World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Comparative Study of Development and Analysis of Antibiotic Tetracycline HCL Tablet with Marketed Preparations

Aditya Sampat Dere [1,*], Prachi Nandkumar Padwal [2], Dhanashri Subhash Jadhav [1], Chaitali Mohan Barhate [1], Harsh Suryakant Chavan [1] and Khushi Sanjay Gourkar [1]

[1] *Student Samarth Institute of Pharmacy, Belhe, Maharashtra, India.*
[2] *Department of Quality Assurance, Samarth Institute of Pharmacy, Belhe, Maharashtra, India.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 24(02), 162-169

Publication history: Received on 26 September 2025; revised on 02 November 2025; accepted on 04 November 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.24.2.0979

Abstract

Tetracyclines are among the earliest broad-spectrum antibiotics that are well-tolerated and easy to administer. They are effective against various diseases, including plague, cholera, typhoid, syphilis, Legionnaire's disease, and anthrax. Additionally, some tetracyclines can be utilized in the treatment of malaria, Lyme disease, tuberculosis, Rocky Mountain spotted fever, and leprosy. Historical evidence suggests that humans first encountered these chemical compounds involuntarily in ancient times, as indicated by the analysis of bone samples that are over 1500 years old.

Following World War II, these antibiotics were "rediscovered" at Lederle Laboratories and Pfizer due to a vigorous search for new antibiotic options. Their bacteriostatic properties arise from the inhibition of protein biosynthesis. Since the structural elucidation by Robert Woodward, Lloyd Hillyard Conover, and others in the 1950s, tetracyclines have become prime targets for the synthesis of natural products. The casual and indiscriminate use of tetracyclines in the initial decades after their introduction, not only in humans but also for veterinary applications and as growth promoters in meat production, quickly led to the development of resistance.

A significant achievement in this field was accomplished by Andrew Myers in 2005 with the convergent total synthesis of (−)-doxycycline, along with various azatetracyclines and pentacyclines, which has motivated chemists in the pharmaceutical industry to seek out novel and highly effective tetracyclines in recent years.

Keywords: Tetracycline; Antibiotic; Total synthesis; Biosynthesis; Plague

1. Introduction

Tetracyclines exhibit a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria. They have been utilized not only in human medicine for treating infectious diseases but also as growth promoters in animal feed. In addition to the pharmacological significance of tetracycline (TC), this compound contains numerous potential metal-binding sites. Consequently, extensive research has been conducted on the chelation of TCs with various metal ions, which have been effectively applied in pharmaceutical analysis.

Depending on the selected experimental parameters such as solvent medium, pH, type of metal ion, and ligand:metal ratio, the O(10), O(11), and O(12) on the BCD-ring, along with O(1), O(3), and N(4) in ring A and at the carboxamide group on the A-ring, are the primary coordination sites frequently identified in the literature. The pharmacokinetics and bioavailability of TC are influenced by its coordination with metal ions like calcium in blood plasma and magnesium

* Corresponding author: Aditya Sampat Dere.

ions in the intracellular environment. By interacting with copper, zinc, iron, and other trace metal elements in enzymes such as collagenase, tetracyclines impede the enzymatic degradation of tissues.

Complexes of (TC) with Co(II), Ni(II), and Fe(III) ions demonstrated greater activity against Bacillus subtilis, Serratia species, and Escherichia coli compared to the original tetracycline hydrochloride. Platinum(II) complexes of tetracycline and doxycycline have been reported to bind to DNA and inhibit the growth of tumoral cells. Although Au(III) is as significant as Pt(II) ions in this regard, there has been limited focus on Au(III) complexes of TC. Environmental investigations on TCs have indicated that the complexation of these drugs with heavy metal cations in natural waters and soils may lead to chronic toxic effects in aquatic plants.

