ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Stability of Oxytetracycline in Different Types of Solutions and Stored at Different Temperatures

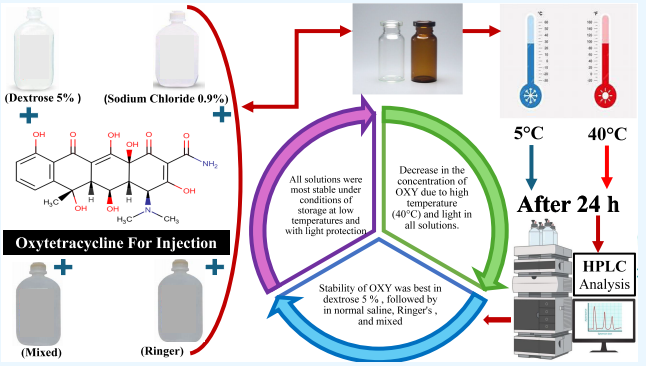
Fatima N. Bashory,* Nisreen H. ALshibeh ALwattar, and Mouhamad R. AL masalma

Cite This: *ACS Omega* 2025, 10, 12037−12042

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Administration of drugs, especially antibiotics, via intravenous injections with different types of solutions is a common and widely applied treatment method in human and veterinary medicine. One of these antibiotics is oxytetracycline, which is a tetracycline. The aim of this article is to study the effect of temperature (5 °C, 40 °C) and light on the stability of oxytetracycline injections after dissolving different types of reconstitution solutions (sodium chloride 0.9%, dextrose 5%, sodium chloride 0.9% with dextrose 5%, Ringer). After 24 h, the concentration of the oxytetracycline was determined by the HPLC method. The results showed a decrease in the concentration of oxytetracycline due to the effect of high temperature (40 °C) and light in all solutions. On the other hand, the decrease in the concentration of oxytetracycline was less than on low temperature (5 °C) and light protection. The effect of the solution reconstitution solution was also evaluated where the stability of oxytetracycline was best in dextrose 5% solution, followed by sodium chloride 0.9%, Ringer's solution, and mixed (dextrose with sodium chloride 0.9%). This paper recommends reconstituting oxytetracycline with 5% dextrose and storage under refrigeration away from light to maintain a better stability of oxytetracycline.



1. INTRODUCTION

Antibiotics are heterogeneous organic compounds with low molecular weights produced by living organisms (microbes, algae, plants, or animals) that have the ability to inhibit the growth of other microorganisms in low concentrations; in a broader sense, they are chemical therapeutic agents that inhibit the growth of microorganisms such as bacteria.[1] Antibiotics were classified according to several criteria, the best of which was classification according to the structure of their organic compounds and their common functional groups.[1]

Tetracycline is one of the most popular classes of antibiotics, a subclass of complex organic compounds known as polyketides that possess the structure of oxyahydrotetracene-2-carboxamide.[2] Tetracycline was first discovered as a fermentation product for soil bacteria.[1] Figure 1 shows the basic structure of all tetracyclines.

Tetracyclines have been widely used in human and veterinary medicine as well as in agriculture for the control of certain bacterial diseases since their discovery,[3,4] as they have shown great effectiveness in the treatment and prevention of many bacterial infections resulting from various bacteria, including chlamydia, mycoplasma, and rickettsia, and interest in these compounds has increased recently due to their use in contemporary medical fields such as neuroscience, tumors, and viruses, in addition to their high effectiveness, which allowed their use in abundance in developing countries with limited health care budgets.[5,6]

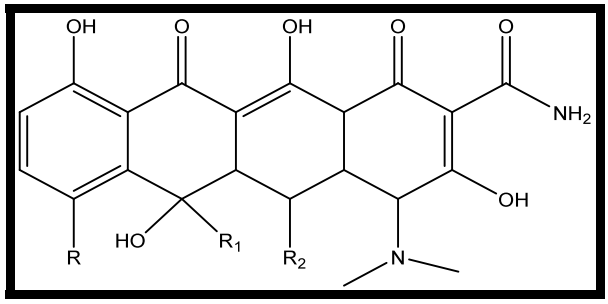
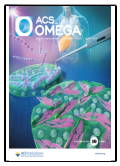


Figure 1. General formula of tetracyclines.

