Tetracyclines Residues in Honey

OLIMPIA DUMITRIU BUZIA[1], GABRIELA PLOSCUTANU[2]*, ALINA MIHAELA ELISEI[3]

[1]Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Department of Morphological and Functional Sciences, 35 Al. I. Cuza Str., 800010, Galati, Romania

[2]Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, 111, Domneasca Str. , 800201, Galati, Romania

[3]Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Research Center in the Field of Medical and Pharmaceutical Sciences, Pharmacology Sciences Department, 35 Al. I. Cuza Str., 800010, Galati, Romania

Antibiotic residues have toxic acute and chronic effects on human health and also reduce the efficacy and quality of honey. This review was conducted aiming to evaluate the tetracyclines (TCs) residues in honey, compared to international standards available in this field. In conclusion, our study demonstrated that a worldwide concerted effort is required to uphold the all-natural, wholesome and clean and green image of honey.

Keywords: honey, tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), doxycycline (DC)

Honey producers and consumers

Tetracyclines (TCs), a family of antibiotics with broad-spectrum activity, are frequently used to treat bacterial infections. Its use as veterinary drug is banned in the EU, but is still widely used in countries like USA, Canada, Australia, India, Argentina [1-3].

Honey is being used as a pure natural and as an ingredient in many foods, pharmaceuticals and cosmetics than ever before, so honey testing has become essential to maintain its healthful characteristics and protect public health. TCs are used for the treatment or prevention of American and European foul broad in bee colonies which are caused especially by two species of bacteria - *Paenibacillus larvae* and *Melissococcus pluton* [4].

Honey is defined as the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in honeycombs to ripen and mature [5]. Through the content of its composites bearing antioxidant effect, the honey could contribute to decreasing/preventing the oxidative stress [6].

Antibiotics can accumulate in beehives and migrate from the hives to honey, propolis, royal jelly and wax, resulting in contamination of these bee products [7].

Honey and bee products have the image of being natural, healthy and clean [8]. The presence of TC and its degradation products in honey may have harmful effects on consumers, such as possible allergic reactions, liver damage, yellowing of teeth and gastro-intestinal disturbance due to the selective pressure of antibiotics on the micro flora of human gut [9].

Muhammad et al. (2009) reported that indirect and long term consequence of the ingestion of low-dose of antibiotics by consumers include microbiological effects, carcinogenicity, reproductive effects and teratogenicity [10].

The consumer is often faced with worthless substitutes but sometimes also with a dangerous cocktail of chemicals such as antibiotics, colourings and hydroxymethyl furfural (HMF) in honey [11].

Good agricultural practices, good beekeeping practices, good hygiene practices, and good manufacturing practices all apply to honey production. The use of these good practices from the supply of inputs through to product distribution promotes quality during production, processing, and packaging and provides quality assurance and accreditation to verify honey quality. Some countries, such as Australia, Canada, New Zealand, USA and Japan, have adopted national best practice guidelines for the production and distribution of honey [12].

Tetracyclines group

The history of TCs involves the collective contributions of thousands of dedicated researchers, scientists, clinicians, and business executives over the course of more than 60 years [13].

TCs produced by *Streptomyces* spp. are broad-spectrum agents, exhibiting activity against wide range of Gram-positive and Gram-negative bacteria, parasites, atypical bacteria (chlamydiae, rickettsiae, mycoplasmas). The members of the TCs group include tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC) and doxycycline (DC) [14].

TCs are broad-spectrum antibiotics that consist of a substituted 2-napthacenecarboxamide molecule. They are widely used in veterinary medicine for cost-effective prophylactic and therapeutic treatment. TC antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex [15]. The structures of TCs are presented in figure 1.

Figure 2 shows TCs 3D spectra in honey, in which optimal resolution can be observed [17].

Epi-tetracycline (ETC), epi-anhydrotetracycline (EATC) and anhydrotetracycline (ATC) (fig. 3) may be present in TC as impurities. These compounds may form during storage under adverse conditions of temperature and humidity. Anhydro derivatives may also be found in out-of-date samples of TC. These compounds are either inactive as antibiotics or toxic. Hence, the permitted concentrations of these impurities in pharmaceutical preparations fixed by the European Pharmacopoeia are 0.5% ETC and 0.05% EATC and ATC [18].

ETC is the major degradation product of TC in honey [19]. Therefore, it was not surprising that TC was found mostly together with ETC. OTC was also found several times though it is known to be chemically unstable in honey [20].

**email: Gabriela.Ploscutanu@ugal.ro*

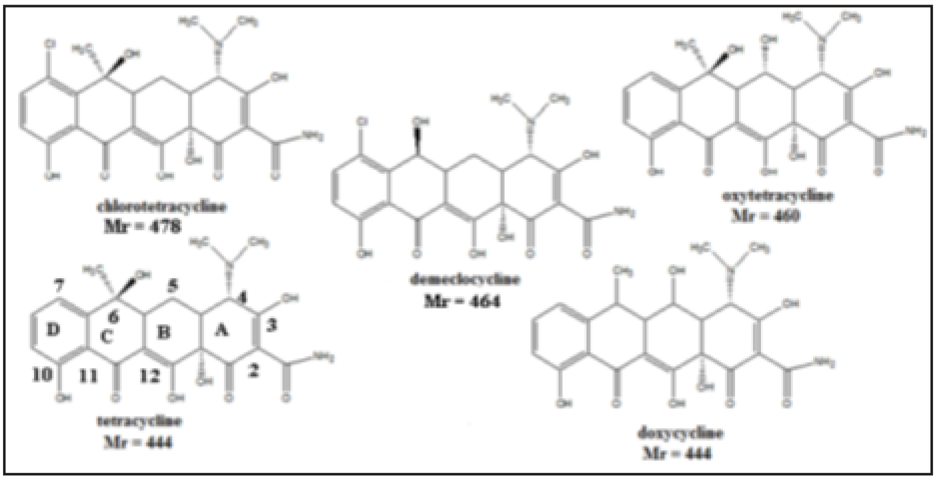

Fig. 1. Chemical structure of TCs [16]

