



## Determination of Tylosin Residues in the Distributed Sausages and Hamburger Products in Tabriz by High-Performance Liquid Chromatography method

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### ABSTRACT

Antibiotic residues in meat products may have public health implications. The aim of this study was to determine the tylosin residue level in meat products (sausages, persian sausages, and hamburgers) in Tabriz. For this study 60 samples (20 samples from each meat product) were collected from food supply stores from March to May 2021, randomly. The tylosin residue levels were detected by the HPLC method. Of all samples, 90 percent of sausages, and 100 percent of hamburgers had tylosin residue. The mean of tylosin residue in sausages, persian sausages (Kalbas), and hamburgers were  $19.34 \pm 2.62$ ,  $22.2 \pm 2.62$ , and  $39.2 \pm 2.62$   $\mu\text{g}/\text{Kg}$ , respectively. The mean of tylosin residue in hamburgers was higher than in other products significantly. In addition, the residual amount of tylosin in all samples was lower than the allowed limit of Codex Alimentarius ( $100 \mu\text{g}/\text{Kg}$ ). Furthermore, the tylosin residue level in hamburgers with 90 percent meat was higher than hamburgers with 70 percent meat significantly. Even though the amount of tylosin residues is low in meat products in Tabriz, continuous control of antibiotic residues in meat products is recommended.

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## 1. Introduction

Today, the use of antibiotics in livestock is inevitable for various reasons such as treatment, prevention of various diseases and increasing the efficiency of the food consumed (increasing the absorption of nutrients due to the thinning of the small intestine wall with the use of antibiotics) [1]. Any chemical substance produced by microscopic living organisms or artificially made to interrupt the vital phenomena of another living organism is called an antibiotic. Antibiotic residue is present in all kinds of meat and milk most of the time, and to control the amount of the residue, it is necessary to use the medicine correctly and observe the drug abstinence time before slaughtering the animal [2]. Despite the benefits mentioned about the use of antibiotics, their residues in food consumed by humans can cause complications and cause the emergence of resistant strains of bacteria. Research conducted in recent years in the world shows the existence of this potential risk in animal products [3]. According to international laws and Iran's National Standard Organization, the amount of antibiotic residue in any food of animal origin should not exceed the permissible limit. Unfortunately, in most of the developing countries, there is no national program to evaluate these residues in animal products, also antibiotic residues in some fermented products can cause the destruction of useful microorganisms and initiator microbes [4]. Many families of antibiotics are used in veterinary medicine, and tylosin is one of them [5]. Tylosin is an antibiotic that belongs to the group of macrolides. Unfortunately, this drug is used as a growth promoter in animal feed. Studies have shown that tylosin-resistant microbial strains have been isolated from animals [6, 7]. In Iran, there is no standard for the residual limit of tylosin in meat and meat products.

The Food Safety Inspection Service of the United States of America has included the control of the remaining antibiotics in meat as part of its annual programs and reports cases where the amount of antibiotics is more than the maximum allowed limit [4].

Today, meat products such as sausages, sausages, and hamburgers play an important role in people's diets due to the increase in the urban population and the change in lifestyle. These products are used in meals due to their

ease of preparation. Nowadays, due to the increase in the price of red meat, chicken meat is used together with red meat in meat products [8].

Several methods have been invented to determine the antibiotic residue in food in the world, and microbiological, safe and chemical methods and physicochemical methods are the most common of them. High performance liquid chromatography method is more important due to its high sensitivity and specificity [7].

Considering that the presence of tylosin residues in beef and poultry can cause the presence of this drug in meat products. In this study, the residual amount of tylosin in sausage, sausage and hamburger products distributed in Tabriz city was determined by high-performance liquid chromatography. According to the search in available sources, this study has been conducted for the first time on the amount of tylosin residues in meat products.

## 2- Materials and methods

### 2-1- Collection of samples

In this study, 60 samples of meat products (including 20 samples of sausages, 20 samples of sausages and 20 samples of hamburgers) were obtained from meat product supply centers in Tabriz city from April to June 1400 by random cluster method and in the second step by simple random sampling. became. Sausage samples were from four commercial brands with a meat percentage above 60%, sausage samples were from four commercial brands with a meat percentage above 60%, and hamburger samples were from four commercial brands with a meat percentage of 70 (10 samples) and 90% (10 samples). The samples were sent to the biochemistry laboratory of Kharazmi University in frozen conditions.