Oxytetracycline (OXY) ($C_{22}H_{24}N_2O_9$) is a broad-spectrum antibiotic yellow crystal powder. It is used in many

Received: October 31, 2024
Revised: February 26, 2025
Accepted: March 5, 2025
Published: March 21, 2025



pharmaceutical forms (injections, tablets, capsules, ointments, and oral suspensions). In 1953, the Food and Drug Administration approved OXY as a feed additive, allowing its use for the therapeutic treatment of numerous poultry diseases on commercial farms. The antibiotic could be added directly to their feed or water or administered via aerosols.[7] OXY is also applied in treating infections in poultry, cattle, sheep, and swine and is used against foulbrood disease in honeybees.[8] Furthermore, its broad antibacterial spectrum and high potency make OXY a commonly used antibiotic for combating bacterial infections in aquaculture, including fish farming and shrimp raised on farms.[9] The overuse of antibiotics in human health, animal husbandry, and food production has compromised their effectiveness due to the rise of bacterial resistance. Therefore, promoting the judicious use of antimicrobials is crucial for ensuring their continued safe and effective application in clinical settings.[10] Oxytetracycline (OXY) is notable among tetracyclines for its wide-ranging efficacy and cost-effectiveness. However, these attributes have led to its widespread misuse, particularly in countries like Brazil, where OXY-containing drugs are dispensed for veterinary purposes with minimal oversight by regulatory bodies such as the Ministry of Agriculture, Livestock and Food Supply and the National Health Surveillance Agency. This unregulated use of OXY in animals risks not only microbial resistance but also environmental pollution, as roughly 20% of the medication is excreted unchanged in feces and urine, ultimately contaminating soil, rivers, and lakes.[11]

Stability is a critical aspect of any active pharmaceutical ingredient (API), which must be taken into account during various stages, such as early formulation, development, production, storage, and commercialization. The chemical and physical stability of an API significantly influences the safety and effectiveness of the drug products. They should remain stable throughout their shelf life, unaffected by environmental factors, such as temperature, moisture, and light. Understanding the effects of these factors is essential for establishing appropriate manufacturing and storage conditions, determining retesting intervals, and assessing the shelf life of an API.[12]

Considering the importance of stability for quality control of pharmaceutical products and the clinical relevance of OXY, mainly in veterinary medicine, this work provides the effect of temperature and light on the stability of oxytetracycline in different types of reconstitution solutions; the reason for selecting these conditions is that oxytetracycline is influenced by environmental factors, such as temperature and light, during the treatment. As for the reconstitution solutions, oxytetracycline in its prepared injection pharmaceutical form is used in intravenous treatments for farm animals where it is administered with these solutions to facilitate treatment.

2. METHODS

2.1. Standard Preparation. Oxytetracycline standard (M_w = 460.434g/mol) was purchased from Hong Kong Lipharma International Company with assay 96.3%. Standard solutions were prepared by dissolving oxytetracycline in deionized water at a concentration of 800 mg/L.

2.2. Control Sample. The control sample was prepared from a commercial product (OXYDIMA 5% oxytetracycline for injection) with deionized water at a concentration of 800 mg/L.

2.3. Sample Preparation. All samples were prepared from OXYDIMA 5% with different diluting solutions: sodium chloride 0.9%, dextrose 5%, sodium chloride 0.9% + dextrose 5%, Ringer's (NaCl 8.6 g/L, KCl 0.30 g/L, $CaCl_2$ 0.33g/L). The pH of the diluting solutions was determined before addition of the medicinal substance.

Stability of OXY in four diluting solutions was tested at 5 °C ± 2 and 40 °C ± 2 °C. The solutions were stored in an air thermostat oven for 24 h. The solutions were placed in transparent glass containers and others in dark glass containers covered with cellophane paper in light protection; artificial light was used to study the effect of light on the stability of oxytetracycline in these solutions. Time 0 was defined as the time of addition of the antibiotic to the diluting solution.

2.4. Assay Method. An optimized high-performance liquid chromatography (HPLC) was performed by the ACDIMA Veterinary Pharmaceutical Laboratory. The isocratic mobile phase consisted of a mixture of HPLC-grade A, consisting of oxalic acid 0.01 M methanol and acetonitrile (55:18:27 v/v), and B, consisting of oxalic acid 0.01 M (A:80 B:20). All solutions for HPLC analysis were filtered through a 0.45 μm microporous membrane before use, at a constant flow rate of 0.6 mL/min. The column was Shim-pack XR-ODS II C18 (3.0 mm i.d. × 100 mm), the ambient temperature of the column during the procedure was around 25 °C, and the pressure was 6.5 MG Pa. Injection volumes of 20 μg were used. To avoid interference from degradation products, PDA detection was performed at a wavelength of 360 nm. Quantitation was performed by integration of the area under the curve and the retention time of about 2.6 min.

3. RESULTS

3.1. Standard. Figure 2 shows the HPLC chromatogram of a standard solution of oxytetracycline.

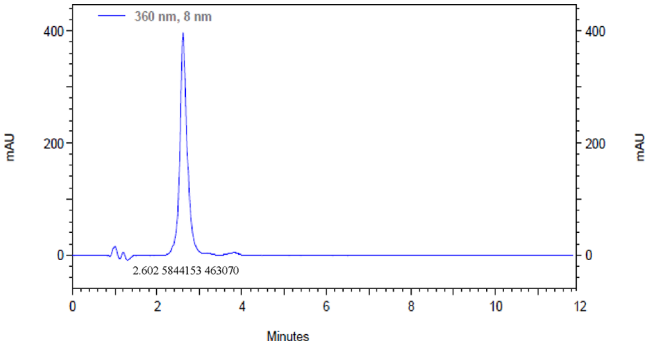


Figure 2. Representative chromatograms of standard solution oxytetracycline.