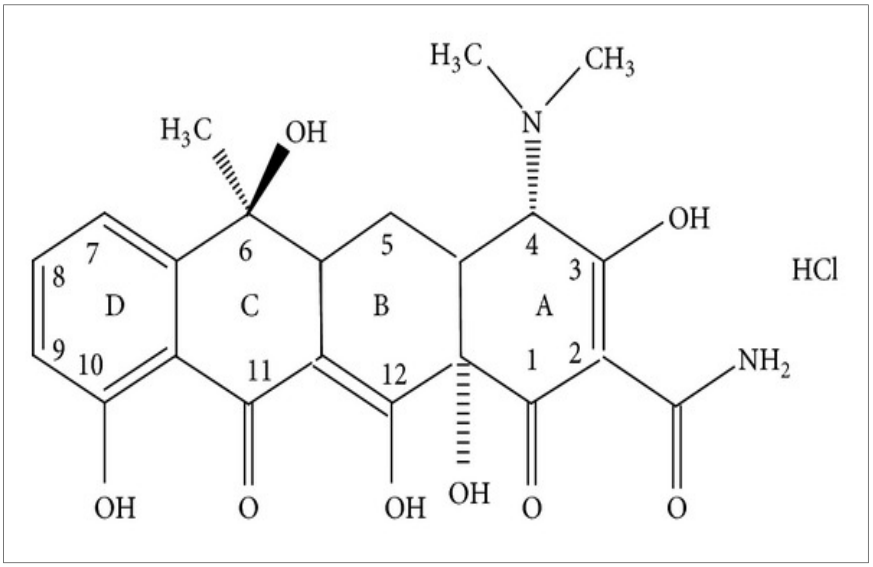

Figure 1 Tetracycline HCL

In this study, we examine the determination of TC in both pure and dosage forms through complexation with gold(III) and mercury(II) ions in solution, utilizing UV-visible and atomic absorption techniques.

2. Materials and Methods

Equipment. A Shimadzu Prominence-i, LC-2030C 3D Plus high-performance liquid chromatography (HPLC) system (Kyoto, Japan), which is equipped with a PDA detector, was

utilized for the chromatographic analysis of the antibiotics under investigation. The operation of the system and data acquisition were conducted on a computer that had Lab solution software installed. Additional equipment used in this study included a Vortex mixer (JEIO TECH, Korea), an Isolab sonicator (Germany), an electronic balance (PTE Ltd, Japan), and a digital centrifuge, DSC-302SD (Taiwan).

Standards and Reagents. Tetracycline (≥98.00%), Oxytetracycline (≥99.0%), and Ciprofloxacin (≥98.00%) were sourced from Sigma® (China), Sigma® (USA), and Fluka® Analytical (China), respectively. HPLC-grade Methanol (≥99.90%) and Acetonitrile (≥99.90%) were obtained from Sigma-Aldrich (Germany). Oxalic acid dihydrate and Trichloroacetic acid were provided by Emsure® (Ultra-pure) (Germany). A Milli-Q water purification system from Millipore (Millipore, Bedford, MA, USA), which operates on the reverse osmosis principle, was employed to purify water to achieve high-purity standards. Reference compounds with a purity exceeding 98.00% were utilized throughout the research.

Samples. The pharmaceutical formulations available in Bangladesh were analyzed to evaluate the method's applicability: Ciprocin-Vet (1000 mg) tablet, Ciprocin (500 mg) tablet, and Tetrax (500 mg) capsule from Square; Cipro-A (500 mg) and Tetravet (500 mg) tablets from ACME; Renamycin (500 mg) tablet from Renata; Beuflox (500 mg) tablet from Incepta; Neofloxin (500 mg) tablet from Beximco; Oxpro (500 mg) tablet from United; Maprocin (500 mg) tablet from Orion; Antibac (500 mg) tablet from FnF; Oxyvet Bolus (500 mg) tablet from Globe; and Vetomycin (500 mg) tablet from Opsonin. A total of fifteen bovine milk samples were collected from various local dairy farms, as well as local and

super shops in different regions of Chittagong, Bangladesh. Among these, eleven samples were raw milk, three were pasteurized, and one was UHT (Ultra-high-temperature processed).

