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PHARMACEUTICAL DEVELOPMENT, VALIDATION, BIOEQUIVALENCE (IN-VITRO) AND COMPATIBILITY STUDY OF SOLID ORAL DOSAGE FORM (HARD CAPSULE) TETRACYCLINE

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ABSTRACT

This study reports the formulation development, analytical validation, and in-vitro comparative evaluation of generic Product Tetracycline 250mg hard gelatin capsules. The formulation was designed using Tetracycline as the active pharmaceutical ingredient, along with pharmacopeial excipients selected based on excipient compatibility studies conducted under accelerated conditions. The finished product met British Pharmacopoeia (B.P.) specifications for assay, dissolution, uniformity of dosage, and water content. Analytical method validation was performed using high-performance liquid chromatography (HPLC), and the method fulfilled all ICH and USP requirements for system suitability, specificity, accuracy, precision (repeatability and intermediate), robustness, and spiking recovery. Comparative dissolution studies were carried out in 0.1N HCl (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) using USP Apparatus II. The test product demonstrated more than 85% drug release within 15 minutes in all media, and exhibited similarity factor (f2) values greater than 50 and difference factor (f1) values below 15 when compared with the reference product Resteclin® 250 mg. These results confirm the pharmaceutical equivalence of

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the test formulation and support its suitability for local production and regulatory approval.

Introduction:

Tetracycline HCl is a semisynthetic derivative of the tetracycline family having chemical name is Tetracycline is N6-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a- pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenylcarbonyl- aminomethyl-L-lysine. Compendial name is Tetracycline with molecular formula is $C_{29}H_{38}N_4O_{10}$ and molecular weight is 602.63, synthesized through the chemical modification of tetracycline by coupling it with L-lysine and formaldehyde, which enhances its solubility and bioavailability [1, 2] [3]. Classified as a second-generation tetracycline, Tetracycline was developed to overcome the limitations of earlier agents like tetracycline and chlortetracycline, particularly in terms of absorption, tissue distribution, and side effect profile [4]. The second-generation tetracyclines, including doxycycline and minocycline, were introduced to provide broader clinical utility and improve tolerability [5].

The history of tetracyclines begins with chlortetracycline (aureomycin), discovered in the late 1940s from Streptomyces aureofaciens, marking the advent of a new era in antimicrobial therapy [6]. Tetracyclines quickly gained prominence due to their ability to inhibit a wide range of pathogens. Over time, structural modifications led to newer agents like doxycycline, minocycline, and Tetracycline, each with improved pharmacokinetic and pharmacodynamic characteristics [7]. These drugs are now used not only for infectious diseases but also for dermatological applications, such as acne and rosacea, due to their dual antimicrobial and anti-inflammatory actions [8].

Mechanistically, tetracyclines—acts as a bacteriostatic agent by inhibiting bacterial protein synthesis. It binds reversibly to the 30S ribosomal subunit, thereby preventing the attachment of aminoacyl-tRNA to the A site of the ribosome, effectively halting peptide elongation. This interruption disrupts bacterial replication and growth, accounting for its efficacy against a broad spectrum of gram-positive and gram-negative organisms[9].

Tetracycline demonstrates activity against several clinically relevant pathogens, including Cutibacterium acnes, Chlamydia trachomatis, Mycoplasma pneumoniae, and Rickettsia species [10]. This broad antimicrobial spectrum underpins its use in managing conditions like respiratory infections, sexually transmitted infections, and dermatologic disorders such as acne vulgaris[11]. One of the distinctive advantages of Tetracycline lies in its pharmacokinetics. It exhibits higher water solubility compared to its parent compound, resulting in more efficient oral absorption and greater bioavailability [12]. Once ingested, Tetracycline undergoes rapid hydrolysis in the gastrointestinal tract to release tetracycline, which is the pharmacologically active moiety, confirming its role as a prodrug [13]. Plasma concentration studies reveal that only tetracycline is present systemically after oral administration of Tetracycline, supporting this metabolic transformation[14]

Additionally, Tetracycline provides better tissue penetration and has a longer half-life