3.2. Control Sample. Figure 3 shows the HPLC chromatogram of the control solution of the pharmaceutical sample. Oxytetracycline retention time was about 2.600 min, the peak area was 5,838,230, and the peak shape was good. The tailing factor was 1.12285, while the RSD% was 0.9% when n = 5.

In order to determine the quality assurance test, the integral of the area under the curve was calculated for both the standard sample (Figure 2) and the pharmaceutical sample (Figure 3). The results show that the concentration of oxytetracycline on the OXYDIMA 5% was 99.90%.

https://doi.org/10.1021/acsomega.4c09760
ACS Omega 2025, 10, 12037−12042

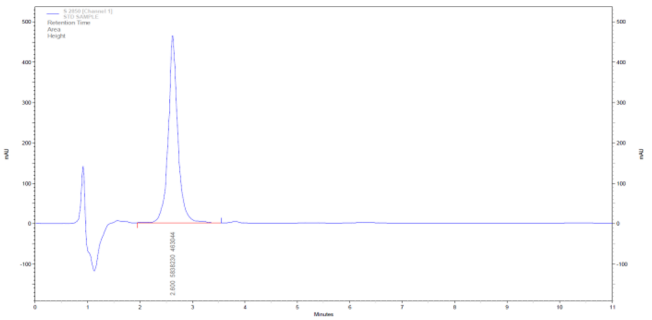


Figure 3. Representative chromatograms of control solution.

3.3. Effect of Sodium Chloride 0.9% Solution. Figure 4 shows the HPLC chromatograms of oxytetracycline in sodium chloride 0.9% at different conditions, A: 40 °C + light − B: 40 °C + without light − C: 5 °C + light − D: 5 °C + without light.

3.4. Effect of Dextrose 5% Solution. Figure 5 shows HPLC chromatograms of oxytetracycline in dextrose 5% solution at different conditions, A: 40 °C + light − B: 40 °C + without light − C: 5 °C + light − D: 5 °C + without light.

3.5. Effect of Sodium Chloride 0.9% + Dextrose 5% Solution. Figure 6 shows HPLC chromatograms of oxytetracycline in sodium chloride 0.9% + dextrose 5% solution at different conditions, A: 40 °C + light − B: 40 °C + without light − C: 5 °C + light − D: 5 °C + without light.

3.6. Effect of Ringer's Solution. Figure 7 shows HPLC chromatograms of oxytetracycline in Ringer's solution at different conditions, A: 40 °C + light; B, 40 °C + without light; C, 5 °C + light; D, 5 °C + without light.

4. DISCUSSION

The results in Table 1 show that the highest decrease in the OXY concentration was at 40 °C in the presence of light, regardless of the solution used. This is consistent with a previous study.[13] To determine the stability of ampicillin and ceftriaxone solutions, it was found that the solutions were stable for 30 h at temperatures of 25 and 30 °C, while none of the stability criteria were achieved at a temperature of 37 °C.

At 5 °C present with light, the decrease in the OXY concentration was much less compared to 40 °C with light: it was 13.6% in the sodium chloride 0.9% + dextrose 5% solution, 9.6% in the Ringer solution, 2.4% in the sodium chloride 0.9% solution, and finally 1.9% in the dextrose 5% solution; this is consistent with the literature.[14] Penicillin diluted in sodium chloride 0.9% and dextrose 5% solution was stable when stored at 5 °C for 21 days with a recovery of no less than 90%. Additionally, another study found that the stability of oxytetracycline-methanol solution is related to the preservation temperature. The solution was more stable under low temperature conditions than at room temperature.[15]

We also note that the highest percentage of decrease in the OXY concentration was in the mixed solution, where it reached 24.9%, followed by 20.7% in the Ringer solution and 17.3% in the sodium chloride 0.9% solution, while it was 5.7% in the dextrose 5% solution, which is the lowest percentage of decrease at a temperature of 40 °C in the presence of light.

Some physical changes were observed on prepared solutions; the OXY in mixed solution precipitate was observed after 24 h. This could explain the decrease of its concentration, since solubility is the most important factor affecting the decrease of