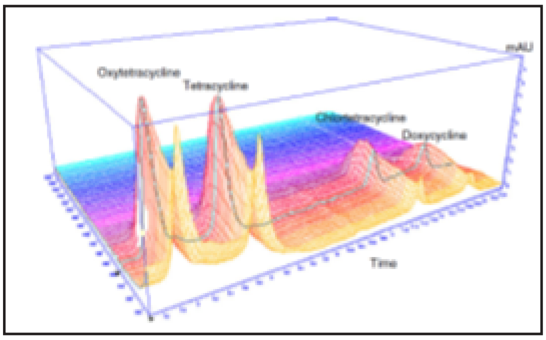

Fig. 2. 3D absorption spectra of OTC, TC, CTC and DC [17]

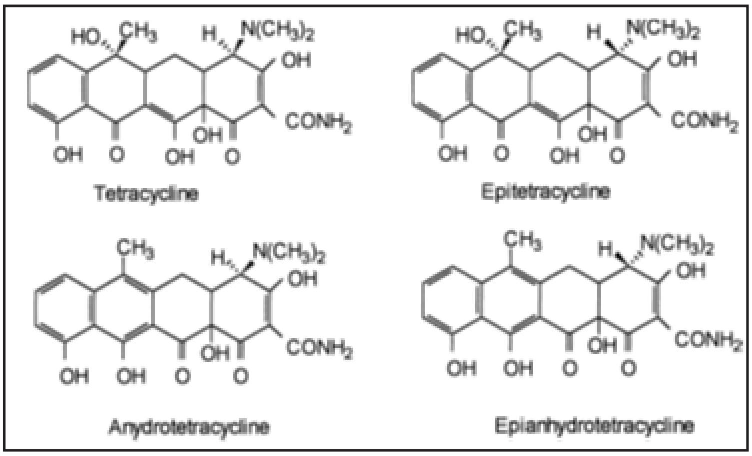

Fig. 3. Structures of TC, ETC, ATC and EATC [18]

Table 1
COMPARISON OF DIFFERENT REGULATION FOR TCs IN HONEY

Country/Regulation	Maximum residue limits (μg/kg)				References
	TC	CTC	OTC	DC	
Codex Alimentarius	-	-	-	-	[23]
EU, European Regulation 37/2010	-	-	-	-	[22]
USA, Code of Federal Regulations - Title 21 Part 556	-	-	300	-	[24]
Canada, List of Maximum Residue Limits for Veterinary Drugs in Foods	-	-	300	-	[25]
Australia/New Zealand, Food Standards 1.4.2 - Schedule 20					[26]
Brazil, Normative Instruction 11/8.03.2017	20	20	20	20	[27]
Japan, The Japanese Positive List System for Chemical Residues in Foods	0.1	-	-	-	[28]
India - EIC, Standards for Honey and prohibition of antibiotics	5	5	-	-	[29]

A challenge in TC determination is their epimerization. In mildly acidic conditions (*p*H = 2 - 6), epimerization occurs at position C-4. Accordingly, European Union MRLs in food are established as sum of TC and its epimer, that is, TC and ETC, OTC and epi-oxytetracycline (EOTC), CTC and epi-chlortetracycline (ECTC) [21].

Legislation of honey

In order to guaranties the nomination of honey and also protect human health, the use of antimicrobials in apiculture is usually strictly regulated or banned. According to Regulation (EC) No 470/2009 [21] and Regulation (EU) No 37/2010 [22], in the European Union, no maximum residue level (MRL) for TC and any other antibacterial substance residues in honey are allowed.

Despite this decision, some countries have established action limits or tolerated levels for TC in honey. For instance, in Belgium, the action limit for the group of TC has been fixed at 20 μg/kg. France applies a nonconformity limit for TC in honey of 15 μg/kg, the reporting limit in Great Britain is 50 μg/kg, while the tolerance level in Switzerland is 20 μg/kg. In Japan, based on microbiological research, a value of 0.1 mg/kg was introduced as the allowed residual quantity of TC in honey. Australia, Indian, American and the US Food and Drug Administration (USFDA) have set MRL for only OTC in honey at 300 ppb [4]. Worldwide limits for TCs in honey can be observed in table 1.

However, it is certainly the case that TCs are authorized for the treatment of honeybees in many third countries. This situation may potentially raise some problems with imports of honey into the EU [17].