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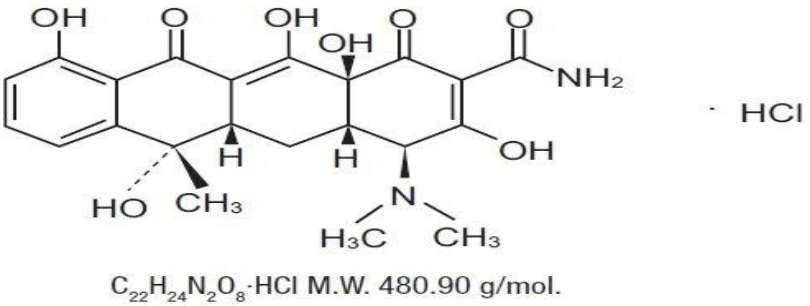
than older tetracyclines, which allows for once-daily or twice-daily dosing and improved patient compliance [15]. Gastrointestinal side effects such as nausea and abdominal discomfort, commonly observed with tetracycline, appear to occur less frequently with Tetracycline, making it more suitable for long-term use[16].

A primary therapeutic application of Tetracycline is in the treatment of moderate to moderately severe acne vulgaris. Acne pathogenesis involves follicular occlusion, sebum overproduction, C. acnes colonization, and inflammation [17]. Tetracycline helps reduce C. acnes levels and exerts anti-inflammatory effects by inhibiting neutrophil chemotaxis and the release of inflammatory cytokines [18]. This dual action enhances its effectiveness in inflammatory acne, especially when topical treatments are insufficient [19].

Beyond acne, Tetracycline has been explored in the management of hidradenitis suppurativa (HS)—a chronic inflammatory skin disorder characterized by recurrent nodules, abscesses, and sinus tracts. Tetracyclines, including Tetracycline, are among the first-line systemic treatments recommended in European S1 guidelines for mild to moderate HS [20]. Their efficacy is attributed to both antimicrobial effects and modulation of the immune response, although high-quality randomized trials are still needed to strengthen the evidence base [21].

Despite these benefits, Tetracycline, like all tetracyclines, carries a risk of adverse effects, notably photosensitivity reactions. Phototoxicity arises when the drug absorbs ultraviolet light, resulting in oxidative damage to the skin [22]. While rare, this reaction necessitates preventive counseling for patients on sun exposure and photoprotection measures. Interestingly, Tetracycline appears to have a lower incidence of such reactions compared to earlier tetracyclines, although comprehensive epidemiological data are still evolving [23].

In conclusion, Tetracycline exemplifies the evolution of tetracycline antibiotics into more patient-friendly formulations with enhanced therapeutic profiles. It combines the antimicrobial spectrum and anti-inflammatory properties of its class with improved pharmacokinetics, reduced side effects, and better patient adherence. These attributes make it an excellent candidate for managing chronic dermatological conditions like acne vulgaris and HS. Continued research may further expand its application into areas such as resistant bacterial infections and immune-mediated inflammatory disorders.



$C_{22}H_{24}N_2O_8 \cdot HCl$ M.W. 480.90 g/mol.

Figure 01. Chemical structure of Tetracycline

Materials, instruments and Chemicals:

Materials:

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The formulation development of Tetracycline Hydrochloride (HCl) hard gelatin capsules was carried out using raw materials procured from authentic and reliable sources. The active pharmaceutical ingredient (API), Tetracycline Hydrochloride, was obtained from Pharmaceutical Grade, supplied as per the British Pharmacopoeia (B.P.) and European Pharmacopoeia (Eur. Ph.) standards. A reference standard of Tetracycline HCl with a certified potency was also employed to ensure accurate quantitative analysis and serve as a primary benchmark for analytical validation.

For excipients, Magnesium Stearate was used as a lubricant to reduce interparticle friction and improve powder flow during capsule filling, while Aerosil-200 (colloidal silicon dioxide) functioned as a glidant to prevent powder agglomeration and improve uniformity of capsule fill weight. The final dosage form was encapsulated in empty gelatin capsule shells, size #0, which are widely available and suitable for accommodating the required fill weight of the developed formulation.

All excipients used were of pharmaceutical grade and complied with pharmacopeial standards. Their selection was based on prior compatibility studies, literature precedence, and established use in oral capsule formulations.

