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STUDY ON IMPACT OF BOILING AND MICROWAVING ON OXYTETRACYCLINE RESIDUES IN RAW COW'S MILK USING THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

By

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ABSTRACT

Antibiotics abundant use in veterinary medicine may result in the presence of their residues in animal products for human consumption, especially in milk at unsafe concentrations that increase the potential health risks to consumers due to their adverse effects. The aim of the current study was carried out to detect the effect of two thermal treatments including, boiling and microwaving on oxytetracycline residues in raw cow's milk using ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) technique. One hundred of raw cow's milk samples were collected from different markets and retail dairy shops in various districts of Damietta governorate. Oxytetracycline residues extraction was carried out by solid phase extraction method. From analytical check performed by HPLC, it was confirmed that 5% of total raw cow's milk samples had different values of oxytetracycline residues ranged from 35.5 to 112 ppb. Referring to the maximum residual level (MRL) that 100 µg/kg for oxytetracycline in milk, only 1% of analyzed samples has been confirmed with value higher than this limit. The observed oxytetracycline residual levels in milk samples were reduced after application of the thermal effect by boiling and microwaving up to 80.5% and to 92.5%, respectively. The obtained results revealed absence of possible transformed metabolites of oxytetracycline because of boiling or microwaving of positive samples concluding 4-epioxytetracycline (4e-OTC), α-apo-oxytetracycline (α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC). Moreover, boiling and microwaving on oxytetracycline residues in raw cow's milk do not guarantee a full elimination but it can reduce its quantities largely. Consequently, will discuss the findings, health risks and economic importance, and those must be recommended.

Keywords:
Oxytetracycline, oxytetracycline epimer metabolites, thermal treatment, boiling, microwaving, HPLC, UPLC-MS/MS, raw cow's milk.

INTRODUCTION

Liquid milk is a common health drink consumed by people of all age groups. A large population in our countries depends on milk from local suppliers. Milk is a product of biological evolution, its role in human nutrition is well known and its biochemical complex matrix which appears to be the only material to function solely as a source of food **(Cashman 2002 and Hoppe *et al.*, 2006)**. Good health starts with good nutrition, which can protect against diseases later in life. Human health is highly attractive world, so food safety remains a major challenge to food producers and to legislators endeavoring to adequate consumer protection. Both man and animals lives under a certain degree of "biological hazard" that occur in food and foodstuffs **(WHO 1999a and Allam *et al.*, 2002)**. Veterinary antibiotics are widely used to treat dairy cattle diseases such as mastitis, or improve feed efficiency or to increase milk yield **(IOM 1989; McEwen and Fedorka-Cray 2002; Erskine *et al.*, 2003; Bogialli and Di Corcia 2009 and Heshmati *et al*., 2013)**. Tetracyclines (TCs) are a group of highly important broad-spectrum antibiotics including chlorotetracycline (CTC), doxycycline (DOC), tetracycline (TTC) and oxytetracycline (OTC) are considered the main antibiotics used **(Botsoglou and Fletouris 2001 and Wang *et al.*, 2012)**. Reliable data on the consumption of veterinary drugs in general and of antibiotics in particular are not easy in developing countries. In order to ensure the safety of human food, most countries have established official standard documents and have strictly regulated the maximum residue levels (MRLs) of veterinary drugs in animal-origin food **(FAO/WHO 1998; WHO 1999b and Companyo *et al.*, 2009)**. MRL for oxytetracycline (OTC) in cow's milk is (0.1 mg/kg-100 ppb) 100 $\mu g/kg^{-1}$ **(Commission Regulation EU/No. 37 2010)**. However, the abuse of antibiotics results in detectable deposition of residues in milk, which, cannot be destroyed with heat treatment and are easily, transferred from the raw milk into milk products **(Kaneene and Miller 1997; Knecht *et al.*, 2004; Wassenaar 2005 and Zorraquino *et al.*, 2011)**. Presence of antibiotic residues in food animal-origin, increase the potential health risks to consumers because of allergic reactions in some hypersensitive individuals which associated with increase in blood histamine, toxicity, carcinogenic effects, disturbance of natural intestinal microflora and possible development of bacterial resistance. The illicit use of antibiotics could increase the risks of foodborne infection with antibiotic resistant pathogenic bacteria contaminating food consumed by human **(van den Bogaard 2001; Wang *et al.*, 2006; Nisha 2008; Messano and Petti 2011 and Heshmati *et al.*, 2013 and 2015)**. In addition, the lack of good veterinary practices and illegal use of veterinary drugs by farmers will increase problem **(McEwen *et al.*, 1991)**. Although, most investigations have been focused on prevention and detection of antibiotic residues, some researches have been conducted on the changes of residues in edible animal products or byproducts during storage and cooking. Almost, no edible animal products or byproducts are consumed raw, but require before consumption some types of heat processing or cooking which, may diminish the activity of antibiotic residues, such as boiling, microwaving, stewing, frying or roasting. Thermal stability of Tetracyclines (TCs) residues in animal food products was considered by number researchers, some of them mentioned that TCs in animal tissues were more stable under heat treatment and were not totally inactivated by cooking conditions or heat processing **(Gratacos-Cubarsi *et al.*, 2007)**. On the other hand, **(Al-Ghamdi *et al.*, 2000)** stated that oxytetracycline (OTC) increased on boiling. While, the concentrations of TCs in heat-treated animal food products were higher than those found before the heat treatment **(Kuhne *et al.*, 2001)**. On the contrary, some studies indicated that TCs in animal tissues were more sensitive to heat treatment and were totally destroyed **(Gratacos-Cubarsi *et al.*, 2007)**.To detect antibiotic residues, different kinds of methods were developed. These consist of screening methods and chromatographic techniques to detect as many antibiotics as possible. Screening methods are generally performed by microbiological, enzymatic and immunological techniques. The screening methods are based on, the various susceptibility of bacteria to different antibiotics. The antibiotic residue detection assays that are currently available use different methods and test microorganisms **(Mitchell *et al.*, 1998)**. Microbiological assays for the detection of antibiotic residues utilize bacteria such as *Bacillus stearothermophilus* because of its high sensitivity to the majority of antibiotics. Both microbiological and chromatographic methods have been described for monitoring tetracyclines and penicillins in milk and animal tissues. Although, the microbiological assay techniques have been recommended as official and conventional methods because of their simplicity, the bioassay methods lack specificity, provide only semi-quantitative measurements of residues detected, and sometimes produce false positives **(Kurittu *et al.*, 2000)**.Therefore,chromatographic techniques, such as TLC and

HPLC with ultraviolet or fluorescence detection, as well as capillary electrophoresis (CE) using laser-induced fluorescence detection have been developed to replace microbiological assays **(Furusawa 1999; Ding and Mou 2000; Cinquina *et al*., 2003; Vinas *et al*., 2004; Zhao *et al*., 2004; Posyniak *et al*., 2005 and Petkovska *et al*., 2006)**. HPLC (high performance liquid chromatography) standard methods were used for the detection and quantification of tetracyclines in milk and milk products samples as described by **(Posyniak *et al*., 1998; Fritz and Zuo 2007 and NaVratiloVa *et al*., 2009)**. In recent years, HPLC was applied to evaluate changes of veterinary drug residues during processing **(Hassani *et al*., 2008 and Heshmati *et al*., 2013 and 2014)**. Goal of the present study was therefore, carried out to detect the effect of two thermal treatments: boiling and microwaving on oxytetracycline residues in raw cow's milk using HPLC technique, and to declare potential public health risks for oxytetracycline residues.