### 2-2- Tylosin extraction from meat products

The liquid-liquid extraction method was used to extract tylosin from the samples. For this purpose, the tissue samples were homogenized with acetonitrile (2:1) at 4 degrees Celsius at a pH of 8, and the dissolved protein was precipitated with three chloroacetic acids. Then, in 2500 rounds of centrifugation, the supernatant was purified by liquid-liquid separation using dichloromethane. This solution was stored with 45 micrometer filter

paper and the final solution at -20 degrees Celsius [10, 9].

### 2-3- HPLC method

20  $\mu$ L of the sample extract was injected into a Crystal-200 (England), then the separation was performed on a 3 x 125 mm column (Purospher, C18, 5 mm), which was protected by a column. The HPLC system was operated with a gradient system with a flow rate of 0.7 mm/min. The mobile phase consisted of two components: detergent A (acetonitrile) and detergent B, which was ammonium acetate at 0.1 ml/liter. In this experiment, a two-step linear elution gradient was used, with initial conditions consisting of A-B (0:100, v/v) for 1 min. In the gradient phase, the percentage of detergent A increased to 30% in 3 min, while detergent B decreased to 70% (v/v). During the gradient step, detergent A reached 95% within 3 min, while detergent B gradually reached 5% (v/v). These conditions were maintained for 4 min and a 3-min process was followed after the test to return to the initial conditions.

A UV detector was used in this experiment. Tylosin was measured at a wavelength of 280 nm and the flow rate was 0.1 ml/min. The time required for the solution containing tylosin to pass through the column was equal to 4.7 minutes [9,10].

### 2-4- Statistical analysis

SPSS version 23 software was used for data analysis. F test was used for analysis of variance. Duncan's test was used to compare the average product type in terms of drug residue. T-test was used to compare between two groups. Also, the significance level in this study was considered less than 0.05 ( $p < 0.05$ ).

## 3. Results and Discussion

Chromatograms related to the collected samples are shown in Figures 1 to 3.

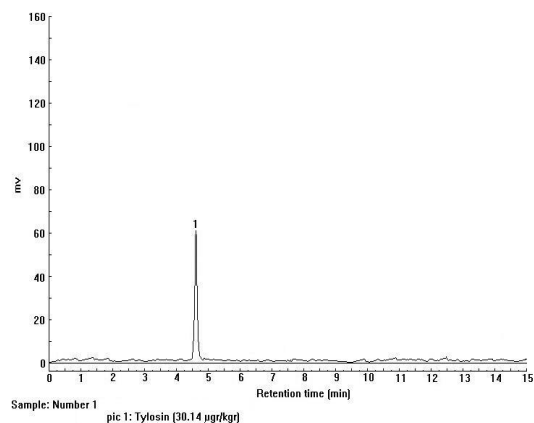


Fig 1 Chromatogram of hamburger sample containing tylosin residue

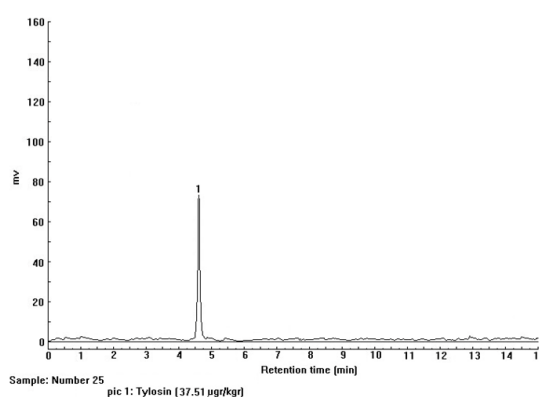


Fig 2 Chromatogram of sausage sample containing tylosin residue

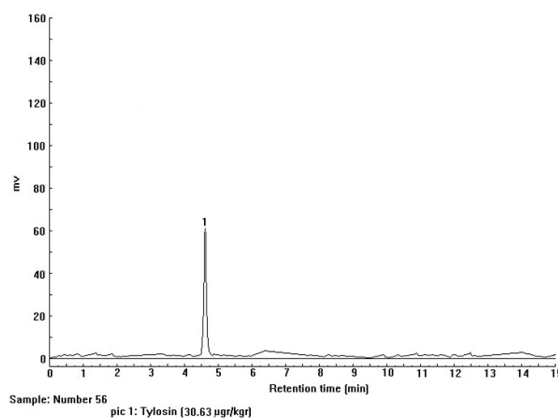


Fig 3 Chromatogram of sausage (Kalbas) sample containing tylosin residue

According to Table 1, the highest amount of tylosin residue was in hamburger. It seems that considering the amount of meat used in hamburgers and the use of meat paste in sausages and sausages produced in the country, this result is justified to some extent.