Chromatographic conditions were established using a reversed-phase Shim-pack GIS column; 5.00 μm, C18; measuring 4.60 × 250.00 mm, to investigate the simultaneous quantification of Tetracycline, Oxytetracycline, and Ciprofloxacin at a column temperature of 30°C. A buffer solution of 0.05 M Oxalic acid dihydrate (C2H2O4·2H2O), along with acetonitrile and methanol, constituted the mobile phase in the gradient system outlined. The flow rate of the mobile phase was maintained at 1.50 mL min−1, and the PDA detection wavelength was configured to 330 nm. An injection volume of 20.00 μL was utilized, resulting in the elution of all drugs within a time frame of 7.39 ± 0.05 min.

Table 1 Optimum gradient program for the proposed HPLC method

Time (Min)	**Solvent**			**Flow Rate (mL/Min)**	**Retention Time (Min)**		
	0.05 M Oxalic acid	ACN	MeOH		OTC	CIP	TC
0.01	84	16	0	1.5	6.38 ± 0.06	6.97 ± 0.05	7.39 ± 0.05
4	80	18	2				
7	58	30	12				
8	84	16	0				
10	Stop						

Standard and working solutions. An appropriate amount of each of tetracycline, oxytetracycline, and ciprofloxacin was dissolved separately in methanol to prepare stock standard solution at a concentration of 100 μg mL−1. All solutions protected from light were stored at 4°C. They used up to 3 months. Working solutions at concentration levels of 0.50, 1.00, 2.00, 4.00, 6.00, and 8.00 μg mL−1 were prepared daily before analysis by the dilution of standard solutions with methanol. Buffer: 0.05 M aqueous solution of Oxalic acid dihydrate buffer was prepared by mixing appropriate weight in Milli Q water, filtered, and sonicated before use.

2.1. New Compounds under Development

Sriram et al. (2007) successfully synthesized tetracycline derivatives exhibiting anti-HIV, antimycobacterial, and HIV-1 integrase inhibitory properties. This synthesis was accomplished through the reaction of specific tetracyclines (minocycline, tetracycline, and oxytetracycline), formaldehyde, and the secondary amine function (piperazine) found in certain fluoroquinolones (norfloxacin, lomefloxacin, ciprofloxacin, gatifloxacin), facilitated by microwave radiation. Compound no. 10, a hybrid of tetracycline and lomefloxacin, illustrated exhibited the most significant effect on HIV-1 replication. These investigations indicate that the combination of tetracyclines with fluoroquinolones has yielded both anti-HIV and anti-tuberculosis activity (Mycobacterium tuberculosis) and holds promising potential for AIDS treatment.

Employing total synthesis, Sun et al. (2015) proposed a series of tetracycline analogues characterized by six fused rings, termed hexacyclines. Their structure retains the classical tetracycline skeleton, with a bicyclic ring EF attached at the D ring level.

The correlation between chemical structure and antibacterial activity was examined, with modifications at positions C7, N8, C9, and C10, assessing the effectiveness of various analogues against a broad spectrum of Gram-positive and Gram-negative bacteria, including tetracycline-resistant or multidrug-resistant strains. Among all compounds analyzed, the most favourable outcomes were observed for C7-fluorohexacycline and C7-trifluoromethoxyhexacycline, demonstrating extensive antibacterial activity in vitro and commendable efficacy in vivo against Pseudomonas aeruginosa. The encouraging findings from this research advocate for the optimization of this structural framework to identify and develop new clinically valuable tetracyclines in the future.

At present, Tetraphase Pharmaceuticals is advancing two compounds, TP-271 and TP-6076, which are in phase I of clinical trials, with their structures represented.

2.2. Method of Preparation

2.2.1. Apparatus

Mortar and pestle, sieve, hand operated capsule filling machine, volumetric flask, pipette, beaker, stop watch, measuring cylinder, filter paper, UV spectrophotometer, Disintegration test apparatus.

