DETERMINATION OF CARCINOGENIC SUBSTANCES IN TURKEY MEAT USING CHROMATOGRAPHIC METHODS

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Abstract: The results of samples of turkey meat grown in Uzbekistan, Russia, Tajikistan, and Germany using liquid chromatography-mass spectrometry (LC-MS/MS) to determine the residues of antibiotics (cancerogenic substances) in turkey meat has been analyzed in this article. While the usage of antibiotics in poultry farming increases efficiency, they pose safety and antimicrobial resistance (antibiotic resistance) problems for consumers.

According to the results of the scientific experiment presented in the article, antibiotic residues were evaluated based on sanitary rules and standards, requirements of standards and technical regulations. The study offers noteworthy conclusions and recommendations for reducing the use of antibiotics, ensuring food safety, and preventing antimicrobial resistance.

Keywords: turkey meat, antibiotic residues, liquid chromatography-mass spectrometry (LC-MS/MS), antimicrobial resistance, benzyl penicillin, tetracycline, bacitracin, food safety, Sanitary rules and regulations (SanPiN), technical regulations, veterinary control.

ОПРЕДЕЛЕНИЕ КАНЦЕРОГЕННЫХ ВЕЩЕСТВ В МЯСЕ ИНДЕЙКИ С ИСПОЛЬЗОВАНИЕМ ХРОМАТОГРАФИЧЕСКИХ МЕТОДОВ

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Аннотация: В статье проанализированы результаты исследования образцов мяса индейки, выращенной в Узбекистане, России, Таджикистане и Германии, с использованием жидкостной хромато-масс-спектрометрии (LC-MS/MS) для определения остатков антибиотиков (канцерогенных веществ). Несмотря на то что применение антибиотиков в птицеводстве повышает эффективность производства, оно создаёт риски для безопасности потребителей и способствует развитию антимикробной резистентности (устойчивости к антибиотикам).

По результатам проведённого научного эксперимента остатки антибиотиков были оценены в соответствии с санитарными правилами и нормами, а также требованиями стандартов и технических регламентов. В исследовании представлены важные выводы и рекомендации по снижению использования антибиотиков, обеспечению пищевой безопасности и предотвращению антимикробной резистентности.

Ключевые слова: мясо индейки, остатки антибиотиков, жидкостная хромато-масс-спектрометрия (LC-MS/MS), антимикробная резистентность, бензилпенициллин, тетрациклин, бацитрацин, пищевая безопасность, санитарные правила и нормы (СанПиН), технические регламенты, ветеринарный контроль.

Introduction

Today, turkey meat is widespread among dieters and supporters of a healthy diet, which is due to its low-fat content, high protein levels, and richness in various vitamins. However, antibiotics are widely used in turkey cultivation to increase efficiency and prevent poultry from contracting diseases. [1].

In poultry farming, antibiotics are primarily used to prevent and treat diseases. At the same time, antibiotics also help to accelerate the growth of turkeys. Through the use of antibiotics, farms will be able to increase feed efficiency and eliminate diseases. For example, in the United States, turkey growers use antibiotics according to veterinary guidelines, in which medications are used only at the right doses and at the right time [2].

According to research conducted worldwide from 2013 to 2021, antibiotics were primarily used to treat respiratory diseases and enteritis. Among short-term treatment methods, antibiotics such as tetracycline, penicillin, benzyl penicillin, and bacitracin and lincomycin were widely used. Furthermore, the duration of the

antibiotic application period was often 4 to 7 days, depending on the type of disease [3].

One of the most important issues related to the use of antibiotics is the emergence of antibiotic resistance (antimicrobial resistance). Some bacteria become resistant to antibiotics, which leads to negative consequences not only for birds, but also for humans. Therefore, in recent years, many countries, including the European Union, have taken strict measures to reduce the use of antibiotics in turkey cultivation and use them only according to veterinary guidelines[4].

In Europe, for example, rules for regulating the use of antibiotics and their implementation under veterinary supervision have been introduced. This ensures that antibiotics can only be used for disease prevention or treatment. As a result, in recent years, the use of antibiotics has decreased, and strategies have been implemented aimed at reducing antimicrobial resistance in poultry.