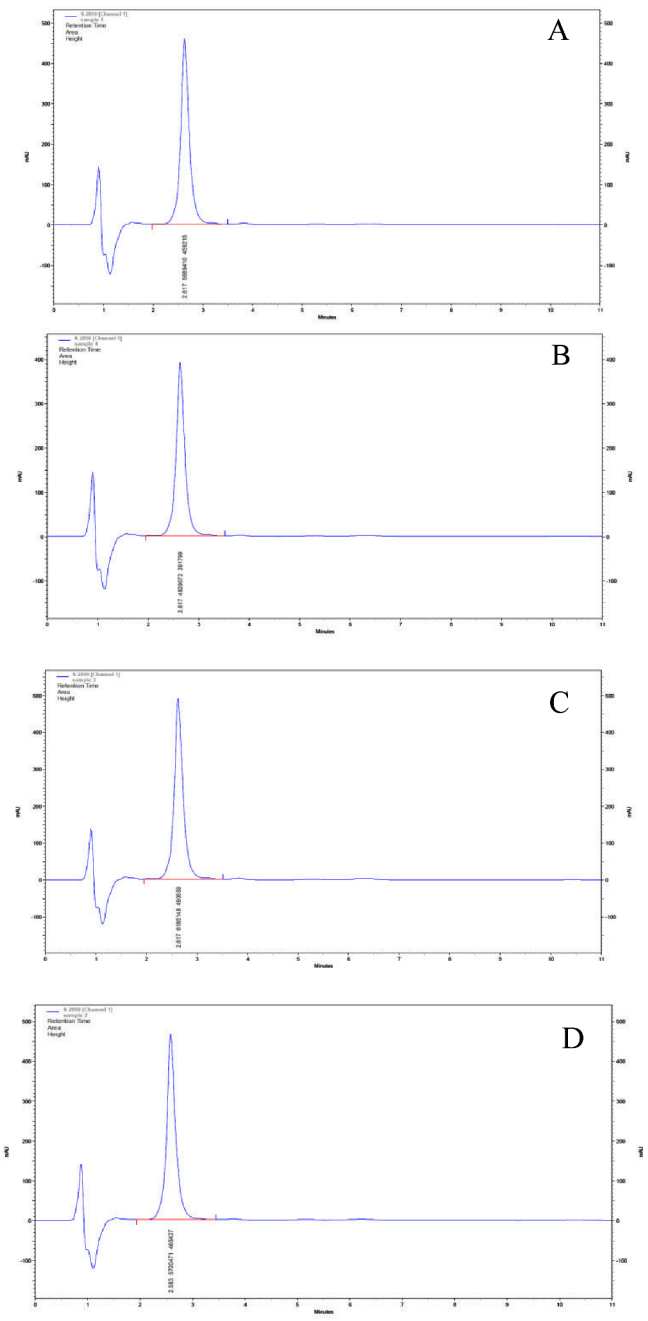


Figure 4. Representative chromatograms of oxytetracycline diluted in sodium chloride 0.9%, A: 40 °C with light; B: 40 °C without light; C: 5 °C with light; D: 5 °C without light.

concentration;[16] also, a color change was observed after 24 h when OXY was mixed with Ringer's solution. This change can be attributed to the binding of OXY to cations in the solution. Tetracycline was considered as a strong chelating agent, and its antibacterial properties and pharmacokinetics are affected by the chelation of metal ions present in food and the biological environment. OXY forms complexes at several positions in its molecule due to the presence of different electron donor groups.[17,18] Figure 8 shows the molecular form of the association of tetracycline with metals.[19]

Our results showed the impact of direct light on accelerated degradation. Samples stored without light protection were unstable even during 24 h. All tetracyclines are sensitive to