MATERIAL AND METHODS

Milk samples:

One hundred samples of non-heat-treated raw cow's milk were randomly collected from different markets and retail dairy shops in various districts of Damietta governorate. The samples were put in an insulated container at about 4.0±0.5°C and then transported immediately to the laboratory were kept refrigerated until analysis.

Methods:

Oxytetracycline standard solutions preparation:

For the preparation of the standard solutions of oxytetracycline, the method of **(NaVratiloVa *et al*., 2009)** was followed with minor modification in the working standard solution concentrations.

EDTA-Mcllvaine buffer:

This solution was freshly prepared weekly according to **(Young and Tran 2013)**.

Determination of oxytetracycline residues in raw cow's milk:

The samples were extracted and analyzed for the presence of oxytetracycline in raw cow's milk using solid phase extraction method then determined and confirmed using HPLC (high performance liquid chromatography) coupled with mass spectrometry technique.

Solid phase extraction (SPE):

This step of analysis was conducted according to **(NaVratiloVa *et al*., 2009)** with minor modification where solid phase extraction columns (6cc-500mg, Agilent Technology Part No. 12102052) were used instead of Oasis-HLB, Waters (Milford, USA).

Study the effect of thermal applications on oxytetracycline residues in milk:

The samples were examined for detection of the effect two thermal treatments: boiling and microwaving on oxytetracycline residues in raw cow's milk.

Positive samples are divided into two parts for thermal treatments as follows:

- Part 1: samples were subjected to boiling (100°C/10 minute) in triplicate.
- Part II: samples were subjected to microwaving (for 75 second) in triplicate using (home microwave model MS-191MC-2450MHz, frequency microwave 1250W-RF output 800W).

Chromatographic analysis of raw cow's milk samples and the positive ones of (part I and II) was the same.

Instrumentation (UHPLC-MS/MS):

UHPLC–MS/MS conditions:

The chromatographic analysis was done according to **(Young and Tran, 2014)** using an ultra-high pressure liquid chromatograph (UHPLC).

The column temperature was maintained at 40ºC. A gradient elution system was used with mobile phase consisted of:

1- Mobile phase A (0.1% formic acid in water).

2- Mobile phase B (0.1% formic acid in acetonitrile) at a total flow rate of 300 µl/minute.

The gradient program was follows:

Time (minute)	Flow rate (µl/min.)	Mobile phase A%	Mobile phase B%	Curve
Initial	300	85	15	Initial
2.5	300	60	40	6
3.9	300	5	95	6
4.9	300	5	95	6
5.0	300	85	15	6
7.0	300	85	15	6
A blank solvent was inserted in the run sequence every 5 samples for column wash purpose				

The autosampler temperature was 10ºC and the injection volume was 10 µl. The total run time for each sample analysis was 7 min.

MS/MS condition:

Mass spectrometric detection was carried out by a tandem quadrupole mass spectrometer using an electrospray technique. The electrospray ionization (ESI) interface was used in the positive ionization mode. Quantitation was performed in the multiple reaction monitoring (MRM) mode using peak areas.

Detection of oxytetracycline epimers metabolites after heat treatments:

To detect epimerase of oxytetracycline that may be produced due to different heat treatments, the same extracted positive milk samples were subjected to analysis using a powerful screening instrument which was an AB SCIEX TripleTOF™ 5600^{+}LC-MS System accompanied with MasterView™, PeakView® and Analyst® Software platforms. The instrument has the ability to detect target and non-target ions through its own library that could detect more than 2300 ions in one single run. The instrument have the ability to investigate complex samples in greater depth that select up to 100 precursors and generate high resolution, high mass accuracy and MS/MS spectra in a second using powerful IDA (information-dependent acquisition) algorithms. A complete digital archive of all precursor and product ions in a sample could be studied in each run. In the same time, the MasterView™ add-in of PeakView® Software was used to compare samples to a control sample using a non-targeted peak-finding algorithm to identify unexpected compounds in suspicious samples. The formula finder and automatic ChemSpider search is a useful and easy-to-use tool for identification, if the MS/MS library search does not return a hit for a compound of interest. The identification method was according to **(Taylor 2015)**, where the compounds of interest were4-epioxytetracycline (4e-OTC), α-apo-oxytetracycline(α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC).

RESULTS AND DISCUSSION

IntelliStart found the following compounds:

Compound	Formula/Mass		Parent m/z	Cone Voltage	Daughters	Collision Energy	Ion Mode
OTC	460.1	1	461.13	26	426.06	18	ES+
		2	461.13	26	200.99	40	ES+

Compound

OTC
(460.1)

Fig. (1): Result of automated tuning function performed with IntelliSearch software to optimize the mass spectrometry parameters for the determination of oxytetracycline residues.

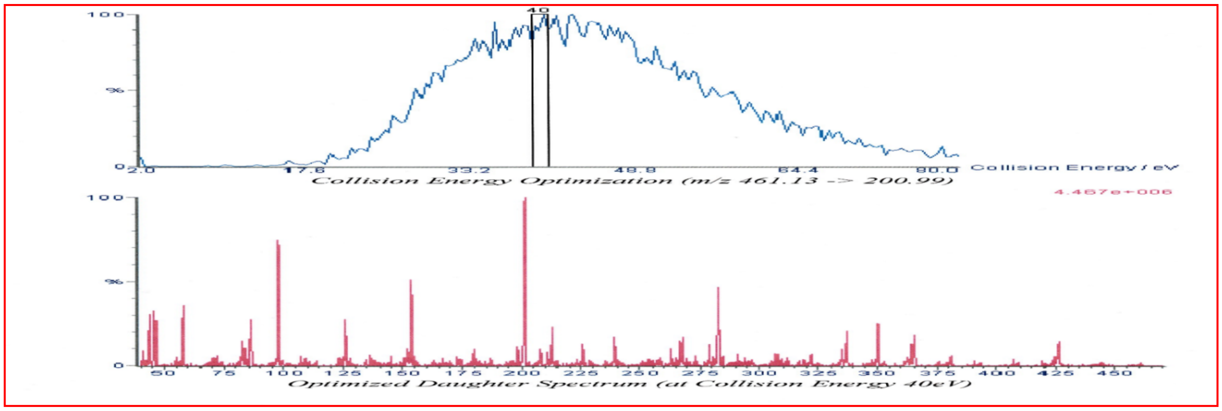

Fig. (2): Collision energy optimization for (m/z 461.13>200.99).

