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# Evaluation of Tetracycline and Enrofloxacin Residues in Bovine Milk in Tehran Utilizing ELISA and HPLC Methods

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## HIGHLIGHTS

- Tetracycline and enrofloxacin in milk samples were lower than the maximum residue level advised.
- Prevalence of tetracycline and enrofloxacin residue in raw milk was higher than the pasteurized milk.
- Tetracycline and enrofloxacin residues in cow milk samples assessed by Enzyme-Linked Immunosorbent Assay and High-Performance Liquid Chromatography methods.

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***Acronyms and abbreviations***
EDTA=Ethylenediaminetetraacetic Acid
ELISA=Enzyme-Linked Immunosorbent Assay
HPLC=High-Performance Liquid Chromatography
LOD=Limit of Detection
MRL=Maximum Residual Limit

## ABSTRACT

**Background:** Milk is regarded as one of the most sources of nutrition in the world and has a high value for individual's health. Milk is consumed by sensitive groups including pregnant women, older adults, and children. Therefore, the significance of antibiotic on human health makes it crucial to monitor their existence in food. This investigation aims to evaluate enrofloxacin and tetracycline in the raw and pasteurized milk in Tehran.

**Methods:** In this cross-sectional study, 112 raw and 112 pasteurized milks were accumulated in spring and winter from six reputable brands and traditional dairy stores in Tehran from December 2021 until May 2022 for six months, and antibiotic residues were examined by Immunosorbent Assay and High-Performance Liquid Chromatography techniques.

**Results**: The findings indicated that the prevalence of tetracycline in raw and pasteurized milk was 41.07 and 26.78% in spring and 85.71 and 35.71% in winter, respectively. The median concentration of tetracycline was 33.27 and 22.65 ppb in spring and 55.81 and 21.91 ppb in winter, respectively. The prevalence of enrofloxacin in raw milk and pasteurized milk samples were 33.92 and 14.28% in spring and 64.28 and 32.14% in winter, respectively. The median concentrations of enrofloxacin were 9.13 and 9.38 ppb in spring and 10.57 and 11.23 ppb in winter, respectively. The raw and pasteurized milk samples collected in winter had higher percentage of antibiotic residue in terms of enrofloxacin than samples collected in spring ($p$<0.05). Furthermore, the quantity of tetracycline antibiotic in raw milk was significantly higher in winter than in spring ($p$=0.002). However, the pasteurized milk fails to have significant difference between two seasons. The finding showed the tetracycline and enrofloxacin in all samples were less than 100 ppb (standard limit), and there is no significant difference with the standard limit.

**Conclusion:** Based on the obtained results, monitoring the antibiotic residues in milk, controlling and minimizing these residues for human health are regarded crucial.



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## Introduction

One of the most nutrient foods is milk that it consumed by humans and has a special position in the human diet, especially children and elderly (Kubicová et al., 2019). Milk consists of protein, fat, lactose, vitamins, and minerals. Minerals in milk include calcium, phosphorus, and magnesium, which cause the growth of skeletons and bones in children and juveniles (Beck and Coad, 2017). Milk also contains vitamins including A, $B_1$, $B_2$, $B_6$, and $B_{12}$ that affected the health of the bones positively (Kubicová et al., 2019). However, milk can be contaminated with microbial agents and chemical substances, which pose serious risks for consumers' health (Sharifi et al., 2021). Milk can be exposed to microbial and chemical contaminations during milking, collection, and transportation. Chemical contaminants in milk include veterinary drugs (antibiotics), insecticides, mycotoxins, and heavy metals that can enter animal foods, and subsequently their residues can be observed in milk (Motarjemi et al., 2014). The presence of antibiotic residues and milk derivatives can be attributed to the use of antibiotics for managing mastitis in dairy animals. The application of antibiotics for these animals can be carried out through various methods, including intramuscular, intravenous, intramammary, intrauterine, oral, and topical techniques. After their applications through all of these routes, antimicrobial agents and their corresponding metabolites permeate into the milk (Ray and Sen, 2019).

