UDC:619:614.31:638.16

DETERMINATION OF TETRACYCLINE RESIDUES IN HONEY

Burlacu Svetlana, PhD Student, svetlanaburlaku24@gmail.com

Technical University of Moldova

Antibiotics from the tetracycline group are widely used for prevention and control of infectious diseases and have a great activity against variety of Gram-positive and Gram-negative bacteria. Due to the widespread use of tetracyclins in animal husbandry, it can lead to an increase the risk of antibiotics remaining in human food. In this study, the degree of contamination of honey from the markets with antibiotics from the tetracycline group was examined. Antibiotics from the tetracycline group: Tetracyclin, Oxitetracyclin and Chlortetracyclin were examined by competitive enzyme immunoassay method for the quantitative analysis using the kits R-biopharm, Biopanda Reagen oxitetracyclin and chlortetracyclin. The principle of the method is to identify the unknown amount of antibiotic by present in the sample and the fixed amount of antibiotic antigens pre-coated on the wells of microtiter strips compete for the anti-antibiotics antibodies, which in turn is detected with enzyme conjugate. After incubation the wells are washed and the bound enzyme is visualised by adding TMB solution. Any *coloured product is measured at 450 nm after adding stop solution. The absorbance value of the developed colour is inversely proportional to the amount of the antibiotics in the sample. The quantity of antibiotics in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.*

Key words: *honey, tetracycline group, contamination, monitoring.*

Introduction. Tetracycline antibiotics are broad-spectrum medicinal drug compounds active against a number of gram-positive and gram-negative bacteria. Its have been successfully used worldwide in both veterinary and human medicine. These antibiotics are widely used as veterinary drugs for food-producing animals, including honeybees, because of their broad-spectrum activity and cost effectiveness [6].

In beekeeping, tetracyclin antibiotics are used to treat bacterial brood diseases such as American Foulbrood caused by *Paenibacillus larvae* and European Foulbrood caused by *Melissococcus pluton*. As these drugs have been widely used for prevention and treatment of diseases, and often have not been followed the label direction in its use, its residues often remain in food [3].

Currently, European regulation No. 37/2010 (Commission Regulation (EU) No. 37/2010) has not established MRLs for antimicrobial substances in honey and therefore the use of antibiotics in beekeeping is not allowed in the European community. The absence of MRLs therefore means “zero tolerance” for antibiotic residues in honey, which corresponds to the detection limit of the analytical method used [6].

Even though antibiotic drugs are not authorized for the treatment of bees, many studies show the presence of residues in honey, raising the suspicion that this is caused mainly by illegal use in beekeeping. During flight, as bees are exposed to various pollutants dispersed in the environment, the antibiotic contamination could result from their presence in the territory. Indeed, several studies show the presence of pharmacological substances in different environmental compartments such as soil and water [5].

Monitoring antibiotic residues in honey helps to avoid potential risk to human health, as it is a natural product widely used for both nutritional and medicinal purposes in all population groups including the most vulnerable, as well as children. Honey can also be used to manage deteriorative oxidation reactions in food like fruit and vegetables and/or lipid oxidation in meat inhibiting moreover the growth of pathogens and microorganisms that cause food decomposition. Potential contamination of honey can derive from different environmental sources, connected to intensive agriculture and industrial activities, but antibiotics are also commonly used by beekeepers to prevent and treat disease among honeybees, even if this practice is prohibited in the European Union [2].

Several methods have been reported for the determination of tetracyclines using a variety of techniques, including high performance liquid chromatography with ultraviolet detection UV-HPLC, and liquid chromatography–mass spectrometry methods. These methods are sensitive and confirmatory, but the expensive instruments may not be available in every laboratory [4].

Comparison with those instrumental methods, ELISA is a low cost and sensitive method capable of screening large amount of samples in a single test. Although the aforementioned analytical methods offer predominant accuracy and high-throughput screening capability, they are relatively resource demanding and multiple steps of these methods hinders their instant and filed applications. Accordingly, it still remains tremendous requirements to construct facile detection methods with satisfactory simplicity, speed and cost, results visible by naked eye, small sample volume requirement, shorter detection time, ease of mass production and portability [1].

Materials and Methods.

Sampling. For research, 12 samples of honey from several districts of the Republic of Moldova were purchased. The honey is produced this year. The types of honey are diverse: acacia, linden, rapeseed, sunflower and polyflora. The honey was collected in 300g glass jars. The jars were well washed and rinsed with distilled water. Until the research was carried out, it was kept at a temperature of 6°C.

Honey sample preparation. From oxitetracyclin: Honey samples (dilution factor 4). Weigh 1.0 g of honey into a centrifuge tube with a volume of 50 ml, add 3.0 ml of solution 4, vortex for 5 min and shake well for 15 min. Centrifuge 5min/4000rpm. For a nalysis used 100 µl.

