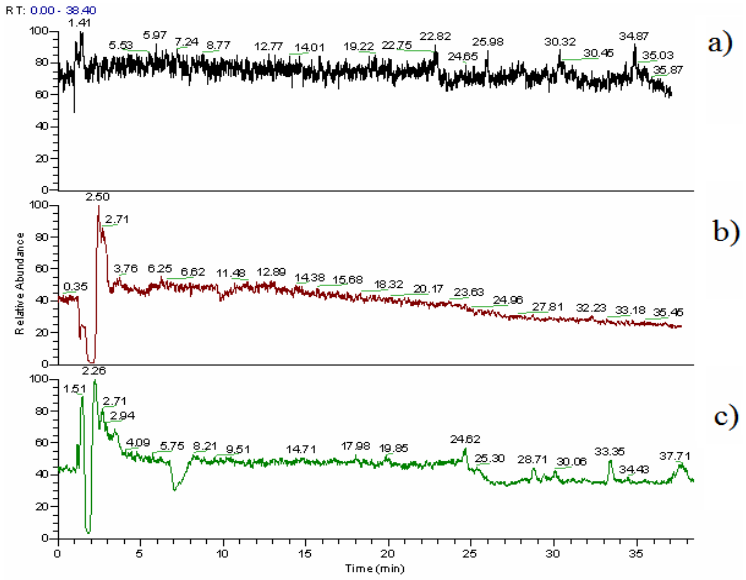

Figure 8. *TIC a) mixed standard solution b) honey sample treated with Florisil cartridge c) honey sample spiked with mixed standard and treated by Florisil cartridge.*

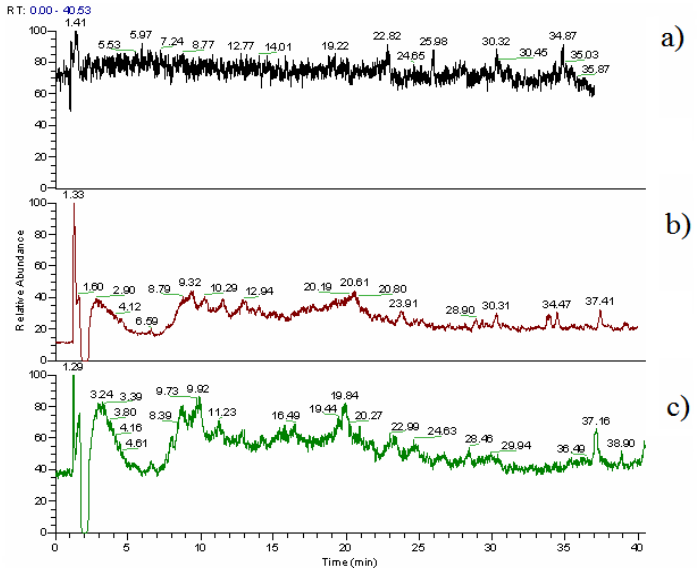

Figure 9. *TIC a) mixed standard solution b) honey sample treated with C_{18} cartridge c) honey sample spiked with mixed standard and treated by C_{18} cartridge*

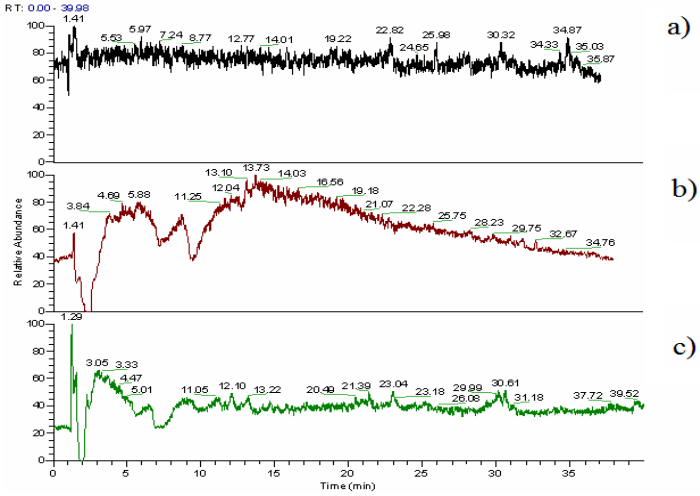

Figure 10. *TIC a) mixed standard solution b) honey sample treated by LLE c) honey sample spiked with mixed standard and treated by LLE*

CONCLUSION

Direct injection of mixed stock standard solution, without any previous treatment, in LC-MS system, enabled optimization of column and mass spectrometer (ionization and ion trap) conditions. All base peaks of studied pesticides and their characteristic fragments were registered in chromatogram and mass spectra. After SPE treatment (Florisil and C_{18}) and LL extraction of the same mixed standard, it was possible to detect only prometrine, cyprodinil and famoxedon. In spiked honey samples, after treatment with Florisil were detected prometrine and famoxadon; after treatment with C_{18}, atrazine and prometrine were detected and after LL extraction famoxedon and pirimicarb were detected.

The obtained results confirmed that methods of sample preparation have very important impact on determination of selected pesticides in real samples. In the specific case, as acceptable sample preparation, method SPE with Florisil cartridge may be proposed, since it enables reliable identification of the most examined pesticides.

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BIOGRAPHY

Marija Ilić was born in Aleksinac, Serbia, in 1983.
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She works as a Research Associate in two projects founded by Serbian Ministry of Education and Science (Grant no. 172051 and 170247).

ODREĐIVANJE ODABRANIH PESTICIDA U MEDU LC-MS-IT METODOM: UTICAJ PRIPREME UZORAKA

Marija Ilić, Ljubica Svilar, Snežana Jovanović, Aleksandra Đorđević, Violeta Mitić, Snežana Nikolić-Mandić, Vesna Stankov-Jovanović

Rezime: *Med se od davnina koristi u ljudskoj ishrani i smatra se jednom od najzdravijih namirnica, koja ima visoku hranljivu vrednost Zbog sve većeg prisustva zagađujućih materija antropogenog porekla, koje imaju visoke vrednosti poluraspada i rasprostranjene su u prirodi, neke od njih mogu biti prisutni u medu.. U uzorke meda dodavane su smeše šest pesticida, koji se najčešće koriste u vreme cvetanja biljaka (pirimikarb, atrazin, prometrin, malation, ciprodinil i famoksadon), razvijene su metode za pripremu uzoraka meda (ekstrakcije čvrstom fazom sa Florisil i C_{18} adsorbensima, kao i tečno-tečnom ekstrakcijom) i uzorci su ubrizgavani u HPLC, povezanim sa masenim spektrometrom sa ESI izvorom jonizacije i IT masenim analizatorom. Metanolni rastvori pesticida u istoj koncentraciji kao i u model uzorcima meda zagađenog pesticidima, korišćeni su kao slepa proba. U uzorcima meda sa dodatkom smeše pesticida, nakon tretmana Florisil adsorbensom detektovani su famoksadon i prometrin, nakon tretmana sa C_{18}, atrazin i prometrin, a nakon tečno-tečno ekstrakcije famoksadon i pirimikarb. U konkretnom slučaju, može se predložiti metoda pripreme uzorka ekstrakcijom čvrstom fazom sa Florisil adsorbensom, kao optimalan način tretiranja uzoraka, jer omogućava pouzdaniju identifikaciju najvećeg broja analiziranih pesticida.*

Ključne reči: med, pesticidi, tečna hromatografija, masena spektroskopija, ekstrakcija čvrste faze