Methods for the determination of TCs residues in honey

In routine honey analysis TCs are generally tested by:

1. screening, determination of positive samples: Charm II Test, ELISA (enzyme-linked immunosorbent assay);

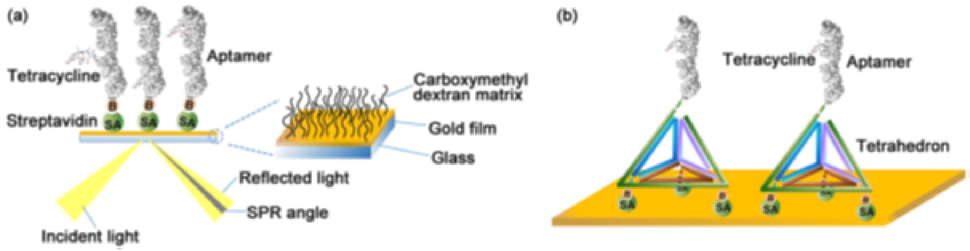

Fig. 4. Scheme of SPR aptasensor for direct capture of tetracycline (a) ss-aptamer-based aptasensor for direct capture of tetracycline; (b) oriented immobilization of aptamer (Apt76) assisted by DNA tetrahedron nanostructure [46]

2. quantitative determination of positive samples by HPLC, LC-MS [8, 30-32].

Different analytical methods are reported for the determination TC residues in honey. These include HPLC with UV detection [33-40], HPLC with fluorescence detection [41, 42] liquid chromatography with mass spectrometry [43, 44]. Nowadays, liquid chromatography coupled with mass spectrometry and tandem mass spectrometry seem to be the techniques of choice for analysis of these groups of antibiotics [45].

For the first time Wanga et al., (2018) report a tetrahedron-assisted aptamer-based SPR (Surface Plasmon Resonance) biosensor for automatic screening of small molecules. The aptasensor was then validated in real world application for tetracycline screening in multiple honey samples, achieving good recovery rates of 80.20-114.3%, intuitive sensorgrams indicating the binding kinetic properties, and high specificity towards tetracycline. Figure 4 illustrates the construction of SPR aptasensor [46].

Because of their polar nature, TCs have the ability to strongly bind to proteins as well as to chelate with divalent metal ions. The analytical steps of each selected method (sample treatment, analytical technique and detection limits) are summarized in tables 2 [47].

International reports of TCs residues in honey samples

There are several international reports of antibiotic residues in honey samples.

A total of 567 Basque honey samples were analyzed with the Charm II system. 24 samples were presumptive positive for TCs [56]. The residues were confirmed by liquid chromatography fluorescence detection (LC-FD) and tandem mass spectrometry (LC-MS/MS), according to the latest EU criteria for the analyses of veterinary drug residues [57]. The TC levels was from 15 to 920 µg/kg. Residues of veterinary drugs were confirmed in a very limited number of honey samples: tetracycline (4.22%) [56]. In another Spanish study by Vidal et al. (2009), in which 251 honey samples was analyzed. 19% of the samples have found to be contaminated by the residue of TC [58].

In a study in which 251 honey samples collected across Greece were analyzed by LC to detect TC - derived residues, 29% of the samples had TC residues. The range of the detected amounts of each observed drug residue in the examined samples was 0.018-0.057µg/kg, 0.023-0.335 µg/kg, 0.018-0.190 ìg/kg and 0.013-0.393 µg/kg for TC, OTC, DC and CTC respectively. The reason for this frequent use could have arisen from easier access connected with pricing, flexibility of use or the need to go above the normal dose in response to dwindling efficacy [59].

Table 2
CONFIRMATORY METHODS FOR TCs [47]

Compounds[a]	Extraction/ clean-up	Separation		Equipment	LOD[c] (µg/kg)	References
		Column	Mobile phase			
CTC, DC, MINO, TC MTC, OTC	MacIlvaine buffer (Na_2EDTA) (pH 4.0)/phenyl-SPE	Discovery RP-Amide C16 (5.0 µm)	Gradient: 0.09% OA (pH 3.0)/CAN	LC-DAD	15-30	[44]
CTC, DC, OTC, TC	50 mM oxalate buffer (pH 4.0)/ Oasis HLB-SPE	Atlantis dC18 (150 × 2.1 mm, 3 µm)	Gradient: 1% FA/ ACN:MeOH (50:50, v/v)	LC-MS/MS	3.3	[48]
CTC, OTC, TC	MacIlvaine buffer (Na_2EDTA) (pH 4.0)/hexane, PLS-2-SPE[b]	Hydrospher C18 HS-301–3 (100 × 4.6 mm, 3.0 µm)	Isocratic: 1M Imidazole buffer/MeOH (82:18, v/v)	LC-FLD	5-9	[49]
CTC, OTC, TC	Citrate buffer, (Na_2EDTA)/ PLS-2-SPE[b]	Tsk-gel ODS-80Ts (150 × 4.6 mm)	Isocratic: 1 M Imidazole buffer/MeOH (75:25, v/v)	LC-DAD	10-20	[50]
CTC, DC, OTC, TC	5% HCl/ MIP-SPE	Restek C18 (150 × 2.1 mm, 5.0 µm)	Isocratic: 100 mM OA/ACN/MeOH (70:20:10, v/v/v)	LC-MS/MS	0.1-0.3	[51]
CTC, OTC, TC	ACN/SPE (home-made sorbent)	ShodexRSpak DE-613 (150 × 6.0 mm)	Isocratic: 0.05% TFA/ACN (60:40, v/v)	LC-MS/MS	3-20	[52]
CTC, OTC, TC	50 mM NH4Ac buffer (pH 5.5)/ MCAC-SPE, Oasis HLB-SPE	Waters Phenyl (100 × 2.1 mm, 3.5 µm)	Gradient: 0.1% FA/0.1% FA in ACN:MeOH (50:50, v/v)	LC-MS/MS	7.2-7.7	[53]
TC	MacIlvaine Buffer (pH 4.0)/ Strata-X-SPE	Symmetry C18 (150 × 2.1 mm, 3.5 µm)	Gradient: 0.05% AcOH/0.05% AcOH in CAN	LC-MS/MS	5.5-9.2	[54]
OTC, TC	Water/chitosan modified graphitized	SB-C18 (50 × 4.6 mm, 5 µm)	Gradient: 0.1% FA/MeOH	LC-HRMS	0.6-10	[55]