https://doi.org/10.1021/acsomega.4c09760
ACS Omega 2025, 10, 12037−12042

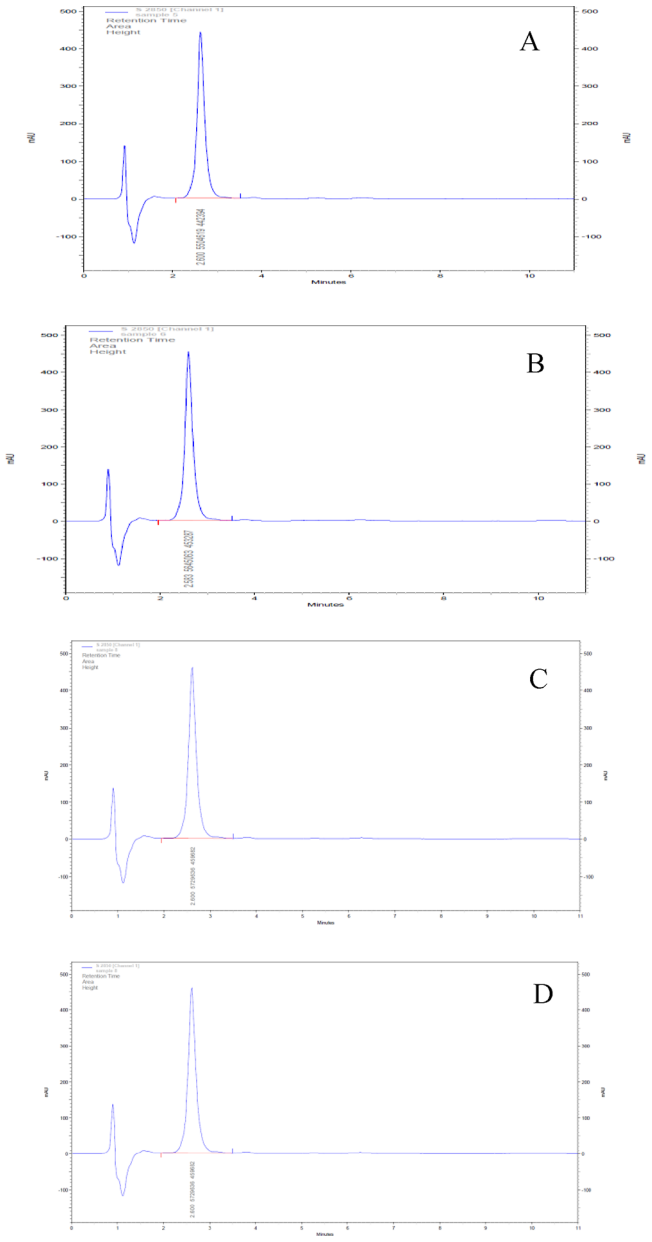


Figure 5. Representative chromatograms of oxytetracycline diluted in 5% dextrose, A, 40 °C with light; B, 40 °C without light; C, 5 °C with light; D, 5 °C without light. Effect of sodium chloride 0.9% + dextrose 5% solution.

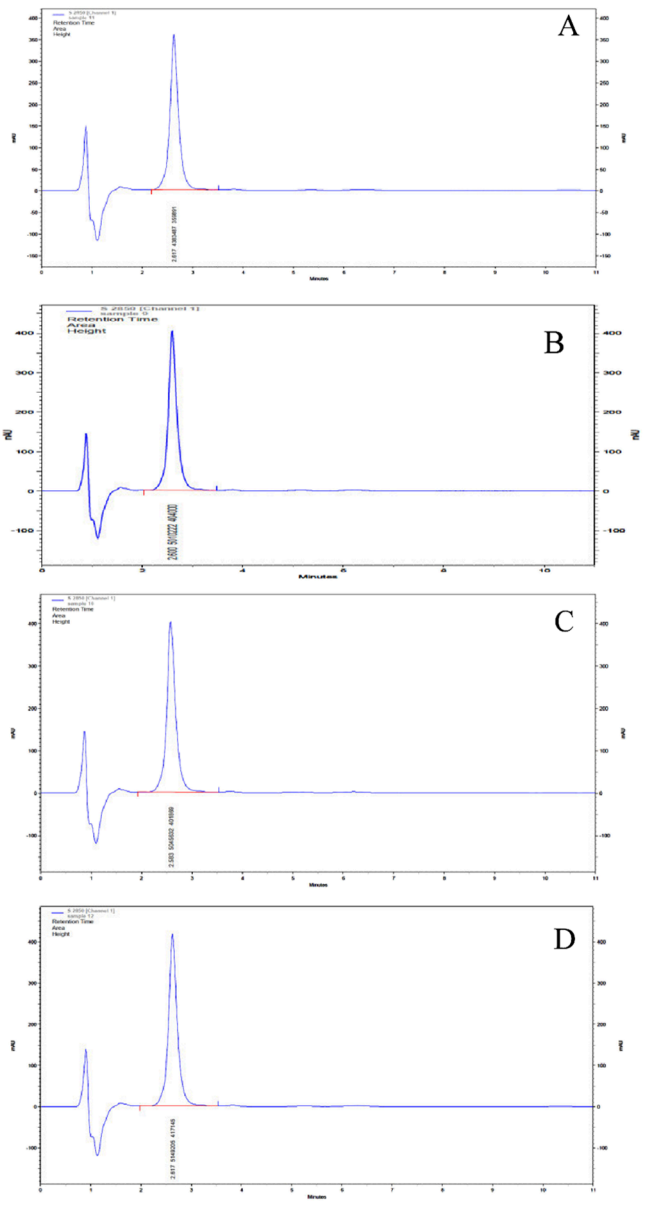


Figure 6. Representative chromatograms of oxytetracycline diluted in sodium chloride 0.9% + dextrose 5%. A: 40 °C with light − B: 40 °C without light − C: 5 °C with light − D: 5 °C without light.

light, high temperatures, and humidity, and aqueous solutions of OXY decompose by light in the absence of oxygen, and high temperature can accelerate photodegradation.[20,21] Scheme 1 shows some of the OXY decomposition products.

The pH of diluting solution also has a role in accelerating the dissolution of OXY, and it forms positive ion (weak acid) in acidic solutions; the aqueous solutions of OXY are stable at pH 1.0 to 2.5 for at least 30 days at 30 °C.[23] However, in alkaline solutions, it ionizes and gives the conjugate anion, which increases the compound's ability to bond with cations.[24] This also explains that OXY is more stable in dextrose solution at pH = 4.45, which is more acidic than sodium chloride 0.9% at pH = 5.95, followed by the Ringer's solution, which is pH = 6.5 OXY reacting with Ca^{2+}. It forms complexes in solutions whose pH is higher than 6.[23] As for the apparent decrease in the mixed solution, even though its pH is lower than the Ringer solution, this is due to its precipitation, which was mentioned previously.