**Table 1** The mean of tylosin residue in meat products and comparing the means of tylosin residue between product types in Tabriz

Sample type	Mean of Tylosin residue(Mean±SEM) µg/Kg	Minimum Tylosin Residue (µg/Kg)	Maximum of Tylosin residue(µg/Kg)
Sausage	19.34 <sup>b</sup>	0	43.63
Sausage(Language)	22.2 <sup>b</sup>	0	38.68
Hamburger	39.2 <sup>a</sup>	28.3	50.19
WHICH		2.62	
P value		0.0001	

Averages that do not have same alphabets have a significant difference (P <0.05)

According to Table 2, tylosin residues were observed in 100% of hamburger samples and 90% of sausage and sausage samples, but the

amount of tylosin residues in the samples was less than the permissible limit.

**Table.2**The number of samples containing tylosin residue exceeds the limit of Codex standard [11].

Sample type	Percent of samples with Tylosin residue	Percent of samples more than MRL(100 µg/Kg ,Codex standard)	Percent of samples less than MRL(100 µg/Kg ,Codex standard)
Sausage	90	0	100
Sausage(Language)	90	0	100
Hamburger	100	0	100

According to Table 3, the amount of residual tylosin in special hamburger samples (90% meat) was significantly more than the residual

tylosin in normal hamburger samples (70% meat), which can be related to the amount of meat in the product.

**Table 3** The mean of tylosin residue in hamburgers and the mean comparison of tylosin residue between hamburgers with different percentage of meat in Tabriz

Sample type	Mean of Tylosin residue(Mean±SEM) µg/Kg	Minimum Tylosin Residue (µg/Kg)	Maximum of Tylosin residue(µg/Kg)
Hamburger(70 percent meat)	34.6±1.10 <sup>b</sup>	28.3	39.69
Hamburger(90 percent meat)	45.06±1.07 <sup>a</sup>	40.11	50.19

Averages that do not have same alphabets have a significant difference (P <0.05)

A study was conducted in Urmia city by Meshak et al. in 2012 on determining the amount of quinolone residue in beef and chicken by ELISA method. Out of a total of 395 samples, 217 samples (54.9%) contained antibiotic residues. Of the total samples, 48.7% of beef samples and 59.2% of chicken meat samples contained quinolone residues. The average quinolone residue in beef and chicken was  $5.51 \pm 1.17$  and  $37.86 \pm 0.57$  micrograms per kilogram, respectively [12].

The results of the study conducted in Urmia city are not consistent with the current study, which can be caused by the type of antibiotic and the method of determining the antibiotic residue in the two studies. The accuracy of the high-performance liquid chromatography method is higher than the ELISA method. Also, in meat products, other ingredients besides meat are added to the composition of the product, which

has an effect on the remaining amount of antibiotics compared to pure meat.

In Tajik et al.'s study (2013), 160 samples including muscle tissues, liver and kidneys of meat poultry carcasses were collected from East Azarbaijan, West Azarbaijan and Ardabil provinces, and the remaining amount of enrofloxacin was determined by four-plate and ELISA methods. 28 samples (17.5%) contained enrofloxacin, and 4 samples (2.5%) of muscle had excess residues [13]. The results of the above study are not consistent with the results of tylosin residues in meat products.

In a study in Mazandaran province, antibiotic residues in industrial poultry carcasses were determined by the four-plate method. In total, out of 815 examined carcasses, 65.4% of the samples had antibiotic residues in at least one of the tested organs (muscle-liver-kidney). The lowest number of positive samples contained

antibiotic residues in muscle [14]. The percentage of samples containing antibiotics was lower than the samples containing tylosin in the current study.

