ABSTRACT

Keywords: bee products, antibiotics apitherapy, sulfonamides, heavy metal contamination, imunoenzymatic assay (ELISA), RIA CHARM II assay, TETRASENSOR, high performance liquid chromatography (HPLC), liquid chromatography with mass spectrometry (LC - MS).

The doctoral thesis "*Quantitative correlation of heavy metals and antibiotics in melliferous flora, honey and bee product, established through modern methods of investigation*" is divided into two distinct parts, the first relies on 218 bibliographical references, includes 74 pages representing 31,9% of the paper work, and the second one, personal contributions, is extended on 158 pages representing 68,1% of the thesis.

The first chapter of the bibliographical research "*Honey and its biological significance*" deals with issues related to the structure, composition, and application of the bee products. It was emphasized the nutritional and therapeutic value of honey, pollen and royal jelly, also mentioning the need for developing and complying a quality standard for these valuable products. This chapter ends by mentioning some aspects related to "organic" food products, an area where bee products can easily fit into.

The second chapter, "*Honeybees-bioindicators of environmental pollution*" shows the potential sources of hive contamination, and how bees can be used for assessing and monitoring environmental pollution. There were emphasized several studies for evaluating the fidelity of such monitoring and most of them concluded that the bees respond well to this desideratum, and the level of environmental contamination can be accurately and promptly assessed.

There have been mentioned also specific situations, which had revealed residues of antibiotics, pesticides, acaricides and heavy metals in bee products collected and analyzed from different geographical areas of the world.

The third chapter, "*Aspects of European Union legislation on residues control*" approaches extensively European legislation in force in beekeeping and food safety. There has been made a comparative presentation of the most important quality parameters, physicochemical characteristics and contaminants enacted by international and European

regulations. The limits imposed by the above regulations are mentioned briefly, but repressive in the tables included in this chapter.

Chapter four "*The current status of the analytical methods for determining antibiotic and heavy metal residues in honey and other bee products*" contains three subsections reviewing the methods used for accurate detection and evaluation of such undesirable compounds in honey and other bee products.

The second part of the thesis - Personal researches - consists of six chapters and includes the results of investigations performed between 2004 and 2009. An extensive survey was conducted for monitoring the honey samples collected from several north-eastern districts of Moldova. For this purpose, there had been used screening tests (competitive ELISA-type methods, RIA Charm II tests, and rapid tests), supplemented with high-performance analytical methods such as HPLC and LC-MS.

Chapter-V "*The purpose and the objectives of the research*" presents a brief scientific argument to support the strategy of the investigations carried out on honey quality.

Objectives:

1. Antibiotic residues screening in honey (tetracycline was determined with an ELISA assay - Ridascreen Tetracycline, and also with a rapid test - Tetrasensor, streptomycin was searched with a competitive ELISA immunoassay and for sulfonamides, CHARM II RIA test was used.)
2. Methodology for the confirmation of the presence of the antibiotics and sulfonamides residues in honey.
3. Methodology for the identification and quantification of the heavy metals (Pb and Cd) residues in melliferous flora (flower of linden, acacia, rape), honey (polyfloral and honeydew) and pollen.

Chapter VI "*Screening for antibiotic residues in honey*" is one of the largest and most complex chapters as there had been used several screening tests.

A competitive ELISA immunoassay (Ridascreen tetracycline) had been used for detection and evaluation of tetracycline, Ridascreen Streptomycin was used to test streptomycine, and sulfonamides were determined via an expedient but sufficiently accurate, Charm II RIA test. The results were presented in a comprehensible manner, such a way their inherent value can be fully expressed and, therefore assessed. 59 samples of honey were collected from private producers from four districts of the Moldova (Iasi, Bacau, Botosani and Suceava) and analyzed between 2004 and 2008 to detect residues of tetracycline by the ELISA assay. Among the analyzed samples, 12, representing 17.92 % were classified based on their tetracycline content, as suspected of contamination, so presumably positive.

TETRASENSOR rapid test was also used for detection of tetracycline residues in honey harvested from the same geographical area. It proved to be at least as sensitive as ELISA method.