5. CONCLUSIONS

According to the results presented here, the stability of oxytetracycline-reconstitution solutions is highly related to the preserved temperature and light. Under the same temperature preservation and light conditions, the oxytetracyclines were more stable when diluted with 5% dextrose, followed by sodium chloride 0.9% and then Ringer, and it was least stable when diluted with mixed solution. Under these conditions, all solutions were most stable under conditions of storage at low temperatures and light protection. Based on the recent results, when the oxytetracycline solution needs to be stored without degradation, it is recommended to use the dextrose 5% solution with oxytetracycline at 5 °C with light protection for

https://doi.org/10.1021/acsomega.4c09760
ACS Omega 2025, 10, 12037−12042

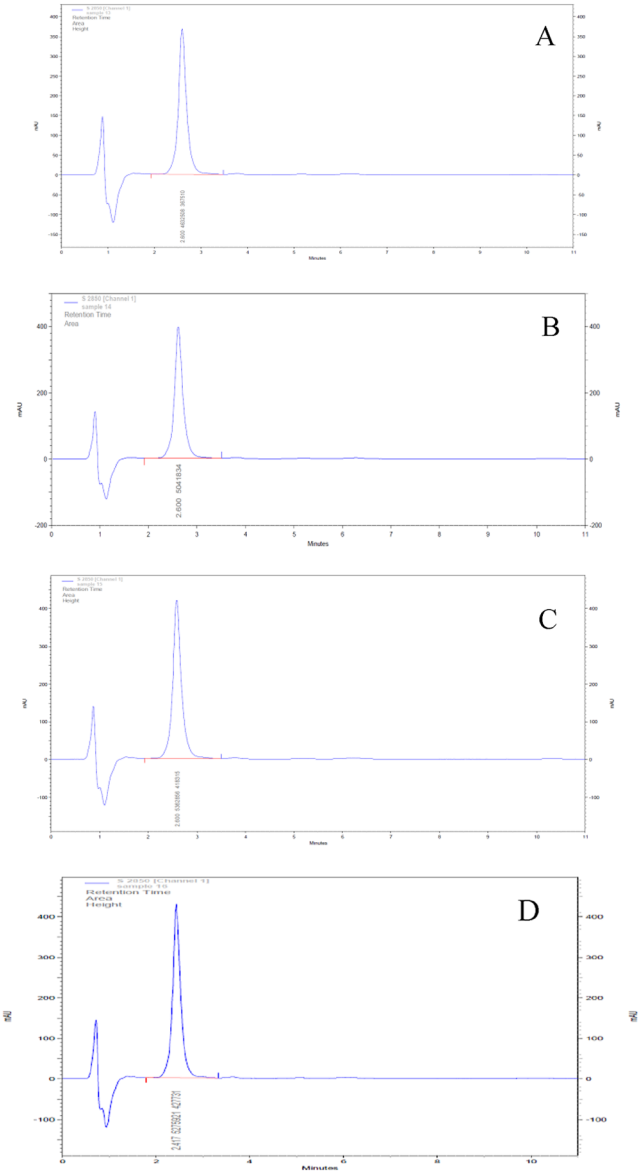


Figure 7. Representative chromatograms of oxytetracycline diluted in Ringer. A: 40 °C with light − B: 40 °C without light − C: 5 °C with light − D: 5 °C without light.

Table 1. Stability of Oxytetracycline after 24 h Diluted in Different Types of Solutions

		40 °C		5 °C	
factors		light	without light	light	without light
sodium chloride 0.9% solution pH = 5.97	recovery	82.7%	97.5%	97.6%	100%
	percentage of decrease	17.3%	2.5%	2.4%	0.0%
dextrose 5% solution pH = 4.85	recovery	94.3%	96.7%	98.1%	99.2%
	percentage of decrease	5.7%	3.3%	1.9%	0.8%
sodium chloride 0.9% + dextrose 5% solution pH = 5.56	recovery	75.1%	85.8%	86.4%	88.2%
	percentage of decrease	24.9%	14.2%	13.6%	11.8%
Ringer's solution pH = 6.5	recovery	79.3%	86.4%	90.4%	91.9%
	percentage of decrease	20.7%	13.6%	9.6%	8.1%