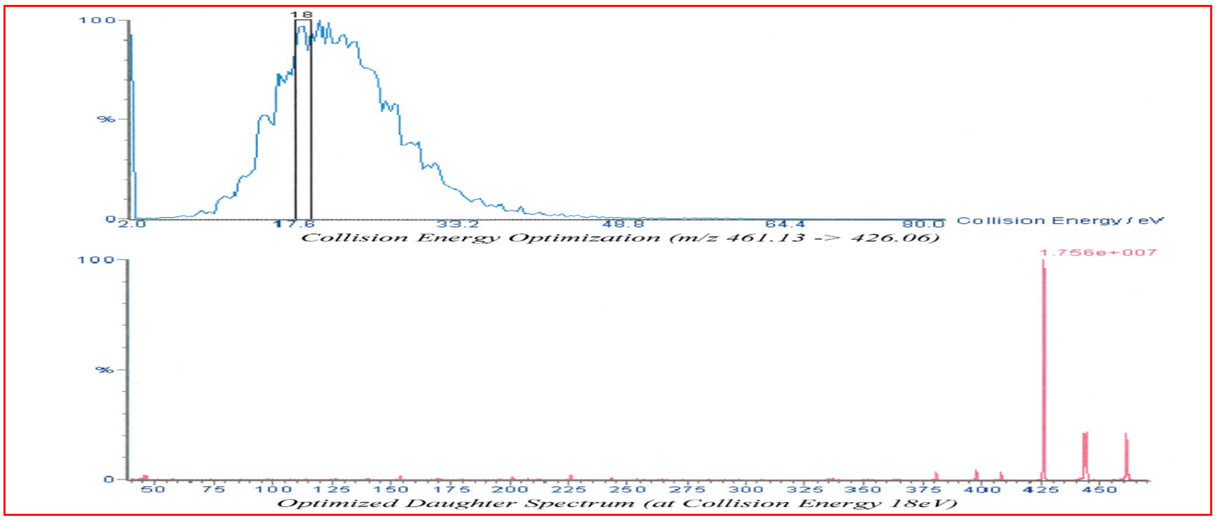

Fig. (3): Collision energy optimization for (m/z 461.13>426.06).

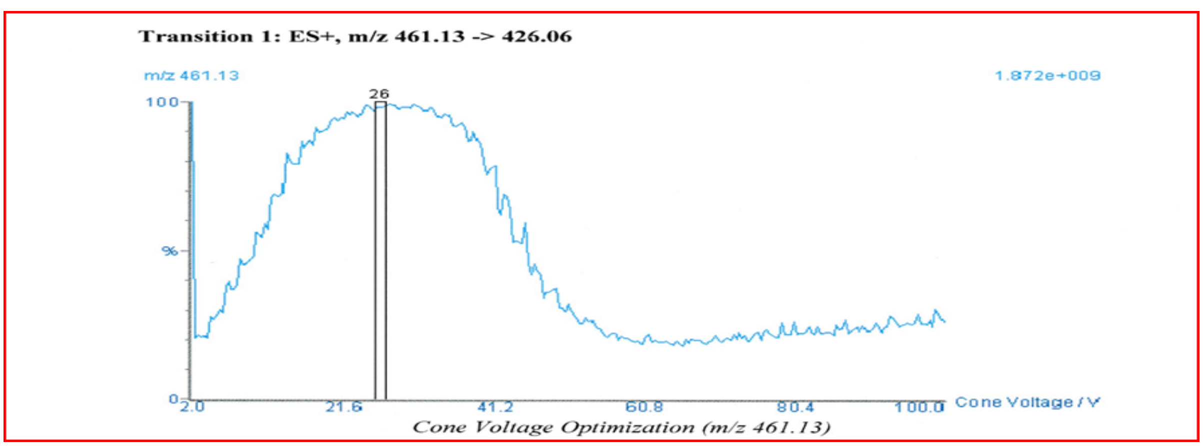

Fig. (4): Cone voltage optimization for (m/z 461.13>426.06).

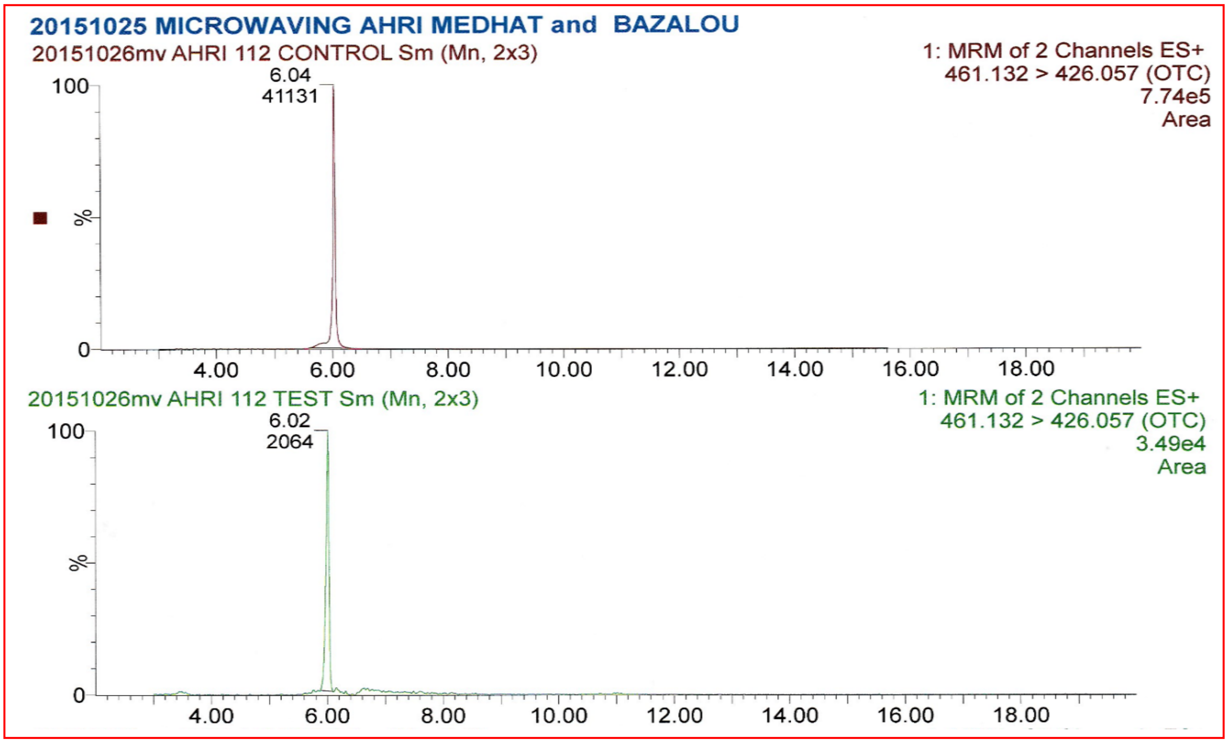

Fig. (5): Representative chromatographs for the effect of boiling on oxytetracycline in milk at a concentration of 100ng/ml (above2chromatograms) and 50 ng/ml(lower two chromatograms).