Many countries, including Uzbekistan, have established acceptable standards for antibiotic residues in food products. These standards were introduced to ensure food safety and limit the use of antibiotics. Therefore, to ensure that turkey meat products meet the requirements and standards of the domestic and international markets, it is important to analyze the antibiotic residues contained in them and check their compliance with regulatory and technical documents.

Antibiotic residues in various samples of turkey meat grown in Russia, Tajikistan, Uzbekistan, and Germany were analyzed using liquid chromatography-mass spectrometry (LC-MS) has been studied. LC-MS is a modern technological device that allows for the study of antibiotic residues in poultry products with high accuracy, allowing for in-depth analysis of quantitative and qualitative aspects of components on a micro scale.

A brief diagram of the procedure for determining antibiotic residues in turkey meat samples using this method is presented in Table 1 below:

Table 1

Method for determining antibiotic residues in turkey meat samples

№	Stage	Description
1	Equipment Set up	Preparation of the LC-MS/MS system with necessary components: mass spectrometer, chromatographic column, and accessories.
2	Preparation of Reagents	Preparation of methanol-water solutions, ammonium sulfate, and standard solution (water/methanol 0.5%, formic acid).
3	Preparation of Calibration Solution	Preparation of standard solutions of benzylpenicillin, tetracycline, and bacitracin with concentrations of 0.1–2 ppb*.
4	Sample Preparation	Homogenization of 5 g of turkey meat, extraction of antibiotics with methanol, followed by purification and centrifugation.
5	Chromatographic Analysis	Introduction of the sample into the LC-MS/MS system, separation and identification of antibiotic

		residues, and data recording.
6	Data Analysis	Comparison of analysis data with the calibration curve to determine the amount of antibiotic residues and verification against regulations.
7	Reporting	Documentation of results and verification of compliance with safety standards for antibiotic residue levels.

Note: 1 microgram/liter (µg/L).

The following regulatory documents on the maximum permissible amount of antibiotic residues in turkey meat are valid in the territory of Uzbekistan [6]:

Sanitary rules and regulations (SanPiN): Order of the Chief State Sanitary Doctor of the Ministry of Health of the Republic of Uzbekistan "Hygienic standards for the safety of food products." This document establishes the maximum permissible amount of harmful substances, including antibiotic residues, in food products.

2. Technical regulation: Resolution of the Cabinet of Ministers of the Republic of Uzbekistan dated January 22, 2018 No. 36 "General technical regulation on the safety of meat and meat products." This regulation includes requirements for the safety of meat products, including antibiotic residues. The maximum permissible levels of antibiotic residues (MPLs) in turkey meat, as specified by the sanitary norms (SanPiN) and technical regulations, are presented in **Table 2**.

Table 2: Maximum Permissible Levels of Antibiotic Residues in Turkey Meat According to SanPiN and the Technical Regulation of Uzbekistan

Antibiotic Type	**SanPiN (MPL) (mg/kg)**	**Technical Regulation (MPL) (mg/kg)**
Tetracyclines	Not permitted, <0.01	Not permitted, <0.01
Bacitracin	Not permitted, <0.02	Not permitted, <0.02
Benzylpenicillin	Not permitted, <0.01	Not permitted, <0.01

Note: 1 ppb = 0.001 microgram/kg.

Both documents set the maximum permissible amount of antibiotic residues in turkey meat at the same level. These requirements are aimed at protecting consumer health and ensuring food safety [7].

