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**Original Article**

# Measuring active matter levels in original and open forms of veterinary preparations containing oxytetracycline stored under varied storage conditions

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ABSTRACT

This study aimed to evaluate the level of active matter in oxytetracycline stored under different conditions. Analyzing the active matter content of the drug was performed in its original packaging and in opened packages that were stored at room temperature, in the dark, and in the refrigerator for varying periods. Oxytetracycline was used for each preparation, and the active matter quantities were analyzed after 3, 6, and 9 months using HPLC. Additionally, considering the drug's life cycle, the opened packages were also analyzed at 24 and 48 hours. Results for the drug, which contained oxytetracycline (traditional) and was stored in a normal temperature room, were respectively: (6.14 ± 0.32, 6.50 ± 0.19), (5.58 ± 0.00, 6.38 ± 0.20), and (6.08 ± 0.10, 6.45 ± 0.32) ppm. Also, the results for the drug kept in the dark and a newly unwrapped drug were respectively: (6.29 ± 0.27, 6.37 ± 0.34), (5.30 ± 0.04, 6.30 ± 0.17), and (5.97 ± 0.13, 5.86 ± 0.06) ppm. Finally, the results for the drug kept in the refrigerator and a newly unwrapped drug were respectively: (7.55 ± 0.15, 7.48 ± 0.11), (5.36 ± 0.05, 5.81 ± 0.20), and (6.06 ± 0.36, 5.72 ± 0.07) ppm. Altogether, the results indicated that several external factors, such as heat, light, humidity, and oxygen, significantly impacted drug stability. Additionally, internal factors such as the internal reaction of the active ingredient, preservatives, and solvents influenced stability. Therefore, these various factors collectively affect the half-life of oxytetracycline.

## اندازه گیری سطوح ماده فعال در اشکال اصلی و باز ترکیبات دامپزشکی حاوی اکسی تتراسایکلین ذخیره شده در شرایط مختلف نگهداری

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**چکیده**

این مطالعه با هدف ارزیابی سطح ماده فعال در اکسی تتراسایکلین نگهداری شده در شرایط مختلف انجام شد. تجزیه و تحلیل محتوای ماده فعال دارو در بسته بندی اصلی آن و در بسته های باز شده که در دمای اتاق، تاریکی و در یخچال که برای دوره های مختلف نگهداری می شدند، انجام شد. برای هر آماده سازی از اکسی تتراسایکلین استفاده شد و مقدار ماده فعال پس از ۳، ۶ و ۹ ماه با استفاده از HPLC مورد تجزیه و تحلیل قرار گرفت. همچنین با توجه به نیمه عمر دارو، بسته‌های باز شده نیز در ساعت‌های ۲۴ و ۴۸ آنالیز شدند. نتایج برای داروئی که حاوی اکسی تتراسایکلین (سنتی) بود و در یک اتاق با دمای معمولی نگهداری می شد، به ترتیب (۶/۱۹±۰/۵۰، ۶/۱۴±۰/۳۲)، (۵/۵۸±۰/۰، ۶/۳۸±۰/۲۰) و (۶/۰۸±۰/۱۰، ۶/۴۵±۰/۳۲) ppm بود. همچنین نتایج برای داروی نگهداری شده در تاریکی و داروی تازه بسته بندی شده به ترتیب (۶/۲۹±۰/۲۷، ۶/۳۷±۰/۳۴)، (۵/۳۰±۰/۰۴، ۶/۳۰±۰/۱۷) و (۵/۹۷±۰/۱۳، ۵/۸۶±۰/۰۶) ppm و در نهایت، نتایج برای داروی نگهداری شده در یخچال و داروی تازه بسته بندی شده به ترتیب (۷/۵۵±۰/۱۵، ۷/۴۸±۰/۱۱)، (۵/۳۶±۰/۰۵، ۵/۸۱±۰/۲۰) و (۰/۰۶±۰/۳۶، ۵/۷۲±۰/۰۷) ppm بود. در مجموع، نتایج نشان داد که عوامل خارجی متعددی مانند گرما، نور، رطوبت و اکسیژن به طور قابل توجهی بر پایداری دارو تأثیر می‌گذارند .علاوه بر این، عوامل داخلی مانند واکنش داخلی ماده فعال، مواد نگهدارنده و حلال‌ها بر پایداری تأثیر می‌گذارند. بنابراین، این عوامل مختلف در مجموع بر نیمه عمر اکسی تتراسایکلین تأثیر می گذارند.