Analytical Reagents and Chemicals:

For qualitative and quantitative analysis, high-purity analytical-grade reagents and solvents were employed. The key chemicals included hydrochloric acid, purified water, sodium acetate, glacial acetic acid, monobasic potassium phosphate, sodium hydroxide, phosphoric acid, 2-methyl-2-propanol, di-potassium hydrogen orthophosphate, tetrabutylammonium hydrogen phosphate, sodium edetate, and sodium metabisulfite. Each reagent was procured from certified suppliers, and only analytical-grade materials were used to ensure accuracy, reproducibility, and compliance with pharmacopeial specifications. These reagents were primarily utilized for preparing mobile phases, buffers, and solutions required in high-performance liquid chromatography (HPLC), UV spectrophotometric analysis, dissolution studies, and Karl Fischer titration for moisture content analysis. The use of amber-colored volumetric flasks minimized light-induced degradation of Tetracycline HCl during solution preparation, given the known photosensitivity of tetracyclines.

Instruments and Equipment:

The formulation and evaluation studies were performed using well-calibrated and validated laboratory instruments. The following equipment played a crucial role:

Refrigerator – Used for the storage of reference standards, working standards, and sensitive reagents at controlled conditions to prevent degradation.

HPLC system – Equipped with a stationary phase (HPLC Column: 4.6 mm × 25 cm, 5 µm packing, L-7/C8 type) for chromatographic separation, quantification, and purity analysis of Tetracycline HCl. The selection of a C8 column ensured adequate retention and resolution of the analyte under optimized chromatographic conditions.

UV-Visible Spectrophotometer – Employed for rapid determination of drug content and calibration curve construction during method validation.

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Dissolution Apparatus (USP Type I, 12-basket assembly) – Utilized for in vitro release testing to evaluate dissolution behavior of formulated capsules under simulated gastrointestinal conditions
Karl Fischer Apparatus – Used for the determination of moisture content in raw materials and final formulations to ensure compliance with pharmacopeial limits.
Glassware – High-quality volumetric flasks (amber-colored), beakers, graduated pipettes, and other standard laboratory glassware were used to prepare solutions with high accuracy.
Each instrument was calibrated according to standard operating procedures (SOPs) before use.

Methodology:
Pre-formulation Studies
Before the actual formulation, pre-formulation evaluations were conducted to study the physical and chemical properties of Tetracycline HCl and its interaction with excipients. These included:
Organoleptic evaluation (appearance, color, and odor) of API
Solubility profile in water and various buffers to guide dissolution method design.
FTIR compatibility studies between the API and excipients (Magnesium Stearate, Aerosil-200) to confirm the absence of any chemical interactions that might affect drug stability.
Micromeritic properties of the blend (bulk density, tapped density, angle of repose, compressibility index, Hausner's ratio) to ensure suitability for capsule filling and good flow behavior

Formulation Development:
The formulation of Tetracycline HCl capsules was developed by direct blending and encapsulation. The weighed quantities of API and excipients were geometrically mixed in a clean, dry stainless-steel container. Aerosil-200 was first blended with the drug to enhance uniformity and flow, followed by addition of magnesium stearate as a lubricant in the final mixing step.
The final homogeneous blend was encapsulated into hard gelatin capsule shells, size #0, using a semi-automatic capsule filling machine. Filled capsules were stored in amber-colored containers to protect from light-induced degradation.
Product Formulation Development:

Table# 01 Scale up of Tetracycline formulation Development:

Sr, #	RAW MATERIALS	Role of Ingredients
1	Tetracycline HCl	**API**
2	Colloidal silicon dioxide	Glidant
3	pregelatinized starch (corn)	Lubricant
4	stearic acid	Dissolution Enhancer
	Empty Gelatin Capsule shell#2 (Green/White)	To enclose medicine

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Post-formulation Evaluation:
The developed capsules underwent various physicochemical evaluations:
Weight Variation Test: Twenty capsules were randomly selected and weighed individually and collectively to check compliance with pharmacopeial specifications.
Disintegration Test: Conducted in purified water at 37 ± 0.5 °C to ensure that capsules disintegrated within the pharmacopeial time limit.
Dissolution Testing: Performed using USP Dissolution Apparatus I (basket method) with appropriate dissolution media. Samples were withdrawn at defined intervals and analyzed by HPLC or UV spectrophotometer.
Drug Content Uniformity: Capsules were assayed individually using validated HPLC methods to ensure uniform distribution of the drug.
Moisture Content: Determined using Karl Fischer titration to confirm stability and prevent degradation due to excessive water content.
Analytical Method Development and Validation:
Quantitative estimation of Tetracycline HCl in the formulated capsules was carried out by HPLC method, optimized with a C8 column and a suitable mobile phase prepared from sodium acetate buffer and organic modifiers. The method was validated in accordance with ICH guidelines for:
Linearity across a specified concentration range
Accuracy and Precision through recovery studies and repeatability testing
Specificity against excipients and potential degradation products
Robustness and Ruggedness by deliberate variations in method parameters
Complementary UV spectrophotometric analysis was employed for rapid estimation and cross-verification of results'

