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The Effect of Thermal Treatment on the Concentration of Common Antibiotics in Cow Milk Samples in Sudan

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Abstract: Antibiotics in veterinary treatment have increased owing to intensive animal breeding for human consumption, resulting in antibacterial residues in food and potential health risks. This study examined how pasteurization and boiling affected the amounts of commonly used antibiotics—Tylosin (TYLO), Enrofloxacin (ENRO), Ampicillin (AMP), and Oxytetracycline (OTC)—in fresh cow milk. Cow milk samples without antibiotics were added with three antibiotic concentrations (3, 6, and 9 ppm). Each concentration was heat-treated twice and analyzed by Liquid Chromatography-Mass Spectrometry to determine antibiotic percentage reduction and kinetic degradation rate constants (k). The findings showed that all thermal treatments greatly reduced the initial antibiotic concentrations, with pasteurization being the most effective overall. Boiling raised k-values, suggesting faster deterioration. Boiling for 10 minutes works better than 5 minutes. For 5 minutes, there was a significant difference between pasteurization and boiling (P=0.002), but there was no significant difference after 10 minutes (P=0.09). TYLO decreased the most dramatically with pasteurization (49.3%-15%) as compared to boiling (30.4%-14.6%) and was much more influenced than OTC (46%-35.3%), ENRO (44.6%-32%), and AMP (30.6%-26.6%). The k-values for TYLO after pasteurization (0.0224 to 0.005) showed worse stability than other antibiotics. Boiling was very effective in lowering OTC concentrations (59%-15.3%), whereas ENRO and AMP showed the smallest decreases (11.6%-6.2% and 30.6%-26.6%, respectively).

In summary, thermal treatments considerably reduced antibiotic concentrations in milk, with boiling displaying higher stability based on k-values, although pasteurization was more effective in terms of overall reduction based on breakdown rates. OTC had the most instability during boiling, while TYLO showed the least stability during pasteurization. AMP and ENRO were more stable over both treatments than OTC and TYLO, indicating that the effectiveness of thermal treatments is dependent on the individual antibiotic employed.

Keywords: Antimicrobial drugs, thermal processing, pasteurization, dairy products, degradation rate.

تأثير المعالجة الحرارية على تركيز المضادات الحيوية الشائعة في عينات حليب الأبقار في السودان

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المستخلص: لقد زادت المضادات الحيوية في العلاج البيطري بسبب التربية المكثفة للحيوانات للاستهلاك البشري، مما أدى إلى بقايا مضادات حيوية في الغذاء ومخاطر صحية محتملة. دراسة هذا البحث تناولت كيفية تأثير البسترة والغليان على كميات المضادات الحيوية المستخدمة بشكل شائع—تيلوسين(TYLO) ، إنروفلوكساسين(ENRO) ، أمبيسيلين(AMP) ، وأوكسي تتراسيكلين—(OTC) في حليب الأبقار الطازج. تمت إضافة عينات حليب الأبقار التي لا تحتوي على مضادات حيوية بثلاثة تركيزات من المضادات الحيوية (3، 6، 9 جزء في المليون). تمت معالجة كل تركيز حرارياً مرتين وتحليله بواسطة الكروماتوغرافيا السائلة مطياف الكتلة لتحديد نسبة تقليل المضادات الحيوية وثوابت معدل التحلل الحركي .(k) أظهرت النتائج أن جميع العلاجات الحرارية قللت بشكل كبير من تركيزات المضادات الحيوية الأولية، حيث كانت البسترة هي الأكثر فعالية بشكل عام. الغليان رفع قيمk ، مما يشير إلى تدهور أسرع. الغلي لمدة 10 دقائق يعمل بشكل أفضل من 5 دقائق. لفترة 5 دقائق، كان هناك فرق كبير بين البسترة والغليان(P=0.002) ، ولكن لم يكن هناك فرق كبير بعد 10 دقائق (P=0.09) انخفض TYLO بشكل كبير مع البسترة (49.3%-15%) مقارنة بالغلي (30.4%-14.6%)، وكان أكثر تأثراً بكثير من(46%-35.3%) OTC ، (44.6%-32%) ENRO، و AMP (30.6%-26.6%).أظهرت قيم k لـ TYLO بعد البسترة (0.0224 إلى 0.005) استقرارًا أسوأ من المضادات الحيوية الأخرى. كان الغليان فعالاً جداً في خفض تركيزات(59%-15.3%) OTC ، في حين أظهرت ENRO و AMP أقل الانخفاضات (11.6%-6.2% و30.6%-26.6% على التوالي). باختصار، خفضت العلاجات الحرارية تركيزات المضادات الحيوية في الحليب بشكل كبير، حيث أظهرت الغليان استقرارًا أعلى بناءً على قيمk ، على الرغم من أن البسترة كانت أكثر فعالية من حيث الانخفاض العام بناءً على معدلات التحلل. كان OTC الأكثر عدم استقرار أثناء الغليان، بينما أظهر TYLO أقل استقرار أثناء البسترة. كان AMP و ENRO أكثر استقرارًا خلال كلا العلاجين من OTC وTYLO ، مما يشير إلى أن فعالية العلاجات الحرارية تعتمد على المضاد الحيوي المستخدم.

الكلمات المفتاحية: الأدوية المضادة للميكروبات، المعالجة الحرارية، البسترة، منتجات الألبان، معدل التحلل.

1. Introduction:

Milk is the fluid secreted by the mammary glands of mammals to nourish their newborns. Milk and dairy products are important sources of animal protein, which contribute significantly to human growth and development (Tona and Olusola, 2014). Milk consumption has been associated with several benefits. For example, bovine milk contains about 87.1% water, 4.0% fat, 3.3% protein, 4.6% lactose, and 0.7% ash (Walstra *et al.*, 2006 & Furlani *et al.*, 2015). These characteristics make milk an essential food item in the human diet, particularly for children and older people. Hence, milk and its related products are unquestionable in a healthy and balanced diet. Raw milk quality is monitored to ensure processed product quality and safety. Raw milk must meet other quality standards including the absence of any antibiotic's residues, added water, and contaminants (Murphy and Boor, 2000).

Contaminated milk is considered unsaleable or unfit for human consumption following treatment of the animal with veterinary products, i.e., antibiotics and other chemical and biological substances (FDA, 2015a). However, if milk is not produced with good dairy farming practices, it can be a source of contaminants that may be deleterious to human health (Furlani *et al.*, 2015). Antibiotics are chemical substances that are either synthetically produced in the laboratory or naturally produced by various species of microorganisms, including bacteria and fungi, Actinomycetes, and Streptomyces, which can kill or inhibit the growth of other microorganisms (Bbosa and Mwebaza, 2013). Antibiotics can be classified according to their effects as either bactericidal or bacteriostatic or according to their range of efficacy as narrow or broad in the spectrum used in the prevention and treatment of bacterial infections in humans and animals (Darwish *et al.*, 2013).

It is estimated that 73% of global antibiotic use is due to the consumption of antibiotics in animals intended for food production. Antimicrobial agents are widely used in dairy cattle management for disease therapy and as growth-promoting agents (Treiber & Beranek,2021). (Kusumaningsih *et al.*, 1996) used of antibiotics in healing the cattle must meet standard criteria: (a) antibiotics must be used for curing diseases, (2) antibiotics dosage must be used suitably according to the drug factory regulation, (3) antibiotic concentration in milk should be less than maximum standards according to SNI 2000; Tetracycline 0.05 mg/kg, Penicillin 0.1 mg/kg and Streptomycin 0.1 mg/kg, (4) the antibiotic should fulfill the drug withdrawal time of 2-5 days' post-treatment The extensive use of antibiotics in veterinary medicine and medicated feed is a common practice, intensive production of animals intended for human consumption, leading to a significant increase in antibiotic resistance and allergic reactions, having essential consequences for public health (Moreno *et al.*, 2017).

2. Literature review

In Africa, as in other parts of the world, antibiotic residues in animal-derived foods have been extensively recorded. In many African countries, these residues have exceeded the WHO maximum residue levels in many cases (Darwish *et al.*, 2013).

Antibiotic residues were found in 38.9% of the milk samples tested in Khartoum State by Said Ahmed *et al.* (2008), with a higher prevalence at retail locations (55.6% vs. 22.2% and 0%) than on farms. El Zubeir and El Owni (2009) found a similar sequence, reporting that 6.66 percent and 12.25 percent of milk samples had antibiotic and sulphonamide contamination. In Khartoum State, it was found that the disease control was not satisfactory as most laborers give the treatment without the consultation of veterinarians (El Zubeir and Ahmed, 2011). Also, they reported that dairy farmers themselves always decide the drugs they administer according to their experience or to the advice of their colleagues whose cows have shown the same symptoms. They attributed that to the high financial cost of the veterinarian personnel and the easiness of dispensing veterinary medicinal products.

2.1 Antibiotic Withdrawal Time: The toxicological residue needs to tolerate the withdrawal time. It is the period between when an animal is taken off the medicine and when it may be slaughtered when antibiotics are administered label and extra-label. Antibiotic withdrawal time varies within 2-30 days, depending on the type of drug, species, genetic factors of cattle, local climate, way of treatment, dosage, the Health status of the cattle, type of animal products, drug residue tolerance, drug formulation (Nisha, 2008). Despite that, not all drug factory communicates withdrawal time in the packaging. The minimum withholding period for milk is 7 days after being treated with antibiotics (Gupta, 2012).

2.2 Antibiotics used as veterinary medications were studied.

Oxytetracycline (OTC), a member of the Tetracycline (T.C.) family, is used in food-producing animals because of its broad-spectrum effect and low cost. OTC has been widely used to treat and control diseases and as a feed additive to improve live weight gain (Chopra and Roberts, 2001; García *et al.*, 2004). This broad-spectrum antibiotic bacteriostatic affects numerous gram-negative and gram-

positive bacteria (Jevinova *et al.*, 2003). Oxytetracycline's MRL in cow's milk is 100 μg/kg (Commission Regulation 37/2010; Kellnerová *et al.*, 2015).

After microwaving, boiling, and roasting, Abou-Raya *et al.* (2013) evaluated Oxytetracycline (OTC), tetracycline (TTC), chlortetracycline (CTC), and doxycycline (DOC) in chicken breasts and thighs. They found the sample's safe cooking time. Heat degrades Tetracycline from 2% to 100%. DOC is the most heat stable in a chicken matrix, whereas OTC is the least. Nguyen *et al.* (2015) investigated male rats' 90-day oral exposure to OTC breakdown products α-apo-OTC and β-apo-OTC. α-apo-OTC was harmless, whereas βapo-OTC caused liver and kidney damage, hepatocyte degeneration, and necrosis.

Enrofloxacin (ENRO): a synthetic fluoroquinolone antimicrobial drug, is extensively used in veterinary medicine to treat Gram-negative, Gram-positive, and mycoplasma-related infectious illnesses. Veterinarians treat gastrointestinal, respiratory, and mammary illnesses in dairy goats using ENRO. The MRL is 30 μg/k. (Beltrán *et al.*,2018) . ENRO residue in the diet might alter the bacteria in the gut. The human gut flora is similarly affected by its major metabolite and ciprofloxacin (Chen *et al.*, 2011). Lolo et al. (2006) found that boiling did not diminish Enrofloxacin residues. Therefore, they remained stable. Boiling removed some quinolones into the broth. Quinolone residues increased in water-loss cooking. Lolo *et al.* (2006) and Junza et al. (2014) observed that Enrofloxacin degraded into ciprofloxacin after heating. Moharana *et al.* (2015) analyzed the veterinary drug Enrofloxacin in cow milk samples obtained from two cities in India. They used reverse-phase HPLC with a limit of detection of 100 μg/. They have verified that 8% of the samples had values above the MRL.