Antibiotics are one of the most critical chemical contaminants in milk. The major groups of antibiotics include beta-lactams, tetracyclines, sulfonamides, macrolides, and aminoglycosides. Antibiotics are generally consumed to control and treat infectious diseases, enhance growth, and improve production efficiency (Moudgil et al., 2019b). Since livestock is one of the essential sources of milk production, in case antibiotics are used in livestock, antibiotics are transferred into the milk and threaten human health (Kurjogi et al., 2019). The consumption of milk and milk products containing antibiotics and long-term exposure to antibiotics cause complications in humans, including drug resistance, allergic reactions, digestive system disorders, cancer, and mutagenesis (Alimohammadi et al., 2020). Antibiotics can prevent the action of starter cultures and cheese ripening, delay the acid production by these bacteria and also destroy beneficial bacteria used in the production of yogurt, cheese, and other fermented (Pirsaheb et al., 2014). According to the studies conducted by researchers, antibiotics such as penicillin, erythromycin, tetracycline, etc. cause an increase in cheese production time and a delay in reducing acidity (Chiesa et al., 2020; Virto et al., 2022).

One of the most crucial antibiotic classifications is tetracyclines. These groups of antibiotics bear bacteriostatic activity and are widely used in livestock to prevent and treat infectious diseases, especially mastitis. Tetracyclines are generally administered orally and in intramammary infusion. After the administration of antibiotics, a slight amount of the drug may remain in the body for a long period and be excreted in milk, and causing harmful effects to the consumer (Mesgari Abbasi et al., 2011; Mohammadzadeh Moghadam et al., 2016). For example, tetracycline and azithromycin can lead to photosensitivity reactions, discoloration of nails, and teeth, as well as disturbances in the functioning of the immune system and cardiovascular system (Kurjogi et al., 2019). Fluoroquinolone antibiotics such as enrofloxacin have been approved for use in livestock. The fluoroquinolone antibiotics treat infectious diseases brought on a wide spectrum of Gram-positive, and Gram-negative bacteria and have intense antibacterial properties against aquatic animal pathogens (Zhang et al., 2023). In general, tetracycline, quinolones, enrofloxacin, lincomycin, and streptomycin are common antibiotics used in dairy cows, and tracking the presence of these antibiotics in consumed milk is considered extremely substantial (Du et al., 2019).

Researchers currently pay particular attention to monitor and control of drug residues in food and establish and implement special regulations in this regard due to the adverse effects of antibiotics on consumer health. In European countries, the limit of antibiotic residues in food products of animal origin for enrofloxacin and tetracycline are 100µg/L (Mahmoudi et al., 2014a).

Numerous European countries have already implemented a prohibition on the addition of antibiotics in animal foods as a preventive measure in relation to the prevention of diseases in livestock populations. Furthermore, the European Union has implemented maximum limits for the presence of various substances in milk. The situation differs in numerous emerging and developing countries. Several factors contribute to the existence of antibiotic residues in meat products, particularly in developing countries. The primary rationales are as follows: (1) there is a lack of certification systems concerning food products of animal origin, and there is a significant absence of regulation in the use of veterinary antibiotics; (2) both breeders and butchers often lack the necessary time for each antibiotic to be metabolized or excreted in the animal body; and they have a limited knowledge about the consequences of excessive dosage and the development of resistance; (3) there is no comprehensive monitoring from prescription to substance usage; and (4) the methods of detection are frequently insufficient or completely unavailable in order to comply with limit values (Schmerold et al., 2023).

A number of techniques, including microbial,

immunochemical, and physicochemical methods have been developed with the aim of screening, identifying, and determining the antibiotic residues in food of animal origin. Among the mentioned methods, physicochemical methods such as spectrophotometry, spectrofluorimetry, and High-Performance Liquid Chromatography (HPLC) are used to evaluate the exact concentration of antibiotic residues (Mohammadzadeh Moghadam et al., 2016). Enzyme-linked Immunosorbent Assay (ELISA) appears to be a quantitative method, and HPLC is a confirmatory method to identify and determine antibiotic residues. Moreover, HPLC method can detect the antibiotic residues less than the respective Maximum Residual Limit (MRL) values (Kurjogi et al., 2019). The choice of these methods depends on factors such as the type of antibiotic, time, sensitivity, and cost of the method (Mahmoudi et al., 2014b).