[a]DMC - demeclocycline; MINO - minocycline; MTC - methacycline.
[b]PLS-2, polystyrene-divinylbenzene polymer (RP-SPE).
[c]LOD - limit of detection.

Table 3
SUMMARY OF REVIEWED STUDIES FOR THE DETECTION OF TCs

Honey samples		Analysis methods	TC		OTC		Reference
			Positive samples		Positive samples		
Country	n		(%)	Range (µg/kg)	(%)	Range (µg/kg)	
Algeria	36	HPLC-MS	-	-	5	0.03-3	[67]
Belgium	72	HPLC-FLD	2,8	10-30	-	-	[31]
Greece	251	HPLC	5	18-57	14	23-335	[59]
India	12	HPLC-FLD	-	-	50	27.10-250.40	[68]
	8	HPLC-UV	-	-	100	0.05-0.96	[40]
Iran	145	HPLC	-	-	23	2.10-120.60	[69]
	145	ELISA	-	-	23	5.32-369.10	[69]
Pakistan	100	HPLC	7	3.67-16.31	-	-	[70]
Romania	10	HPLC-UV	30	20-23	-	-	[71]
	12	ELISA	50	15.47- 60.67	-	-	[72]
	130	HPLC-FLD	-	-	-	-	[66]
Spain	567	LC-MS/MS	4	15-920	-	-	[56]
Thailand	6	HPLC-FLD	33	0.1-14.01	66	32.53-106.9	[42]
Turkey	50	LC-ESI-MS	-	-	-	-	[61]
Yemen	16	HPLC-UV	12	2.33-2.85	31	3.4-13.8	[56]

HPLC (high performance liquid chromatography); HPLC-UV (HPLC with ultraviolet detector); HPLC-FLD (HPLC with fluorescence detection); ELISA (enzyme-linked immuno-sorbent assay); LC-FD (liquid chromatography fluorescence detection); LC-MS/MS (liquid chromatography mass spectrometry); LC-ESI-MS (LC electrospray ionization mass spectrometry); - not detected.

Reybroeck (2003) monitored 248 samples of locally produced and imported honey on the Belgian market for the presence of residues of antibiotics in the period 2000-2001. According to them residues of antibiotics were found in a very limited number of honey samples produced in Belgia and TC was detected in 2 out of 72, samples [31].

In China the near infrared spectrum detection technology (NIR) has been used in the detection of TCs residues in 153 honey samples. The TC content in honey was very low (10^{-7} - 10^{-9}), in 41 samples [60].

50 honey samples collected from Southern Marmara region in Turkey were analyzed for the presence of OTC residues by using LC-MS system. Samples were free from residues [61].

30 German and 47 imported non-European honeys were analyzed for TCs. 22 of the imported honeys contained residues (in most cases more than one), whereas 29 of the 30 German samples were free of residues [62].

A total of 16 samples of honey were collected from Ethiopia. TC analysis was done using a Tetrasensor test. No TC residues were detected [63].

In France, TC residues were detected in honey after a treatment in hives, indicating their persistence and diffusion into the apiary. These results showed that the TC must be used with precaution in honey production [64].

In UK, a study aimed to assess OTC residue levels in honey after treatment of honeybee colonies with two methods of application in liquid sucrose and in powdered icing sugar. The samples of honey were extracted up to 12 weeks after treatment. It was demonstrated that the method of application of OTC in liquid form results in high residue levels in honey with residues of 3.7 mg/kg, eight weeks after application [65].

The results obtained in antibiotic analysis of 130 samples shows that honey have no content of TCs. This shows that Romanian polyflora honey is superior than others, the most valuable honey is harvested from Transylvania region, an area of the mountain, which raises the value and prestige of Romanian honey [66].

Table 3 summarizes the analytical performance for the detection of tetracyclines reported in the literature.

Residual levels of contaminants cannot be changed through various production techniques; therefore, adequate monitoring is required [73, 74].

Conclusions

Honey is a natural product that is widely used for both nutritional and medicinal purposes. TCs consumed along with honey can produce resistance among bacteria in the consumers and consequently, there is difficulty in treating many infections in humans. There are studies [75,76] that demonstrate bacterial resistance to antibiotics as a result of their abusive or coincidental use such as the use of honey with significant content in antibiotics such as tetracycline.

It is useful, under specific circumstances, that new substances - perhaps plant derived products of limited adverse reaction potential - and non-aggressive alternative treatments to be investigated and used [77-80] for the prevention and management of chronic diseases.

TCs residues in honey originate mostly from improper beekeeping practices and not from the environment. Beekeepers should aware and reduce the use of antibiotics in bee honey in order to control honey quality and for the safety of consumers.