Results and Discussion:
Formulation Development:

Sr, #	RAW MATERIALS	Role of Ingredients	Percentage of Ingredients/cap.	Scale mg/capsule
1	Tetracycline HCl	**API**	93.45%	257.0*
2	Colloidal silicon dioxide	Glidant	2.90%	8.0**
3	pregelatinized starch (corn)	Lubricant	1.8%	5.000
4	stearic acid	Dissolution Enhancer	1.8%	5.000
	Empty Gelatin Capsule shell#2 (Green/White)	To enclose medicine	----	65
Total weight of Contents/ capsule				275mg
Total weight of capsule with shell				340mg

Quantity of API is subjected to potency adjustment to 100 % according to the API Contents.
Adjustment of difference in quantity of API after potency adjustment should be deducted from Colloidal silicon dioxide.
Step-1

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Figure-02 Tetracycline Raw Material (API)

Step-2

Figure-03 Tetracycline formulation development (at Final Mix/ Bulk stage)

Step-3

Figure-04 Tetracycline formulation development (at Intermediate/ After encapsulation)

Step-4

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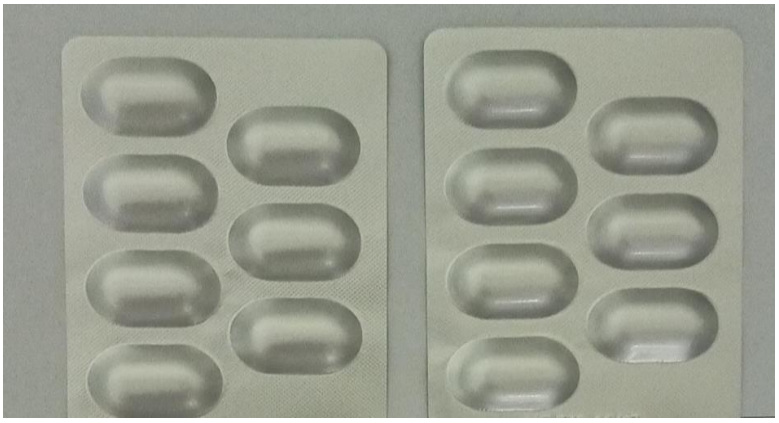

Figure-05 Tetracycline Finished Pharmaceutical Product (After Packaging) Packed in Alu-Alu Foil.

Analytical method Development (Contents By HPLC):

Preparation of Diluted: (10% NaOH):

In 10.0g of the Sodium hydroxide pellets in 90mL volumetric flasks and dilute up to the mark with water.

Mobile Phase:

In 80.0g of 2-methyl-2-propanol and 3.5g of Di-potassium hydrogen orthophosphate in 1000mL volumetric flask dilute with 250ml of water adjust the pH 8.0 with dilute phosphoric acid now add 2.0g tetrabutylammonium hydrogen phosphate dilute with 200ml of water adjust the pH 8.0 with diluted sodium hydroxide, and 0.4g of sodium Edetate dilute with 10ml of water adjust the pH 8.0 with diluted sodium hydroxide and make up the volume up to the mark with water.

Chromatographic Conditions:

Detector: UV 254 nm
Column: 4.6mm × 25cm; Packing (L-7/C8) (8µm)
Column Temperature: 40ºC ±1ºC
Flow Rate: 1.2mL/min
Injection Volume: 20 µL
Retention Time: About 5.6 Mint

Preparation of Sodium Meta-bisulfite: (4% w/v):

In 4.0g of the Sodium meta-bisulfite in 96mL volumetric flasks and dilute up to the mark with water

Preparation of 0.05M Hydrochloric Acid:

In 2.2mL Hydrochloric Acid (usually 37%) in 50mL water, now dilute up to 500mL with water and mix well.