**واژه های کلیدی:** سطوح ماده فعال، اکسی تتراسایکلین، شرایط نگهداری مختلف

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## INTRODUCTION

Oxytetracycline (OTC), a broad-spectrum antibiotic belonging to the secondgeneration tetracycline group, is derived from the bacteria Streptococcus rimosus. It was first discovered in 1953 and has since been used to effectively treat a wide range of infections. This discovery also paved the way for the development of another widely used tetracycline in the same group, known as doxycycline [1, 2]. OTC is a broad-spectrum antibiotic commonly utilized in veterinary medicine to treat gastrointestinal and respiratory diseases primarily caused by aerobic microorganisms. This includes Gram-positive and Gram-negative bacteria, Rickettsia, Mycoplasma, and Chlamydia species. The drug is available in long-acting (LA) formulations, which come in different solvents. The LA formulations allow for a dosage interval of 3 to 5 days, providing sustained therapeutic effects [3]. This drug can be administered through various routes including oral, topical, parenteral, breast, and intravaginal methods. In monogastric animals such as calves, lambs, young goats, and birds, it is commonly administered orally [2, 4]. In cases requiring parenteral administration, intravenous injection is the preferred method. For intramuscular administration, it is important to ensure 5topical anesthetics like procaine and lidocaine can be included when administering oxytetracycline via intramuscular injection. Intravenous administration of long-term oxytetracycline is not recommended. Oxytetracycline exerts its bacteriostatic effects by interfering with bacterial protein production, thereby hindering bacterial growth [5-7]. OTC is utilized for the treatment of various infections in humans, cattle, sheep, and pigs. For instance, Moka et al. conducted a study on a herd of 500 cows affected by pneumonia, where 68 animals were treated with OTC. This treatment resulted in the survival of 29 animals, accounting for 42.6% of the treated group [7]. Additionally, antibiotics are commonly employed as preventive measures against diseases in healthy organisms. For instance, OTC is injected into cattle as a preventive measure for anaplasmosis, even in the absence of an actual infection [7]. The spectrum and mechanisms of action for antibiotics in the tetracycline group are generally similar. Therefore, many aspects related to oxytetracycline can also apply to other types of tetracycline antibiotics. Oxytetracycline is categorized as a broad-spectrum antibiotic that can affect various types of bacteria and organisms including gram-negative bacteria [7], gram-positive chlamydia bacteria (responsible for psittacosis in parrots, conjunctivitis, and trachoma in humans), spirochetes (such as the bacteria causing Lyme disease), actinomycetes, mycoplasma, and rickettsia. It is worth noting that gram-positive bacteria tend to be more sensitive to oxytetracycline compared to gram-negative bacteria. Bacterial sensitivity or resistance to the drug can be determined based on its concentration in plasma: a bacterium is considered sensitive if the drug concentration affects it at less than 2-4 µg/ml, while it is deemed resistant if it is affected at concentrations higher than 16 µg/ml [2, 3]. OTC is an effective treatment for various infections involving the respiratory system, sinusitis, otitis media, soft tissue infections, and urinary tract infections. It also demonstrates efficacy in the treatment of anthrax, brucellosis, tularemia, and cholera. Additionally, oxytetracycline is commonly used as a preferred medication for the treatment of acne [6, 8-9]. Also, it is frequently employed as a systemic therapy to treat clinical metritis; however, information regarding its penetration into the uterus and uterine secretions is currently unavailable. In a