Tylosin (TYLO): is a popular macrolide antibiotic (Ji et al., 2014; Soliman and Sedeik, 2016). To protect food safety, the European Union has defined maximum residue limits (MRLs) for erythromycin, spiramycin, and Tylosin in cow's milk. Muñoz *et al.* (2014) found the most significant TYLO residual in egg yolks after 3 days of withdrawal in laying hens. TYLO oral bioavailability, distribution, molecular weight, and solubility substantially affected their outcomes. According to Commission Regulation 37/2010, the MRL for Tylosin is 50 μg/kg.

Ampicillin (AMP): is effective against gram-positive organisms like streptococci and enterococci (unless they exhibit βlactamases) and gram-negative organisms such as Hemophilus species, E. coli, Salmonella, and Shigella—many gram-negative organism's express plasmid-mediated β-lactamases, making them ampicillin-resistant(Montero *et al.*, 2005). Ampicillin's MRL in milk is 4 μg/kg (Commission Regulation 37/2010).

2.3 The Use of Antibiotics in Animal Health Care.

The improper administration by farmers and veterinarians without observing the withdrawal time for treated animals can result in the accumulation of antibiotic residues in milk and its products. Therefore, antimicrobial residues in animal-originated food have been a critical problem in many countries. Because drug residues might result in various Health Hazards, both actual incidence of reactions and potential hazards perceived by the public (Heshmati, 2015; Roba, 2023). The extensive use of antibiotics in veterinary medicine and medicated feed is a common practice, leading to a significant increase in antibiotic resistance and allergic reactions, having meaningful consequences for public health (Moreno *et al.*, 2017). Also, milk with antibiotic residues used to produce fermented products can interfere with fermentation by affecting desired lactic acid bacteria. Usually, this is just a technical problem resulting in financial loss. For these reasons, many countries have regulations prohibiting the sale of milk from cows being treated for mastitis, and milk is routinely tested for antibiotic residues (Darwish *et al.*, 2013).

The likelihood of milk residues is more significant in developing nations than in developed countries. This might be due to a lack of detection facilities and regulatory organizations that govern drug residue levels in foods via maximum residue limits (MRLs) (Kebede, 2014). Milk and dairy products that require time/temperature control for safety, Tetracycline to limit pathogenic microorganism growth or toxin formation, and also includes heat-treated, pasteurized, or ultra-pasteurized at the end (FDA, 2015a). Heat treatment for antibiotic residues in milk may produce new chemical compounds with higher toxicity levels than the parent compound (Botsoglou and Fletouris, 2001). Heshmati (2015) stated that the stability of antibacterial residues during heating is different. The changes in antibacterial drug residues during further cooking degrade several antibacterial drug residues; depending on the amount of heat treatment involved in cooking time and temperature; in some cooking procedures, sufficient heating temperature and time can reduce several antibacterial drug residues, although it does not generally provide an additional margin of safety for consumers. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations, comprised of the Codex Alimentarius Commission,

have stated that the scientific literature about the influence of processing on veterinary drug residues in food is inadequate to consider precise determination. Therefore, additional studies are needed in this area because veterinary drug residues vary in their susceptibility to degradation by heating (CAC, 2017).

Martins *et al.* (2007) stated that analysis of antibiotics in milk by LC-MS/MS. It is more specific and more reliable. Simultaneous determination of 14 antibiotics from different classes includes five β-lactams, four sulfonamides, three Tetracycline, one macrolide, and one cephalosporin by (LC-ESI) and triple quadrupole mass spectrometry (MS/MS) (Jayalakshmi *et al.*, 2017).

(Rinky et al., 2025) tested 27 Dhaka milk samples for five veterinary antibiotics. Oxytetracycline (OTC) was present in all samples, along with varied amounts of CTC, ENR, TC, and DOX. Raw milk contained fewer OTC residues than UHT and flavored milk. 37.04% exceeded residual limits, mostly TC. Tests found moderate to significant positive relationships among TC. Moreover (Negi et al., 2025) Detected and quantified amoxicillin and enrofloxacin in raw milk from North-Western Himalayan Yaks, Dzomo, and indigenous hill cattle by timing and assessing a solid phase extraction-HPLC technique. With antibiotic selection and measurement constraints, the method was linear and accurate. Two antibiotics over maximum residual levels were found in 170 dzomo and hill cow milk samples.

2.4 Public Health Risk:

Antibiotic residues may cause problems such as toxic effects, transfer of antibiotic-resistant bacteria to humans, contribute to the development of microbial drug resistance and the spread of resistant bacteria, pathological immune effects, carcinogenicity (e.g., sulfamethazine, Oxytetracycline, and furazolidone), mutagenicity, nephropathy (e.g., gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g., chloramphenicol), and allergy (e.g., penicillin) (Nisha, 2008; Bilandzic *et al.*, 2011; Jayalakshmi *et al.*, 2017), it has other significant implications for public health. Some key effects include:

- Antibiotic Resistance: Prolonged exposure to low levels of antibiotics through food consumption can contribute to the development and spread of antibiotic resistance. Resistant bacteria can enter the human body through contaminated food, potentially leading to treatment failures when antibiotics are needed to combat bacterial infections (Bilandzic *et al.*, 2011; Woolhouse *et al.*, 2015). Also, consuming food containing antibiotic residues may reduce the effectiveness of antibiotics in treating infections. Antibiotic-resistant bacteria can transfer their resistance genes to other bacteria, creating a reservoir of resistant strains that are challenging to control and treat (Madigan *et al.*, 2010).
- Allergic Reactions: Individuals with known allergies or hypersensitivity to specific antibiotics may experience adverse reactions if exposed to food containing residues of those antibiotics. This can range from mild allergic reactions to more severe responses. Many allergic reactions, including anaphylaxis and serum sickness, have been related to antibiotic residues, especially penicillin. (Treiber & Beranek,2021).
- Disruption of Microbiota: Antibiotic residues in food can disrupt the natural balance of beneficial bacteria in the human gut. This disturbance in the gut microbiota can affect digestion, nutrient absorption, and overall gut health (Fritz and Zuo, 2007).
- Environmental Impact: Antibiotic residues from food can enter the environment through the drug manufacturing process, throwing of unused drugs and containers, or application of manure and waste slurries, wastewater, and agricultural runoff, contributing to environmental contamination. This contamination can potentially affect ecosystems and the development of antibiotic-resistant bacteria in the environment. Also, animals excrete a significant proportion of antibiotics (17-90%) directly into urine and feces as parent compounds or toxic metabolites because many of the 28 administered antibiotics are not completely absorbed from the gut (Chee-Sanford *et al.*, 2012; Massé *et al.*, 2014).

2.5 Antibiotic residues detection methods

Microbiological assays for identifying antibiotic residues include bioassays that use antibiotic-sensitive bacterial strains for quantitative or qualitative outcomes, as well as microbial screening tests that use genetically modified bacteria that exhibit fluorescence in response to medicines. Immunoassays such as ELISA use antibodies for the sensitive and specific identification of antibiotic residues, while Lateral Flow Immunoassays (LFIA) provide fast qualitative findings, making them appropriate for on-site testing. Chromatographic techniques, including High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC), are proficient in detecting antibiotic residues in intricate matrices, with HPLC serving as a robust instrument in residue analysis. Gas Chromatography-Mass Spectrometry (GC-MS) is optimal for analyzing volatile antibiotics. Mass spectrometry methods, such as LC-MS

and GC-MS, facilitate the detection, identification, and quantification of antibiotic residues. Biosensors, including electrochemical and optical varieties, identify antibiotic residues by interactions with bio-receptors or by assessing fluorescence and surface plasmon resonance. Molecular techniques such as PCR may detect antibiotic resistance genes, so implicitly suggesting the presence of antibiotic residues, while DNA microarrays enable the high-throughput detection of many resistance genes. The selection of a detection technique is contingent upon the kind of antibiotic, the complexity of the sample, sensitivity requirements, and available resources, with researchers and regulators often using a mix of these technologies for precise identification of antibiotic residues.

3. Materials and Methods

An experimental investigation was carried out to determine the impact of heat treatment on specific antibiotic concentrations in fresh cow milk. Milk samples were collected from a healthy cow not treated with antibiotics for at least eight months at Khartoum University Farm.

3.1 Preparation of stock standards and working

The pure antibiotic powders of TYLO, ENRO, and OTC were obtained from PASH-PHARMA CO.LTD. whereas AMP was obtained from AMI-PHARMA CO.LTD.To prepare standard stock solutions of antibiotics, 10 mg of TYLO, OTC, ENRO, and AMP were dissolved in methylene chloride, HCL, or water, depending on the solubility characteristics of the antibiotics. The resulting solution was adjusted to 50 ml in a volumetric flask and stored in a refrigerator at 4ºC to achieve a concentration of 200 ppm. A mixture of antibiotics was prepared by taking an aliquot from each antibiotic and mixing it with the standard stock solution, with some modifications made based on the method outlined by Loksuwan (2002). Additionally, different concentrations of antibiotics (3 ppm, six ppm, and nine ppm) were prepared by taking another aliquot to spike milk samples.

3.2 Preparation of milk samples spiked with antibiotics

Three concentrations (3 ppm, 6 ppm, and 9 ppm) were selected to prepare different antibiotics in milk. The amount of stock solution required to obtain each concentration was determined based on the desired final volume of the mixture. The preparation details for each concentration are shown in Table (1) below:

Table (1): The preparation of milk samples spiked with antibiotics

Concentration of Antibiotics	Stock solution (ml)	Milk (ml)
3 ppm	**7.5**	**42.5**
6 ppm	**15**	**35**
9 ppm	**22.5**	**27.5**

(ml = milliliter)

For example, to prepare a mixture with a concentration of 3 ppm, 7.5 ml of the stock solution was added to 42.5 ml of milk. The same procedure was followed to prepare combinations with 6 ppm and 9 ppm concentrations, using the appropriate amounts of stock solution and dairy.

3.3 Thermal Treatment

Three different thermal treatments were implemented, including pasteurization at 63 ºC for 30 min, boiling at 87 ºC for 5 and 10 min, on fresh cow milk spiked with three different concentrations of antibiotics (3 ppm, 6 ppm, and 9 ppm). The temperature was monitored with a thermometer, and the duration of heating was recorded. Pasteurized samples were rapidly cooled to 4 ºC. All heat-treated samples were stored at 4 ºC until analysis by LC-MS. The experimental models were prepared at the Central Laboratory Shambat Campus U of K and later sent to the University of Medical Science Technology (UMST) for analysis. (Loksuwan, 2002).