Many studies have been performed to assay and identify antibiotic residues in Iran and other countries, and all of them indicate their presence in animal products through which antibiotics residues are investigated, e.g., milk and meat (Alimohammadi et al., 2020; Kurjogi et al., 2019; Mesgari Abbasi et al., 2011; Moudgil et al., 2019b). However, it is of utmost significance to observe the quantity of these remnants in order to assess the influence of regulatory actions on the milk industry over various years. Therefore, due to the escalating apprehension regarding antimicrobial resistance and food safety matters on a global scale, our study has been conducted with a particular emphasis on identifying antibiotic residues in milk in Tehran city.

## Materials and methods

### *Milk samples*

According to this descriptive cross-sectional study, 112 raw samples of milk and 112 pasteurized samples of milk obtained from six reputable brands and traditional milk stores, half in winter and the rest in spring in Tehran from December 2021 to May 2022 for six months. Based on 22 regions, the city of Tehran was divided into four sections: North, South, East, and West, with two chosen areas from each segment. The selected regions are North (regions 2 and 3), south (regions 16 and 19), east (regions 8 and 13), and west (regions 21 and 22). Sampling was conducted in selected regions. The highest selling brands in each area were chosen, through which pasteurized milk was obtained. Additionally, raw milk was collected randomly from traditional dairy stores located within the aforementioned selected regions.

The sample size was determined based on the research conducted by Alimohammadi et al. (2020). According to the research, milk tetracycline residue prevalence was 29%, the confidence level was 95%, and the precision appeared to be 5% (Alimohammadi et al., 2020). According to the following formula, where $Z_{1-\alpha/2}$ is equal to 1.96; P=0.29; 1-P=0.71; and d=0.05, the required sample size was calculated as 224 samples.

$$n = \frac{\left(Z_{1-\alpha/2}\right)^2 \times \mathrm{P}(1-P)}{\mathrm{d}^2}$$

Cold storage was used to transport all samples to the microbiology laboratory of Amol University of Special Modern Technologies, Faculty of Veterinary Medicine. We stored all samples at 4 °C to the time of analysis.

### *Analysis of milk samples utilizing the ELISA method*

Tetracycline and enrofloxacin levels were measured with an ELISA test kit (Europroxima BV, Arnhem, Netherlands), 5,091TC, and 5,101ERFX, respectively. Analyses, sample and reagent preparation were conducted in accordance with instructions provided by the manufacturer. The cream was collected from the top layer of homogenized milk after centrifuging at 10 °C (3,000×g, 10 min). Four hundred fifty µl of dilution buffer was combined with 50 µl of cream-free samples. A 96-well ELISA microplate was filled with 50 µl of each standard solution or prepared sample. Each well was also injected with 50 µl of antibody. Once the wells had been incubated at 20-25 °C for 60 min, a wash with buffer 250 µl was performed (The washing step was repeated two times), afterward, each well received 100 µl of the enzyme conjugate solution. Fifteen min were spent maintaining the plate at 20-25 °C. The last washing step was fulfilled twice with 250 µl of the buffer. Subsequently, 100 µl of the substrate was applied to each well and permitted to incubate at 20-25 °C for 15 min. At 450 nm, microplate reader was used to determine the absorbance following the addition of 100 µl of stop reagent to each well (Biotek, ELx 800, USA). Both tetracycline and enrofloxacin have low Limit of Detections (LODs) of 0.4 and 6 ng/g, respectively. The outcomes were ascertained by acquiring optical density values and computing the percent mean relative absorbance through the given formula. Following this status, a calibration standard curve was constructed, establishing a correlation between standard concentrations, and relative absorbance (%).

$$Relative\ absorbance\ (\%) = \frac{Absorbance\ of\ standard\ or\ sample}{Absorbance\ of\ zero\ standard} \times 100$$

### *Verification of tetracycline and enrofloxacin levels in milk through HPLC analysis*

*-Making solutions with standard concentrations*

Standard stock solutions for tetracycline and enrofloxacin (Sigma-Aldrich, Germany) were formulated from 1 µg of each compound dissolved in 1 ml of methanol. Storage of the solutions was accomplished at -20 °C. Using stock solutions, calibration curves were prepared for concentrations of 100, 50, 250, 500, and 1,000 ng/ml.

According to Moudgil et al. (2019a), sodium Ethylenediaminetetraacetic Acid (EDTA)-McIlvaine buffer was prepared. For the buffer preparation, 11.8 g of citric acid monohydrate (Merck, Germany), 13.72 g of disodium hydrogen phosphate dehydrate (Merck, Germany), and 33.62 g of EDTA disodium salt (Merck, Germany) were mixed in 1 L of water, followed by filtration through a 0.45 µm membrane filter.