Standardized and updateable analytical protocols need to be established to determine contamination of honey.

References

1.CHOPRA, I., ROBERTS. M., Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance, Microbiol. Mol. Biol. Rev., 65, 2001, p. 2320-2326.

2.KREPPER, G., PIERINI, G. D., PISTONESI, M. F., DI NEZIO, M. S., In-situ antimony film electrode for the determination of tetracyclines in Argentinean honey samples, Sens. Actuators B, 241, 2017, p. 560-566.

3.CASEWELL, M., FRIIS, C., MARCO, E., MCMULLIN, P., PHILLIPS, I., The European ban on growth-promoting antibiotics and emerging consequences for human and animal health, J. Antimicrob. Chemother., 52, no 2, 2003, p. 159-161.

4.SALEH, S. M. K., MUSSAED, A. M., AL-HARIRI. F. M., Determination of tetracycline and oxytetracycline residues in honey by High Performance Liquid Chromatography, J. Agr. Sci. Tech. B, 6, 2016, p. 135-139.

5.COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 relating to honey, p. 1-9.

6.DOBRE, I., GADEI, G., PATRASCU, L., ELISEI, A. M., SEGAL, R. The antioxidant activity of selected Romanian honeys, The Annals of the University Dunarea de Jos of Galati Fascicle VI - Food Technology, 34, no. 2, 2010, p. 67-73.
7.BIE, M., LI, R., CHAI, T., DAI, S., ZHAO, H., YANG, S., QIU, J., Simultaneous Determination of tetracycline antibiotics in beehives by Liquid Chromatography - Triple Quadrupole Mass Spectrometry, Adv. Appl. Sci. Res., 3, no. 1, 2012, p. 462-468.
8.BOGDANOV, S., Contaminants of bee products, Apidologie, 37, no. 1, 2006, p. 1-18.
9.THANASARAKHAN, W., KRUANETR, S., DEMING, R. L., LIAWRUANGRATH, B., WANGKARN, S., LIAWRUANGRATH, S., Sequential injection spectrophotometric determination of tetracycline antibiotics in pharmaceutical preparations and their residues in honey and milk samples using Yttrium (III) and cationic surfactant, Talanta, 84, no. 5, 2011, p. 1401-1409.
10.MUHAMMAD, F., AKHTAR, M., ZIAUR-RAHMAN, I. J., ANWAR, M. I., Role of veterinarians in providing residue-free animal food, Pak. Vet. J., 29, 2009, p. 42-46.
11.ZABRODSKA, B., VORLOVA, L., Adulteration of honey and available methods for detection - a review, ACTA VET. BRNO, 83, 2014, p. S85-S102.
12.*** AHBIC (Australian Honey Bee Industry Council) National best management practice for beekeeping in the Australian environment. Sydney, Australia, 2007. Available at: https //www.honeybee.org.au/pdf/NBPFBIAE.pdf (accessed 23 July 2018).
13.NELSON, M. L., LEVY, S. B., The history of the tetracyclines, Ann. N. Y. Acad. Sci., 1241, 2011, p. 17-32.
14.CHERLET, M., SCHELKENS, M., CROUBELS, S., DE BACKER, P., Quantitative multi-residue analysis of tetracyclines and their 4-epimers in pig tissues by high-performance liquid chromatography combined with positive-ion electrospray ionization mass spectrometry, Anal. Chim. Acta, 492, 2003, p. 199-213.
15.ONAL, A., Overview on liquid chromatographic analysis of tetracycline residues in food matrices, Food Chem., 127, no. 1, 2017, p. 197-203.
16.DASENAKI, M., Development of methods for the determination of veterinary drugs in food matrices by Liquid Chromatography - Mass Spectrometry, Doctoral Thesis, National and Kapodistrian University of Athens, 2015, Available at: https://repository.edulll.gr/edulll/retrieve/11410/3596_1.54_%CE%94%CE%94_2_2_15.pdf (accessed 3 September 2018).
17.MOLINO, F., LAZARO, R., PEREZ, C., BAYARRI, S., CORREDERA, L., HERRERA A., Effect of pasteurization and storage on tetracycline levels in honey, Apidologie, 42, 2011, p. 391-400.
18.PENA, A., PALILIS, L. P., LINO, C. M., SILVEIRA, M. I., CALOKERINOS, A. C., Determination of tetracycline and its major degradation products by chemiluminescence, Anal. Chim. Acta, 405, 2000, p. 51-56.
19.ULAKOVA, V., KISS, E., KUSINOVA, J., SILHAR, S., Kinetics of degradation of oxytetracycline and tetracycline in honey, J. Food Nutr. Res., 47, no. 3, 2008, p. 139-143.
20.MUNSTEDT, T, RADEMACHER, E, PETZ, M., Chlortetracycline and oxytetracycline residues in honey after administration to honey-bees. In: Proceedings 4th International Symposium on Hormone and Drug Residue Analysis, Antwerp, Belgium, June 4-7, 2002, p. 147.