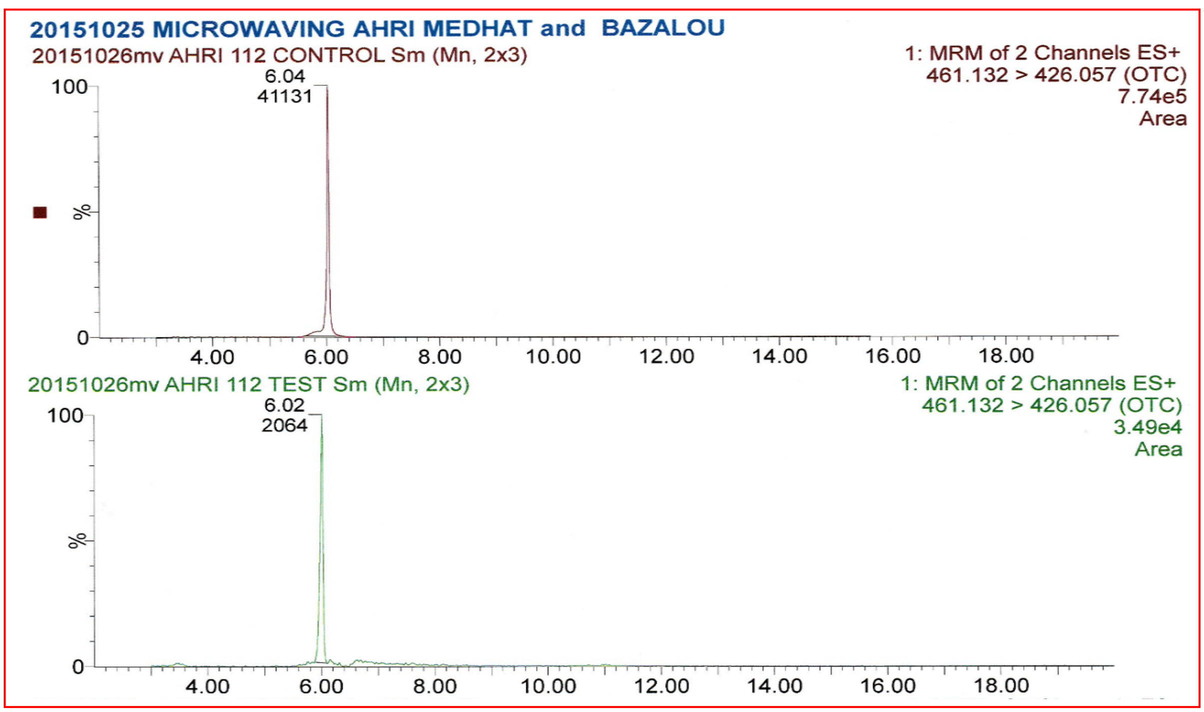

Fig. (6): Representative chromatograph for the effect of microwaving on oxytetracycline in milk at a concentration of 112 ng/ml (response area was deceased from 41131 to 2064 area units).

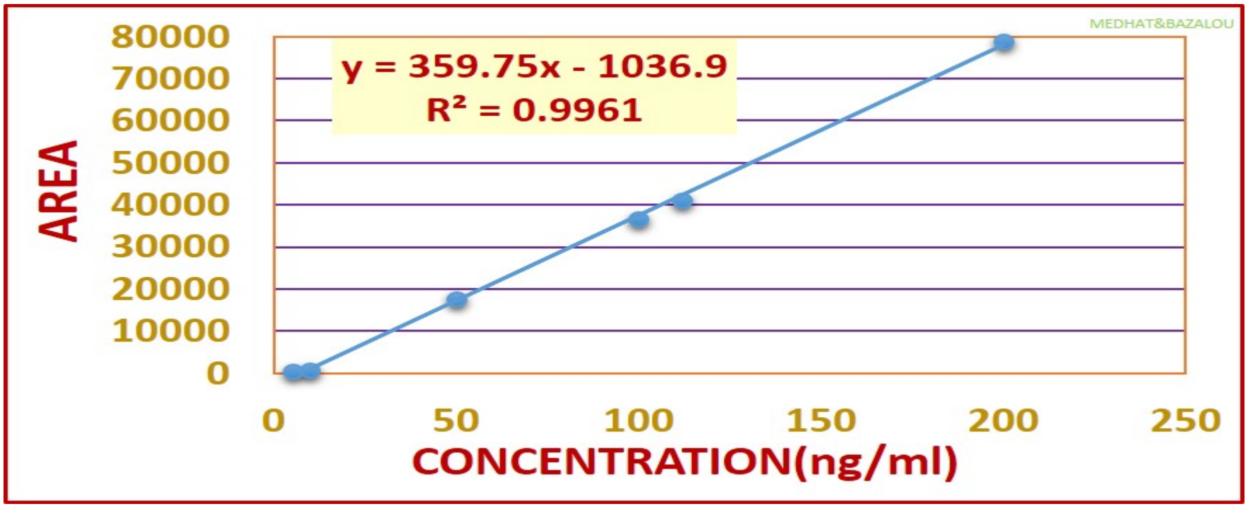

Figure (7): Standard matrix matched calibration curve of oxytetracycline in cow's milk.

Table (1): The samples and its relations to maximum residual limit (MRL).

Total number of samples	Number of positive samples (%)	Number of negative samples (%)	Maximum Residue Limit (MRL) (100 µg/kg (ppb))***	
			Over MRL (%)	Less MRL (%)
100	5 (5%)	95 (95%)	1 (1%)* (20%) **	99 (99%)* (80%) **

***Percentage in relation of total examined samples (100)**
****Percentages in relation to positive samples (5)**
***** (Commission Regulation EU/No. 37/2010)**

Table (2): Oxytetracycline residues of positive samples.

Serial of positive samples	Oxytetracycline residues concentration	Comparison with MRL (100 µg/kg (ppb))*	Judgment
Sample No. 1	48.00 µg/kg (ppb)	Lower than MRL by 52%	Accepted
Sample No. 2	35.50 µg/kg (ppb)	Lower than MRL by 64.50%	Accepted
Sample No. 3	100.00 µg/kg (ppb)	Same as MRL	Accepted
Sample No. 4	112.00 µg/kg (ppb)	Exceed MRL by 12%	Rejected
Sample No. 5	50.00 µg/kg (ppb)	Lower than MRL by 50%	Accepted

***(Commission Regulation EU/No. 37/2010)**

Table (3): Effect of boiling and microwaving on oxytetracycline residues in milk.

Serial of positive samples	Residues concentration in raw milk µg/kg (ppb)	Mean± concentration after boiling µg/kg (ppb)	Mean± concentration after microwaving µg/kg (ppb)
Sample No. 1	48.00	12.62±0.66	7.17±0.26
Sample No. 2	35.50	12.48±0.84	7.04±1.02
Sample No. 3	100.00	21.96±1.18	9.09±0.42
Sample No. 4	112.00	21.54±0.84	8.37±0.72
Sample No. 5	50.00	12.93±0.38	7.56±0.50

Table (4): The thermal effect of boiling treatment on oxytetracycline residues in milk.

Thermal effect of boiling on oxytetracycline residues in milk and its reduction percentages					
Original concentration µg/kg (ppb)	48.00	35.50	100.00	112.00	50.00
Concentration after boiling µg/kg (ppb)	12.62	12.48	21.96	21.54	12.93
Reduction percentage	73.71%	64.84%	78.04%	80.76%	74.14%

Table (5): The thermal effect of microwave treatment on oxytetracycline residues in milk.

Thermal effect of microwaving on oxytetracycline residues in milk and its reduction percentages					
Original concentration µg/kg (ppb)	48.00	35.50	100.00	112.00	50.00
Concentration after microwaving µg/kg (ppb)	7.17	7.04	9.09	8.37	7.56
Reduction percentage	85.06%	80.17%	90.91%	92.53%	84.88%