Residues of three antibiotic names: benzylpenicillin, tetracycline, and bacitracin were analyzed using a chromatographic method (LC-MS/MS) for turkey meat samples grown in different regions (Fig. 1). The following results were obtained based on the spectrogram. The residual amount of antibiotics in turkey meat samples was determined using a high-performance liquid chromatography water Xevo TQ-S cronos (LC-MS/MS) +ESI Mass detector in various modes using an ACQUITY UPLC BEH C18 2.1×100 mm, 1.7 µm column. Below are standard

samples of the antibiotics Benzylpenicillin, Tetracycline, and Bacitracine in Table 3:

Table 3:

Chromatograms of Standard Samples of Benzylpenicillin, Tetracycline, and Bacitracin

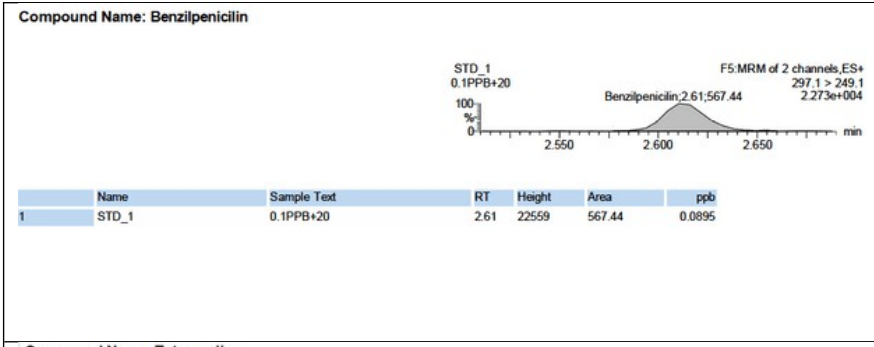
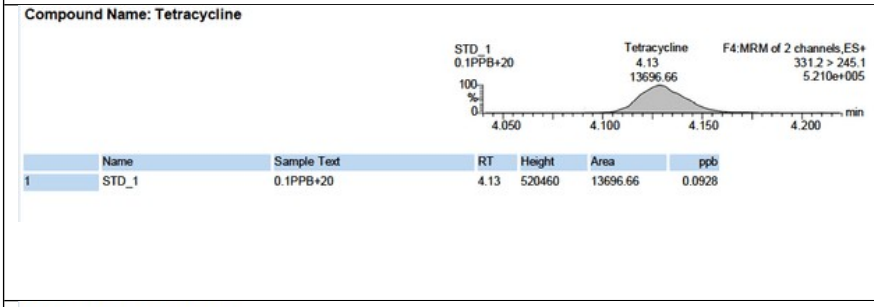
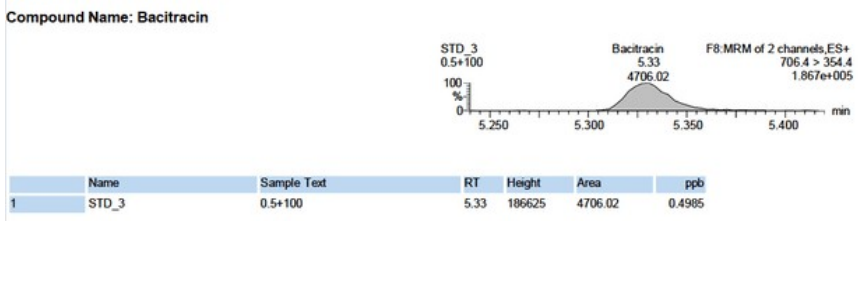
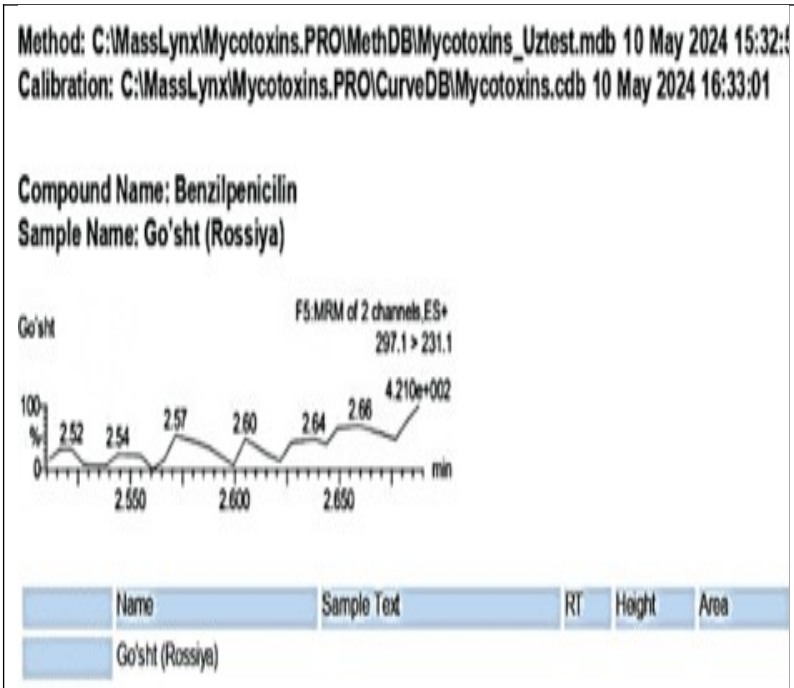
	Standard Sample of Benzylpenicillin: **Retention Time (RT)**: 2.61 minutes **Wavelength**: 297.1 > 249.1 nm **Signal Height**: 22,559 **Signal Area**: 567.44
	Standard Sample of Tetracycline: **Retention Time (RT)**: 4.13 minutes **Wavelength**: 331.2 > 245.1 nm **Signal Height**: 20,460
	Standard Sample of Bacitracin: **Retention Time (RT)**: 5.33 minutes **Wavelength**: 706.4 > 354.4 nm **Signal Height**: 186,625 **Signal Area**: 4,706.02