3.4 Sample Extraction

Following the method described by Gauguin *et al.* (2009) with some modifications, a 0.5 ml spiked milk sample was mixed with 2 ml of diluent factor containing 20% acetonitrile and 0.1% formic acid in 80% water. The mixture was vortexed for 5 seconds and then centrifuged at 13000 RPM for 5 minutes. The supernatant obtained was brought to a final volume of 10 ml using the diluent factor

in a volumetric flask and filtered through 0.45-micron syringe filters. An aliquot of 15 µL was then injected and subjected to analysis using a Liquid Chromatography-Mass Spectrophotometer (LC-MS) instrument.

3.5 LC-MS Condition

The LC-MS analysis was performed using a binary gradient mode, with pumps A and B (LC-20 AD) at a B concentration of 10.0%. The autosampler was set to -20°C, while the oven model was CTO-20AC, and the column used was C18 with a length of 150 mm and an inner diameter of 4.6 - 3 km. The run time for each injection was 11 minutes with an injection volume of 15 µL. The column temperature was maintained at 30°C throughout the analysis process.

3.6 Degradation percentage (D.P.) and degradation rate constant (k) Calculation

In chromatography-based analysis, degradation efficiency is determined using Equation (1), where C0 and C final represent the chemical concentrations before and after heating, respectively.

$$Degradation\ Efficiency\ (DP) = \left(1 - \frac{C_{final}}{C0}\right) x\ 100 \qquad (\mathbf{1})$$

C0 represents the initial concentration of the antibiotic compound. The hypothesis suggests that the degradation of antibiotic compounds follows a first-order model. Equation (2) was applied to study antibiotic residues using k, which is based on the Equation developed by Martin (1993):

$$\frac{d[C]}{dt} = -k * [C] \qquad (\mathbf{2})$$

Here, t is the heating time, and [C] is the compound concentration in the sample at a specific point in time t. Integrating Equation (2) leads to:

$$\ln[C] = \ln[C0] - k\ x\ t \qquad (\mathbf{3})$$

Equation (2) can be written as Equation (4), where the k value can be computed for each antibiotic from experimental data. Examining k values enables a straightforward comparison of the stability of antibiotics in food across studies under specific conditions independent of time.

$$K = \frac{\ln(C0) - \ln(C)}{t} \qquad (\mathbf{4})$$

3.7 Data Analysis

This study calculated k values and degradation percentages using Microsoft Excel. Statistical analysis was performed using ANOVA and independent t-tests utilizing SPSS software. A significance level of P<0.05 was considered statistically significant.

4. Results & Discussion

Information concerning the thermal stability of drug residues in food is toxicologically essential. Most food of animal origin is not eaten raw but requires heat treatment: boiling or poaching, frying, roasting, or stewing. These culinary processes may lead to protein denaturation, an increase in temperature, water, and fat loss, and changes in pH, which in turn may result in changes in the residues' concentration, chemical structure, and chemical reactions, as well as to their loss of solubility. For the past 50 years, many researchers have been interested in evaluating whether antibiotic residues can be destroyed by cooking procedures, pasteurization, or canning processes (Hassani *et al.*, 2008).

This research aims to determine the Effect of pasteurization and boiling on the concentration of some commonly used veterinary antibiotics (TYLO, ENRO, AMP, and OTC) in fresh cow milk. Secondly, the study was intended to find which type of antibiotic is more affected by the thermal treatment as per kinetic degradation. In general, the result obtained in the present study showed that all kinds of antibiotics (TYLO, ENRO, AMP, and OTC) were decreased in their all-tested concentrations (3, 6, and 9 ppm) at both types of thermal treatment (pasteurization and boiling for 5 and 10 mins) as shown in Figure (1, 2, 3 4 and 5). Moreover, according to the k- k-

values, the reduction was significantly different (p = 0.008) between thermal treatments for all antibiotics, representing the k-value trend at three thermal therapies. The pasteurization treatment was more effective in reducing antibiotic concentration than the boiling treatment. In addition, long-time boiling (10 mins) is better than short (5 mins). These findings were similar to those obtained by Roca *et al.* (2013) and Ezenduka (2014) when they compared the data for milk processing; long-time boiling is better than short-time treatment in degrading the antibiotic residues in milk. Whereas the degradation rate constant (k) at pasteurization treatment was significantly different (p= 0.002) when compared with boiling for 5 minutes, and no significant difference between pasteurization and boiling for 10 minutes (p =0.09). More specifically, in the present study, it was observed that TYLO is more affected by pasteurization (49.3%-15%) at concentrations 3, 6, and 9 ppm than OTC (46%-35.3%), ENRO (44.6%-32%) and AMP (30.6%-26.6%) as shown in Figure (6). Tylosin showed higher k values at pasteurization (0.0224 to 0.005) and boiling (0.04 to 0.016) when compared with other antibiotics under studies Figure (5). That means Tylosin is the most degraded and hence less stable one. In contrast, TYLO was more affected by pasteurization (49.3 %-15 %) than boiling (30.4%-14.6%), as shown in Figure (6). The (k) values showed a non-significant difference (p = 0.394) between k values for TYLO at pasteurization and boiling (5/10); this may reflect that all thermal treatments showed the same Effect on TYLO degradation. This is similar to the results obtained by (Zorraquino *et al.*, 2011), who found that TYLO residue was reduced during chicken cooking. Reduction percentage is an insignificant and positive correlation with cooking time, sample weight percent, and center temperature. Despite this, a different result was observed by Abdurrahman (2001), who indicated the absence of any effect of heating or boiling on Tylosin. In addition, it was observed that pasteurization reduced OTC concentration (46 %-35.3 %).

However, a more pronounced reduction (59.7 %-15 %) was observed at boiling treatment than TYLO (30.4%-14.6%), AMP (29.3%-23.3%), and ENRO (11.6%-6.2%), as shown in Figure (6). The range of k value for OTC (0.122-0.089) at boiling (5/10) was higher than the other antibiotics. The author concluded that OTC had a faster breakdown and was less stable at (87OC/ 10 and 5 mins). This observation agrees with (Aulton & Taylor, 2017), who said that the higher the value of k, the faster the breakdown and the less stable the product, whereas a small value of k means a slow rate of study; hence a sturdy product. The multiple Comparisons analysis (ANOVA) showed a significant difference (p=0.023) between TYLO and OTC p-value—also, a highly significant difference (p=0.008) between the k value for OTC and ENRO. The author observed that ENRO was more labile than OTC. This finding agrees with (Rose *et al.*, 1996) observation; OTC appears to be very heat labile, as it can be almost wholly degraded during boiling for half an hour in water. Also, agree with the finding of Abou-Raya *et al.* (2013), who examined the effects of heating T.C.s such as Oxytetracycline (OTC) and Tetracycline (TTC) in the microwave, simmering, and roasting procedures, Chlortetracycline (CTC) and Doxycycline (DOC) in chicken breast and thigh under heat treatments, the degradation percentages of tetracycline range from 2 to 100%. Their studies have demonstrated that DOC is the most heat-stable of the four compounds, while OTC is the least in a chicken matrix. Despite this, the author observed a non-significant difference between the k value for Oxytetracycline at different thermal treatments (p =value 0.223) was observed. This finding agrees with Ezenduka (2018), stating that cooking methods generally reduce the concentration of Oxytetracycline (OTC) in the meat while boiling is more effective in reducing the concentration of OTC in muscle while roasting is more effective in reducing the concentration of liver samples. Since some of the reductions in OTC concentrations were, although OTC concentrations were not statistically significant, it is possible that not statistically significant, there is a possibility that OTC concentrations after cooking were still above the MRL.

Also, it does not comply to some extent with Abdurrahman's 2001 observation of a very slight influence of boiling on OTC. In contrast, a reverse pattern was observed by Loksuwan (2002), who studied the change by heating residues of OTC, T.C., and CTC in milk by using HPLC with a U.V. detector. The milk spiked with OTC, T.C., and CTC at 200, 200, and 400ppb are heated to 63oC for 30 min. OTC residues were significantly (p< 0.05) reduced by 79.36-86.17%.

T.C. residues were significantly (p < 0.05) reduced by 22.97-54.15%. Non-significant (p >0.5) reduction of CTC was found. In general, he concluded that a standard pasteurization procedure (630 C for 30 min) causes a decrease in OTC, T.C., and CTC residues in milk, but it does not eliminate all the detritus from milk. Also, the same observation was obtained by Ezenduka (2014) when he studied the effect of pasteurization treatment in milk spiked with OTC, which was significant ($p \leq 0.05$). The present study demonstrated that ENRO was the antibiotic with the lowest efficacy. When compared to the other antibiotics in the research, its boiling impact ranged from 11.6% to 6.2% for 10 minutes, and the (k) values demonstrated a statistically significant difference between the two (p = value 0.031) as shown in Figure (3). Also, there was approximately a considerable difference (p =0.055) between TYLO and ENRO; this finding is similar to that reported by Lolo *et al.* (2006), who observed that cooking procedures did not reduce Enrofloxacin residues; therefore, this residue retained its stability during heating. Whereas during boiling treatment, some quinolones were extracted into broth. Nevertheless,

quinolone residues were increased during the cooking procedure with water loss. While Lolo *et al.* (2006) and Junza *et al.* (2014) found that Enrofloxacin was less stable than ciprofloxacin, as Enrofloxacin could degrade into ciprofloxacin during heating.

Also, the author observed that AMP is the lowest type of antibiotic in its effect with pasteurization (30.6%-26.6%) when compared with other types of antibiotics in this study, as shown in Figure (2). Also, there was a significant difference (P=0.018) between AMP and TYLO. These findings were similar to the observation obtained by Bakhit (2006), who investigated the stability of four different concentrations of penicillin added to milk under sterilization, pasteurization, and boiling using the cup plate diffusion method. The result showed that the pasteurization and boiling treatments did not affect the penicillin activity, while the sterilization treatment decreased the penicillin activity. On the other hand, several β-lactams such as penicillin (PCN) G, Ampicillin (AMP), and amoxicillin (AMX) appear partially heat-labile (Traub and Leonhard, 1995).