In a study in Kerman province, 474 chicken meat samples were collected from slaughterhouses in the province from June 2014 to December 2015, and the percentage of samples containing antibiotic residues was determined by the four-plate method. In total, 8.02 samples contained antibiotic residues. Fluoroquinolone, macrolide, cephalosporin, beta-lactam, tetracycline, sulfonamide and aminoglycoside were observed in the samples [15]. The percentage of samples containing antibiotics in Kerman is much lower than the current study, which seems to be low in the accuracy of the method chosen in the study conducted in Kerman.

In the study of Dabbagh Moghadam et al., 70 samples, including chicken meat and eggs, were sampled from army cold stores in Tehran province, and the amount of antibiotic residue was determined by ELISA test. Out of 35 chicken meat samples, 35 (100%), 30 (85.71%), and 28 (80%) samples contained fluoroquinone, tetracycline, and sulfonamide, respectively, and the average antibiotic residue in the samples was  $72.59 \pm 4.30$ , respectively. It was  $15.35 \pm 1.6$  and  $36.52 \pm 3.61$  micrograms per kilogram [16].

The above results are somewhat consistent with the results of the present study, despite the different type of antibiotics examined. In terms of the average antibiotic residue, the highest average amount in the above study was in fluorquinone, which is more than in the present study in tylosin, but the average values of other antibiotics were similar to the amount of tylosin in meat products.

In a study in Tehran city, 90 chicken meat and liver samples were taken from 13 poultry slaughterhouses in 2017 and the amount of antibiotics was determined by liquid chromatography with mass spectrometer. Lincomycin from the macrolide family and enrofloxacin and ciprofloxacin from the quinolone family were observed in the samples. The highest frequency belonged to enrofloxacin, lincomycin and ciprofloxacin. In 24 and 16% of cases, muscle and liver samples contained only one type of antibiotic [17]. The type of antibiotic observed in the above study was not consistent with the present study and the percentage of samples containing

antibiotics was much less than in the present study.

Babapour et al. (2010) in a study they conducted in Ardabil in 2018 and 2019 used the four-plate method to determine the percentage of samples containing antibiotics. Out of a total of 500 samples of beef and sheep meat collected from the sales level in the city, 22.8 and 14% of beef and sheep contained antibiotic residues [18]. The results of the above study did not agree with the results of the present study, which seems that the type, precision of the work method and formulation of meat products have a great influence on the results.

In a study in Turkey, the residual amount of enrofloxacin, doxycycline and tylosin in distributed chicken meat was determined by high-performance liquid chromatography. Out of the total 300 samples collected, 3.6% of the samples contained the remaining amounts exceeding the permissible limit of Turkey (100 to 150 micrograms per kilogram). 2% of the samples contained enrofloxacin, 1% contained doxycycline and 0.6% contained tylosin beyond the permissible limit. According to the above results, the amount of antibiotic residues in chicken meat consumed in Turkey is less than the permissible limit [19]. In the above study, more than the permissible limit was observed, but in the current study, the samples were within the permissible limit.

A study was conducted in the city of Erbil, Iraq by Al-Mashhadani. In this study, 250 beef samples were taken from the sales level in the city, and the antibiotic residue in the samples was checked by the four-plate method. 11.8% of the total samples contained antibiotic residues. In this study, cooking meat for 30 minutes was suggested as a solution to reduce the risks of consuming beef containing antibiotic residues, which seems to be not a general recommendation considering the nature of the type of antibiotic and some antibiotics are not sensitive to high temperature [20]. The results of the above study were not consistent with the results of the present study.

In a study in Dohok region of Iraq, 88 samples of sheep meat were collected from the slaughterhouse of the region and the remaining amount of antibiotics was determined by ELISA method. In the rectus muscle, tetracycline residue in 40.9% of samples with an average of 138.45, penicillin G residue in 40.9% of samples with an average of 355.64, streptomycin residue in 45.45% of samples

with an average of 227.19, and gentamicin residue in 63 13.0% of samples with an average of 897.03 micrograms per kilogram. According to the Iraqi researchers of the above study, in the country of Iraq, antibiotics are used in livestock herds regardless of the period of abstinence from consumption by livestock farmers, which confirms the results of the study conducted in Iraq [21]. The average amount of remaining antibiotics in the above study is more than was ready

In a study in Shanghai, China, 125 samples including red meat, chicken meat, milk and seafood were collected. In total, 35.3% of red meat samples, 22.2% of chicken meat samples, 10.6% of milk samples and 52.1% of seafood samples contained antibiotics. Roxithromycin was observed in 5.9% of red meat samples [22]. The results of the above study were not consistent with the current study, it seems that in China, more antibiotic residues are observed in marine products.