2.2.2. Procedure:

- Weigh the required quantity of drugs and other excipients.
- Moisture content should be less than 1.5%.
- Starch should be dried and sieved through 100*, moisture less than 1.5%
- Talcum should be dried and sieved through 100S, moisture less than 1%.
- Mix all the ingredients uniformly using a mortar and pestle.

Category: Antibiotic Tablets

Dose: Tetracycline is taken by mouth as a capsule or liquid, typically two to four times a day for seven to 14 days.

The usual dose of tetracycline for adults is 1-2 grams per day in two or four divided doses.

You should take tetracycline on an empty stomach with a half glass of water. Take the drug at least one hour before or two hours after meals or snacks.

Storage: Store in a tightly closed container in a cool and dry place.

Packaging: In a plastic bottle.

2.3. Evaluation of Tablets

- **Disintegration Time**: One capsule was placed in each of six tubes of assembly and the assembly was suspended in water. Discs were added to each tube, the temperature was maintained at 37±2°C and assembly was operated for 60 min.
- **Drug Content:** Weigh an amount of the granules equivalent to 50 mg of losartan potassium was dissolved in 100 ml of phosphate buffer pH 6.8, filtered, diluted suitably and analyzed for the drug content at 246 nm using a UV-visible spectrophotometer.
- **In-vitro Drug Release Study**: The release rate of losartan potassium from granules was determined using IP Dissolution Test Apparatus Type II (basket type). Granules were first incorporated in an empty hard gelatin capsule of size #3 and then placed in a dry basket at the beginning of each test. Lower the basket in the dissolution medium and the apparatus was run at 50 rpm, The dissolution test was performed using 900 ml of phosphate buffer pH 6.8, at 37±0.5ºC and 50 rpm. 5 ml were withdrawn at time intervals of five minutes for 60 minutes. This was maintained at the same temperature and was added to the bulk. The samples were filtered through Whatman filter paper no. 41. The absorbance of these solutions was measured using UV- visible spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

At present, Tetraphase Pharmaceuticals is advancing two compounds, TP-271 and TP-6076, which are in phase I of clinical trials, with their structures represented.

3. Experimental Work

3.1. UV-Visible Spectrophotometry Complex Formation

To 1 mL aliquots of metal ion solutions (5.08 × 10−3 M) in a series of 10 mL volumetric flasks, varying volumes of TC. HCl solution (5.08 × 10−3 M) were added: 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 mL, and the total volumes were adjusted to the marks. All experimental conditions were optimized, and the resulting complexes were extracted from the aqueous solutions using ethyl acetate in a 5:1 v/v ratio of aqueous to ethyl acetate prior to measuring the absorbance.

3.2. Determination of Antibiotic Complexes by Direct Method

Solution mixtures containing 5 to 70 μg/mL of TC. HCl and either 10 μg/mL of Au(III) ion or 15 μg/mL of Hg(II) standard solutions were prepared by adding varying volumes of the antibiotics' standard solutions (100 μg/mL) to 1 mL or 1.5 mL, respectively, of the metal ion standard solutions (100 μg/mL) in 10 mL volumetric flasks. The volumes were brought to the mark, and the experimental conditions were fine-tuned to the respective optimum values of concentration, pH, temperature, and liquid extraction with ethyl acetate (EA) in a 5:1 v/v ratio of aqueous to EA. The absorbance of the complexes was measured.

4. Therapeutic Applications of the New Tetracyclines

Historically, tetracyclines have been extensively utilized for a range of genitourinary, gastrointestinal, respiratory, and dermatological conditions. Nevertheless, the significant rise in bacterial resistance, along with the introduction of new antibacterial agents, has reduced the spectrum of infections for which tetracyclines are regarded as the primary therapeutic choice.

The efficacy of tigecycline has been assessed in multiple in vivo clinical trials involving human participants, leading to the establishment of three FDA-approved indications. In the management of hospitalized patients suffering from complicated skin and soft tissue infections, tigecycline has demonstrated not only effectiveness but also a favourable pharmacokinetic profile. Additionally, in a phase 2 open-label clinical trial that included patients with complicated intra-abdominal infections (such as gangrenous perforated appendicitis, cholecystitis, diverticulitis, and peritonitis), tigecycline proved to be a safe and effective treatment option. Furthermore, tigecycline is indicated and has received approval for the treatment of community-acquired pneumonia. Throughout all studies, tigecycline satisfied all non-inferiority criteria.