*-Sample preparation*

The tube of centrifuge was filled with 5 ml of sample of milk. The sample was supplemented with 2 ml of Sodium EDTA-McIlvaine buffer and 20 ml of 20% Trichloroacetic Acid (TCA). Vortexing was performed for 5 min, followed by centrifugation at 7,000 rpm for 15 min. After filtering through whatmanTM filter paper, the supernatant was utilized for the next stages. Cartridge C18 was washed with 3 ml of distilled water and 2 ml of methanol prior to Solid Phase Extraction (SPE). Cartridges were deployed to pass sample extracts through. Following elution with methanol, the bound compounds were removed from the cartridge. The eluted samples were dried with N2 in preparation for HPLC analysis, and flowed through a 0.45 µm membrane filter. Finally, 100 µl was injected into the HPLC system (Dabbagh Moghaddam et al., 2014).

*-HPLC analysis*

Samples with values exceeding the ELISA kit's LOD were analyzed using HPLC. The chromatographic setup included a Triathlon-type 900 autosampler (Germany) and dual KNAVER Wellchrom HPLC pumps (model K-1,001, Germany). The mobile phase was composed of oxalic acid-acetonitrile (0.01 M) with a volume ratio of 15:85, flowed at a rate of 1.5 ml/min during the chromatographic process. The withdrawn sample was introduced into a Phenomenex ODS C18 column (250×4.60 mm, 5 µm) with an injection volume of 20 microliters. A UV detector (RF-10AXL, KNAUER, Germany) configured to operate at a wavelength of 365 nm was employed to detect tetracycline and enrofloxacin.

*Statistical analysis*

Analysis of data was conducted using SPSS software version 26 (SPSS Inc., Chicago, IL, USA). The normality of the data was assessed through the Kolmogorov-Smirnov test. To examine the antibiotic concentration variations across various milk types, the Mann–Whitney U test was applied.

**Results**

As illustrated in Table 1, the prevalence of tetracycline and enrofloxacin residues in raw and pasteurized milk samples using the ELISA method. The residual values for tetracyclines were 63.39 and 31.35% in raw and pasteurized milk. The residual values for enrofloxacin were 49.10 and 23.21% in raw and pasteurized milk. According to the obtained results, a higher prevalence of tetracycline and enrofloxacin residues in raw milk than in pasteurized milk. Table 2 shows the results of seasonal prevalence and the average concentration of antibiotic residues in milk. The obtained results from the ELISA method revealed that the prevalence of tetracycline in raw and pasteurized milk samples were 41.07 and 26.78% in spring and 85.71 and 35.71% in winter, respectively. The prevalence of enrofloxacin in raw and pasteurized milk samples was 33.92 and 14.28% in spring and 64.28 and 32.14% in winter, respectively. Further, based on Table 2 for tetracycline, the highest and lowest levels of antibiotic residue as detected by ELISA were respectively greater than 88 ppb and 1.57 ppb. The median concentration of tetracycline in raw and pasteurized milk samples was 33.27 and 25.65 ppb in spring and 55.81 and 21.91 ppb in winter, respectively. Based on the finding of ELISA method, the median concentration of enrofloxacin in raw and pasteurized milk samples seemed to be 9.13 and 9.38 ppb in spring and 10.57 and 11.23 ppb in winter, respectively. The collected raw and pasteurized milk samples in winter had a higher percentage of antibiotic residue in terms of enrofloxacin than samples collected in spring ($p$<0.05). In addition, the amount of tetracycline antibiotic in raw milk was significantly higher in winter than in spring ($p$=0.002), however, the pasteurized milk samples had no significant differences between the two seasons. Figures 1 and 2 illustrate the standard calibration curve for tetracycline and enrofloxacin by ELISA, respectively. HPLC was used to measure seven samples with tetracycline values above the kit LOD in the ELISA method (Table 3). None of the samples indicated values of enrofloxacin higher than the kit's LOD. The acquired finding from the ELISA and HPLC methods showed that the tetracycline and enrofloxacin in all samples were lower than 100 ppb, which is the maximum residue level advised based on the standards of the Iranian Union, Europe, and Codex.