study conducted on six cows with clinical metritis, uterine secretions and milk samples were collected for microbiological analysis. The cows were treated with long-acting oxytetracycline (20 mg/kg) via intramuscular injection. Samples of plasma, milk, and uterine secretions were then collected to determine the concentrations of the antibiotic using HPLC-PDA analysis. The study aimed to describe the pharmacokinetics of the antibiotic and to predict its penetration into the uterus using in silico methods [10]. OTC is extensively utilized in human medicine, veterinary medicine, as well as in the livestock and poultry breeding industries. As a result, numerous research studies have been conducted to explore its various applications. Moreover, advanced technology is employed to ensure the production of a higher quality product. The drug is subject to regular supervision, quality testing, and monitoring throughout its production, distribution, and administration processes. It is crucial to store this drug under appropriate conditions as improper storage can lead to a reduction in its active ingredient content, resulting in diminished efficacy in treating diseases or even contributing to antibiotic resistance in bacteria. In addition, veterinary clinicians often make long-term use of the drug after opening the package. Considering these factors, our study aims to investigate the active ingredient of oxytetracycline under different storage conditions [4, 10].

## MATERIALS AND METHODS

In this research study, two oxytetracycline 10% (100 mg/ml active ingredient) products from different pharmaceutical companies (referred to as A and B) were examined. Five products with the same serial number were selected from each company and subjected to three different storage conditions: a normal room at 25 °C, a dark room at 25 °C, and a refrigerator at 4°C. The active ingredient of the samples was assessed at six different time intervals: immediately after opening the package, 24 hours later, 48 hours later, at the 3rd, 6th, and 9th month. During each testing cycle, the first unsealed product was resampled, and a new sealed drug was also evaluated for comparison. This process was repeated consistently until the end of the testing period. All the evaluated samples were compared against the amount of active ingredient present in the standard sample, which was initially assessed at the beginning of the study. To create the standard sample, 30 mg of pure powder and %99 purity of oxytetracycline were dissolved in 30 ml of water and preserved as a control sample throughout the study. The methods proposed by Mileva et al (2020) were employed to measure the quantity of active ingredient. In addition to measuring and comparing the active ingredient of the two samples, any changes in the shape and appearance of the drugs were also evaluated. The samples were measured and evaluated at the following concentrations: 0.5 ppm, 1 ppm, 2 ppm, 4 ppm, 8 ppm, and 10 ppm [10].

*The High-Performance Liquid Chromatography (HPLC) specifications for this study are as follows:*

- Detectors: The photo diode – The Interstitial Detector

- Column: ODS-4 C18 Column (dimensions: 150 mm X 4.6 mm X 5 μm)

- Wavelength: 360 nm

- Flow Rate: 1 ml/min

*The mobile phase used for the HPLC analysis consists of the following components:*

- 1.26 g of oxalic acid Merc

- 1.08 g of sodium octane sulfonate

- Acetonitrile

- Deionized water

The ratio of these components in the mobile phase is 2:30:68 (V/V/V).

**RESULTS**

In this study, the oxytetracycline products from two different companies were evaluated. Initially, the control and standard groups were assessed at concentrations ranging from 0.5 ppm to 10 ppm. Subsequently, the results were used to generate a chromatography curve. Finally, the preservation results at various time intervals were presented in Tables 1 and 2, with corresponding figures in Figures 1-5, displaying the values in the ppm scale.

**Table 1.** Oxytetracycline active agent containing the active ingredient levels depending on the time of A and B Preparations (ppm)

| Time / Storage | Start | 24th hour | 48th hour | 3rd month | 6th month | 9th month |
|---|---|---|---|---|---|---|
| **Normal Room A** | 8.35 ± 0.26 | 8.10 ± 1.02 | 7.50 ± 0.58 | 6.14 ± 0.32 | 5.58 ± 0.00* | 6.08 ± 0.10 |
| **Normal Room B** | 7.77 ± 0.55 | 7.82 ± 0.20 | 7.17 ± 0.36 | 6.88 ± 0.20 | 4.90 ± 0.16 | 6.08 ± 0.05 |
| **Dark Room A** | 8.76 ± 0.43 | 8.02 ± 0.61 | 8.14 ± 0.08 | 6.29 ± 0.27 | 5.30 ± 0.04* | 5.97 ± 0.13 |
| **Dark Room B** | 8.42 ± 0.25 | 7.65 ± 0.48 | 7.07 ± 1.28 | 6.37 ± 0.10 | 6.07 ± 0.26 | 5.40 ± 0.72 |
| **Refrigerator A** | 7.24 ± 0.49 | 9.07 ± 0.27 | 7.54 ± 0.62 | 7.55 ± 0.15 | 5.36 ± 0.05 | 6.06 ± 0.36 |
| **Refrigerator B** | 7.33 ± 0.36 | 8.20 ± 0.00* | 7.23 ± 0.25 | 6.94 ± 0.40 | 6.23 ± 0.03* | 5.26 ± 0.19 |