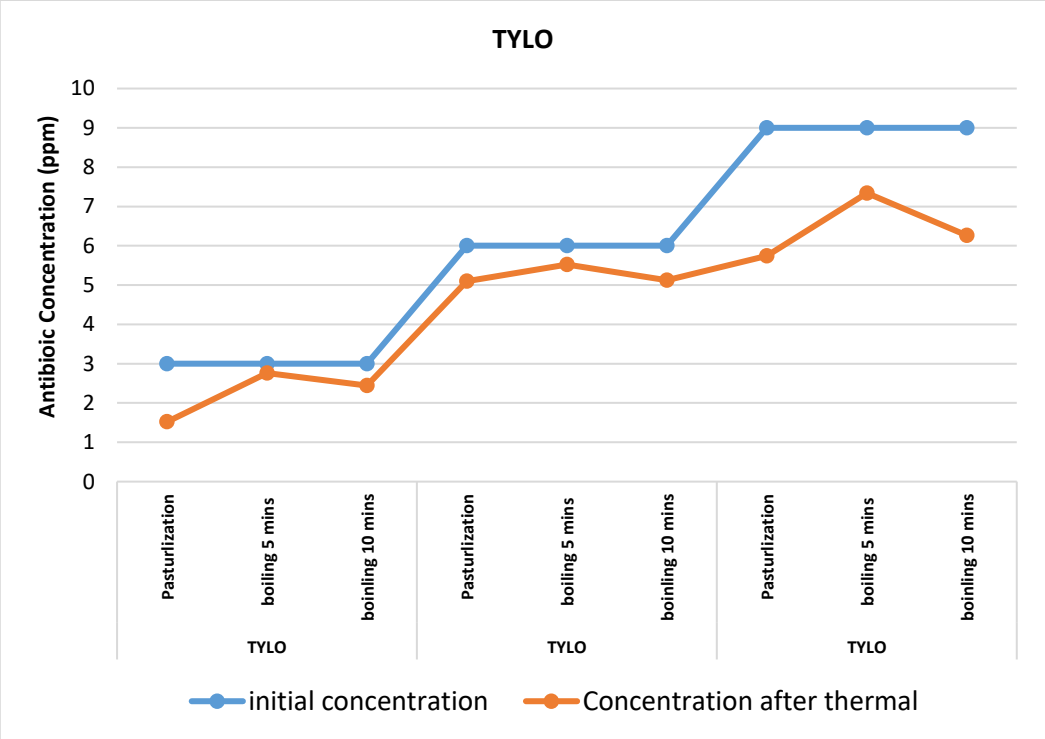

Figure (1): The Effect of thermal treatment on the different Concentration of Tylosin

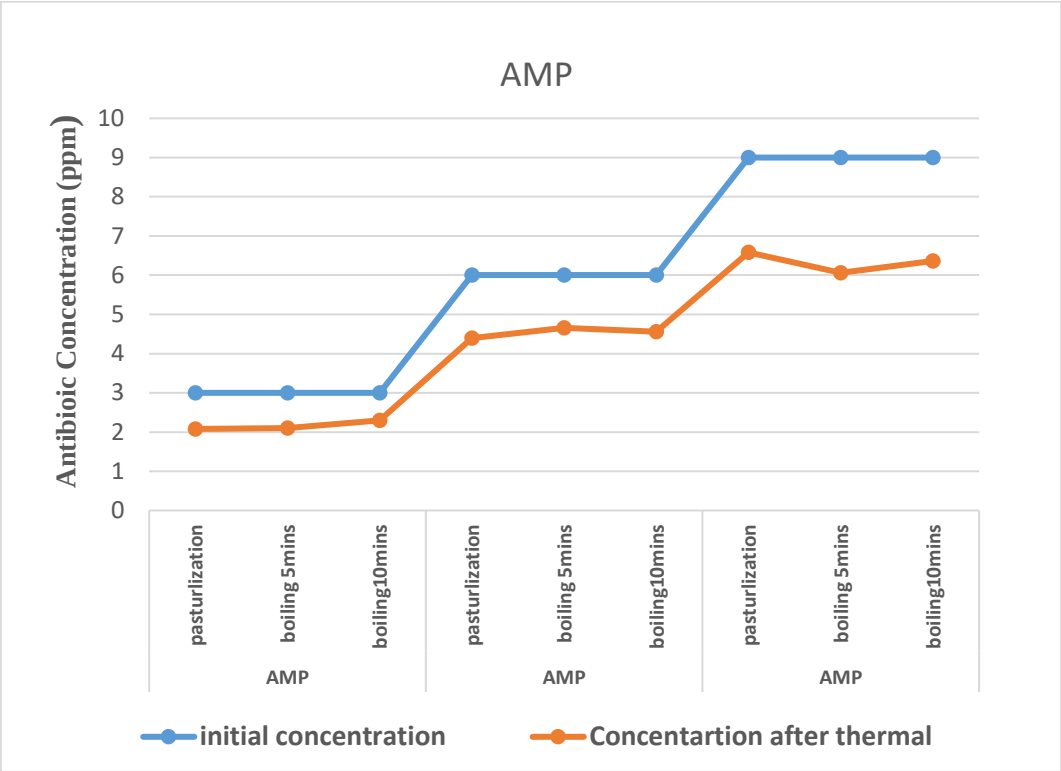

Figure (2): The Effect of thermal treatment on the different concentrations of Ampicillin

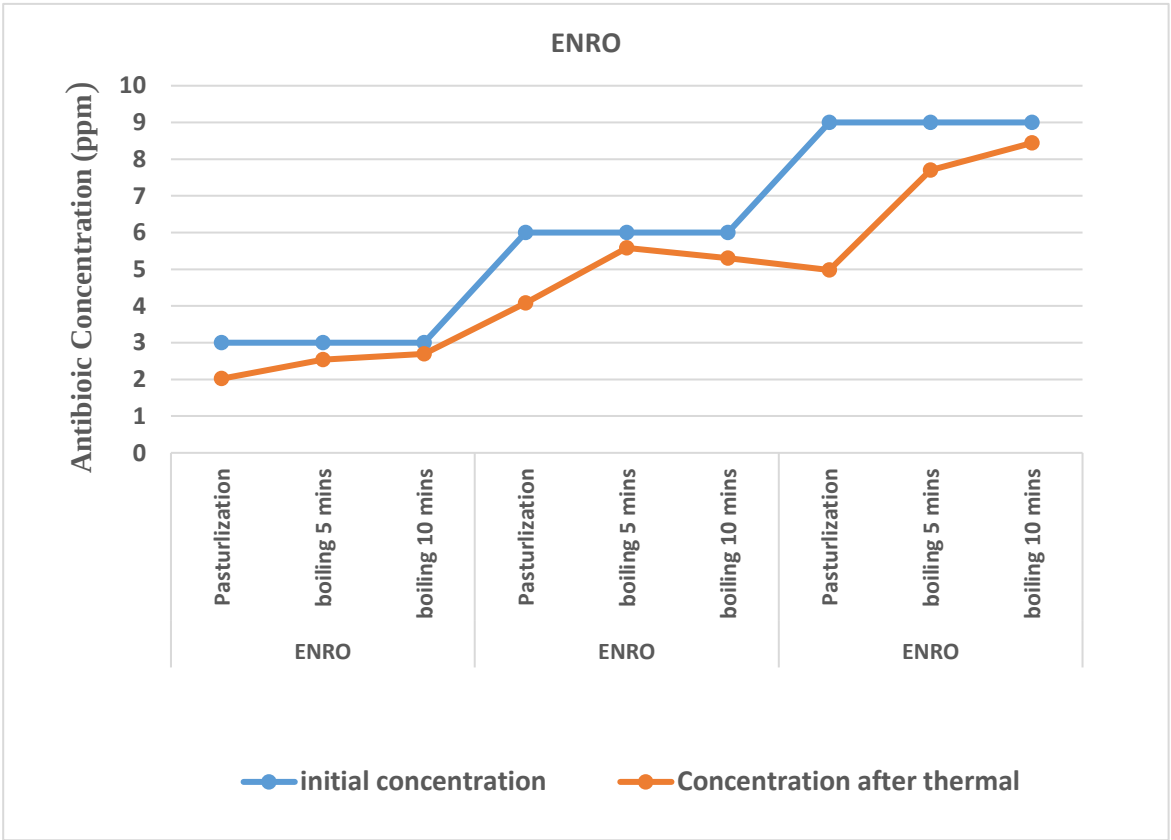

Figure (3): The Effect of thermal treatment on the different concentrations of Enrofloxacin.

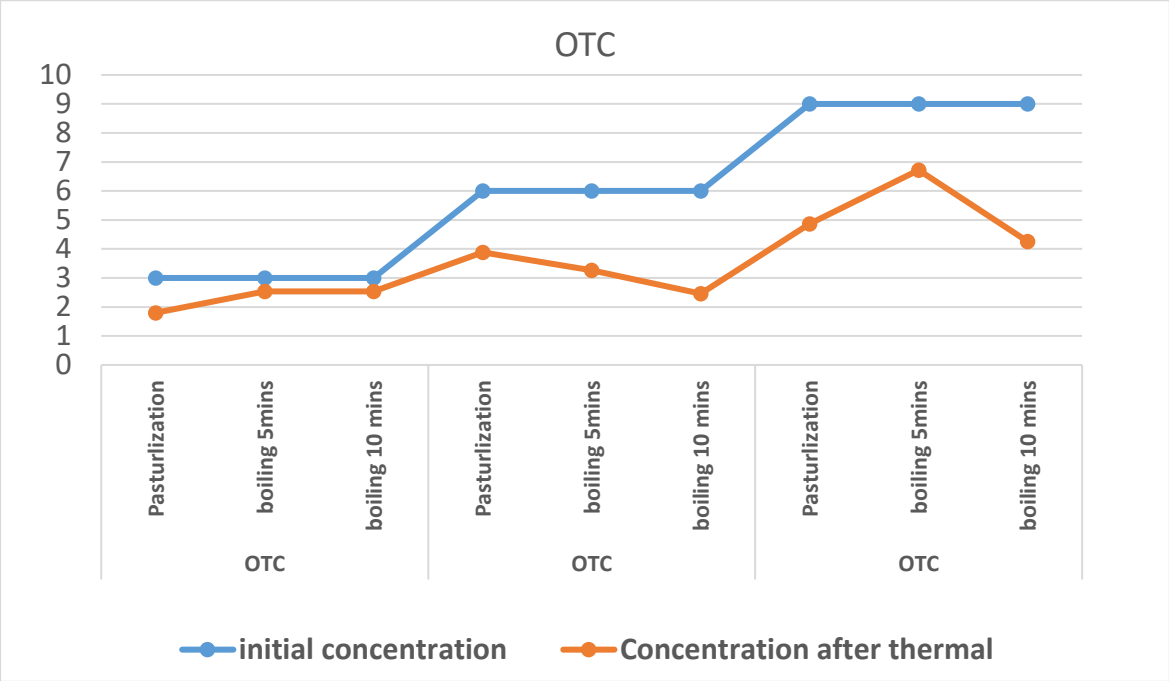

Figure (4): The Effect of Thermal Treatment on the Concentration of Oxytetracycline.