Figure 1: Chromatogram of the Turkey Meat Sample Produced in Russia.

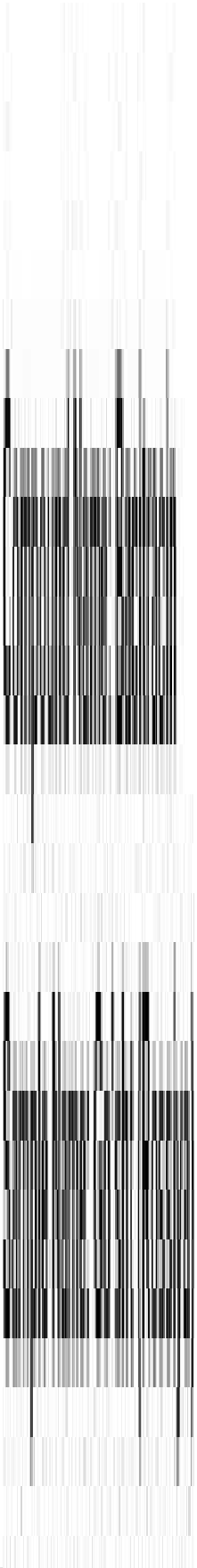


Benzylpenicillin:

- **Wavelength:** 297.1 > 234.1 nm
- **Retention Time (RT):** 2.62 minutes
- **Signal Area:** 4.210e+002
- **Concentration:** Not determined, but signal detected.

Tetracycline:

- Wavelength: 331.2 > 257.1 nm
- Retention Time (RT): 4.13 minutes
- Signal Height: 104,372
- Signal Area: 123,015.27
- Concentration (ppb): 89.106



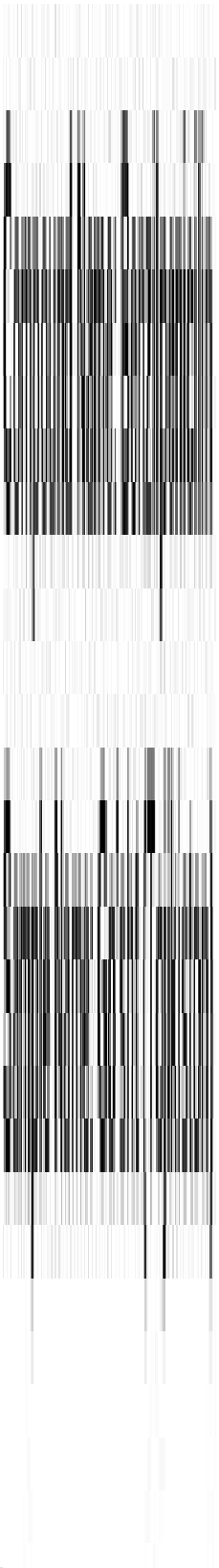
Bacitracin:

- Wavelength: 712.2 > 352.1 nm
- Retention Time (RT): 5.33 minutes
- Signal Height: Not determined
- Signal Area: 1.610e+004
- Concentration: Not determined, but signal detected.