**Table 1:** The frequency of positive samples and the amount of tetracycline and enrofloxacin in raw and pasteurized milk samples in Tehran using the Enzyme-Linked Immunosorbent Assay (ELISA) method (December 2021-May 2022)

| Antibiotic | Type of milk samples | Sample (N) | Positive sample (%) | Median concentration (ppb) |
|---|---|---|---|---|
| Tetracycline | Raw | 112 | 71 (63.39) | 50.69 $^{A}$ |
| | Pasteurized | 112 | 35 (31.25) | 23.40 $^{B}$ |
| Enrofloxacin | Raw | 112 | 55 (49.10) | 9.87 $^{A}$ |
| | Pasteurized | 112 | 26 (23.21) | 10.29 $^{A}$ |

$^{*A-B}$ Different letters in the column indicate significant differences ($p$<0.05) between raw and pasteurized milk samples.

**Table 2:** The amount of tetracycline and enrofloxacin residues in raw and pasteurized milk samples taken in spring and winter in Tehran using the Enzyme-Linked Immunosorbent Assay (ELISA) method (December 2021-May 2022)

| Antibiotic | Type of milk samples | Season | Sample (N) | Positive sample (%) | Median concentration (ppb) | Minimum concentration (ppb) | Maximum concentration (ppb) | Exceed legal limit [1] (N) |
|---|---|---|---|---|---|---|---|---|
| Tetracycline | Raw | Spring | 56 | 23 (41.07) | 33.27 $^{A}$ | 10.76 | >88 | 0 |
| | | Winter | 56 | 48 (85.71) | 55.81 $^{B}$ | 11.60 | >88 | 0 |
| | Pasteurized | Spring | 56 | 15 (26.78) | 25.65 $^{A}$ | 6.23 | 70.65 | 0 |
| | | Winter | 56 | 20 (35.71) | 21.91 $^{A}$ | 12.35 | 72.61 | 0 |
| Enrofloxacin | Raw | Spring | 56 | 19 (33.92) | 9.13 $^{A}$ | 1.57 | 12.67 | 0 |
| | | Winter | 56 | 36 (64.28) | 10.57 $^{B}$ | 2.10 | 13.68 | 0 |
| | Pasteurized | Spring | 56 | 8 (14.28) | 9.38 $^{A}$ | 2.10 | 12.38 | 0 |
| | | Winter | 56 | 18 (32.14) | 11.23 $^{B}$ | 2.10 | 13.54 | 0 |

[1] The permissible limit of tetracycline and enrofloxacin antibiotics is 100 ppb based on the standards of the Iranian Union, Europe, and Codex in raw and pasteurized milk.
$^{A-B}$ Different letters in the column indicate significant differences ($p$<0.05) between seasons in each type of milk for each antibiotic.

**Table 3:** The amount of tetracycline residue in raw and pasteurized milk samples taken in spring and winter in Tehran using the High-Performance Liquid Chromatography (HPLC) method (December 2021-May 2022) (n=7 samples)

| Number of samples | Type of milk | season | Concentration (ppb) |
|---|---|---|---|
| 1 | Raw | Spring | 0.99 |
| 2 | Raw | Winter | 35.47 |
| 3 | Raw | Winter | 17.88 |
| 4 | Raw | Winter | 4.93 |
| 5 | Raw | Winter | 24.16 |
| 6 | Pasteurized | Spring | 8.96 |
| 7 | Pasteurized | Winter | 15.13 |