From clortetracyclin: BIOPANDA Reagents, honey (dilution factor – 50). Weigh 1 g of honey in a 15 ml centrifuge tube and add 9 ml of deionized water, vortex for 5 minutes, centrifuge for 10 min/5000g/room temperature (20-25ºC), transfer 1 ml of the supernatant to another tube and add 1 ml of n-hexane, vortex for 5 min, centrifuge for 10 min/ 5000g/ room temperature (20-25ºC), remove the upper layer of n-hexane, transfer 100µl of the lower layer solution into a 1.5 ml centrifuge tube and add 400 µl of diluted assay diluent II.

From tetracyclin: R-biopharm manufactures.Weigh 1 g of honey in a screw top glass vial 80ml, dilute 1:50 (1+49) with 20mM PBS buffer. Mix intensively for 2min on a vortex. Before use in the test shake the upside down briefly. Use 50µl per well in the assay.

Chemicals and reagents. Ridascreen kits from R-biofarm, and Biopanda reagents were used for the enzyme-linked immunoassay method. The standard solutions and all reagents used were of high purity.

Results and discussion. Following the validation of the methods in the laboratory, the limits of detection and quantification were established. The decision limit (CCα) and the detection capacity (CCβ) were also calculated (Table 1). The given parameters allow us to demonstrate what the residue content is and if it is necessary to receive a decision regarding the product with the given quantity of the substance.

Table 1.**Value calculated LOD, LOQ, CCα, CCβ**

Antibiotic	matrix	MRL/MRPL	LOD	LOQ	CCα	CCβ
		ppb	ppb	ppb	ppb	ppb
Tetracyclin	honey	10,0	6,069	9,57	7,33	7,66
Oxitetracyclin	honey	10,0	1,13	2,56	-	7,87
Chlortetracyclin	honey	10,0	1,38	2,76	-	7,79

European regulation No. 37/2010 and International Food Standards Codex Alimentarius established MRLs for tetracyclin/oxitetracyclin/chlortetracycline in muscle cattle, pig, sheep, poultry, fish 200µg/kg, in eggs 400µg/kg, in milk 100µg/kg. For honey is not established MRLs.

MRL/MRPL were established by Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results for tetracyclin antibiotics residues 10ppb.

From the 22 investigated samples, in 2 samples tetracycline was detected with a content of 23ppb and 18ppb, and in 4 samples oxytetracycline was detected in various concentrations. Chlortetracycline was not detected (Table 2).

Table 2. **Antibiotic concentrations in honey**

Honey	**Tetracyclin**	**Oxitetracyclin**	**Chlortetracyclin**
	ppb	ppb	ppb
1 - acacia	23,0	< LOQ	< LOQ
2 - linden	< LOQ	32,0	< LOQ
4 - rapeseed	< LOQ	15,0	< LOQ
5 - rapeseed	< LOQ	21,0	< LOQ
10- sunflower	18,0	< LOQ	< LOQ
12- polyflora	< LOQ	27,0	< LOQ

Concluzions. Out of 12 honey samples, antibiotics were detected in 6, which is 50% of the total number of samples. Of the types of honey, tetracycline was detected in acacia and sunflower, and oxytetracycline was detected in linden, rapeseed and polyflora. The antibiotic-free honey samples are 1 linden sample, 3 sunflower samples and 2 polyflora samples.

References:

1. Svetlana Burlacu, Valentina Stici, Valeriu Enciu, Evaluation of the presence of nitrofurans in meat and chicken eggs, International Scientific Conference on Microbial Biotechnology 5th edition, Institute of Microbiologi and Biotechnologi, Republic of Moldova, 12-13 october 2022, p.78-81, https://doi.org/10.5 2757/imb22.54

2. Elisabetta Bonerbaa, Sara Panserib, Francesco Ariolib, Maria Nobileb, Valentina Terioa, Federica Di Cesareb, Giuseppina Tantilloc, Luca Maria Chiesab, Determination of antibiotic residues in honey in relation to different potential sources and relevance for food inspection, Journal Food Chemistry, 2021 Jan 1; 334 :127575;

3. Moonsun Jeon, Insook Rhee Paeng, Quantitative detection of tetracycline residues in honey by a simple sensitive immunoassay, Journal Analytica Chimica Acta 626, 2008, 180–185;

4. Nuria Pastor-Navarro, Sergi Morais, Angel Maquieira, Rosa Puchades, Synthesis of haptens and development of a sensitive immunoassay for tetracycline residues Application to honey samples, Journal Analytica Chimica Acta, Volume 594, Issue 2, 2 July 2007, Pages 211-218;

5. Hendrik De Ruyck, Herman De Ridder, Determination of tetracycline antibiotics in cow's milk by liquid chromatography/tandem mass spectrometry, Rapid Communications in Mass Spectrometry. 2007; 21: 1511–1520 Published online in Wiley InterScience, DOI: 10.1002/rcm.2991;

6. Alessandra Emilia Savarino, Valentina Terio, Roberta Barrasso, Edmondo Ceci, Sara Panseri, Luca Maria Chiesa, Elisabetta Bonerba, Occurrence of antibiotic residues in Apulian honey: potential risk of environmental pollution by antibiotics, Italian Journal of Food Safety 2020; p.14, 9:8678 doi:10.4081/ ijfs . 2020.8678.