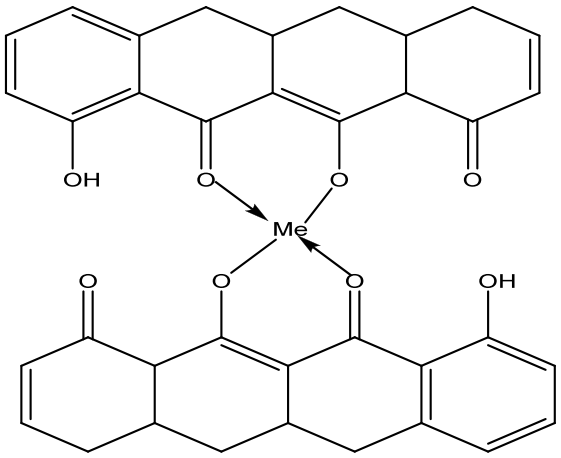
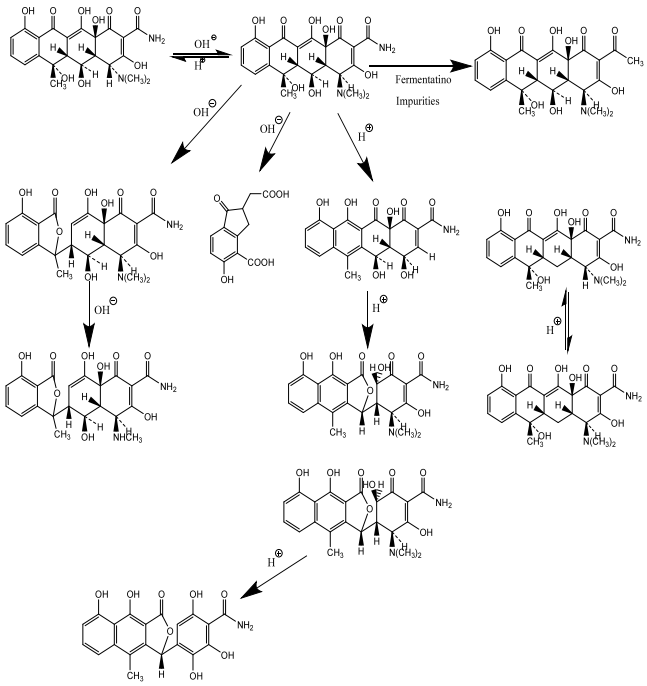


Figure 8. Chemical formula of the association of tetracycline with metal.

Scheme 1. Chemical Structures and Degradation Pathway of Oxytetracycline Degradation Products[a22]



[a]OXY, oxytetracycline; 4-EOXY, 4-epioxytetracycline; 2-ADOXY, 2-acetyl-2- decarboxamidooxytetracycline; TC, tetracycline; ETC, 4-epitetracycline; ISO-OXY, iso-oxytetracycline; NDMOXY, *N*-desmethyl-OXY; AOXY, anhydrooxytetracycline; a-APOXY, a-apooxytetracycline; b-APOXY, b-apooxytetracycline; TL, terrinolidine; TA, terranoic acid. Source: adapted from ref 22.

24 h. However, further studies are necessary to better understand the oxytetracycline degradation process as well as the biological effects and other properties of the degradation products.

AUTHOR INFORMATION

Corresponding Author

Fatima N. Bashory − *Faculty of Science - Department of Chemistry, Damascus University, Damascus, Syrian Arab Republic;* orcid.org/0009-0009-5009-6120;

https://doi.org/10.1021/acsomega.4c09760
ACS Omega 2025, 10, 12037−12042

Email: Fatima.bashory@damascusuniversity.edu.sy, fafa1415@hotmail.com

Authors

Nisreen H. ALshibeh ALwattar − *Faculty of Science - Department of Chemistry, Analytical Chemistry, Damascus University, Damascus, Syrian Arab Republic*

Mouhamad R. AL masalma − *Daraa - Faculty of Veterinary Medicine - Microbiology, Damascus University, Damascus, Syrian Arab Republic*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c09760

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the research affairs of Damascus University for the financial support.

■ REFERENCES

(1) Arrhashmi, M.; Ajittvarma, V. *Soil Biology Antibiotics and Antibiotics Resistance Genes in Soils Monitoring, Toxicity, Risk Assessment and Management*. http://www.springer.com/series/5138.

(2) Yuwono, M.; Indrayanto, G. Oxytetracycline: Analytical Profile. In *Profiles of Drug Substances, Excipients and Related Methodology* (Vol. *32*, pp 97−117). Academic Press Inc. 2005.

(3) Petković, H.; et al. Genetics of Streptomyces rimosus, the Oxytetracycline Producer. *Microbiol. Mol. Biol. Rev.* **2006**, *70* (3), 704−728.

(4) McManus, P. S.; Stockwell, V. O.; Sundin, G. W.; Jones, A. L. Antibiotic use in plant agriculture. *Annual Review of Phytopathology* **2002**, *40*, 443−465.

(5) Askari Rizvi, S. F. Tetracycline: Classification, Structure Activity Relationship and Mechanism of Action as a Theranostic Agent for Infectious Lesions-A Mini Review. *Biomedical Journal of Scientific & Technical Research* **2018**, *7* (2), 5787.

(6) Kogawa, A. C.; Regina, H.; Salgado, N. DOXYCYCLINE HYCLATE: A REVIEW OF PROPERTIES, APPLICATIONS AND ANALYTICAL METHODS. *Int. J. Life Sci. Pharm. Res.* **2012**, *2* (4), 11−25.