In contrast to tigecycline and eravacycline, omadacycline offers the benefit of an oral formulation, which enhances patient adherence and reduces hospitalization expenses. Omadacycline is recommended for the treatment of complicated skin and soft tissue infections as well as community-acquired pneumonia, although ongoing research is exploring its potential use in urinary tract infections.

Eravacycline, owing to its extensive antibacterial spectrum, in vitro efficacy, and superior tolerability compared to tigecycline, represents a suitable option for managing complicated intra-abdominal infections in adults, particularly when the pathogen exhibits resistance mechanisms to other tetracyclines or antibiotic classes. The effectiveness of eravacycline has been evaluated in comparison to the beta-lactam antibiotic meropenem.

The FDA-approved sarecycline is utilized for the treatment of acne vulgaris in individuals aged nine years and older, showing effectiveness against moderate-to-severe, inflammatory, or non-inflammatory (comedonal) types. Furthermore, owing to its specific action on Cutinebacterium acnes and minimal penetration into the blood-brain barrier, sarecycline boasts a favorable safety profile (with minimal side effects), a low likelihood of inducing bacterial resistance, and potentially a reduced impact on gut microbiota when compared to the broad-spectrum antibiotics doxycycline and minocycline.

Pharmacokinetic properties. Tigecycline exhibits a high binding affinity to plasma proteins and possesses a substantial volume of distribution (exceeding plasma volume), indicating its concentration within tissues. Additionally, tigecycline is swiftly distributed throughout tissues, with the highest concentrations found in the bone marrow, thyroid gland, salivary glands, spleen, and kidneys. Tigecycline undergoes metabolism independently of cytochrome P450 enzymes, albeit not extensively. As a result, tigecycline does not disrupt the metabolism of other substances that are mediated by the six cytochrome P450 isoforms (1A2, 2C8, 2C9, 2C19, 2D6, and 3A4). The pharmacokinetics of tigecycline are linear—this may be affected by the coadministration of P-glycoprotein inhibitors or inducers, as tigecycline serves as a substrate for these. Likewise, omadacycline demonstrates a low likelihood of interactions via transport mechanisms. The absorption rate of omadacycline diminishes if a high-fat meal is ingested two hours prior. Therefore, omadacycline should be taken following a fasting period of at least 4 hours, succeeded by 2 hours without the intake of food and beverages (excluding water), as well as 4 hours without the use of antacids, multivitamins, and dairy products. The liver metabolizes eravacycline; however, none of the resulting metabolites exhibit pharmacological activity. Consequently, caution is advised with CYP3A4 inducers to enhance the extent of eravacycline metabolism to a clinically significant level.

5. Result

5.1. UV-Visible Spectrophotometry

The UV-visible spectra of TC. HCl, metal ions, and their complexes (50 μg · mL−1) in aqueous solutions are illustrated in Figure 2. The drug's spectrum displayed a multiplet with peak absorption maxima at λ 235, 270, and 370 nm, primarily corresponding to π → π* transitions [18]. The spectrum of the yellow gold(III) aqueous solution showed two absorption bands, with a doublet appearing at λ 240 and 295 nm, along with a low-intensity band at 385 nm, which were attributed to ligand to metal charge transfer and 1 A 1 g → 1 Eg transitions of the square planar tetrachloroaurate(III) anion [AuCl4]−The spectrum of the Hg(II) ion revealed a single high-intensity band at λ 285 nm, assigned to ligand → metal charge transfer. The spectrum of TC. HCl in conjunction with the Au(III) ion exhibited a hypsochromic shift of the π → π* transitions band, along with the emergence of two new absorption bands at λ max 350 and 425 nm, which were assigned to 1 A 1 g → 1 Eg and 1 A 1 g → 1 B 2 g transitions of square planar gold(III) complexes, respectively. The spectrum of the Hg(II) complex solution displayed shifts of ligand bands to shorter wavelengths and the emergence of an additional band at λ max 320 nm, attributed to ligand to metal charge transfer transition.