Antibiotic residues are on the top of priority for the public health authorities all over the world. Rare researches have been carried out regarding the impact of heat treatments on antibiotic residues in raw milk in developing countries. Although, most investigations have been focused on the detection of antibiotic residues, where 2.3% (6/262) of total raw cow's milk samples had different values of oxytetercycline residues, referring to maximum residual level (MRL) for 1.6% (4/262) of analyzed samples has been confirmed with values higher than permissible limit **(Kamberi and Sulaj 2014)**. In addition, several studies in Europe carried out for detection of oxytetracycline residues in raw cow's milk reported values of incidence from 0.1% to 2.9% **(Allara *et al*., 2002)**. While, 2 (14.3%) of raw milk samples had higher oxytetercycline residues than the recommended WHO standard MRL 100 µg/kg **(Abbasi *et al*., 2011)**. Elsewhere, total Tetracyclines (TCs) in commercial cow's milk samples was determined to be 2.5 times, which is greater than MRL set by **(FAO/WHO Food Standards Codex Alimentarius 2007 and 2012)** oxytetracycline had the highest share 86.71% of the determined contamination **(Aalipour *et al*., 2015)**. Additionally, oxytetracycline residues were detected in 50.6% of analyzed raw cow's milk samples studied by **(NaVratiloVa *et al*., 2009)**. It is noteworthy to mention that similar studies were conducted on other food matrixes other than milk. Some authors as **(Botsoglou and Fletouris 2001; Loksuwan 2002; Hassani *et al*., 2008 and Hsieh *et al*., 2011)** used different temperatures and methods for thermo-stability evaluation to declare the impact of heat treatments on residues of different antibiotics including oxytetracycline. The changes studied by various cooking procedures including roasting, boiling and microwave on tetracyclines (TCs) including oxytetracycline (OTC) in chicken meat and obvious that among various procedures studied, microwaving was the most effective one, to destroy 90% of initial level **(Abou-Raya *et al*., 2013)**. Elsewhere, the impact of processing on antibiotic residues in chicken meat using various heat processing treatments, oxytetracycline was decreased significantly by 72.37% in microwave processing **(Khan *et al*., 2015)**. Oxytetracycline residues in cattle liver and muscles and sheep muscles was reduced during cooking processes including microwaving, boiling, roasting, grilling, braising and frying about 35-94% **(Rose *et al*., 1996)**. In study of thermo-stability of oxytetracycline, tetracycline and doxycycline in foods, at ultrahigh temperature, regarding sterilization, whereas low temperature long time treatments (conventional sterilization) would destroy >98% of the initial concentration of the residues of the three antibiotics, high temperature short time treatments would leave unaltered residues in the 50-90% range **(Hassani *et al*., 2008)**. Recently, liquid-chromatography coupled with mass spectrometry (LC-MS) is a powerful technique **(Posyniak *et al*., 2005 and Gajda *et al*., 2013)** but tandem mass spectrometry (LC-MS/MS) seems to be the technique of choice for analysis of tetracyclines **(Charlet *et al*., 2003 and Gajda *et al*., 2013)**. Fig. (1), showed the results of automated tuning function performed with the IntelliStart software included in the UPLC-MS/MS controlling program to optimize the very spectrometric parameters for the determination of oxytetracycline. The results indicated that precursor ion (m/z) of oxytetracycline was 461.13 and produced two daughter ions of positive mode, the first m/z was 426.06 that used as quantifier ion meanwhile, and the other ion (m/z) was 200.99. Fig. (2, 3&4), confirmed the results obtained from Fig. (1), where cone voltage optimization for both (m/z 461.13>426.06 and 461.13>200.99) was 26 volt. In the same time the collision energy optimization for (m/z 461.13>200.99) was 40 electron volt (eV) and it was 18 electron volt for (m/z 461.13>426.06). The findings of the present research showed as indicated from Fig. (5,6), that thermal treatments such as boiling had a reducing effect on oxytetracycline residues concentration in milk and the same response was observed in case of the thermal treatment by microwaving. It was concluded from the obtained results that number of positive samples was 5 out of 100 examined samples (5%) and one of these samples (1%) was higher than the maximum residue level (MRL) 100 µg/kg. When considering the positive samples it was found that (one sample out of five) had a concentration exceeding the MRL to be rejected whereas, the remaining positive samples (80%) were lower than the MRL level to be accepted for human consumption (Table 1,2) Some research has been conducted on the changes of residues in edible tissues during cooking. Consequently, before consumption most animal-originated food is submit to heat processing or cooking such as pasteurization, sterilization, boiling, microwaving, stewing, frying or roasting or undergo various processing such as food additive treatments to increase digestibility, sensory properties, appetizing characteristic and shelf-life. From (Table 3), it was observed that thermal treatment of milk samples using the microwave was more profound than that of boiling. This result was more explained by the findings in (Table 4,5), which showed that mean reduction percentages in case of microwaving were 85.06, 80.17, 90.91, 92.53 and 84.88% for 48, 35.50, 100, 112 and 50 µg/kg respectively, while the corresponding results in case of boiling were 73.71, 64.84, 78.04, 80.76 and 74.14% for the same concentration respectively. Nearly similar finding was declared by **(Tremonte *et al.*, 2014)**, who mentioned that microwaving could be an excellent alternative to boiling as it guarantees the hygienic quality of milk. Indeed, the study of the presence of any chemical transformation products of oxytetracycline because of boiling or microwaving of milk samples contained residues of oxytetracycline; it was not found any of 4-epioxytetracycline (4e-OTC),α-apo-oxytetracycline (α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC) produced due to those mentioned thermal treatments as indicated from AB

SCIEX 5600+ Software searches archive. To some extent nearly similar finding was reported by **(Spisso *et al*., 2010)**, who surveyed commercial pasteurized milk samples sold in the metropolitan area of Rio de Janeiro, Brazil and fined that the presence of 4-epioxytetracycline in positive samples contained the parent drug oxytetracycline (OTC) could not be confirmed. In any case, the transformation of the tetracyclines (TCs) to their 4-epimers is resolved **(Blasco *et al*., 2009)**, because most regulations have defined tetracycline residues as the sum of the parent compound and the4-epimers **(FDA 1975;Commission Regulation 508/1999/EC 1999 and FAO/WHO Food Standards Codex Alimentarius 2007)**.On the same concern, **Nguyen *et al*., 2015** studied experimentally the formation of oxytetracycline (OTC) degradation products in chicken and pork under boiling or microwave treatment. Where samples of chicken and pig muscles previously dosed with OTC residues were analyzed for the presence of α-apo-oxytetracycline (α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC) after boiling or microwave treatment where residues were extracted in a mixture of citrate buffer-MeOH (75:25 v/v), then analyzed by high performance liquid chromatography with photodiode array detection using a XBridge™ C18 reverse-phase chromatographic column. Thermal treatment resulted in the degradation of OTC and the concentrations of the degradation products α-apo-oxytetracycline (α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC) in muscle samples were calculated as 0.7 to 1.2% of the initial OTC content. The toxic effects of the degradation products of oxytetracycline, α-apo-OTC and β-apo-OTC were studied in rats. Male rats received oral doses of 10mg/kg body weight/day of either α-apo-OTC or β-apo-OTC for 90 days. The results of this study suggested that the toxic effects of β-apo-OTC treatment could damage liver and kidney tissues of rats, as well as degeneration and necrosis in the hepatocytes. However, the dose given in that study was far away by more than 100 folds from that could be intake by a normal person through milk where the maximum residue limit of oxytetracycline in milk is 0.1mg/kg (milk) and the studied toxic dose was 10mg/kg BW. As far as a very little available literatures dealing with the impact of boiling and microwaving on oxytetracycline residues in raw cow's milk. Article states that higher residues of antibiotics are found in raw milk and that pasteurization will reduce concentrations **(Baron *et al*., 2014)**. In addition, the effect of pasteurization of raw milk on oxytetracycline residues decreased by 15.3% and this was highly significant (P≤0.01) **(Kellnerova, *et al*., 2014)**. Likewise, OTC residues were significantly (p≤0.05) reduced 79.36-86.17% **(Loksuwan 2002)**. While, a studies draw attention to heating milk as important for sanitation, and retain a long shelf life;