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Volume 3, Issue 9 (2025)

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http://amresearchreview.com/index.php/Journal/about

Reference Solution:
Weigh accurately 325mg of Tetracycline Hydrochloride Reference **(B.P)** (Equivalent to 250mg of tetracycline approx.) in 100ml volumetric flask contains 5ml of water. Add 1ml solution of sodium metabisulfite and allow to standing in dark place at 20 to 25° C for 20 to 24hrs.without stirring.
Now add 50mL 0.05M hydrochloric acid to dissolve the precipitate and dilute up to 100ml with water.
In 5mL of solution in 100mL of volumetric flask and dilute up to the mark with water and filter the solution with 0.45µ nylon filter
(Proposed Dilution: 325mg/100mL x 5mL/100mL)

Sample Solution:
In the contents of NLT 20 Capsule of Tetracycline and weigh accurately 500mg (Equivalent to 250mg of tetracycline) in 100ml volumetric flask contains 5ml of water. Add 1ml solution of sodium metabisulfite and allow to standing in dark place at 20 to 25° C for 20 to 24hrs.without stirring.
Now add 50mL 0.05M hydrochloric acid to dissolve the precipitate and dilute up to 100ml with water.
In 5mL of solution in 100mL of volumetric flask and dilute up to the mark with water and filter the solution with 0.45µ nylon filter
(Proposed Dilution: 500 mg/100mL x 5mL/100mL)

Analysis:
Separately inject six replicate injections (provided 0.45µ syringe filter) of standard preparation and two replicate injections of sample preparation into the chromatograph and record the responses of the major peaks. Calculate the percentage of tetracycline by comparison of major peak responses of standard and sample solutions.
System suitability requirements are met if tailing factor of major peak of analyte in standard solution is NMT 2.0, Number of theoretical plates NLT 2000 and relative standard deviation for six replicate injections of standard preparation should not be more than 1.0%.

Calculation:

$$\%age\ (Tetracycline) = \frac{Avg.Area\ of\ Sample}{Avg.\ Area\ of\ Standard} \times \frac{Dil.of\ Standard}{Dil.of\ Sample} \times \frac{0.9241}{1} \times 100$$

Note: This Method (section 3.2) is also applicable for Tetracycline Raw Material (API)

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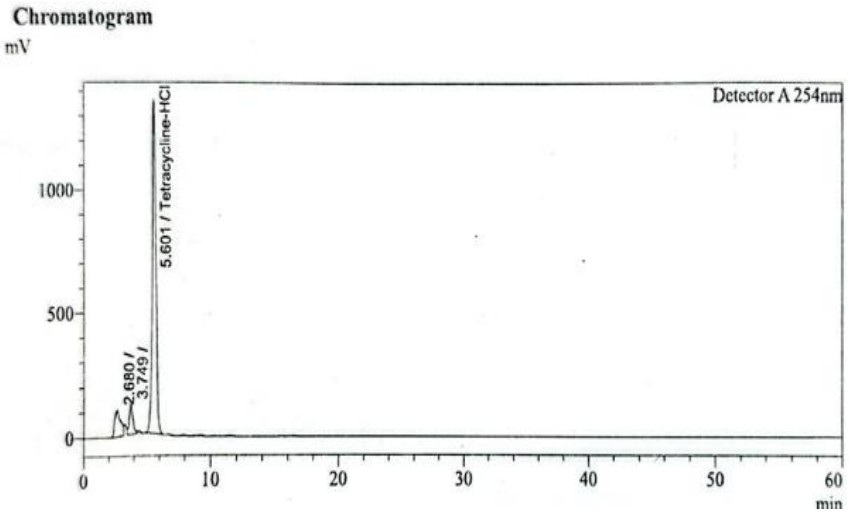