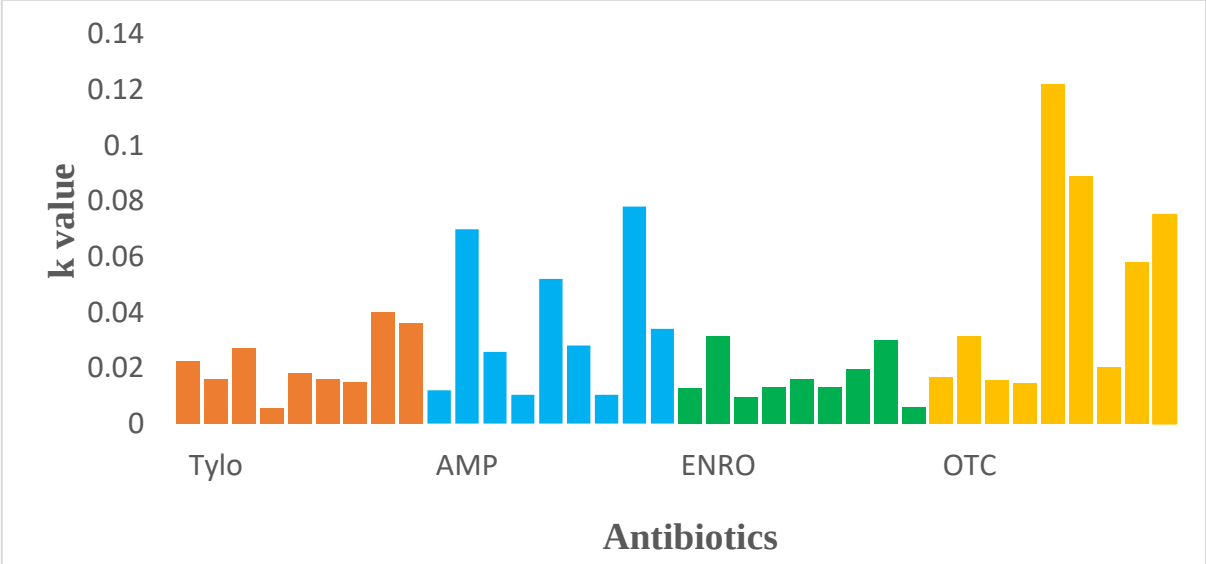

Figure (5): The Range of k values for antibiotics

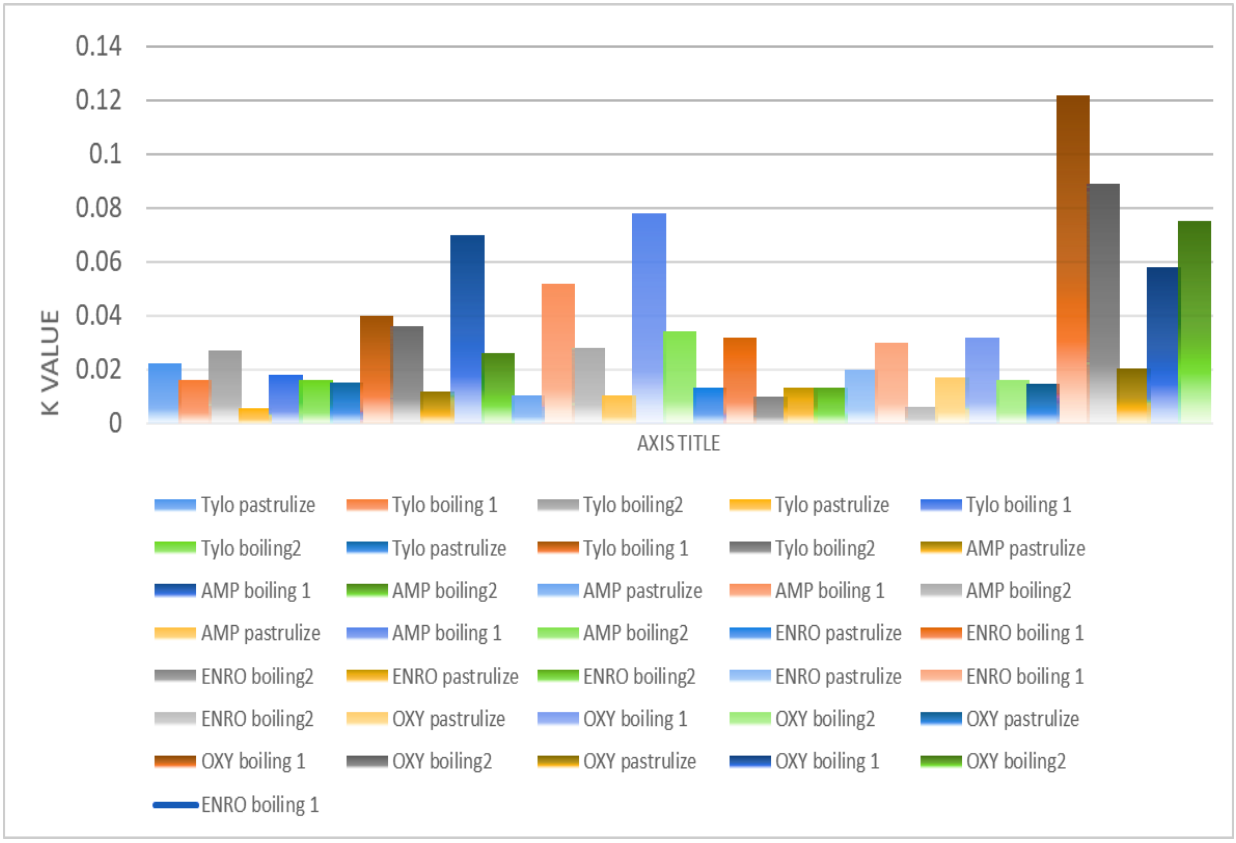

Figure (6): Compared the k values for all Antibiotics at different thermal treatments

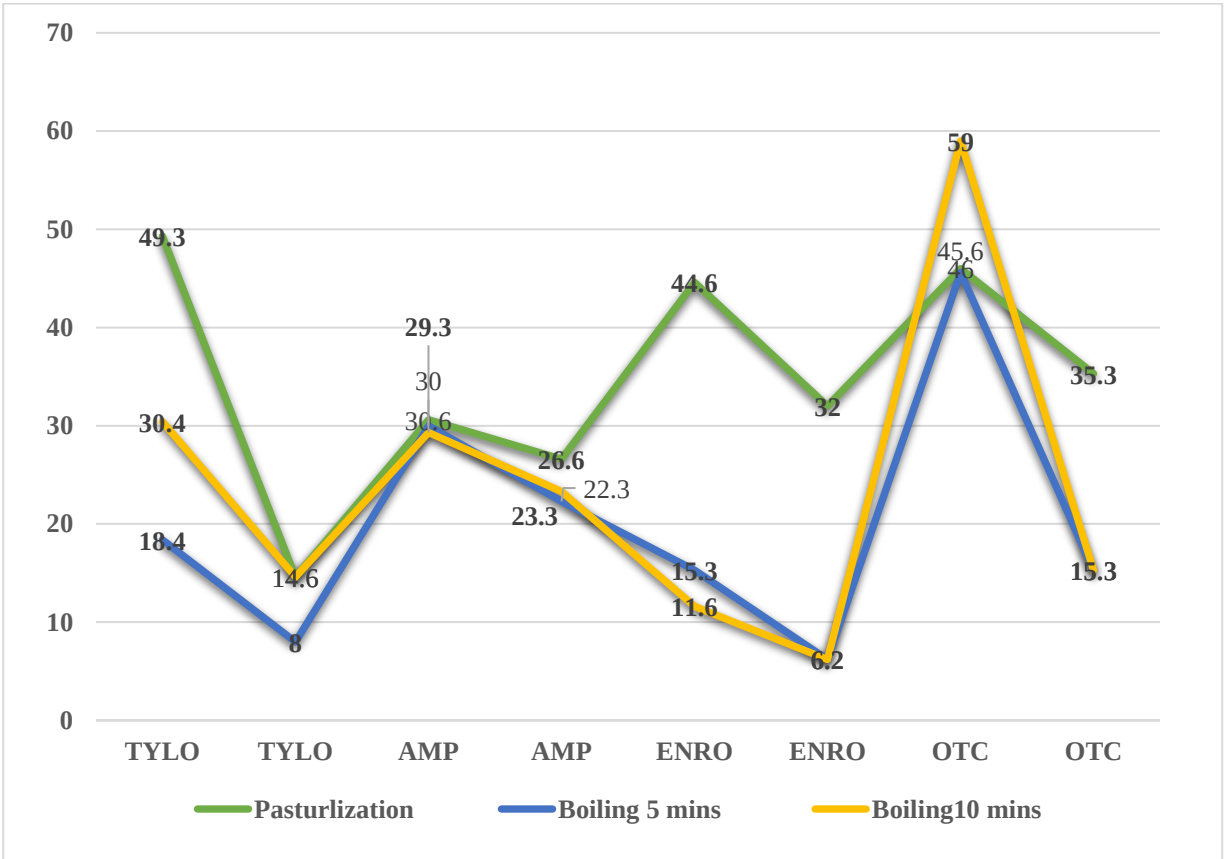

Figure (7) Degradation percentage range of antibiotics at different thermal treatment

5. Conclusion:

The study concluded that thermal treatments reduced the concentrations of all types of antibiotics added to the milk. According to the K value, the results revealed that boiling treatment had a better effect in reducing antibiotic residues. However, according to D.P., pasteurization had a higher percentage of all antibiotics. On the other hand, OTC was the most affected and least stable; it had a higher k value and higher degradation percentage at both boiling treatments (87 ºC / 5 and 10 mins) than all antibiotics. Also, TYLO had a higher k value and higher degradation percentage at pasteurization than boiling; therefore, it is the least stable. Also, AMP and ENRO were more stable at thermal treatments than other antibiotics under study. Thus, according to the type of antibiotic, type of thermal treatment, kind of food and its physicochemical properties, extraction methods, and detecting instruments, results may vary from one study to the other. Hence, no unique or absolute resolution could be obtained to reduce the antibiotic effect when added to food items.