suggest that microwave treatment should be investigated as an alternative to boiling **(Clare *et al*., 2005 and Tremonte *et al*., 2014)**.In addition, heat treatment of cow's milk only brought about a partial reduction of Tetracyclines residue concentration and that total elimination did not take place as mentioned and confirmed by **(NaVratiloVa *et al*., 2009)**. Finally, antibiotic residues in milk are of great concern to consumers, dairy farmers and milk processors. Recently, it was found that, the excessive or improper use of Tetracyclines resulted in presence of residues in edible animal tissues. This could cause harmful effects on consumer's public health as allergic reactions associated with increase in blood histamine, liver damage, yellowing of children teeth and gastrointestinal disturbance **(FAO/WHO 2002 and Jing *et al*., 2009)**. Furthermore, toxicity, carcinogenic effects, disturbance of natural intestinal microflora and possible development of bacterial strains resistance **(Cerniglia and Kotarski 2005 and Wang *et al*., 2006)**. Therefore, nowadays it is necessary to take into account the relatively high probability of acquired Tetracyclines resistance **(Michalova *et al*., 2004)**. The resistance could be transferred from nonpathogenic microorganisms to pathogenic ones, which would then no longer respond to normal drug treatment, making treatment of human infections more difficult **(Filazi *et al*., 2005 and Heshmati e*t al*., 2015)**. Additionally, Tetracyclines have been reported to cause hypouricemia, hypokalemia, proximal and distal renal tubular acidosis in humans **(Goldfrank *et al*., 2002)**. Antibiotics not only threaten public health, but also it has economic effects.As its presence in milk used in dairy industry results in adverse effects, .Those included acidophilus milk, soured cream, yogurt and cheeses through interfering with the fermentation process by inhibiting the desirable lactic acid bacteria **(Suhren 2002; Alica *et al*., 2003 and Mohsenzadeh and Bahrainpour 2008)**. Indeed, this is just a technical problem resulting in economic losses for producers and manufacturers of milk and dairy products **(WHO 1999b)**.

CONCLUSION

The present study concluding that heat treatment of milk by boiling is currently the optimal solution to ensure the hygienic quality of raw milk. While, microwaving could be an excellent alternative to boiling as it guarantees the hygienic quality of raw cow's milk. Moreover, boiling and microwaving on oxytetracycline residues in raw cow's milk do not guarantee a full elimination but it can reduce its quantities largely. While, the obtained results revealed absence of possible transformed metabolites of oxytetracycline as a result of boiling or microwaving impact.

RECOMMENDATION

Antibiotic residues are on the top of priority for the public health authorities all over the world. Ultimately, all measures must be taken for preventing or decreasing antibiotic residues in animal originated food .However, further studies are required to identify and standardize the process variables and to guarantee safe domestic handling. In order to ensure the safety of human food, most countries have established official standard documents. As they strictly regulated the maximum residue levels (MRLs) of veterinary drugs in animal-origin food, and monitor their amount in milk**(FAO/WHO1998;Brito and Junqueira 2006 and Commission Regulation EU/No. 37/ 2010)**. It is recommended and suggested that, the whole food chain needs to be restructured and stakeholder should work together in controlling the use of antibiotics. They should be aware of using drugs banned by the international authorities, even if it is still permissible on our country. Veterinarians might be careful in prescribing any antibiotic, as there are many rules for the selection of certain drugs, putting in consideration public health significance of the drug residues as harmful effects, drug compatibility, drug interaction and drug synergism. Milk consumption from cows treated with antibiotics before withdrawal time should be prevented. Many countries have regulations prohibiting the sale of milk from cows being treated for any infection especially mastitis and milk is routinely tested for the presence of antibiotic residues. It is essential to determine the effect of processing on all veterinary drugs when considering human exposure to drug residues in animal food products **(Botsoglou and Fletouris 2001)**. Proper using of antibiotics should be adopted by good diagnosis to diseases by experienced veterinarians, using of modern techniques laboratory tests for confirming the diagnosis should be adjusted, using sensitivity test for choosing the effective antibiotics should be carried out, completing the course of antibiotic not less than five days and follow the withdrawal time directions. On presence of the antibiotic residues in raw milk, they must be lower than the permissible limit of antibiotic residues; some antibiotics have a great dangerous effect on public health so, it must be prevented from usage as growth promoters **(Jahed Khaniki 2007 and Marshall and Levy 2011)**. In few words it could concluded that residues of antibiotic agents may be of toxicological significance for the consumer and may influence the technological properties of milk used for manufacturing fermented products. To guarantee consumers safe and high quality dairy products, raw milk is regularly analyzed for the presence of antibiotic residues.

If the milk from a single cow undergoing treatment accidentally enters the herd bulk milk, this may be sufficient to make the content of a tanker unsuitable for human consumption **(McEwen *et al*., 1991)**. Further investigations and more researches are required to determine the kinetics of antibiotics metabolites biodegradation during cooking conditions or heat treatment processing of raw cow's milk.

REFERENCES

Aalipour, F.; Mirlohi, M.; Jalali, M. and Leila, A. (2015): Dietary exposure to tetracycline residues through milk consumption in Iran, Journal of Environmental Health Science and Engineering, 13: 80.

Abbasi, M.M.; Babaei, H.; Ansarin, M.; Nourdadgar, A.S. and Nemati, M. (2011): Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method, Advanced Pharmaceutical Bulletin, 1 (1): 34 -39.

Abou-Raya, S.; Shalaby, A.R.; Salama1, N.A.; Emam, W.H. and Mehaya, F.M. (2013): Effect of ordinary cooking procedures on tetracycline residues in chicken meat. Journal of Food and Drug Analysis, 21: 80-86.

Al-Ghamdi, M.S.; Al-Mustafa, Z.H.; El-Morsy, F.; Al-Faky, A.; Haider, I. and Essa, H. (2000): Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. Public Health, 114: 300-304.

Alica, D.; Jennifer, M.; Shannon, R. and Frederick, J. (2003): Public health consequences of use of antimicrobial agents in food animals in the United States, Journal of Microbial Drug Resistance, 9: 1-7.

Allam, S.M.; Soliman, A.A.; Ahmed, M.E. and Shebl, M.A. (2002): Aflatoxicosis in Egyptian sheep. 2- clinical chemistry and carcass quality., Proc. 2nd Conf. Foodborne Contamination and Egyptians' Health, 23-24 April, El-Mansoura, Egypt.

Allara, M.; Izquiierdo, P.; Torres, G. and Rodriguez, B. (2002): Penicillin G in pasteurized milk produced in Zulia State Venezuella, Revista Cientifica Facultad de Ciencias Veterinarias, 12: 683-687.

Baron, P.A.; Love, D.C. and Nachman, K.E. (2014): Pharmaceuticals and personal care products in chicken meat and other food animal products: A market-basket pilot study. Sci Total Environ. 490: 296-300.

Blasco, C.; Di Corcia, A. and Picó, Y. (2009): Determination of tetracyclines in multi-specie animal tissues by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. Food Chemistry, 116: 1005-1012.

Bogialli, S. and Di Corcia, A. (2009): Recent applications of liquid chromatography-mass spectrometry to residue analysis of antimicrobials in food of animal origin, Anal. Bioanal. Chem., 395: 947-966.

Botsoglou, N.A. and Fletouris, D.J. (2001): "Drug Residues in Foods" Pharmacology, food safety, and analysis. Marcel Dekker, New York, 1194 p.

Brito, R.B. and Junqueira, R.G. (2006): Determination of beta lactams residues in milk by HPLC. Brazilian Archives of Biology and Technology, 49: 41-48.