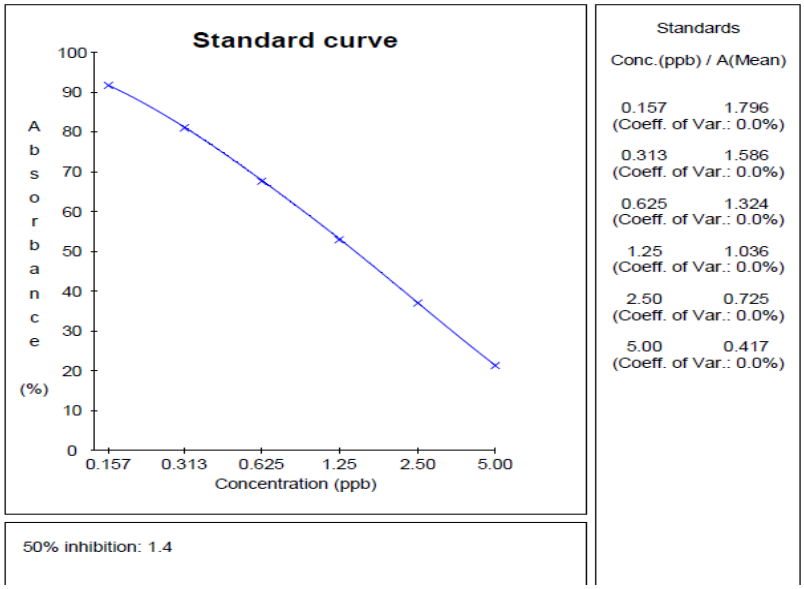


**Figure 1:** Standard calibration curve for enrofloxacin by Enzyme-Linked Immunosorbent Assay (ELISA)

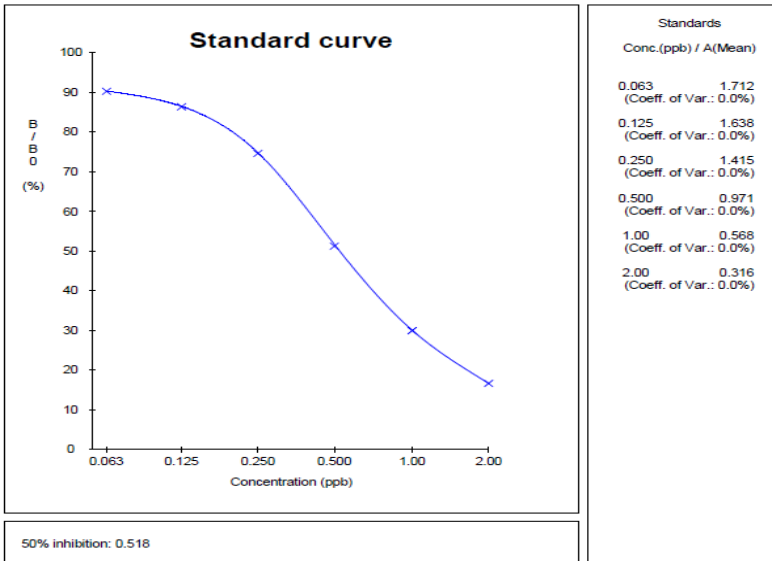


**Figure 2:** Standard calibration curve for tetracycline by Enzyme-Linked Immunosorbent Assay (ELISA)

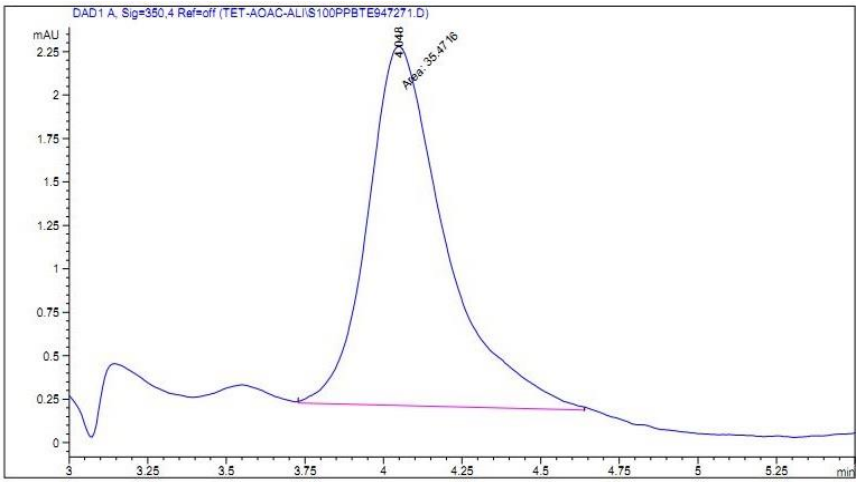


**Figure 3**: The High-Performance Liquid Chromatography (HPLC) of the mixed standard solution of tetracycline (100 ng/g)