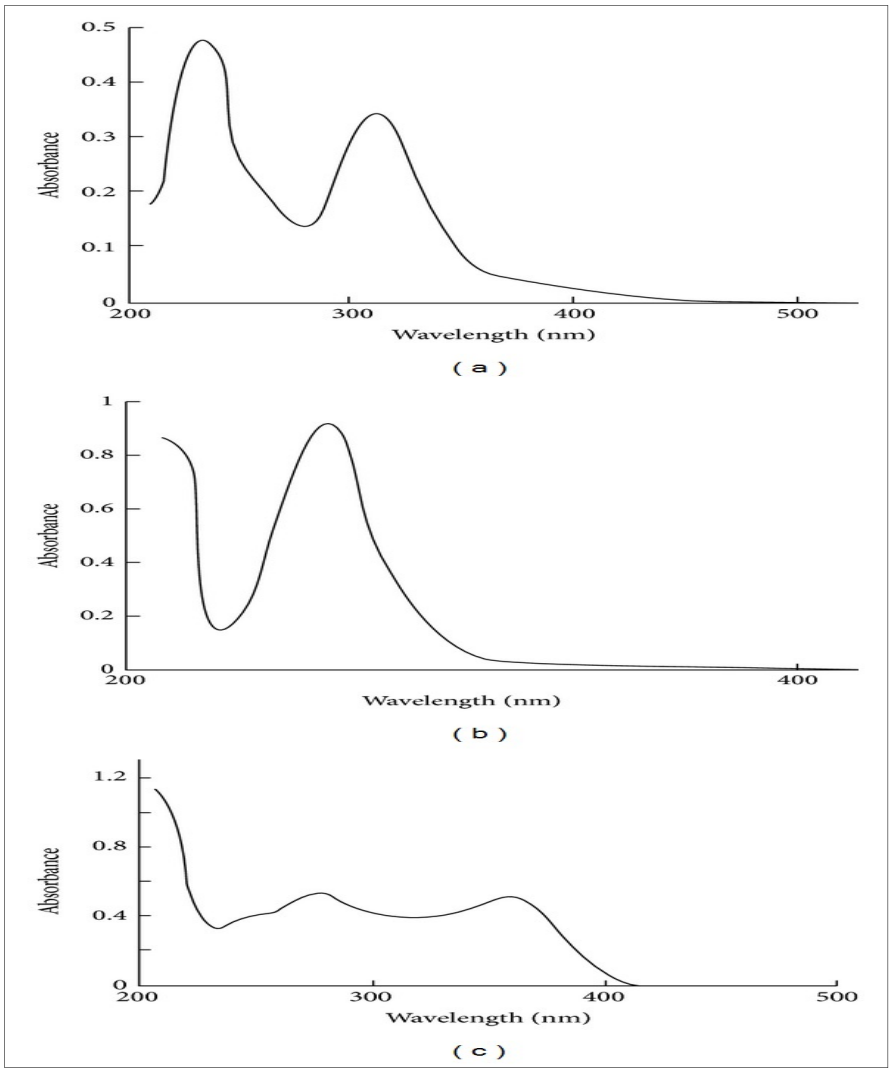

Figure 2 UV-visible spectra of (a) gold(III) ion, (b) mercury(II) ion, (c) TC. HCl, (d) TC-Au(III), and (e) TC-Hg(II) complexes in aqueous solutions (50 μg/mL each)