Cashman, K.D. (2002): Macrominerals in milk and dairy products, nutritional significance. In: H. Roginski, P.F. Fox and J.W. Fuquay, Editors, Encyclopedia of Dairy Sciences, Academic Press, London, UK, pp. 2051-2058.

Cerniglia, C.E. and Kotarski, S. (2005): Approaches in the safety evaluations of veterinary antimicrobial agents in food to determine the effects on the human intestinal microflora. Journal of Veterinary Pharmacology and Therapeutics, 28: 3-20.

Charlet, M.; Schelkens, M.; Croubels, S. and De Backer, P. (2003): 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 Act., 492: 199-213.

Cinquina, A.; Longo, F. and Anastasi, G. (2003): Validation of a high performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. J. Chromatography A., 987: 227-233.

Clare, D.A.; Bang, W.S.; Cartwright, G.; Drake, M.A.; Coronel, P. and Simunovic, J. (2005): Comparison of sensory, microbiological, and biochemical parameters of microwave versus indirect UHT fluid skim milk during storage. J. Dairy Sci., 88 (12): 4172-82.

Commission Regulation 508/1999/EC (1999): Amending Annexes I-IV to Council Regulation (EEC) No. 2377/90 laying down Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal of European Communities, L224, 16, (http://europa.eu.int).

CommissionRegulationEU/No.37 (2010): Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. O. J. L.,15: 1-77.

Companyo, R.; Granados, M.; Guiteras, J. and Prat, M.D. (2009): Antibiotics in food: legislation and validation of analytical methodologies. Anal. Bioanal. Chem., 395: 877-891.

Ding, X. and Mou, S. (2000): Ion chromatographic analysis of tetracyclines using polymeric column and acidic eluent. J. Chromatography A., 897: 205-214.

Erskine, R.J.; Wagner, S. and De Graves, F.J. (2003): Mastitis therapy and pharmacology. Veterinary Clinics of North America-food Animal Practice., 19 (1): 109-138.

FAO/WHO "Joint Food and Agricultural Organization and World Health Organization" (1998): Expert Committee on Food Additives.

FAO/WHO "Joint Food and Agricultural Organization and World Health Organization" (2002): Evaluation of certain veterinary drug residues in food: fifty eighth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO technical report series, No. 911. FAO, Rome, p. 33.

FAO/WHO Food Standards Codex Alimentarius (2007): Veterinary drug residues in food. Maximum residue limits. (Available at: (http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp).

FAO/WHO Food Standards Codex Alimentarius (2012): Maximum Residue Limits for Veterinary Drugs in Foods, Updated as at the 35th Session of the Codex Alimentarius Commission, 1- 40. (Available at: http://www.codexalimentarius.net/vetdrugs/data/vetdrugs/index.html?lang=en).

FDA "Food and Drug Administration" (1975): Titled 21 - Food and drugs. Chapter I - Food and Drugs Administration Department of Health and Human Services, Subchapter E - Animal drugs, feed and related products.Part 556. Tolerances for residues of new animal drugs in food. (Available at: http: //www.accessdata. fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.0063fm CFRPart=556).

Filazi, A.; Sireli, U.T. and Cadirci, O. (2005): Residues of gentamicin in eggs following medication of laying hens. Br. Poult. Sci., 46: 580-583.

Fritz, J.W. and Zuo, Y. (2007): Simultaneous determination of tetracycline, oxytetracycline and 4-epitetracycline in milk by high performance liquid chromatography. Food Chem., 105: 1297-1301.

Furusawa, N. (1999): Rapid liquid chromatographic determination of oxytetracycline in milk. J. Chromatogr. A., 839 (1-2): 247-251.

Gajda, A.; Posyniak, A.; Żmudzki, J. and Tomczyk, G. (2013): Determination of doxycycline in chicken fat by liquid chromatogarphy with UV detection and liquid chromatography-tandem mass spectrometry. J. Chromatogr B., 928, 113 -120. 10.

Goldfrank, L.R.; Flomenbaum, N.E.; Lewin, N.A.; Howlan M.A.; Hoffman, R.S. and Nelson, L.S.(2002): Goldfrank's Toxicologic Emergencies (The McGraw-Hill Companies, New York).

Gratacos-Cubarsi, M.; Fernandez-Garcia,A.; Picouet, P.; Valero-Pamplona,A.;García-Regueiro, J.A. and Castellari, M.(2007): Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions.J. Agric. Food Chem., 55: 4610 - 4616.

Hassani, M.; Lazaro, R.; Perez, C.; Condon, S. and Pagan, R. (2008): Thermostability of oxytetracycline, tetracycline, and doxycycline at ultrahigh temperatures. Journal of Agricultural and Food Chemistry, 56: 2676-2680.

Heshmati, A.; Kamkar, A.; Salaramoli, J.; Hassan, J. and Jahed G.H. (2013): The effect of two methods cooking of boiling and microwave on tylosin residue in chicken meat .Iranian Journal of Nutrition Sciences and Food Technology, 8: 61-71.

Heshmati, A.; Kamkar, A.; Salaramoli, J.; Hassan, J. and Jahed G.H. (2015): Effect of deep-frying processing on tylosin residue in meat. Iranian Journal of Nutrition Sciences and Food Technology, 12: 61-71.

Heshmati, A.; Salaramoli, J.; Kamkar A.; Hassan, J. and Jahed, G.H. (2014): Experimental study of the effects of cooking methods on tilmicosin residues in chicken. Journal of Veterinary Research, 69: 283-290.

Hoppe, C.; Mølgaard, C. and K.F. Michaelsen (2006): Cow's Milk and Linear Growth in Industrialized and Developing Countries., Annual Review of Nutrition, 26: 131-173.

Hsieh, M.K.; Shyu, C.L.; Liao, J.W.; Franje, C.A.; Huang, Y.J.; Chang, S.K.; Shih, P.Y. and Chou, C.C. (2011): Correlation analysis of heat stability of veterinary antibiotics by structural degradation, changes in antimicrobial activity and genotoxicity. Vet Med-Czech, 56: 274-285.

IOM "Institute of Medicine" (1989): Human health risks with sub-therapeutic use of penicillin or tetracyclines in animal feed. Committee on human health risk assessment of using Subtherapeutic antibiotics in animal feeds, National Academy Press, Washington DC.

Jahed Khaniki, Gh.R. (2007): Chemical Contaminants in Milk and Public Health Concerns: A Review. International Journal of Dairy Science, 2: 104-115.

Jing, T.; Gao, X.D.; Wang, P.; Wang, Y.; Lin, Y.F.; Hu, X.Z.; Hao, Q.L.; Zhou, Y.K. and Mei, S.R. (2009): 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: 2009-2018.

Kamberi, M. and Sulaj, K. (2014): Study on oxytetracycline residues in cow's milk samples collected in tetovo, macedonia from 2012 to 2013 Stockbreeding, 68 (2): 59 - 63.

Kaneene, J.B. and Miller, R. (1997): Problems associated with drug residues in beef from feeds and therapy. Review of Scientific Technology Office of International Epizootics, 16: 694 -708.

Kellnerova, E.; Navrátilova, P. and Borkovcova, I. (2014): Effect of pasteurization on the residues of tetracyclines in milk, ACTA VET. BRNO, 83: 21-26.

Khan, A.A.; Randhawa, M.A.; Butt, M.S. and Nawaz, H. (2015): Impact of Various Processing Techniques on Dissipation Behavior of Antibiotic Residues in Poultry Meat, Journal of Food Processing and Preservation, 40 (1): 76 - 82.