As can be seen from the results of Figure 1, the content of tetracycline in the chromatogram of turkey meat samples grown in Russia is 89.106 ppb (0.089 mg/kg), which is higher than the maximum permissible amount (10 ppb or 0.01 mg/kg) established by the requirements of SR&N and TR. This result indicates that the meat does not meet regulatory requirements.

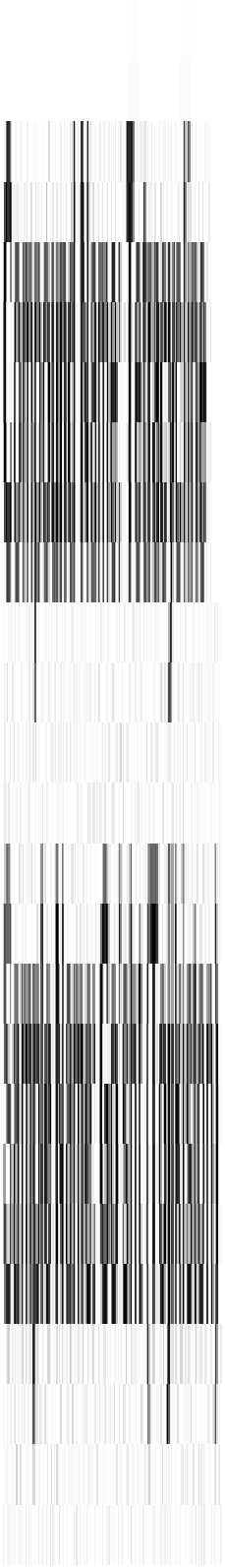
Figure 2

Chromatogram of an example of turkey meat grown in Tajikistan



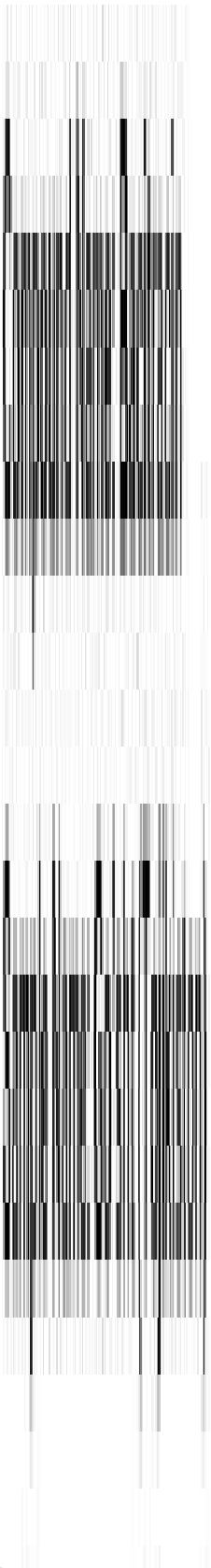
Benzylpenicillin:

- Wavelength: 297.1 > 231.1 nm
- Retention Time (RT): 2.61 minutes
- Signal Height: 182,650
- Signal Area: 43,243.59
- Concentration (ppb): 101.522



Tetracycline:

- Wavelength: 331.2 > 257.1 nm
- Retention Time (RT): 4.31 minutes
- Signal Height: Not determined
- Signal Area: 2.41e+002
- Concentration (ppb): Not determined