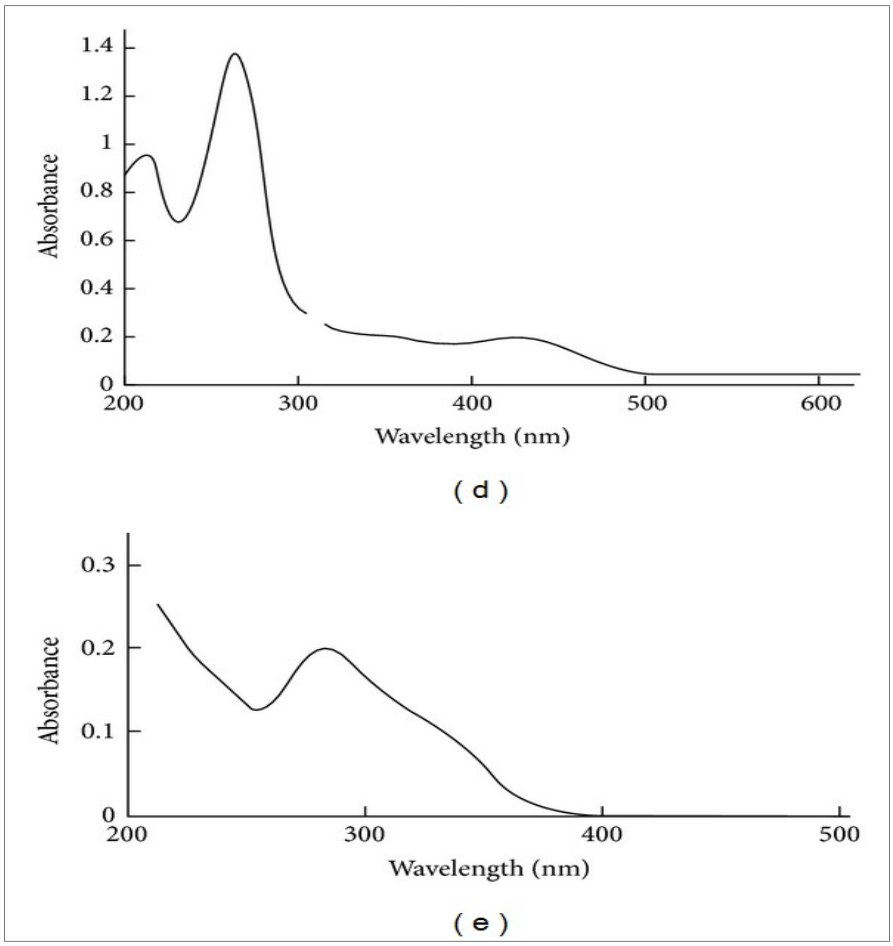

Figure 3 UV-visible spectra of (a) gold(III) ion, (b) mercury(II) ion, (c) TC. HCl, (d) TC-Au(III), and (e) TC-Hg(II) complexes in aqueous solutions (50 µg/mL each)

6. Conclusion

The present study successfully developed and evaluated Tetracycline Hydrochloride tablets and compared their physicochemical and dissolution characteristics with those of marketed formulations. The prepared tablets exhibited acceptable parameters for weight variation, hardness, friability, disintegration time, and drug content, meeting official pharmacopoeial specifications.

Comparative dissolution studies demonstrated that the in-house formulation achieved a drug release profile comparable to, and in some cases superior to, the marketed preparations, indicating effective formulation design and process optimization. Statistical analysis confirmed that there was no significant difference ($p > 0.05$) in the dissolution behavior, suggesting bioequivalence in vitro.

Overall, the study concludes that the developed Tetracycline HCl tablet formulation is pharmaceutically equivalent to existing marketed products and can serve as a cost-effective and therapeutically reliable alternative. Further in vivo bioavailability studies are recommended to establish complete therapeutic equivalence.

Compliance with ethical standards

Acknowledgments

I would like to express my sincere gratitude to my guide, Ms. Padwal Prachi Nandkumar, for their invaluable guidance, constant support, and insightful suggestions throughout the preparation of this review paper. Their expertise and encouragement have been instrumental in shaping the quality and direction of this work.

I am also thankful to all my co-authors, Jadhav Dhanshri Subhash, for their collaborative efforts, constructive discussions, and contributions that greatly enhanced the depth and clarity of this paper.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Tetracyclines: a pleiotropic family of compounds with promising therapeutic properties. Review of the literature (Griffin et al., 2010) — a comprehensive review of the therapeutic properties of tetracyclines.