21.*** COMMISSION REGULATION (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council, Official Journal of the European Communities L152, 2009, p. 11-22.
22.*** COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009. Official Journal of the European Communities L15, 2010, p. 1-72.
23.*** FAO/WHO Codex Alimentarius: Veterinary Drugs Residues in Food Maximum Residue Limits, 2008, Available at: http://www.fao.org/fao-who-codexalimentarius/standards/veterinary-drugs-mrls/en/ (accessed 30 August 2018).
24.*** FDA 21 CFR 556 - Food Drug Administration Code of Federal Regulations. Tolerances for residues of new animal drugs in food, Title 21, Part 556 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=556 (accessed 30 August 2018).
25.*** GOVERNMENT OF CANADA. List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Food. Available from: http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau-eng.php (accessed 3 September 2018).
26.*** FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ) Code Standard 1.4.2. Schedule 20. Available at: https://www.legislation.gov.au/Series/F2008B00619 (accessed 30 August 2018).
27.*** MINISTERIO DA AGRICULTURA, PECUARIA E ABASTECIMENTO (MAPA), Secretaria Defesa Agropecuaria (SDA). Instrucao Normativa SDA No. 11/8.03.2017. Available at: http://www.agricultura.gov.br/assuntos/inspecao/produtos-animal/plano-de-nacional-de-controle-de-residuos-e-contaminantes/documentos-da-pncrc/pncrc-2017.pdf (accesse 1 September 2018).
28.*** THE JAPAN FOOD CHEMICAL RESEARCH FOUNDATION. The Japanese Positive List System for Agricultural Chemical Residues in Foods Available at: https://www.ffcr.or.jp/en/zanryu/the-japanese-positive/the-japanese-positive-list-system-for-agricultural-chemical-residues-in-foods-enforcement-on-may-29-.html (accessed 1 September 2018).
29.*** FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA (FSSAI). Standards for Honey and prohibition of antibiotics. Available at: http://www.assuranceindia.com/industry-standards/fssai-advisory-on-standards-for-honey-and-prohibition-of-antibiotics/ (accessed 30 August 2018).
30.BOGDANOV, S., Current state of analytical methods for the detection of residues in bee products, Apiacta, 38, 2003, p. 399-409.
31.REYBROECK, W., Residues of antibiotics and sulfonamides in honey on the Belgian market, Apiacta, 38, 2003, p. 23-30.
32.JAKSIC, S. M., RATAJAC, R. D., PRICA, N. B., APIC, J. B., LJUBOJEVIC, D. B., ZEKIC STOSIC, M. Z., ZIVKOV BALOS, M. M., Methods of determination of antibiotic residues in honey, J. Anal. Chem., 73, no. 4, 2018, p. 317-324.
33.BONTA, V., MARGHITAS, L. A., DEZMIREAN, D., MOISE, A., BOBIS, O., MAGHEAR, O., Optimization of HPLC method for quantifying tetracycline residue in honey, Bull. Uni. Agri. Sci. Veter. Med., 63, 2007, p. 86-190.
34.LI, J., CHEN, L., WANG, X., JIN, H., DING. L., ZHANG, K., ZHANG, H., Determination of tetracyclines residues in honey by on-line solid-phase extraction high performance liquid chromatography, Talanta, 75, no. 5, 2008, p. 1245-1252.
35.CARRASCO, P. A., CASADO, T. S., SEGURA, C. A., FERNANDEZ, G. A., Reversed-phase high-performance liquid chromatography coupled to ultraviolet and electrospray time-of-flight mass spectrometry on-line detection for the separation of eight tetracyclines in honey samples, J. Chromatogr. A, 1195, no.1, 2008, p. 107-116.
36.HAKUTA, T., SHINZAWA, H., OKAZI, Y., Practical method for the detection of tetracyclines in honey by HPLC and derivative UV-Vis spectra, Analytical Sciences, 25, 2009, p. 1149-1153.
37.ZANG, Y., DONG, H. C., YI, Z., HONG, T. H., Analysis of tetracyclines (TCs) residues in honey with HPLC-UV, Advan. Mat. Res., 159, 2010, p. 89-94.
38.PAGLIUCA, G., GAZZOTTI, T., SERRA, G., SABATINI, A. G., A scientific note on the determination of oxytetracycline residues in honey by HPLC with UV detection, Apidologie, 3, no. 6, 2002, p. 583-584.
39.GAJDA, A., POSYNIAK, A., BOBER, A., BLADEK, T., ZMUDZKI, J., Oxytetracycline residues in honey analyzed by liquid chromatography with UV detection, J. Api. Sci., 57, no. 1, 2013, p. 25-32.
40.RAO, C. R. M., KUMAR, L. C. A., SEKHARAN. C. B., Quantitative analysis of oxytetracycline residues in honey by High Performance Liquid Chromatography, Int. Res. J. Biological Sci., 4, no. 5, 2015, p. 59-65.