Figure-08 HPLC Report/ Chromatogram for Tetracycline HCl Reference

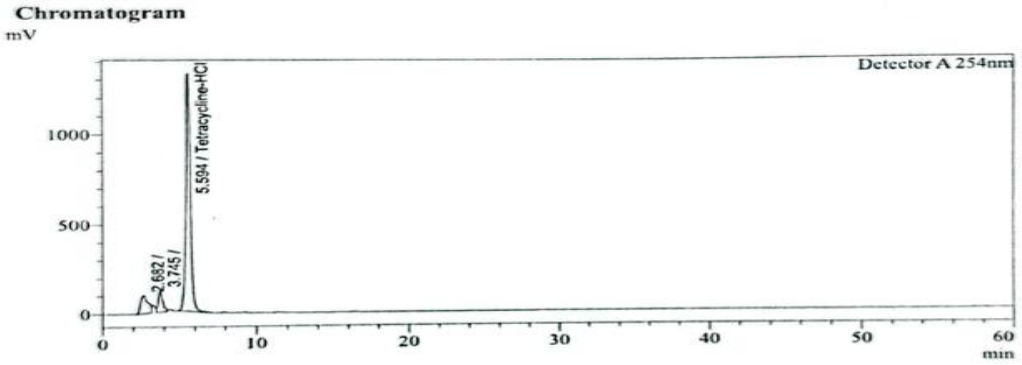

Figure-09 HPLC Report/ Chromatogram for Tetracycline Test Sample

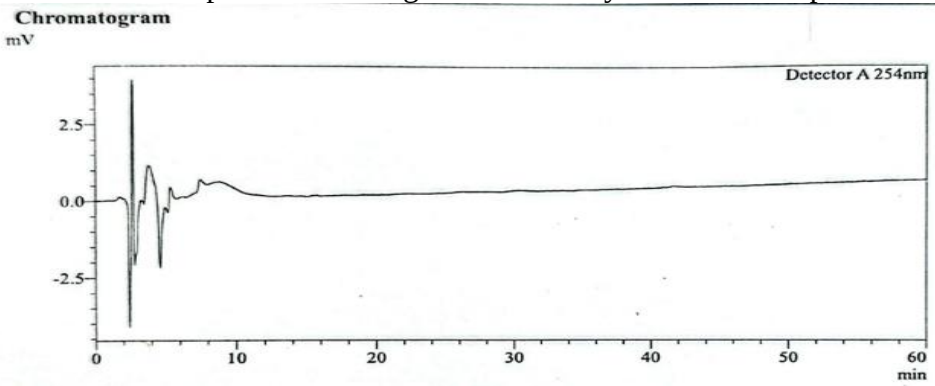

Figure-10 HPLC Report/ Chromatogram Blank Solution (Diluent)

The recovery of Tetracycline ranged from 95.0 to 105.0%; the RSD is of Tetracycline 0.8%. and tailing factor is less the 2.0 The precision as well as the reproducibility of this method was satisfactory.

Dissolution (By UV Spectrophotometer):

Preparation of Dissolution medium 0.1M Hydrochloric Acid:

In 51mL of Hydrochloric Acid (usually 37%) in 1000mL water, now dilute up to

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Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

6000mL with water and Mix well.

Parameters

- Apparatus : 2 (U.S.P-II)
- RPM : 75 RPM
- Medium : 900 mL (0.1M Hydrochloric Acid)
- Time : 60 Min
- Temperature : 37 + 0.5 ºC

Procedure:

Carry out the procedure protected from light. Add 900 mL of 0.1M Hydrochloric Acid in each of six vessels and equilibrate its temperature at 37 +0.5 ºC. Transfer one Capsule contains 250mg of tetracycline HCl equivalent to 250mg of Tetracycline in each of six vessels. Immediately run the apparatus for 60 minutes.

Sample Preparation:

After specified time withdraw approximately 20 mL of sample and filter it with Whatman filter paper.

In 5ml of sample in 100mL of volumetric flask with 50mL of water and 5mL of 5M sodium hydroxide and dilute up to the mark with water and measure the absorbance exactly after 6 mints adding the 5M sodium hydroxide (Proposed Dilution: 250mg / 900mL x 5mL / 100mL)

Standard Preparation:

Transfer accurately 0.027g of Tetracycline Hydrochloride working standard 100 mL volumetric flask, add 70 mL of 0.1M Hydrochloric Acid to dissolve active by stirring / sonication and, make volume up to the mark with the same, mix and filter it by using Whatman filter paper.

In 2ml of sample in 100mL of volumetric flask with 50mL of water and 5mL of 5M sodium hydroxide and dilute up to the mark with water and measure the absorbance at the maximum at λ380nm exactly after 6 mints adding the 5M sodium hydroxide (Proposed Dilution: 27mg / 100mL x 5mL / 100mL)

Analysis:

Calculate the percentage of Tetracycline in the medium from the absorbance obtained and declared the content of Tetracycline Hydrochloride.

Each mg of Tetracycline Hydrochloride is equivalent to 0.9241 mg of Tetracycline.