*.The difference between the time indicated by the A and B drugs for the same storage conditions was significant ($p<0.05$).

**Table 2.** Oxytetracycline agent contains the active ingredient due to different storage conditions and the time of the A and New Drug levels (ppm)

| Time / Storage | 3rd month | 6th month | 9th month |
|---|---|---|---|
| **Normal Room A drug** | 6.14 ± 0.32 | 5.58 ± 0.00* | 6.08 ± 0.10 |
| **Normal Room New** | 6.50 ± 0.19 | 6.38 ± 0.20 | 6.45 ± 0.32 |
| **Dark Room A drug** | 6.29 ± 0.27 | 5.30 ± 0.04* | 5.97 ± 0.13 |
| **Dark Room New** | 6.37 ± 0.34 | 6.30 ± 0.17 | 5.86 ± 0.06 |
| **Refrigerator A drug** | 7.55 ± 0.15 | 5.36 ± 0.05 | 6.06 ± 0.36 |
| **Refrigerator New** | 7.48 ± 0.11 | 5.81 ± 0.20 | 5.72 ± 0.07 |

* The difference between A and the new drug in the period for the same storage conditions as indicated by significant ($p<0.05$).

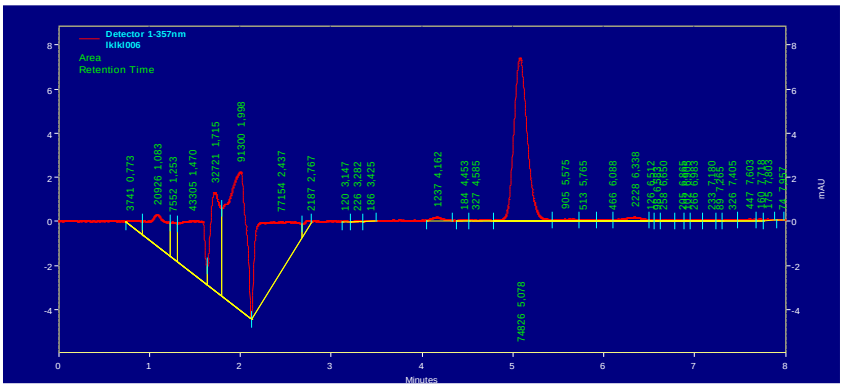


**Figure 1.** The start time for the standard operation of oxytetracycline.

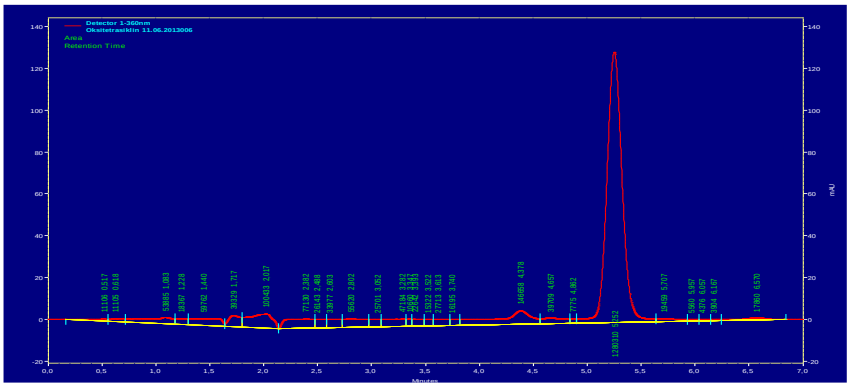


**Figure 2.** Chromatogram for preparations containing oxytetracycline (beginner time) (HPLC chromatogram).

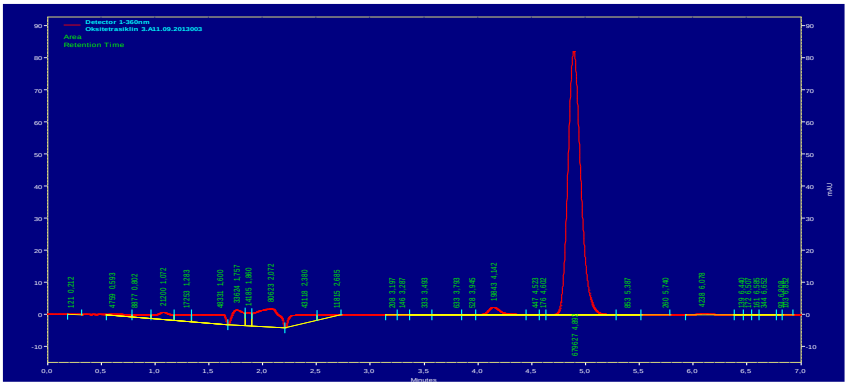


**Figure 3.** Chromatogram for preparations containing oxytetracycline (3rd month) (HPLC chromatogram).

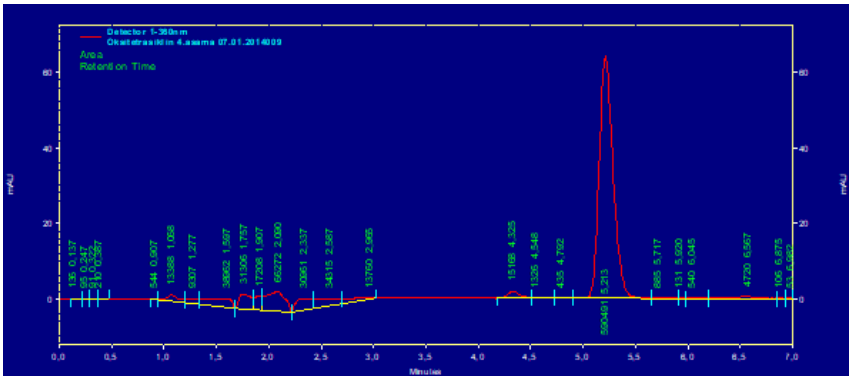


**Figure 4.** Chromatogram for preparations containing oxytetracycline (6th month) (HPLC chromatogram).

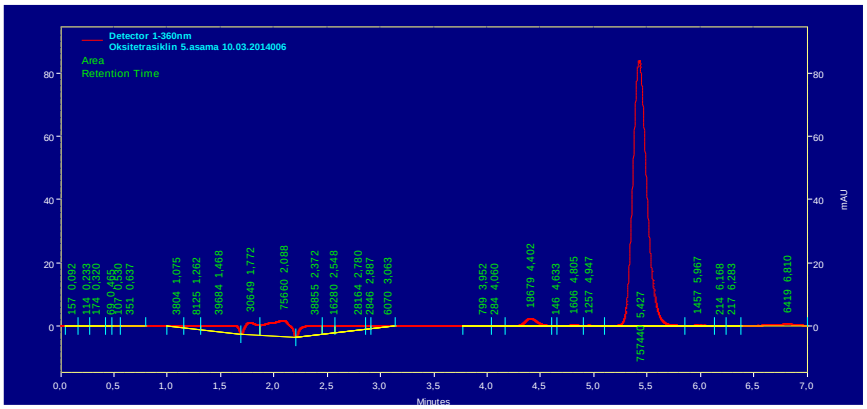


**Figure 5.** Chromatogram for preparations containing oxytetracycline (9th month) (HPLC chromatogram).

## DISCUSSION

The presence of a consistent and justifiable dosage of active ingredient in a synthesized drug is the fundamental principle of drug stability [11, 12]. However, various internal and external factors can lead to changes in the active ingredient over time, potentially resulting in a decrease in potency or even deterioration of the drug. Drug deterioration can alter the pharmacological effects of the drug and may even make it toxic [4, 13]. Drug stability encompasses not only the active ingredient but also the additives in the medicine, such as flavors and colors. Preserving both the chemical and biological features of the drug is crucial in discussions about drug stability. Internal and