Out of 29 polyfloral honey samples analyzed, nine were considered non-compliant, leading to a 31.2% non-compliance rate.

Highest level of contamination was recorded for Bacau County, and the lowest degree of pollution with tetracycline, assessed through the number of presumed positive samples, was registered for Botosani County.

During the period 2006-2008, production of honey from three counties, Iasi, Vaslui and Harghita, was monitored in terms of contamination with sulfonamides using for this purpose as a screening method, CHARM II RIA test.

Out of 69 samples analyzed, 14 were detected as presumed positive samples, representing 20.3%. In terms of regional distribution, the percentage of non-compliant honey samples was as follows: Iasi with 23%, Harghita County, 18% and Vaslui County with 17% presumed positives samples.

The time distribution of the contaminated samples revealed a manifested regression phenomenon, meaning that the non-compliant rate decreased from 21.4% in 2006, 20%, in 2007, and to 6.3% in 2008.

Highest degree of contamination with sulfonamides has been recorded in Iasi County, where the presumed positive honey samples reached 19%, and the lowest rate of pollution had been registered in Vaslui County with a percentage of 14%.

Chapter VII "*Methods for the confirmation of the antibiotic residues in honey*", deals first with a number of theoretical issues on the functional aspects of the HPLC with fluorescence and ultraviolet detectors, closely related to the exploration of a such a device, justifying and explaining in the same time, the necessity for highly trained operators.

The 14 honey samples detected as being suspected of contamination by the CHARM II assay were further analyzed for residues of sulfonamides using the HPLC technique with a fluorescence detector. The results have shown outstanding performance of this highly accurate methodology. 10 out of 14 samples were found negative for sulfonamide analysis (sulfathiazole, sulfamethazine, sulfamerazine, sulfacetamide). Only four have been confirmed as contaminated samples, three of them containing 150 ppb sulfathiazole and one with a lower degree of contamination, sulfathiazole content being above 100 ppb.

Analysis of oxytetracycline residues in honey by HPLC with UV detection, as a confirmatory method, demonstrates the superiority of this analytical method to an ELISA immunoassay or to the Tetrasensor rapid test. 5 out of 12 samples considered presumptive

positive by ELISA assay or Tetrasensor test were confirmed by the HPLC method, three of them containing oxytetracycline above 150 ppb and two samples with the same antibiotic values ranging between 75 and 150 ppb.

Chapter VII presents liquid chromatography - mass spectrometry (LC-MS) as a new method for quantification of sulfonamides residues in honey.The chapter begins with some notions related to mass spectrometry and its application in analytical chemistry in general, and sulfonamides residues detection in particular.

The Chapter "*Liquid chromatography with mass spectrometry method for sulfonamides residues detection in honey"* details the necessary reagents, working stages and operating conditions for confirmation of the above mentioned residues in honey.

The same 14 honey samples analyzed during 2006-2008 period by the RIA Charm II test and detected as suspicious, were reanalyzed with LC-MS method. After examining, four samples were detected positives for sulfathiazole, with a residue content between 100 ppb and 150 ppb, and 10 samples were considered negative in terms of their sulfathiazole content. The conclusion that emerges from these investigations is that the LC-MC method, although it is a costly investment, it has a higher accuracy compared to HPLC technics.

Chapter IX. "Method for heavy metal residues detection, namely flame atomic absorption spectrometry (FAAS)" requires special reagents, rigorous working method and working parameters optimization according to the nature of the item being evaluated.

During the period 2007-2009, there were analyzed 145 samples of melliferous flora, honey and bee product, with a recorded 11 non-compliant samples for lead and none for cadmium. There have been analyzed the factors that influenced the results: atmospheric pollution caused by road traffic, and the moment of the harvest. Somehow similar to the situation of antibiotic residues, but due to other factors, a decrease in heavy metal contamination for all categories of analyzed samples was recorded.

Despite this downward trend of heavy metal contamination of the bee products, this parameter remains an important point in the national plan for residues monitoring because of the increasing importance the beekeeping has gained the last years.