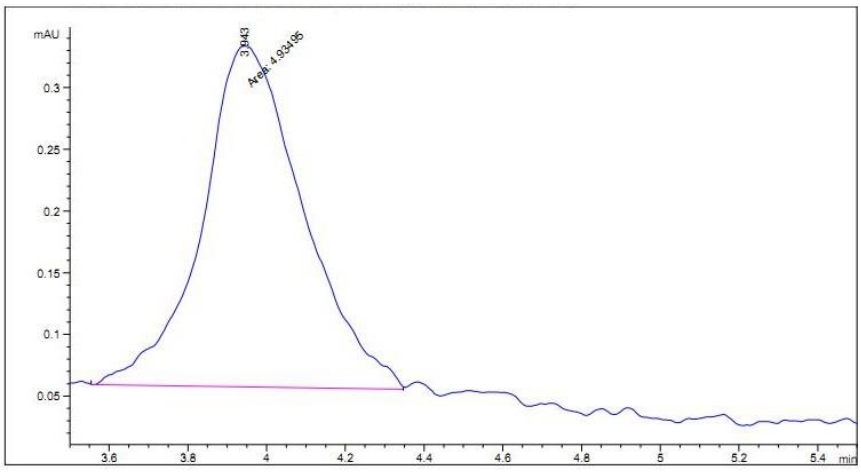


**Figure 4:** The High-Performance Liquid Chromatography (HPLC) of one milk sample containing tetracycline residues

## Discussion

Milk is regarded as one of the most sources of nutrition in the world and has a high value for individual's health. Milk is consumed by sensitive groups including pregnant women, older adults, and children (Yao et al., 2023). Therefore, the presence of contamination, such as antibiotic residues, can endanger the health of consumers. The presence of antibiotic residues in milk can cause complications such as drug resistance and allergic reactions in humans. For this reason, monitoring and inspection of antibiotic residues in milk is essential (Roba, 2023). In our study, the results of the ELISA test showed that the raw and pasteurized milk taken in the spring and winter seasons contained tetracycline and enrofloxacin residues. The results of ELISA and HPLC manifested that the quantity of antibiotic residues in all samples was less than the maximum residue level advised (100 ppb). Additionally, on the basis of the results, prevalence of tetracycline and enrofloxacin residue in raw milk was higher than the pasteurized milk, which can be due to the thermal process and the effect of pasteurization.

The results of the study by Alimohammadi et al. (2020) on 82 milk samples (raw and pasteurized milk) collected from Neyshabour in two seasons (winter and fall) using the Four-Plate Test (FPT) showed that 58.3% of the raw milk in the factory, 25% of the pasteurized milk and 67.65% of the raw milk collection centers were contaminated with antibiotic residues including tetracycline, sulfonamide, enrofloxacin, aminoglycoside, and macrolide. The results reveal that, 68.3% of the milk samples collected in winter and 36.6% of the milk samples accumulated in fall were positive. The analysis of milk samples suggested that the antibiotic residue content in the milk samples taken in winter was higher than the taken samples in fall ($p$<0.05), which is consistent with the present study that indicated a higher antibiotic contamination of milk samples in winter.

The results of the assessment of antibiotic residues on 251 consumed milk, including pasteurized milk packets distributed in schools, raw milk collection centers, and pasteurized milk production factories, collected from southern Khorasan-e Razavi province in spring, summer, fall, and winter, showed that 28.7% (41 samples) of the pasteurized milk packet distributed in schools, 21.4% (18 samples) of the raw milk collection centers, and 12.5% (3 samples) of the pasteurized milk production factories had antibiotic residues. Additionally, the findings demonstrated that there was no significant difference between the contaminated milk collected during various seasons (Mohammadzadeh Moghadam et al., 2016), which is not consistent with the present study. In the study of Alipour et al. (2013), out of 154 pasteurized milk samples and 33 sterilized milk samples prepared in the cold and warm months of the year, they stated that 37 samples (19.8%) were higher than the European Union MRLs and 28 (14.97%) samples included antibiotic residues below the European Union MRLs. Moreover, in this study, the results unveiled that a variation of weather temperature with seasons had no significant effect on contamination prevalence of milk samples, however a higher prevalence of antibiotic residues has been observed with increasing temperature during the warm season attributed to prescribed antibiotics for diarrhea and is inconsistent with this investigation. In the present study, the antibiotic contamination of milk samples was higher in the winter, which can be related to weather conditions and livestock-keeping conditions. In the winter season, due to the cold weather, diseases increase in livestock, hence antibiotics are used more widely in the treatment of livestock (Alimohammadi et al., 2020).