41.PENA, A., PELANTOVA, N., LINO, C. M., SILVEIRA, M. I. N., SOLICH, P., Validation of an analytical methodology for determination of oxytetracycline and tetracycline residues in honey by HPLC with fluorescence detection, J. Agri. Food Chem., 53, no. 10, 2005, p. 3784-3788.
42.TAOKAENCHAN, N., SANGSRICHAN, S., HPLC-Fluorescence detection method for quantitative determination of tetracycline antibiotic residues in honey, Naresuan Uni. Science J., 6, no. 2, 2010, p. 147-155.
43.ISHII, R., HORIE, M., MURAYAMA, M., MAITANI, T., Analysis of tetracyclines in honey and royal jelly by LC/MS/MS, Shokuhin Eiseigaku Zasshi, 47, no. 6, p. 277-283.
44.VINAS, P, BALSALOBRE, N, LOPEZ-ERROZ, C, HERNANDEZ-CORDOBA M., Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey, J. Chromatogr. A, 1022, 2004, p. 125-129
45.PATYRA, E., KWIATEK, K., Analytical procedure for the determination of tetracyclines in medicated feeding stuffs by liquid chromatography-mass spectrometry, J. Vet. Res., 60, 2016, p. 35-41.
46.WANGA, S., DONGB, Y., LIANGA, X., Development of a SPR aptasensor containing oriented aptamer for direct capture and detection of tetracycline in multiple honey samples, Biosens. Bioelectron., 109, 2018, p. 1-7.
47.MORETTI, S., SALUTI, G., GALARINI, R., Residue determination in honey. In: Honey analysis, De Toledo V. A. (eds.), IntechOpen, 2017, p. 325-365.
48.KHONG, S. P., HAMMEL, Y. A., GUY, P. A., Analysis of tetracyclines in honey by high-performance liquid chromatography/tandem mass spectrometry, Rapid Commun, Mass Sp., 19, 2005, p. 493-502.
49.FUJITA, K., ITO, H., ISHIHARA, M., INUKAI, S., TANAKA, H., TANIGUCHI, M.. Analysis of trace of tetracyclines in dark-colored honeys by high-performance liquid chromatography using polymeric cartridge and metal chelate affinity chromatography, J. Food Hyg. Soc. Jpn., 49, no. 3, 2008, p. 196-203.
50.HAKUTA, T., SHINZAWA, H., OKAZI, Y., Practical method for the detection of tetracyclines in honey by HPLC and derivative UV-Vis spectra, Analytical Sciences, 25, 2009, p. 1149-1153.
51.JING, T., GAO, X. D., WANG, P., WANG, Y., LIN, Y. F., HU, X. Z., HAO, Q. L., ZHOU, Y. K., MEI, S. R., Determination of trace tetracycline antibiotics in foodstuffs by liquid chromatography-tandem mass spectrometry coupled with selective molecular-imprinted solid-phase extraction. Anal. Bioanal. Chem., 393, 2009, p. 2009-2018.
52.TSUKAMOTO, T, YASUMA, M, YAMAMOTO, A, HIRAYAMA, K, KIHOU. T, KODAMA, S, INOUE, Y., Evaluation of sulfobetaine-type polymer resin as an SPE adsorbent in the analysis of trace tetracycline antibiotics in honey, J. Sep. Sci., 32, 2009, p. 3591-3595.
53.GIANNETTI, L., LONGO, F., BUIARELLI, F., RUSSO, M. V., NERI, B., Tetracycline residues in royal jelly and honey by liquid chromatography tandem mass spectrometry: validation study according to Commission Decision 2002/657/EC, Anal. Bioanal. Chem., 398, 2010, p.1017-1023.
54.TARAPOULOUZI, M., PAPACHRYSOSTOMOU, C., CONSTANTINOU, S., KANARI, P., HADJIGEORGIOU, M., Determinative and confirmatory method for residues of tetracyclines in honey by LC-MS/MS, Food Addit. Contam. Part A, 30, no. 10, 2013, p. 1728-1732.
55.XU, J. J., AN, M., YANG, R., TAN, Z., HAO, J., CAO, J., PENG, L. Q., CAO, W., Determination of tetracycline antibiotic residues in honey and milk by miniaturized solid phase extraction using chitosan-modified graphitized multiwalled carbon nanotubes, J. Agric. Food Sci., 64, 2016, p. 2647-2654.
56.BONVEHI, J. S., GUTIEERREZ, A. L., Residues of antibiotics and sulfonamides in honeys from Basque Country (NE Spain), J. Sci. Food Agric.,89, 2009, p. 3-72.
57.*** COMMISSION DECISION 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Union, L221, 2002, p. 8-36.
58.VIDAL, J. L. M., MDEL, A. L. M., GONZALEZ, R. R., FRENICH, A. G., Multiclass analysis of antibiotic residues in honey by ultra-performance liquid chromatography tandem mass spectrometry, J. Agric. Food Chem., 57, no. 5, 2009, p. 1760-1767.
59.SARIDAKI-PAPAKONSTADINOU. M., ANDREDAKIS, S., BURRIEL A., TSACHEV, I. Determination of tetracycline residues in Greek honey, Trakia J. Sci., 4, no. 1, 2006, p. 33-36.
60.CHEN, H., TU, Z., QING, Z., QIU, X., MENG, C., Feasibility study of veterinary drug residues in honey by NIR detection, Springer, IFIP Advances in Information and Communication Technology, 392, 2013, p. 150-156.
61.GUNES, M. E, GUNES, N., CIBIK, R., Oxytetracycline and sulphonamide residues analysis of honey samples from southern Marmara region in Turkey, Bulg. J. Agric. Sci., 15, no. 2, 2009, p. 163-167.
62.NAUMANN, G., MAHRT, E., HIMMELREICH, A., MOHRING, A., FRERICHS H., Traces of contamination - well preserved in honey, J. Verbr. Lebensm., 7, 2012, p. 35-43.
63.BELAY, A., SOLOMON, W. K., BULTOSSA, G., ADGABA, N., MELAKU, S., Botanical origin, colour, granulation, and sensory properties of the Harenna forest honey, Bale, Ethiopia, Food Chem., 167, 2014, p. 213-219.
64.MARTEL, A.C., ZEGGANE, S., DRAJNUDEL, P., FAUCON. J. P., AUBERT, M., Tetracycline residues in honey after hive treatment, Food. Addit. Contam., 23, no. 3, 2006, p. 265-273.
65.THOMPSON, H. M., WAITE, R. J., WILKINS, S., BROWN, M. A., BIGWOOD, T., SHAW, M., RIDGWAY, C., SHARMAN, M., Effects of European foulbrood treatment regime on oxytetracycline levels in honey extracted from treated honeybee (Apis mellifera) colonies and toxicity to brood, Food Add. Cont. Part A, 22, no. 6, 2005, p. 573-578.
66.IANCU, R., OPREAN, L,, TI£A, M. A., LENGYEL, E., CODOI, V., BOICEAN, A. G., SCHNEIDER, A. O., Physical-chemical analysis and antibiotic content of polyflora honey in Romania, Bulletin UASVM, 69, no. 1-2, 2012, doi.org/10.15835/buasvmcn-asb:69:1-2:8493 (accessed 3 July 2018).
67.DRAIAIA, R., CHEFROUR, A., DAINESE, N., BORIN, A., MANZINELLO, C., GALLINA, A., MUTINELL, F., Physicochemical parameters and antibiotics residuals in Algerian honey, Afr. J. Biotechnol., 14, no. 14, 2015, p. 1242-1251.
68.JOHNSON, S., JADON, N., Antibiotic Residues in Honey. New Delhi, India: Center for Science and Environment, 2010, Available at: https://cdn.cseindia.org/userfiles/Antiboitics_Honey.pdf (accessed 3 July 2018).
69.MAHMOUDI, R., MOOSAVY, M., NORIAN, R., KAZEMI, S., NADARI, M. R. A., MARDANI, K., Detection of oxytetracycline residues in honey samples using ELISA and HPLC methods, Pharmaceutical sciences, 19, no. 4, 2014, p. 145-150.
70.ZAI, I. U. M., REHMAN, K., HUSSAIN, A., SHAFQATULLAH, Detection and Quantification of Antibiotics Residues in Honey Samples by Chromatographic Techniques, Middle-East J. Sci. Res., 14, no. 5, 2013, p. 683-687.
71.MOISE, A., MARGHITAS, L. A., CORRADINI, D., GREGO, S., Tetracycline determination from honey using HPLC method, Bulletin USAMV-CN, 60, 2004, Available at: http://dspace.unitus.it/dspace/handle/2067/1777?mode=full (accessed 1 September 2018).
72.CARA, M. C., DUMITREL, G. A., GLEVITZKYAND, M., PERJU. D., Stability of tetracycline residues in honey, J. Serb. Chem. Soc., 77, no. 7, 2012, p. 879-886.
73.AL-WAILI, N., SALOM, K., AL-GHAMDI, A., ANSARI, M, J., Antibiotic, pesticide, and microbial contaminants of honey: human health hazards, The Scientific World Journal, 2012, doi:10.1100/2012/930849.
74.CHITESCU, C. L., LUPOAE, M., ELISEI, A. M., Pharmaceutical Residues in the Environment - New European Integrated Programs Required, Rev. Chim. (Bucharest), **67**, no.5, 2016, p. 1008-1013.
75.DRAGANESCU M, IANCU A.V., FIRESCU D, DUMITRIU BUZIA.O., DIACONU C, REBEGEA L, Trends in antimicrobials consumption and antimicrobial resistance in an infectious diseases hospital from the south-eastern,Farmacia,2016,Vol 64,5,pag.770-774