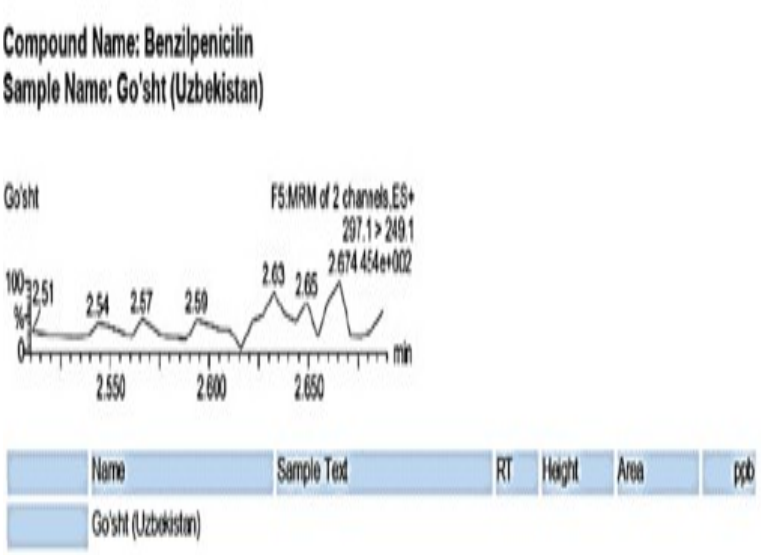
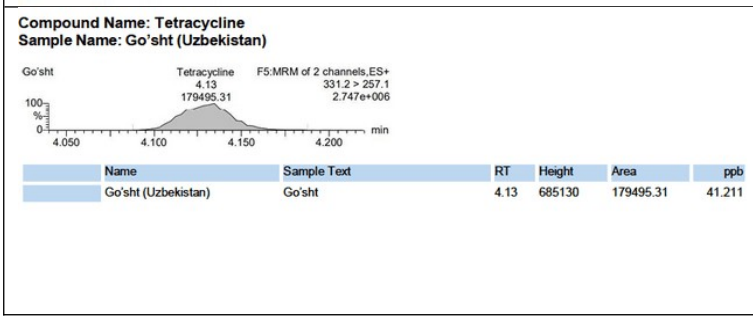
Bacitracin:

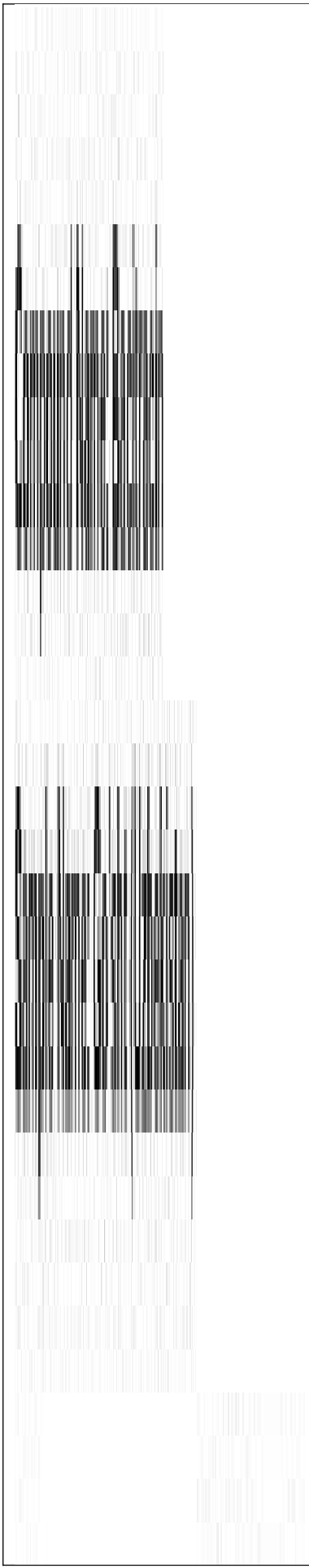
- Wavelength: 706.2 > 335.7 nm
- Retention Time (RT): 5.41 minutes
- Signal Height: Not determined
- Signal Area: 1.025e+003
- Concentration (ppb): Not determined, but signal detected

A sample of turkey meat grown in Tajikistan showed that the residual doses of the antibiotics Benzyl penicillin, Tetracycline, and Bacitracin, identified in Figure 2, were twice as high as the normative requirements in practical regulatory documents for Benzyl penicillin, i.e., 101.522 ppb, exceeding the normative limits. The results of this analysis were adapted for evaluation in accordance with the requirements of the SR&N and international organizations: 1 ppb = 0.001 mg/kg.

Benzyl penicillin: Amount determined by analysis: 101.522 ppb = 0.101 mg/kg.