Calculation:

$$\%\text{age of Tetracycline} = \frac{\text{Absorbance of Sample}}{\text{Average Absorbance of Standard}} \times \frac{\text{Dil.of Standard}}{\text{Dilution of Sample x}} \times \frac{0.9241}{1} \times 100$$

Invitro Study (Comparative Dissolution Profile):

Recommendation / Requirement:

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Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

The dissolution measurements of the two products (e.g. test and comparator) to be made under the same test conditions
Use at least 12 units for determination of each profile using Apparatus-II (Paddle) at 100rpm.
A minimum of three time-points to be included, the time-points for both reference (comparator) and test product being the same
For Immediate Release Product;
Studies to be performed in media covering the physiological range, e.g. pH 1.2 (0.1M) hydrochloric acid Medium, pH 4.5 acetate buffer & pH 6.8 phosphate buffer.
Mean dissolution values to be used to estimate the similarity factor, f2
If both the test and reference (comparator) products show more than 85% dissolution in 15 minutes the profiles are considered similar (no calculations required).
For product to be considered similar, f2 values should be close to 100. Generally, greater than 50 (50-100) ensure sameness or equivalence of the two dissolution curves and, thus, of the performance of the test and reference products.

Table # 02 Preparations of Dissolution Medium:

▪ Acid Buffer (pH 1.2):	Prepare the 0.1N HCl by taking 102.0mL of HCl (37%) in 12000mL of purified water.
▪ Acetate Buffer (pH 4.5):	In 35.88g of sodium acetate and 19.2 mL acetic acid in 2000mL of purified water mix well and make volume up to 12000mL with purified water adjust the pH 4.5 with acetic acid.
▪ Phosphate Buffer (pH 6.8):	Dissolve 81.6g of monobasic potassium phosphate and 10.9g of sodium hydroxide in 12000 mL of water. Adjust with 6N sodium hydroxide or dilute phosphoric acid to a pH of 6.8.

Procedure:
Carry out the procedure protected from light. Add 900 mL of dissolution medium in each of six vessels and equilibrate its temperature at 37 +0.5 ºC. Transfer one Capsule in each of six vessels. Immediately run the apparatus for 60 minutes.

Sample Preparation:
After specified time withdraw approximately 20 mL of sample and filter it with Whatman filter paper.
In 5ml of sample in 100mL of volumetric flask with 50mL of water and 5mL of 5M sodium hydroxide and dilute up to the mark with water and measure the absorbance exactly after 6 mints adding the 5M sodium hydroxide (Proposed Dilution: 250mg / 900mL x 5mL / 100mL)

Standard Preparation:
Transfer accurately 0.090g of Tetracycline Hydrochloride working standard 100 mL volumetric flask, add 70 mL of dissolution medium to dissolve active by stirring /

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sonication and, make volume up to the mark with the same, mix and filter it by using Whatman filter paper.
In 2ml of sample in 100mL of volumetric flask with 50mL of water and 5mL of 5M sodium hydroxide and dilute up to the mark with water and measure the absorbance exactly after 6 mints adding the 5M sodium hydroxide (Proposed Dilution: 90mg / 100mL x 2mL / 100mL)

Analysis:
Calculate the percentage of Tetracycline in the medium from the absorbance obtained and declared the content of Tetracycline Hydrochloride.
Each mg of Tetracycline Hydrochloride is equivalent to 0.9241 mg of Tetracycline.
Then multiply the content of Tetracycline by 1.356 to obtain the content of Tetracycline.

Calculation:

$$\%age\ of\ Tetracycline = \frac{Absorbance\ of\ Sample}{Average\ Absorbance\ of\ Standard} \times \frac{Dil.of\ Standard\ x\ 0.9241}{Dilution\ of\ Sample} \times Potency\ of\ W.S$$

Mean ($\bar{X}$) = $\sum x$ (Sum of all the readings) / N (total number of readings)
% Co-efficient of Variation (CV or RSD) = Std. Dev. / Mean × 100
Difference Factor (f1) = $\{[\sum^{n}_{t=1} (R_t - T_t)] / [\sum^{n}_{t=1} R_t]\} \times 100$
Similarity Factor (f2) = $50 \times LOG \{[1+(1/n) \times \sum^{n}_{t=1} (R_t - T_t)^2]^{-0.5} \times 100\}$

Acceptance Criteria:
% Coefficient of Variation (CV) < 20% for time points up to 10 minutes and < 10% for other time points
Both Reference product & Test product shows more than 85% mean drug release within 15 minutes (no calculation required)
Difference Factor (f1) value ≤ 15
Similarity Factor (f2) value ≥ 50 (50 – 100)

Tablet # 03 Product Description for in-vitro study both reference and test product