76.DRAGANESCU, M., BAROIU, N., BAROIU, L.,DIACONU, C.,DUMITRIU BUZIA,O.,Efficeint administration of human albumin in clostridiumDifficile infection, Rev. Chim.(Bucharest), **68**, no. 3 , 2017, p.602-604
77.DUMITRIU BUZIA,O.,FASIE,V., MARDARE,N., DIACONU,C., GURAU, G.,TATU, A.L.,Formulation,preparation,physico-chimical analysis, microbiological peculiarities and therapeutic challenges of extractive solution of Kombucha, Rev.Chim. (Bucharest), **69**, no. 3, 2018, p. 720-724.
78.NWABUDIKE, L.C., ELISEI,A. M., BUZIA, O. D., MIULESCU, M., TATU, A. L., Statins. A review on structural perspectives, adverse reactions and relations with non-melanoma skin cancer, Rev. Chim. (Bucharest), **69**, no. 9, 2018, p. 2557-2562.
79.ROBU S., CHESARU B. I., DIACONU C., DUMITRIU BUZIA. O., TUTUNARU D., STANESCU, U., LISA, E. L., Lavandula hybrida: microscopic characterization and the evaluation of the essential oil, Farmacia, vol. 64, 2016, p. 6.914-6.917.
80.TATU,A.L.,CIOBOTARU,O.R., MIULESCU, M., DUMITRIU, BUZIA. O.,ELISEI, A.M., MARDARE,N., DIACONU,C., ROBU,S., NWABUDIKE, L.C., Hydrochlorothiazide: chemical structure, therapeutic, phototoxic and carcinogenetic effects in dermatology, Rev. Chim., (Bucharest), **69**, no. 8, 2018, p. 2110-2114.

Manuscript received: 21.12.2018