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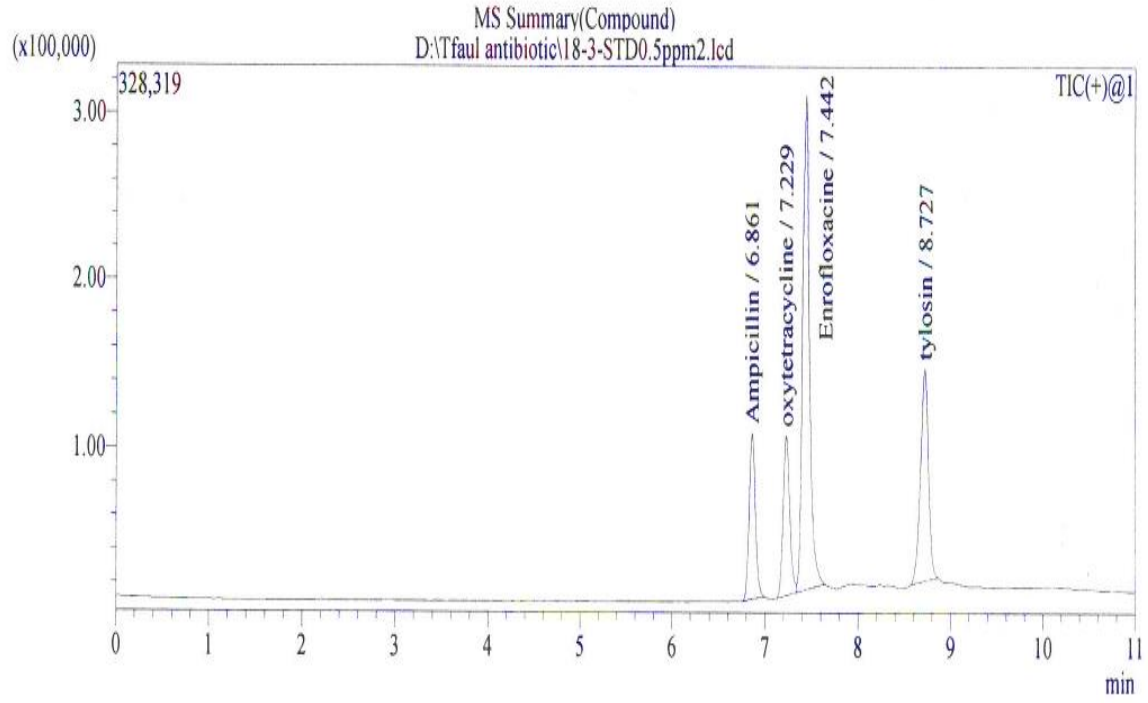
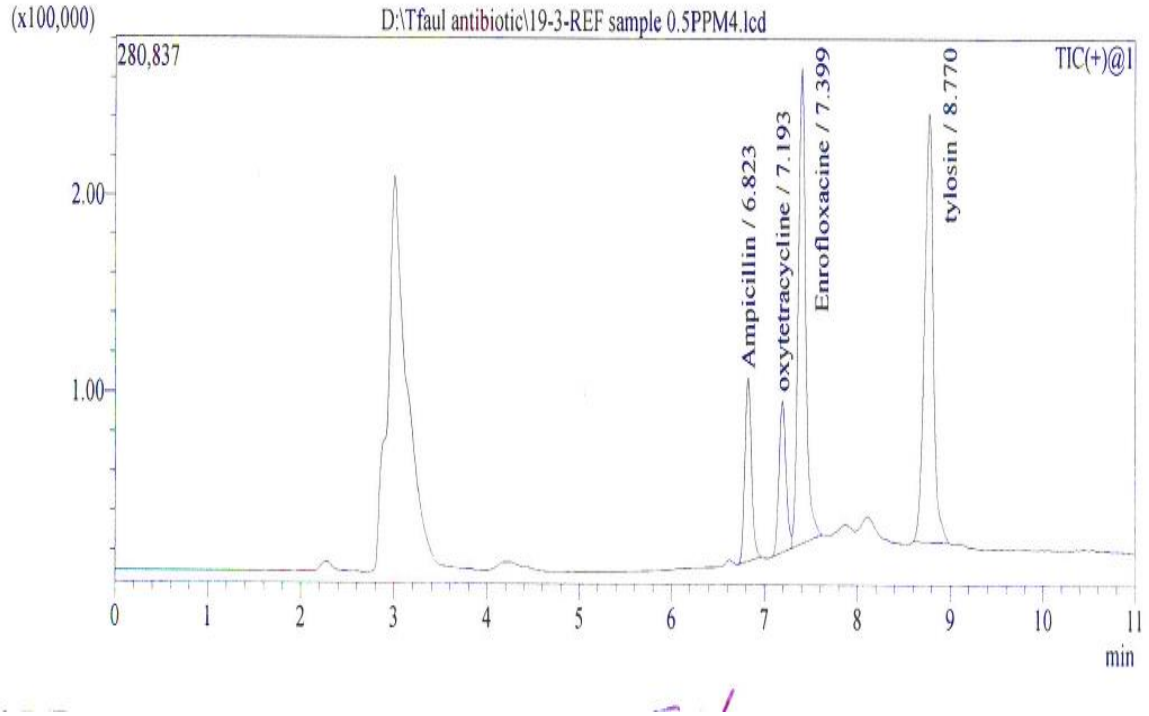
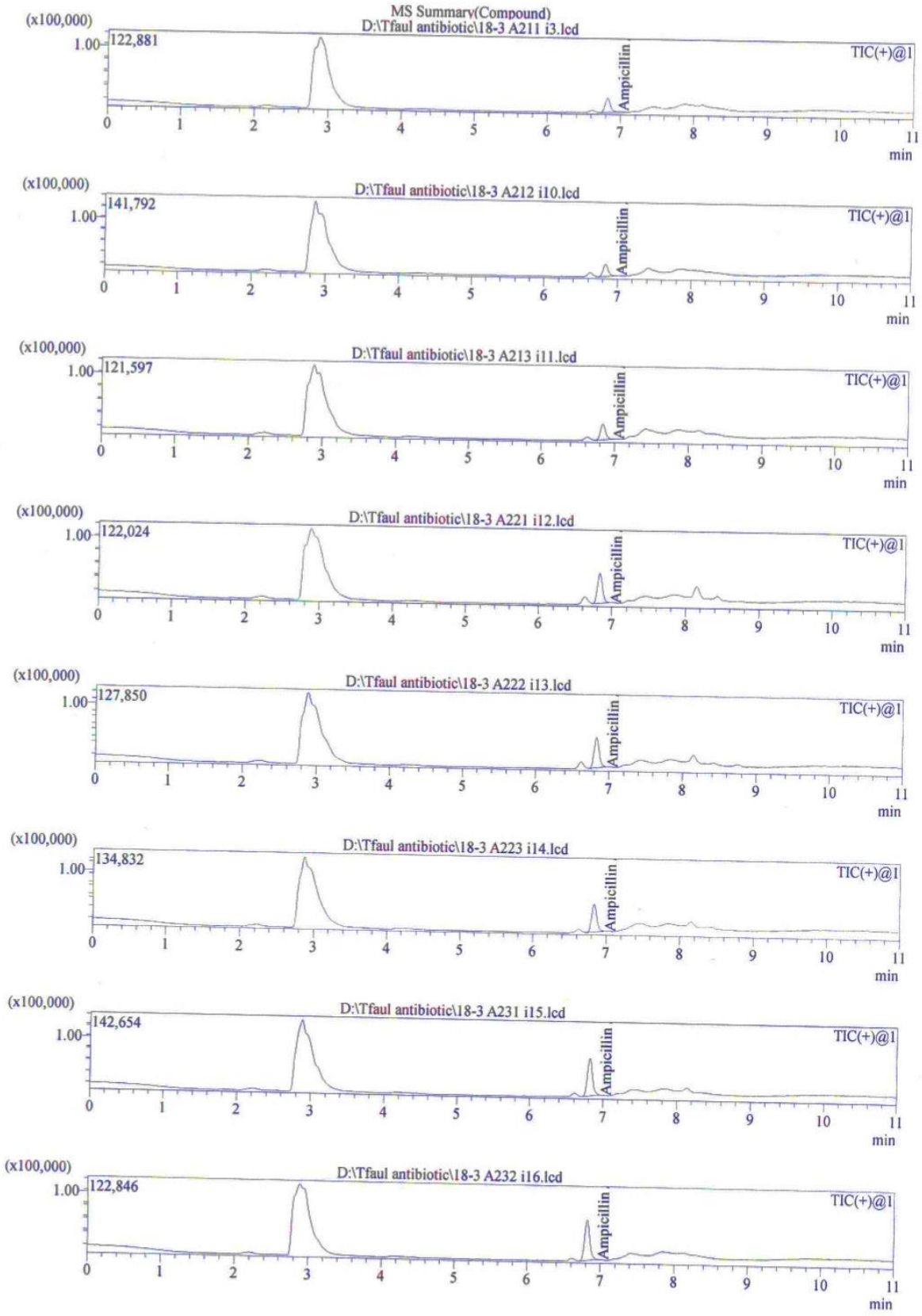
Appendixes:

LC-MS results for four standard antibiotics after different thermal treatments



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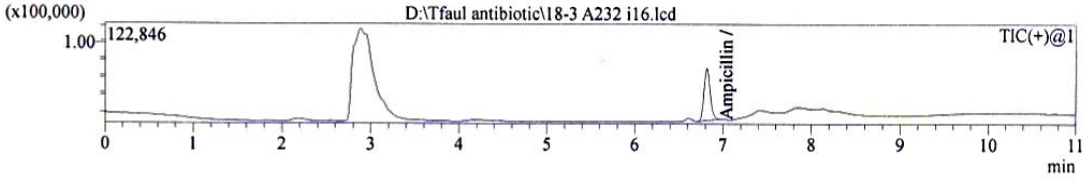
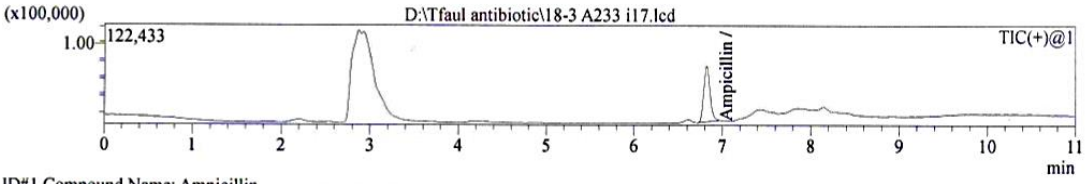
TIC(+)@1
Ampicillin
0 1 2 3 4 5 6 7 8 9 10 11
min





ID#1 Compound Name: Ampicillin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 A211 i3.lcd	6.824	104751	21375	0.104	ppm
D:\Tfaul antibiotic\18-3 A212 i10.lc	6.836	105077	21408	0.105	ppm
D:\Tfaul antibiotic\18-3 A213 i11.lc	6.837	115194	23394	0.115	ppm
D:\Tfaul antibiotic\18-3 A221 i12.lc	6.833	221059	44891	0.220	ppm
D:\Tfaul antibiotic\18-3 A222 i13.lc	6.829	234123	47520	0.233	ppm
D:\Tfaul antibiotic\18-3 A223 i14.lc	6.834	228447	46447	0.228	ppm
D:\Tfaul antibiotic\18-3 A231 i15.lc	6.815	330515	66935	0.329	ppm
D:\Tfaul antibiotic\18-3 A232 i16.lc	6.818	303738	60919	0.303	ppm
D:\Tfaul antibiotic\18-3 A233 i17.lc	6.823	319224	64737	0.318	ppm
Average	6.828	218014	44181	0.217	
%RSD	0.116	41.908	41.644	41.908	
Maximum	6.837	330515	66935	0.329	
Minimum	6.815	104751	21375	0.104	
Standard Deviation	0.008	91366	18399	0.091	