external factors can affect the synthesized drug and its active ingredient [3-6]. Important external factors impacting drug stability include heat, light, humidity, and oxygen, while internal factors may involve the reaction of the active ingredient, preservatives, and solvents used in the drug formulation [3-6]. All these factors influence the drug's half-life, which is the duration between opening the package's cap and the last day the drug retains its characteristics. Preserving drug stability aims to maintain the active ingredient and physical appearance of the drug from its production until its expiration date (when sealed) and during the period of patient use (when unsealed) [12]. Different parameters are considered to assess the stability of a drug formulation, including physical parameters such as shape (appearance), durability, viscosity, and solubility. In drug production, it is important not only to identify the factors that contribute to drug deterioration but also to understand the mechanisms underlying this deterioration. Alongside quantifying the deterioration of the drug, evaluating its quality is also essential [14]. The true indicator of drug stability is ensuring that the active ingredient remains at or above the standard level throughout the usable period of the drug after it has been unsealed (up to the expiration date). Understanding the factors responsible for drug deterioration is crucial for proper storage on pharmacy shelves and during medication administration. The study of drug stability encompasses three main aspects: physical, chemical, and microbial [12]. These aspects collectively contribute to the assessment and preservation of the drug's efficacy and quality over time. In this research study, two oxytetracycline 10% (100 mg/ml active ingredient) products from two different pharmaceutical companies (A and B) were investigated. Five products with the same serial number were selected from both companies and stored under three different conditions: a normal room at 25°C, a dark room at 25°C, and a refrigerator at 4°C. The active ingredient of the samples was assessed at six different time intervals: immediately