[2] Tetracycline Antibiotics and Resistance (Grossman T.H., 2016) — a review focused on tetracycline resistance mechanisms.

[3] Tetracyclines—An Important Therapeutic Tool for Dermatologists (Orylska-Ratynska et al., 2022) — review of tetracyclines from a dermatologic/therapeutic angle.

[4] Tetracyclines: four rings to rule infections through resistance and disease tolerance (JCI article, 2024/2025) — discussing mechanistic aspects of tetracyclines beyond just antibiotic action.

[5] Hamilton, L.A., & Guarascio, A.J. (2019). "Tetracycline Allergy." Pharmacy, 7(3), 104.

[6] Kochling, J., Wu, W., Hua, Y., Guan, Q., & Castaneda-Merced, J. (2016). "A platform analytical quality by design (AQbD) approach for multiple UHPLC-UV and UHPLC–MS methods development for protein analysis." Journal of Pharmaceutical and Biomedical Analysis, 125, 130–139.

[7] Yabré, M., Ferey, L., Somé, I., & Gaudin, K. (2018). "Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis." Molecules, 23(5), 1065.

[8] Palm GJ, Lederer T, Orth P, et al. Specific binding of divalent metal ions to tetracycline and to the Tet repressor/tetracycline complex. Journal of Biological Inorganic Chemistry. 2008;13(7):1097–1110. doi: 10.1007/s00775-008-0395-2

[9] Obaleye JA, Lawal A. A synthesis and antibacterial activity of some tetracycline metal complexes. Centrepoint. 2007;14(1-2):1–6.

[10] Sultan SM, Alzamil IZ, Alarfaj NA. Complexometric-spectrophotometric assay of tetracyclines in drug formulations. Talanta. 1988;35(5):375–378. doi: 10.1016/0039-9140(88)80030-4.

[11] El Said Mahgoub A, Khairy EM, Kasem A. Complex formation of uranyl acetate with tetracycline and its utilization for their microdetermination. Journal of Pharmaceutical Sciences. 1974;63(9):1451–1455. doi: 10.1002/jps.2600630926.

[12] Kang J, Liu H, Zheng Y-M, Qu J, Chen JP. Application of nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, UV-Visible spectroscopy and kinetic modeling for elucidation of adsorption chemistry in uptake of tetracycline by zeolite beta. Journal of Colloid and Interface Science. 2011;354(1):261–267. doi: 10.1016/j.jcis.2010.10.065.

[13] John, M.; Beale, J.H.B. Wilson and Giswold's Organic Medicinal and Pharmaceutical Chemistry, 12th ed.; Lippincott Williams & Wilkins, Wolters Kluwer Health: Baltimore, MD, USA, 2011; pp. 301–307.

[14] Schmitt, M.O.; Schneider, S. Novel Insight into the Protonation–Deprotonation Equilibria of Tetracycline, Sancycline and 10-Propoxy-Sancycline in Aqueous Solution. I. Analysis of the pH-Dependent UV/vis Absorption Spectra by the SVD Technique. Z. Phys. Chem. 2006, 220, 441–475.

[15] Grenier, D.; Huot, M.-P.; Mayrand, D. Iron-Chelating Activity of Tetracyclines and Its Impact on the Susceptibility of Actinobacillus actinomycetemcomitans to These Antibiotics. Antimicrob. Agents Chemother. 2000, 44, 763–766

[16] Chopra, I.; Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. Microbiol. Mol. Biol. Rev. 2001, 65, 232–260.

[17] Al-Kadhumi ASH. Atomic absorption, HPLC and spectrophotometric determination of some antibiotics in pharmaceutical preparations via complexes formation with Au(III) and Hg(II) ions [Ph.D. thesis] Baghdad, Iraq: Department of Chemistry, College of Science, Baghdad University; 2011

[18] Morrison GH. Solvent Extraction in Analytical Chemistry. Butter Worth; 1972