Rassouli et al. (2010) investigated the tetracycline residue in 90 pasteurized milk samples utilizing HPLC method. The results exhibited that seven samples had tetracycline residues, however, the amount of tetracycline residue was lower than 100 ppb and is consistent with the present study. In the investigation of enrofloxacin residues in 168 raw milk samples by ELISA test, 4.8% of samples were found positive for enrofloxacin. Furthermore, 1.7% of samples were above the MRL established by the Europen Union/Codex Alimentarius Commission for enrofloxacin. The mean residue level of enrofloxacin was 87.9±44.0 ng/kg (Moudgil et al., 2019a). The results of the study by Mahmoudi et al. (2014a) on 96 raw and pasteurized milk samples, 28 (29.16%) were positive. A significant difference in antibiotic residue content was observed between milk samples taken in spring and winter. Antibiotic residue contamination in raw milk samples (30.76%) was significantly greater than in pasteurized milk samples (22.2%), which is consistent with the present study by using the HPLC method to analyze the tetracycline residues in pasteurized milk samples obtained in Tehran. The results revealed that presence of several antibiotics in 33.93% of the samples. HPLC identified that more than the maximum residue level of tetracyclines was available in 26.3% of positive samples (8.93% of all samples) (Mohammadzadeh Moghadam et al., 2016). The identification and the dietary exposure assessment for tetracyclines and penicillin residues in liquid milk, yogurt or labneh have been performed in a cross-sectional study conducted in Lebanon. The result demonstrated that the detection rate for tetracycline in 22 milk, 12 labneh, and yogurt samples was 86.4, 50, and 50%, respectively. The mean residue values of milk, labneh, and yogurt samples were 1.16±0.70, 1.76±0.40, and 0.63±0.12 µg/kg, respectively. None of the samples exceeded the maximum residue levels (Kabrite et al., 2019). Maklati et al. (2022) investigated the β-lactam and tetracycline residues in 445

milk samples obtained from the North-Central Algerian Dairies by rapid screening test and Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS). The results displayed that 90.4% of the milk samples had antibiotic residues. Moreover, the amount of antibiotic residue in 55.3% of them was higher than the maximum residue level. The difference between the findings of other researches and the present study can be attributed to the long-term and excessive use of antibiotics among farmers (Zeina et al., 2013).

Alternatively, the lack of adequate and necessary control of milk production and milk collection centers for the determination of antibiotic residues during the delivery of milk in Iran, as well as the lack of adequate and necessary control of livestock holdings, has led to a lack of adequate and necessary control of milk production and milk collection centers. Further, inadequate monitoring of livestock and animal husbandry, non-compliance with health standards and the inappropriate physical environment of livestock cause animals to get ill, in case the ranchers have no other choices except to use antibiotics for treatment, thus necessary training to the ranchers, monitoring, and controlling the construction of the livestock providing physical space by the relevant organizations and also preventing from distribution of contaminated milk to dairy factories will be extremely effective in reducing antimicrobial residues. It is possible to use new and efficient processes including thermal treatment, absorption, electrochemical, gamma rays, biorefining, ozonation as well to remove antibiotics from milk and other foods (Liu et al., 2022).

## Conclusion

The obtained results in this study reveal that tetracyclines and enrofloxacin residue were taken from raw milk as well as pasteurized samples in spring and winter. The results of ELISA and HPLC methods have identified that in all specimens tetracyclines and enrofloxacin are below 100 ppb, which is generally the maximum residue level recommended. However, a potential risk to public health could arise from the presence of these antibiotics in raw and pasteurized milk. Therefore, monitoring and inspecting programs on antibiotic residues in milk and dairy products should be conducted regularly. In view of the fact that this survey covers raw and pasteurized milk, additional investigations should be carried out to establish the presence of antibiotics in other dairy products. Moreover, inspection and control programs on antibiotic residues in milk and dairy products should be conducted regularly. Since this study only applied to consumed milk, further investigations should be possible to conduct additional tests on antibiotic residues in various dairy products.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Author contributions

S.A.S. and F.T. designed the study; R.N. and S.S. conducted the experimental work; S.A.S. analyzed the data; S.A.S. and Z.P. wrote the manuscript, and all authors contributed to the editing the article, which was approved as a final manuscript.

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