after opening the package's cap, 24 hours later, 48 hours later, at the 3rd month, 6th month, and 9th month. The active ingredient quantities were compared to the initial amount of active ingredient on the first day of the study, and the results are displayed in Table 1. Additionally, the unsealed drug was evaluated at the 3rd, 6th, and 9th months under the same storage conditions as the initial unsealed drug. The results were compared to the drug that was unsealed for the first time and preserved in the same conditions. The findings from this evaluation are presented in Table 2. These tables provide a comprehensive overview of the changes in the active ingredient content over time and under different storage conditions in the study [15]. In 2004, Hismiogullari conducted a study to investigate the active ingredient of various drugs available in the market and pharmacies under different storage conditions. Hismiogullari's study shares similarities with our own research. Specifically, Hismiogullari focused on the injectable, oral powder, oral suspension, and oral solution forms of medications such as gentamicin, neomycin, kanamycin, streptomycin, dihydrostreptomycin, sulphadimidine, sulfadiazine, sulfamethoxazole, and trimethoprim. These drugs were stored in three different conditions: a normal room, a refrigerator, and an autoclave. The spectrophotometer was used to assess the drugs' efficacy at the 3rd, 6th, 9th, and 12th months. In 2006, Ozdemir and Yildrim conducted a similar study, focusing on two different forms of the drug oxytetracycline (normal and long-term) administered to sheep via injection. They measured the concentration of the active ingredient in the blood plasma at various time intervals [2, 16].

## CONCLUSION

A common finding across all of these research studies is the gradual decrease in the concentration of the active ingredient observed in the drug over time. In the present study, the decline in active ingredient content initiates in the 3rd month and reaches its maximum reduction by the 6th month. Notably, the rate of decline is more rapid for unsealed drug. The significant contribution of this research lies in providing clinicians with valuable insights into the alterations in active ingredient levels that occur following the unsealing and prolonged preservation of the drug.

## ETHICS

Approved.

## CONFLICT OF INTEREST

None.

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