ID#2 Compound Name: oxytetracycline

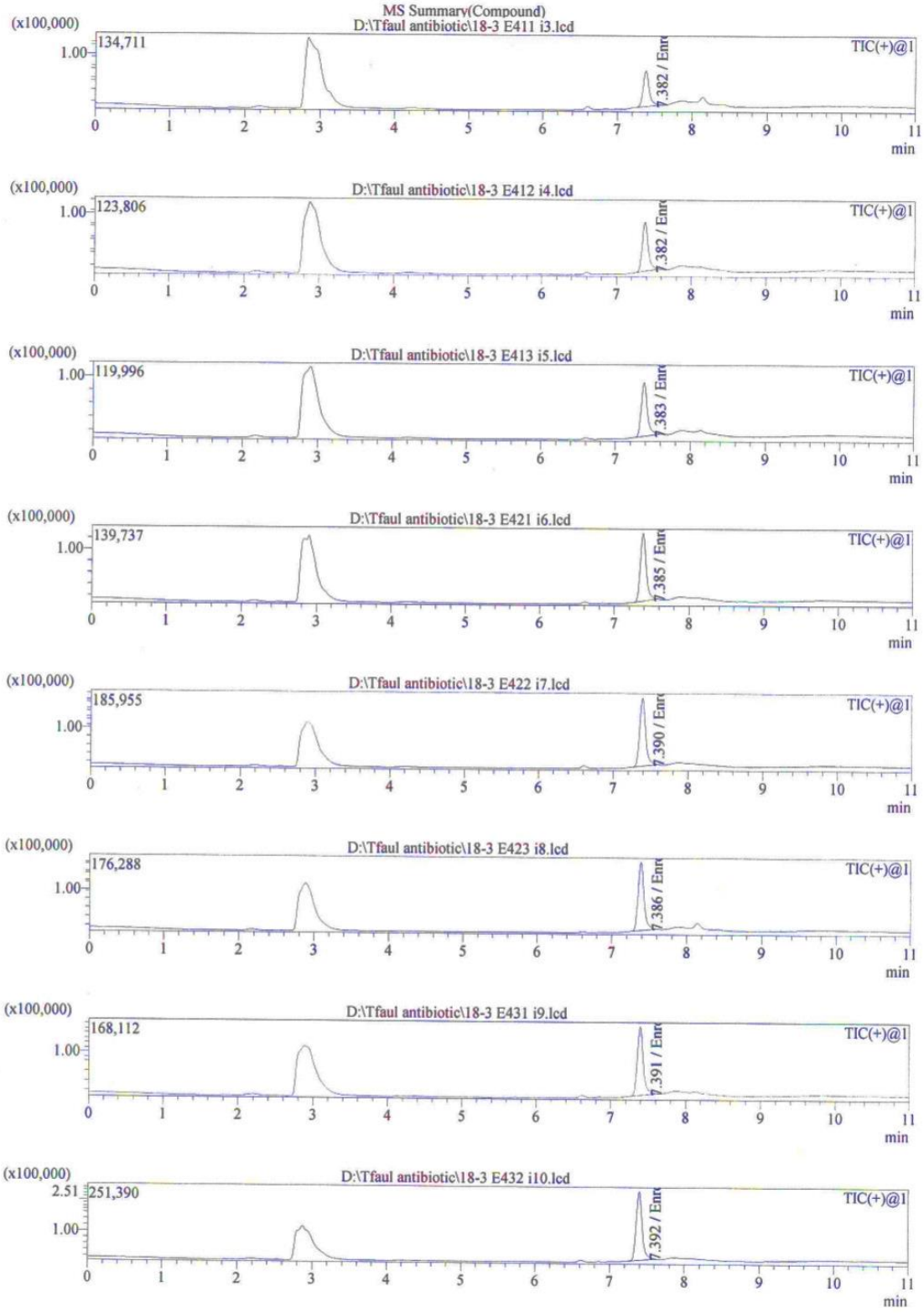
Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 A211 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A212 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A213 i11.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A221 i12.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A222 i13.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A223 i14.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A231 i15.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A232 i16.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A233 i17.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

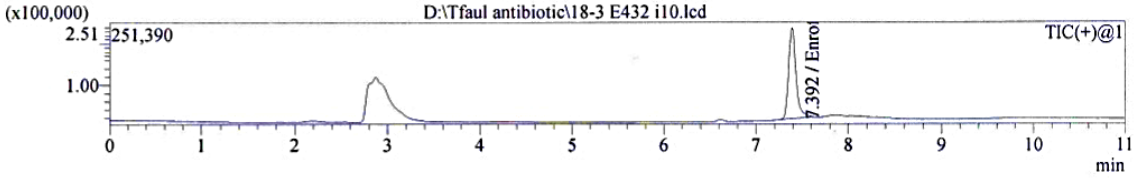
ID#3 Compound Name: Enrofloxacine

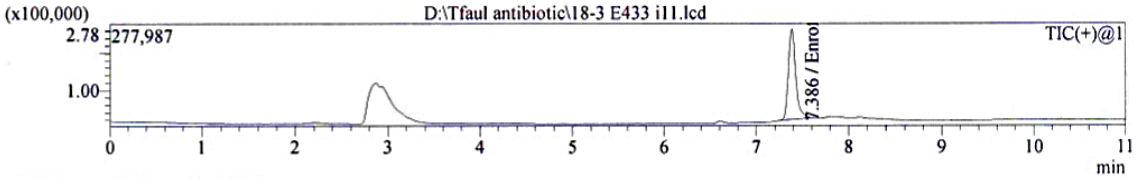
Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 A211 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A212 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A213 i11.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A221 i12.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A222 i13.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A223 i14.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A231 i15.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A232 i16.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A233 i17.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#4 Compound Name: tylosin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 A211 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A212 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A213 i11.lc	--	--	--	N.D.(W/B)	--
D:\Tfaul antibiotic\18-3 A221 i12.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A222 i13.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A223 i14.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A231 i15.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A232 i16.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 A233 i17.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	


MS Summary(Compound)
D:\Tfaul antibiotic\18-3 E411 i3.lcd
(x100,000)
134,711
TIC(+)@1
D:\Tfaul antibiotic\18-3 E412 i4.lcd
123,806
D:\Tfaul antibiotic\18-3 E413 i5.lcd
119,996
D:\Tfaul antibiotic\18-3 E421 i6.lcd
139,737
D:\Tfaul antibiotic\18-3 E422 i7.lcd
185,955
D:\Tfaul antibiotic\18-3 E423 i8.lcd
176,288
D:\Tfaul antibiotic\18-3 E431 i9.lcd
168,112
D:\Tfaul antibiotic\18-3 E432 i10.lcd
251,390
min





ID#1 Compound Name: Ampicillin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 E411 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E412 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E413 i5.lcd	--	--	--	N.D.(W/B)	--
D:\Tfaul antibiotic\18-3 E421 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E422 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E423 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E431 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E432 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E433 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#2 Compound Name: oxytetracycline

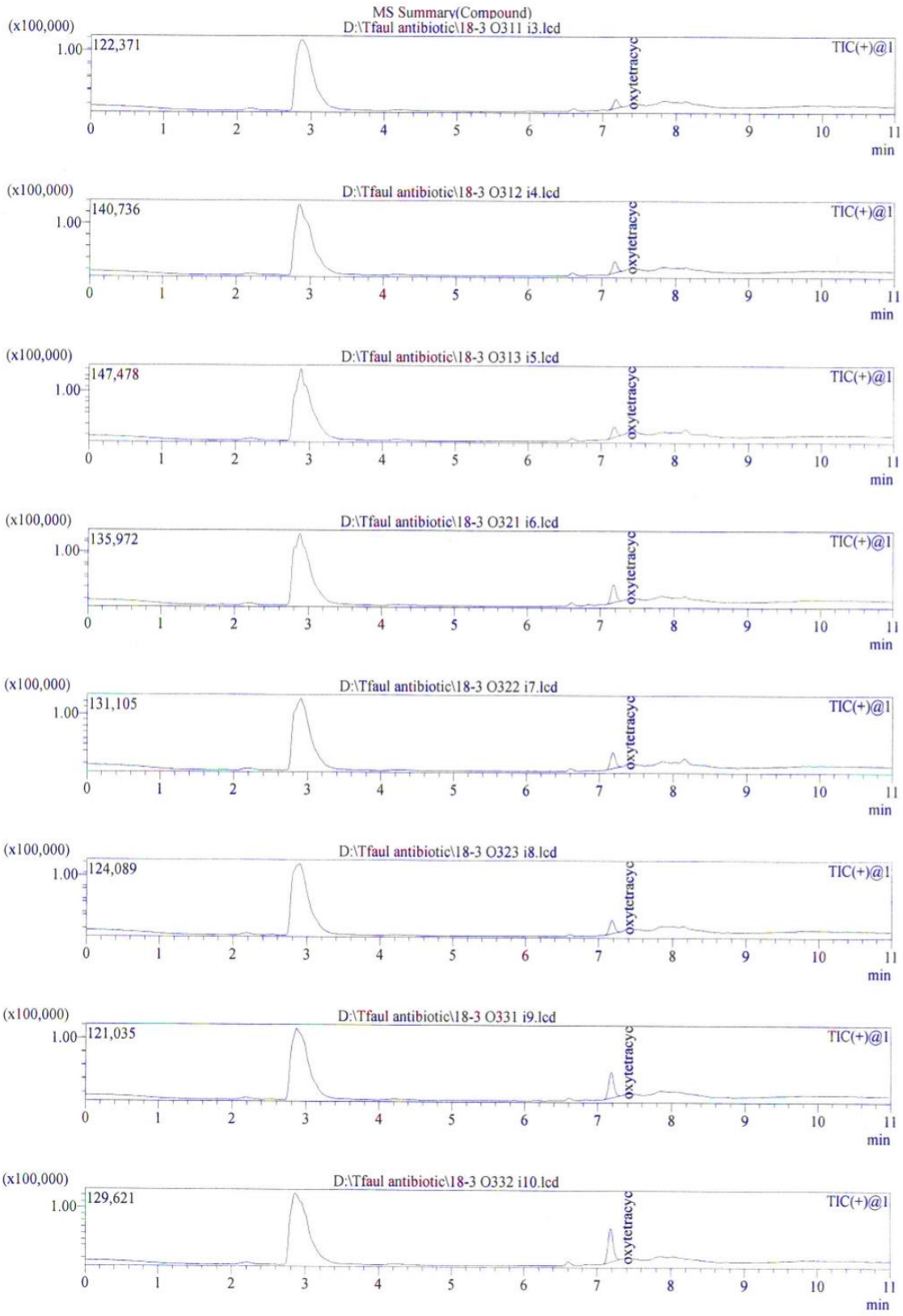
Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 E411 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E412 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E413 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E421 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E422 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E423 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E431 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E432 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E433 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

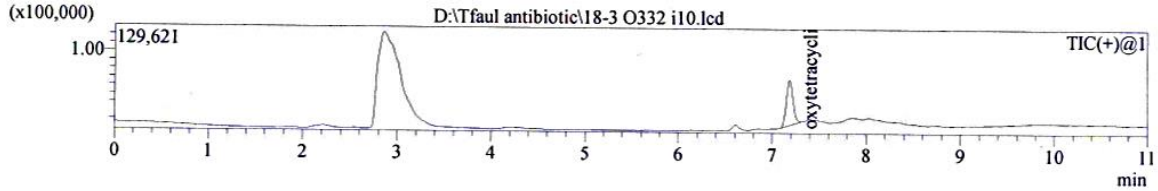
ID#3 Compound Name: Enrofloxacine

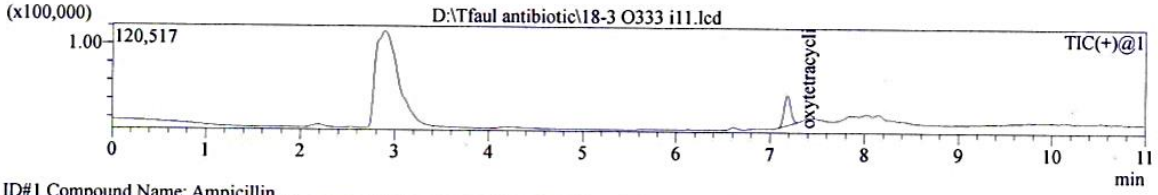
Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 E411 i3.lcd	7.382	307903	57109	0.101	ppm
D:\Tfaul antibiotic\18-3 E412 i4.lcd	7.381	386698	71836	0.127	ppm
D:\Tfaul antibiotic\18-3 E413 i5.lcd	7.382	408123	76129	0.135	ppm
D:\Tfaul antibiotic\18-3 E421 i6.lcd	7.385	618171	115623	0.204	ppm
D:\Tfaul antibiotic\18-3 E422 i7.lcd	7.390	845087	156766	0.279	ppm
D:\Tfaul antibiotic\18-3 E423 i8.lcd	7.386	804675	149490	0.265	ppm
D:\Tfaul antibiotic\18-3 E431 i9.lcd	7.391	755871	140716	0.249	ppm
D:\Tfaul antibiotic\18-3 E432 i10.lc	7.392	1169051	216685	0.385	ppm
D:\Tfaul antibiotic\18-3 E433 i11.lc	7.386	1280895	238319	0.422	ppm
Average	7.386	730719	135852	0.241	
%RSD	0.056	46.607	46.522	46.607	
Maximum	7.392	1280895	238319	0.422	
Minimum	7.381	307903	57109	0.101	
Standard Deviation	0.004	340564	63201	0.112	