Knecht, B.G.; Strasser, A.; Dietrich, R.; Martlbauer, E.; Niessner, R. and Weller, M.G. (2004): Automated microarray system for the simultaneous detection of antibiotics in milk. Analytical Chemistry, 76 (3): 646 - 654.

Kuhne, M.; Hamscher, G.; Korner, U.; Schedl, D. and Wenzel, S. (2001): Formation of anhydrotetracycline during a high-temperature treatment of animal-derived feed contaminated with tetracycline. Food Chem., 75: 423-429.

Kurittu, J.; Lo¨nnberg, S.; Virta, M. and Karp, M. (2000): Qualitative detection of tetracycline residues in milk with a luminescence based microbial method: The effects of milk composition and assay performance in relation to an immunoassay and a microbial inhibition assay. J. Food Prot., 63: 953-957.

Loksuwan, J. (2002): The effect of heating on multiple residues of tetracyclines in milk. Thammasat. Int. J. Sc. Tech., 7: 17-21.

Marshall B.M. and Levy, S.B. (2011): Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev., 24: 718-733.

McEwen, S.A.; Meek A.H. and Black, W.D. (1991): A dairy farm survey of antibiotic treatment practices, residue control methods and associations with inhibitors in milk. J. Food Prot., 6: 454 - 459.

McEwen, S.A. and Fedorka-Cray, P.J. (2002): Antimicrobial use and resistance in animals. Clinical Infectious Diseases, 34 (3): 93-106.

Messano, G.A. and Petti, S. (2011): Antibiotic resistance as a public health problem. The case of genital mycoplasmoses Ig Sanita Pubbl., 67 (6): 697-706.

Michalova, E.; Novotna, P. and Schlegelova, J. (2004): Tetracyclines in veterinary medicine and bacterial resistance to them. Vet. Med-Czech., 49: 79-100.

Mitchell, J.L.; Giffits, M.W.; Mcewen, S.A.; Mcnab, W.B. and Yee, A.J. (1998): Antimicrobial drug residues in milk and meat: Causes, concerns, prevalence, regulations, tests and test performance. J. Food Prot., 61: 742-756.

Mohsenzadeh, M. and Bahrainpour, A. (2008): The detection limits of antimicrobial agents in cow milk by a simple yoghurt test. Pakistan Journal Biological Science, 11: 2282-2285.

NaVratiloVa, P.I.B.; DračkoVá, M.; JaNštoVá, B. and VorloVá, L. (2009): Occurrence of Tetracycline, Chlortetracycline, and Oxytetracycline Residues in Raw Cow's Milk. Czech J. Food Sci., 27 (5): 379-385.

Nguyen, V.H.; Nguyen, V.T.; Li, C.B. and Zhou, G.H. (2015): The degradation of oxytetracycline during thermal treatments of chicken and pig meat and the toxic effects of degradation products of oxytetracycline on rats. Journal of Food Science and Technology, 52 (5): 2842-2850.

Nisha, A.R. (2008): Antibiotic residues-a global health hazard. Veterinary World, 1: 375-377.

Petkovska, E.; Slaveska-Raicki, R. and Rafajlovska, V. (2006): Determination of tetracycline, oxytetracycline and chlortetracycline in milk by TLC and column chromatography using Amberlite XAD-2. Chemia Analityczna, 51: 275-283.

Posyniak, A.; Zmudzki, J.; Semeniuk, S.; Niedzieiska, J. and Ellis, R. (1998): Determination of tetracycline residues in animal tissue by liquid. Chromatography, 12: 294-299.

Posyniak, A.; Mitrowska, K.; Zmudzki, J. and Niedzielska, J. (2005): Analytical procedure for the determination ofchlortetracycline and4-epi-chlortetracycline in pig kidneys.J. Chromatography A., 10: 169-174.

Rose, M.D.; Bygrave, J.; Farrington, W.H.H. and Shearer, G. (1996): The effect of cooking on veterinary drug residues in food: 4. Oxytetracycline. Food Additives and Contaminants, 13: 275-286.

Spisso, B.F.; Monteiro, M.A.; Pereira, M,U.; Ferreira, R.G.; Da Costa, R.P.; Cruz, T.A. and Da Nóbrega, A.W. (2010): Pilot survey of commercial pasteurized milk consumed in the metropolitan area of Rio de Janeiro, Brazil, for tetracyclines residues, including the 4-epimers of oxytetracycline, tetracycline and chlortetracycline. Food Addit. Contam. B, 3: 220-227.

Suhren, V.G. (2002): Inhibitors and residues of veterinary drugs in milk legal basis, detection methods and detection systems. Kieler Milchwirtschaftliche Forschungsberichet, 54: 35-71.

Taylor, A.M. (2015): Accurate Mass Screening Workflows for the Analysis of Novel Psychoactive Substances. TripleTOF® 5600+ LC/MS/M System with MasterView™ Software. AB SCIEX document number: RUO-MKT-02-1884-A.

Tremonte, P.; Tipaldi, L.; Succi, M.; Pannella, G.; Falasca, L. and Capilongo, V.; Coppola, R. and Sorrentino, E. (2014): Raw milk from vending machines: Effects of boiling, microwave treatment, and refrigeration on microbiological quality. Journal of Dairy Science, 6 (97): 3314-3320.

van den Bogaard, A.E. (2001): Human health of antibiotic use in food animals. A review: Tijdschr Diergeneeskd, 15; 126 (18): 590-595.

Vinas, P.; Balsalobre, N.; Lopez-Erroz, C. and Hernandez-Cordoba, M. (2004): Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residue in honey. J. Chromatography A., 1022: 125-129.

Wang, J.; Leung, D. and Lenz, S.P. (2006): Determination of five macrolide antibiotic residues in raw milk using liquid chromatography-electrospray ionization tandem mass spectrometry. J. Agric Food Chem., 54 (8): 2873-2880.

Wang, J.; Mac Neil, J.D. and Kay, J.F. (2012): Chemical Analysis of Antibiotic Residues in Food. John Wiley & Sons, New Jersey, 384 p.

Wassenaar, T.M. (2005): Use of antimicrobial agents in veterinary medicine and implications for human health. Critical Reviews in Microbiology, 31 (3): 155-169.

WHO "World Health Organization" (1999a): Basic Food Safety for Health Workers. M. Adams and Y. Motarjemi, Geneva. WHO/SDE/PHE/FOS/99.1.

WHO "World Health Organization" (1999b): Technical Report Series. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives), No. 888.

Young, M.S. and Tran, K.V. (2013): HPLC/UV Determination of Tetracyclines in Milk Using Mixed-Mode SPE and eXtended Performance [XP] 2.5 µm Columns. Waters Application Note, 720005053EN.

Young, M.S. and Tran, K.V. (2014): Multiresidue LC-MS/MS Screening of Veterinary Drugs in Milk Using the Ostro Pass-through Sample Preparation Plate. Waters Application Note, 720004582EN.

Zhao, F.; Zhang, X. and Gan, Y. (2004): Determination of tetracyclines in bovine milk by high performance liquid chromatography with coulometric electrode array system. J. Chromatography A., 15: 109 -114.

Zorraquino, M.A.; Althaus, R.L.; Roca, M. and Molina, M.P. (2011): Heat treatment effects on the antimicrobial activity of macrolide and lincosamide antibiotics in milk. J. Food Prot., 74 (2): 311-315.