According to regulatory restrictions of TR and SR&N, the maximum permissible amount of benzyl penicillin in food products is typically set at 0.01 mg/kg (10 ppb). The amount found as a result of this analysis shows that it is ten times higher than the established threshold.

	Benzylpenicillin: • Wavelength: 297.1 > 249.1 nm • Retention Time (RT): 2.61 minutes • Signal Area: 2.674e+002 • Concentration (ppb): Not determined
	**Tetracycline:** • Wavelength: 331.2 > 257.1 nm • Retention Time (RT): 4.13 minutes • Signal Height: 685,130 • Signal Area: 179,495.31 • Concentration (ppb): 41.211

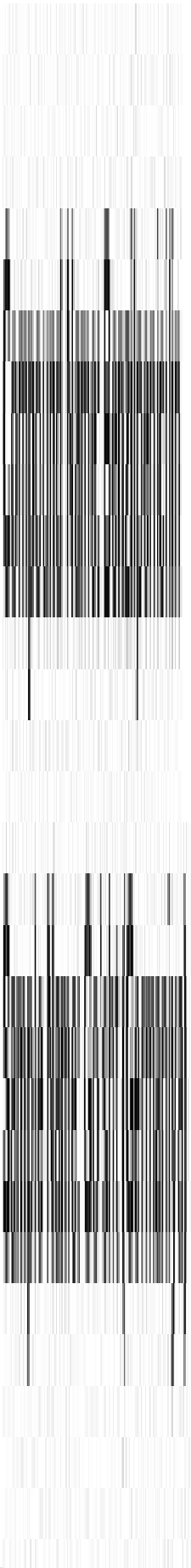


Bacitracin:

- **Wavelength**: 706.1 > 335.4 nm
- **Retention Time (RT)**: 5.35 minutes
- **Signal Area**: 4.689e+003
- **Concentration (ppb)**: Not determined

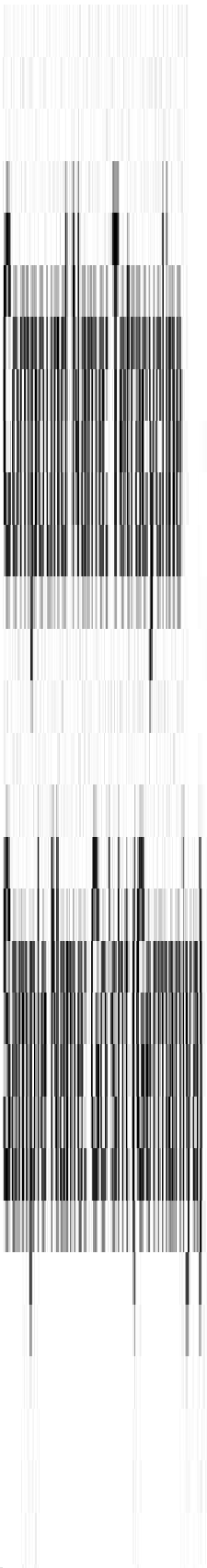
The residual content of tetracycline in meat (Fig. 3) was 41.211 ppb. This is a very low level, which is significantly lower than the regulatory limits compared to the permissible doses (0.041211 mg/kg) in the requirements of the SR&N and TR. Therefore, this quantity indicates that meat is safe for consumption.

Figure 4
Chromatogram of a German turkey meat sample
Figure 5: Chromatogram of the Turkey Meat Sample Produced in Germany.



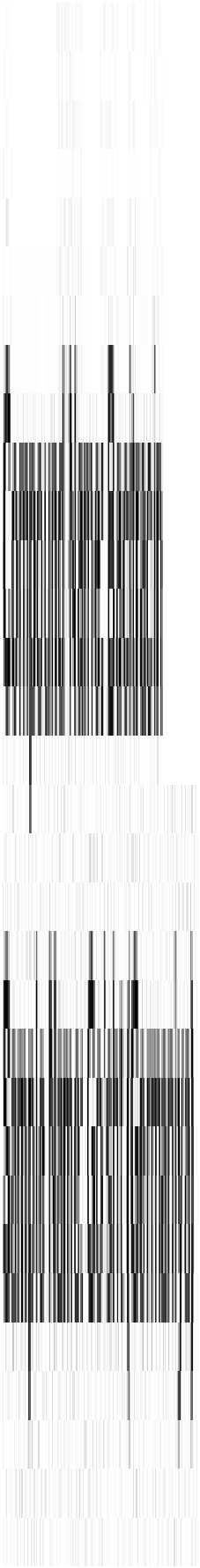
Benzylpenicillin:

- **Retention Time (RT):** 4.05 minutes
- **Signal Height:** 460
- **Signal Area:** 4,610.00
- **Concentration (ppb):** Not determined, but signal detected



Tetracycline:

- **Retention Time (RT):** 4.20 minutes
- **Signal Height:** 957.00
- **Signal Area:** 9.567e+002
- **Concentration (ppb):** Not determined, but signal detected



Bacitracin:

- **Retention Time (RT):** 5.37 minutes
- **Signal Height:** 712.4
- **Signal Area:** 4.466e+003
- **Concentration (ppb):** Not determined, but signal detected

Conclusions and suggestions. We can see that the residues of benzyl penicillin, tetracycline, and bacitracin antibiotics in turkey meat grown in Germany (Fig. 4) are below the permissible minimum doses established by the TR and SR&N documents.

The results of the analysis of turkey meat samples grown in Uzbekistan, Russia, Tajikistan, and Germany may differ from each other, and these differences are related to production practices, veterinary regulations, and antibiotic use strategies in different countries. The following conclusions can be drawn from the analysis of turkey meat samples grown in these countries:

1. The presence of antibiotic residues:

Analysis of turkey meat samples grown in Uzbekistan, Russia, and Tajikistan revealed the presence of various antibiotic residues:

- Russia: The detected amount of tetracycline exceeded the limiting amount established by the regulatory documents of Uzbekistan. Concentrations for the antibiotics benzylpenicillin and bacitracin were not determined, but there were retention times and signal signs for them, indicating the presence of residual substance traces.

- Tajikistan: Although the concentration of benzylpenicillin (0.101522 mg/kg) was higher than the permissible antibiotic residue, as well as for the antibiotics Tetracycline and Bacitracin, it was not determined, but the presence of signal traces was detected. This is a turkey raised in Tajikistan. This indicates that antibiotics are more widely used in turkey meat products grown in Tajikistan.

- Germany: Antibiotic residues were not detected in turkey meat samples, but their signals are present. Antibiotic residues are below the minimum norm established by the TR and SanPiN documents.

- Uzbekistan: Tetracycline residues have been identified, but due to very low concentrations, they meet the standards established by the TR and SanPiN requirements.

2. Compliance with international and domestic regulatory documents [8, 9]:

- Comparison of the analysis results with the international maximum permissible doses (IMP) showed that the amount of antibiotics in the sample of turkey meat grown in Tajikistan exceeded the permissible norms in some cases. This situation can lead to problems related to the export of this product and consumer safety.

3. Local production and control:

The relatively low antibiotic residues in turkey meat samples in Uzbekistan indicate that the country complies with veterinary control and the rules for the use of antibiotics in accordance with established procedures.

4. Methods of analysis and differences in results:

- While samples of turkey meat were examined using different analytical methods in each country, the accuracy of each method and the data processing rules may vary. For this reason, although the presence of some antibiotics has been established, their concentrations are not indicated.

- Also, the differences in the results of the analysis depend on the strictness of the rules for the use of antibiotics and control measures.

5. General recommendations: High levels of antibiotic residues in turkey meat grown in Tajikistan indicate the need for increased control over the use of antibiotics in this country.

• Residues found in turkey meat samples from Uzbekistan, Russia and Germany are below the limits set by international and national regulations, which allows us to conclude that these products are safe for consumers.

Based on the above, it can be concluded that although antibiotics are an important stimulant for increasing efficiency in turkey meat production, their excessive use increases the risk of antimicrobial resistance. Therefore, producers should use antibiotics carefully and strengthen biosecurity measures.

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