ID#4 Compound Name: tylosin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 E411 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E412 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E413 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E421 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E422 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E423 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E431 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E432 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 E433 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	


MS Summary(Compound)
D:\Tfaul antibiotic\18-3 O311 i3.lcd
(x100,000)
1.00
122,371
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O312 i4.lcd
(x100,000)
1.00
140,736
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O313 i5.lcd
(x100,000)
1.00
147,478
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O321 i6.lcd
(x100,000)
1.00
135,972
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O322 i7.lcd
(x100,000)
1.00
131,105
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O323 i8.lcd
(x100,000)
1.00
124,089
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O331 i9.lcd
(x100,000)
1.00
121,035
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 O332 i10.lcd
(x100,000)
1.00
129,621
TIC(+)@1
oxytetracyc
0 1 2 3 4 5 6 7 8 9 10 11
min





ID#1 Compound Name: Ampicillin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 O311 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O312 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O313 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O321 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O322 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O323 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O331 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O332 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O333 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#2 Compound Name: oxytetracycline

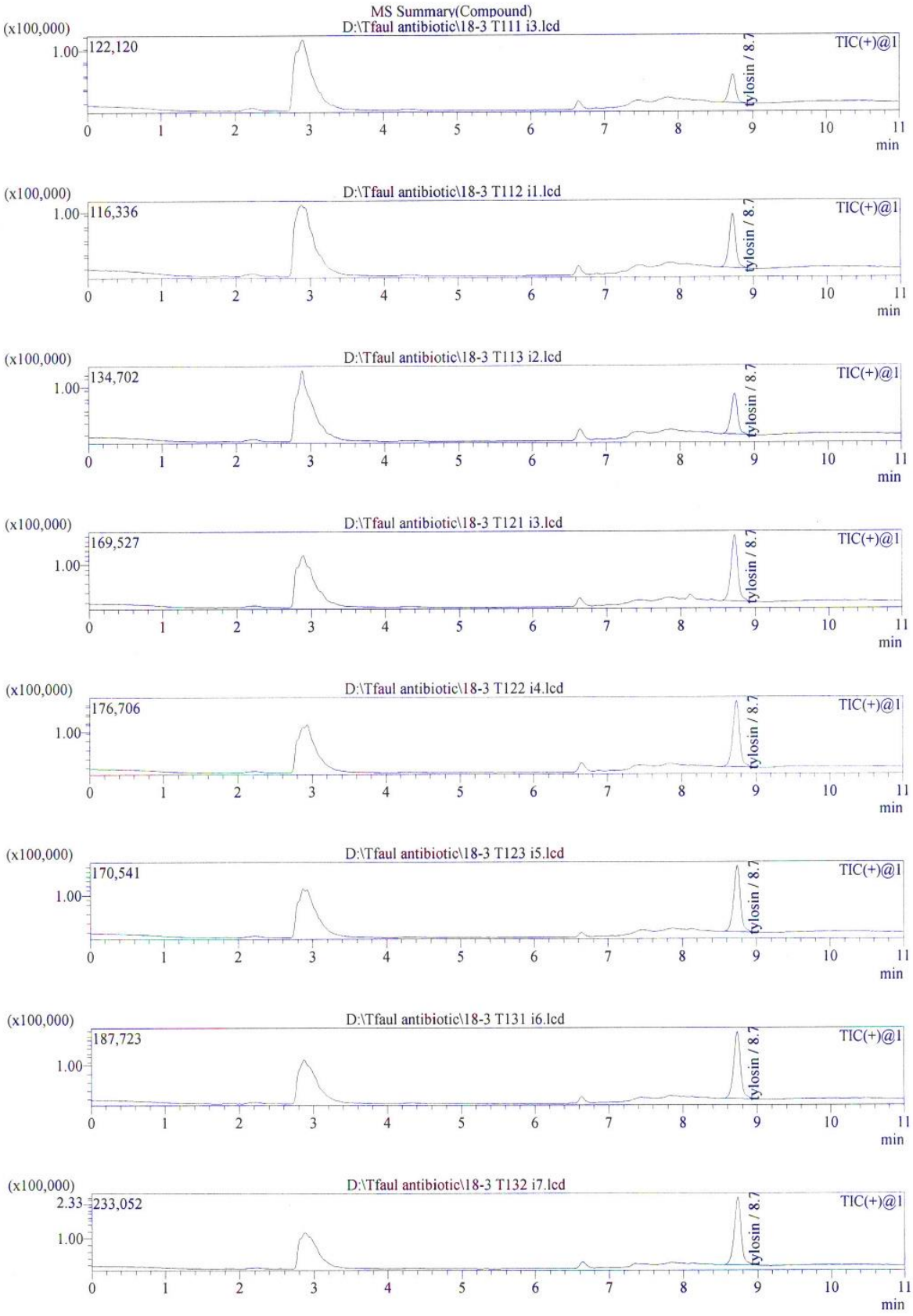
Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 O311 i3.lcd	7.180	79094	14863	0.090	ppm
D:\Tfaul antibiotic\18-3 O312 i4.lcd	7.178	111436	21121	0.127	ppm
D:\Tfaul antibiotic\18-3 O313 i5.lcd	7.178	111144	21019	0.127	ppm
D:\Tfaul antibiotic\18-3 O321 i6.lcd	7.179	169506	31768	0.194	ppm
D:\Tfaul antibiotic\18-3 O322 i7.lcd	7.182	142620	26793	0.163	ppm
D:\Tfaul antibiotic\18-3 O323 i8.lcd	7.179	115052	21401	0.132	ppm
D:\Tfaul antibiotic\18-3 O331 i9.lcd	7.182	212625	39755	0.243	ppm
D:\Tfaul antibiotic\18-3 O332 i10.lc	7.182	293840	55410	0.336	ppm
D:\Tfaul antibiotic\18-3 O333 i11.lc	7.182	186153	34813	0.213	ppm
Average	7.180	157941	29660	0.181	
%RSD	0.029	41.898	41.957	41.898	
Maximum	7.182	293840	55410	0.336	
Minimum	7.178	79094	14863	0.090	
Standard Deviation	0.002	66174	12445	0.076	

ID#3 Compound Name: Enrofloxacine

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 O311 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O312 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O313 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O321 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O322 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O323 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O331 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O332 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O333 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#4 Compound Name: tylosin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 O311 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O312 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O313 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O321 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O322 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O323 i8.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O331 i9.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O332 i10.lc	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 O333 i11.lc	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	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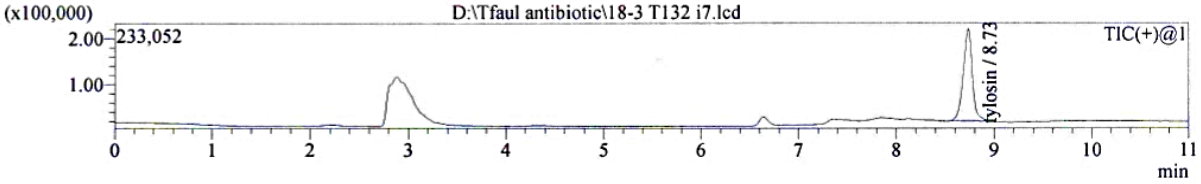
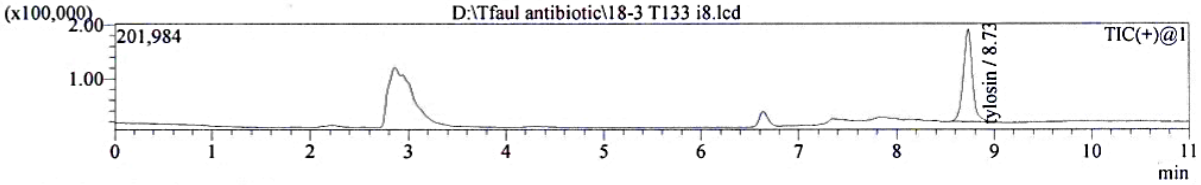
1.00
122,120
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T112 i1.lcd
(x100,000)
1.00
116,336
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T113 i2.lcd
(x100,000)
1.00
134,702
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T121 i3.lcd
(x100,000)
1.00
169,527
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T122 i4.lcd
(x100,000)
1.00
176,706
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T123 i5.lcd
(x100,000)
1.00
170,541
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T131 i6.lcd
(x100,000)
1.00
187,723
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min
D:\Tfaul antibiotic\18-3 T132 i7.lcd
(x100,000)
2.33
1.00
233,052
TIC(+)@1
tylosin / 8.7
0 1 2 3 4 5 6 7 8 9 10 11
min





ID#1 Compound Name: Ampicillin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 T111 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T112 i1.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T113 i2.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T121 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T122 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T123 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T131 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T132 i7.lcd	--	--	--	N.D.(W/B)	--
D:\Tfaul antibiotic\18-3 T133 i8.lcd	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#2 Compound Name: oxytetracycline

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 T111 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T112 i1.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T113 i2.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T121 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T122 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T123 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T131 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T132 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T133 i8.lcd	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#3 Compound Name: Enrofloxacine

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 T111 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T112 i1.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T113 i2.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T121 i3.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T122 i4.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T123 i5.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T131 i6.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T132 i7.lcd	--	--	--	N.D.(Peak)	--
D:\Tfaul antibiotic\18-3 T133 i8.lcd	--	--	--	N.D.(Peak)	--
Average	0.000	0	0	0.000	
%RSD	--	--	--	--	--
Maximum	0.000	0	0	0.000	
Minimum	0.000	0	0	0.000	
Standard Deviation	--	--	--	--	--

ID#4 Compound Name: tylosin

Title	Ret. Time	Area	Height	Conc.	Unit
D:\Tfaul antibiotic\18-3 T111 i3.lcd	8.733	268073	43601	0.076	ppm
D:\Tfaul antibiotic\18-3 T112 i1.lcd	8.724	486814	77421	0.138	ppm
D:\Tfaul antibiotic\18-3 T113 i2.lcd	8.734	429380	68718	0.122	ppm
D:\Tfaul antibiotic\18-3 T121 i3.lcd	8.727	899186	140827	0.255	ppm
D:\Tfaul antibiotic\18-3 T122 i4.lcd	8.740	943022	147693	0.267	ppm
D:\Tfaul antibiotic\18-3 T123 i5.lcd	8.742	903186	142114	0.256	ppm
D:\Tfaul antibiotic\18-3 T131 i6.lcd	8.734	1014155	158580	0.287	ppm
D:\Tfaul antibiotic\18-3 T132 i7.lcd	8.733	1288859	201243	0.365	ppm
D:\Tfaul antibiotic\18-3 T133 i8.lcd	8.734	1103912	172117	0.313	ppm
Average	8.733	815176	128035	0.231	
%RSD	0.064	41.926	41.137	41.926	
Maximum	8.742	1288859	201243	0.365	
Minimum	8.724	268073	43601	0.076	