In a study in Nigeria, out of 50 meat samples taken in the slaughterhouse, 44% of the samples contained antibiotic residues and penicillin was the most abundant among the samples, which results were not consistent with the current study [23].

In a study in South Korea, Lee et al collected 58 chicken meat samples from five provinces. In total, 45% of the samples contained antibiotic residues. Antibiotic amounts in the samples were within the permissible limits. Amoxicillin was the most frequent among the samples, followed by enrofloxacin and sulfamethoxazole [24]. The results of the above study show that the percentage of samples containing antibiotic residues is lower than the current study.

In the meat products sold in Iran, sausage and sausage products are placed under 70 degrees Celsius in the factory, and hamburgers are prepared and used by frying in oil. In a study, Heshmati et al investigated the effect of temperature in reducing the amount of tylosin. It seems that frying has an effect in reducing the amount of tylosin in meat, but it is not a reliable method to reduce or remove the remaining tylosin from meat [6].

According to the presented materials, the most important problem in the consumption of food products containing antibiotic residues is the occurrence of antibiotic-resistant microbes, which can cause the drug to be withdrawn from the cycle of use in the treatment of diseases. This problem is observed more in countries

where excessive use of antibiotics is observed in human and animal populations. Today, it is recommended to observe the period of avoiding consumption in animals treated with antibiotics. Also, in some fermented food industries where starter microbes are used, the presence of antibiotics in raw materials can prevent the growth of microbes and cause many economic damages [25].

#### 4- General conclusion

According to the results of the current study, in the current conditions, the amount of tylosin in all kinds of meat products distributed in Tabriz city is within the permissible limit, but the high percentage of samples containing tylosin indicates the presence of this antibiotic in the meat consumed in these products. Among the hamburger meat products, especially the special hamburger with 90% meat had the highest amount of tylosin. It seems that the regulatory authorities should continuously monitor and determine the presence of antibiotic residues in all types of meat and meat products in order to prevent future problems.

#### 5- Appreciation and thanks

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تعیین باقیمانده تایلوزین در فرآورده‌های سوسیس، کالباس و همبرگر توزیعی در شهر تبریز به روش

کروماتوگرافی مایع با کارایی بالا

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باقی مانده های آنتی بیوتیک در گوشت می تواند باعث بروز پیامدهای نامطلوبی در سلامت عمومی گردد. هدف از این مطالعه تعیین میزان باقی مانده تایلوزین در فرآورده های گوشتی (سوسیس، کالباس و همبرگر) در شهر تبریز بود. برای این مطالعه ۶۰ نمونه (شامل ۲۰ نمونه از هر فرآورده گوشتی) از فروشگاه های عرضه مواد غذایی از فروردین ماه تا خردادماه ۱۴۰۰ به صورت تصادفی جمع آوری گردید. میزان تایلوزین در نمونه ها به روش کروماتوگرافی مایع با کارایی بالا تعیین گردید. از کل نمونه های اخذ شده، باقیمانده تایلوزین در ۹۰ درصد نمونه های سوسیس و کالباس و ۱۰۰ درصد نمونه های همبرگر مشاهده گردید. میانگین باقی مانده تایلوزین در نمونه های سوسیس، کالباس و همبرگر به ترتیب  $۱۹/۳۴ \pm ۲/۶۲$ ،  $۲۲/۶۲ \pm ۲/۶۲$  و  $۳۹/۲ \pm ۲/۶۲$  میکروگرم در کیلوگرم بود. میانگین باقی مانده تایلوزین در همبرگر به صورت معنی داری بیش از سایر فرآورده های گوشتی بود. علاوه بر این، میزان باقی مانده تایلوزین در نمونه ها کم تر از حد مجاز کدکس (۱۰۰ میکروگرم در کیلوگرم) بود. همچنین میزان باقی مانده تایلوزین در همبرگر با ۹۰ درصد گوشت به صورت معنی داری بیش تر از همبرگر با ۷۰ درصد گوشت بود. اگرچه میزان باقی مانده تایلوزین در فرآورده های گوشتی در شهر تبریز پائین می باشد، کنترل مداوم فرآورده های گوشتی توصیه می گردد.