(7) Chopra, I.; Hawkey, P. M.; Hinton, M. Review Tetracyclines, molecular and clinical aspects. *J. Antimicrob. Chemother.* **1992**, *29*, 245.

(8) levy, S. B. *The Antibiotic Paradox: How Miracle Drug Are Destroying The Miracle*. (1993) https://academic.oup.com/ajhp/article-abstract/50/8/1768/5182242.

(9) Reed, L. A.; Siewicki, T. C.; Shah, J. C. Pharmacokinetics of oxytetracycline in the white shrimp, Litopenaeus setiferus. *Aquaculture* **2004**, *232* (1−4), 11−28.

(10) Chopra, I.; Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews* **2001**, *65* (2), 232−260.

(11) Halling-Sørensen, B.; Sengeløv, G.; Ingerslev, F.; Jensen, L. B. Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquindox due to environmental processes. *Arch. Environ. Contam. Toxicol.* **2003**, *44* (1), 7−16.

(12) Bueno, M. S.; Longhi, M. R.; Garnero, C. Pharmaceutical Systems as a Strategy to Enhance the Stability of oxytetracycline Hydrochloride Polymorphs in Solution. *Pharmaceutics* **2023**, *15* (1), 192.

(13) Herrera-Hidalgo, L.; López-Cortes, L. E.; Luque-Márquez, R.; Gálvez-Acebal, J.; De Alarcón, A.; López-Cortes, L. F.; Gutiérrez-Valencia, A.; Gil-Navarro, M. V. Ampicillin and ceftriaxone solution stability at different temperatures in outpatient parenteral antimicrobial therapy. *Antimicrob. Agents Chemother.* **2020**, *64* (7), No. e00309-20.

(14) Hossain, M. A.; Friciu, M.; Aubin, S.; Leclair, G. Stability of penicillin G sodium diluted with 0.9% sodium chloride injection or 5% dextrose injection and stored in polyvinyl chloride bag containers and elastomeric pump containers. *American Journal of Health-System Pharmacy* **2014**, *71* (8), 669−673.

(15) Wang, W.; Wu, N.; Yang, J.; Zeng, M.; Xu, C.; Li, L.; Li, L. Stability studies of oxytetracycline in methanol solution. In *IOP Conference Series: Earth and Environmental Science* (Vol. *113*, No. 1, p. 012110). IOP Publishing. 2018.

(16) Newton, D. W. *Drug compatibility and stability in dextrose vs Sodium Chloride 0.9%*; 2011, www.IJPC.com.

(17) Chartone-Souza, E.; Loyola, T. L.; Bucciarelli-Rodriguez, M.; Menezes, M. Ã. D. B. C.; Rey, N. A.; Pereira-Maia, E. C. Synthesis and characterization of a tetracycline-platinum (II) complex active against resistant bacteria. *Journal of Inorganic Biochemistry* **2005**, *99* (5), 1001−1008.

(18) Duarte, H. A.; Carvalho, S.; Paniago, E. B.; Simas, A. M. Importance of tautomers in the chemical behavior of tetracyclines. *J. Pharm. Sci.* **1999**, *88* (1), 111−120.

(19) Priya, S. S.; Radha, K. V. *BRIEF REVIEW OF SPECTROPHOTOMETRIC METHODS FOR THE DETECTION OF TETRACYCLINE ANTIBIOTICS*; 2014 www.chm.bris.ac.

(20) Jin, X.; Xu, H.; Qiu, S.; Jia, M.; Wang, F.; Zhang, A.; Jiang, X. Direct photolysis of oxytetracycline: Influence of initial concentration, pH and temperature. *J. Photochem. Photobiol., A* **2017**, *332*, 224−231.

(21) Al-Momani, I. F.; Kanan, S. J. Flow-Injection Spectrophotometric and LC Determination of Doxycycline, oxytetracycline and Chlortetracycline in Biological Fluids and Pharmaceutical Preparations. *Int. J. Flow Injection Anal.* **2008**, *25* (1), 29.

(22) Halling-Sørensen, B.; Sengeløv, G.; Tjørnelund, J. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. *Arch. Environ. Contam. Toxicol.* **2002**, *42* (3), 263−271.

(23) Sversut, R. A.; da Silva, A. A.; Cardoso, T. F. M.; Kassab, N. M.; Do Amaral, M. S.; Salgado, H. R. N. A Critical Review of Properties and Analytical Methods for the Determination of Oxytetracyline in Biological and Pharmaceutical Matrices. In *Critical Reviews in Analytical Chemistry* (Vol. *47*, Issue 2, pp 154−171). Taylor and Francis Ltd.; 2017 .

(24) Carlotti, B.; Cesaretti, A.; Elisei, F. Complexes of tetracyclines with divalent metal cations investigated by stationary and femtosecond-pulsed techniques. *Phys. Chem. Chem. Phys.* **2012**, *14* (2), 823−834.