Description	Reference / Comparator Product	Test Product
Product Name	Resteclin 250mg Capsule	Lume 250mg Capsule
Composition	Each hard capsule contains: Tetracycline Hydrochloride equivalent to 250mg tetracycline.	Each hard capsule contains: Tetracycline Hydrochloride equivalent to 250mg tetracycline.
Lot #	4010	T-001

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
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Figure-07. Reference Product used for in-Vitro Study/Bioequivalence

Comparative Dissolution Profile Results:

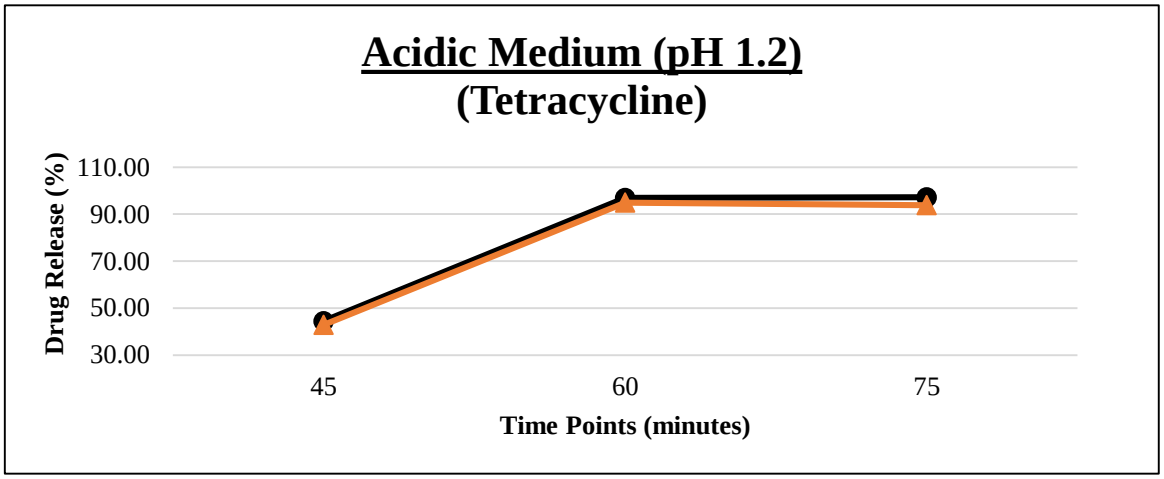
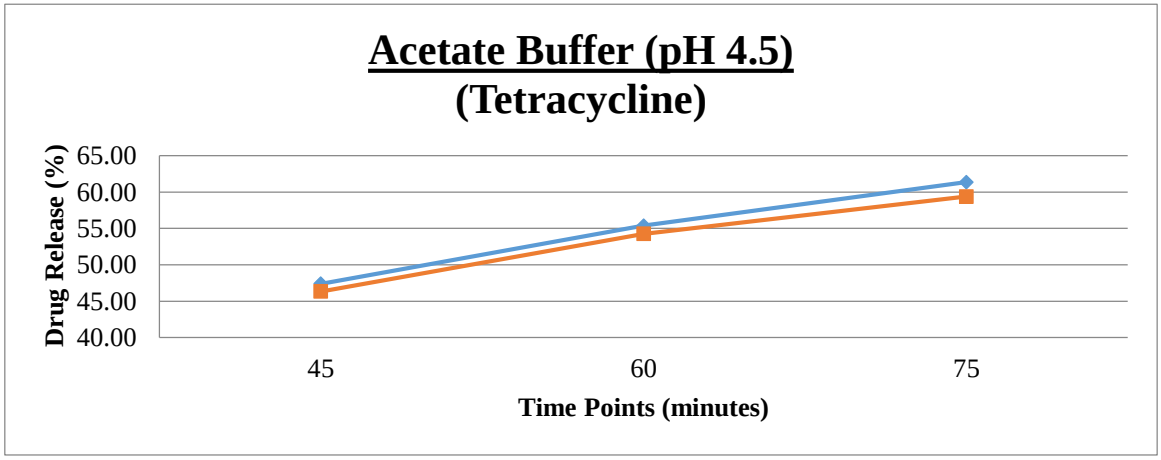


Figure-11 Graphically representation of CDP for reference and test product (pH 1.2)



Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
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http://amresearchreview.com/index.php/Journal/about

Figure-12 Graphically representation of CDP for reference and test product (pH 4.5)

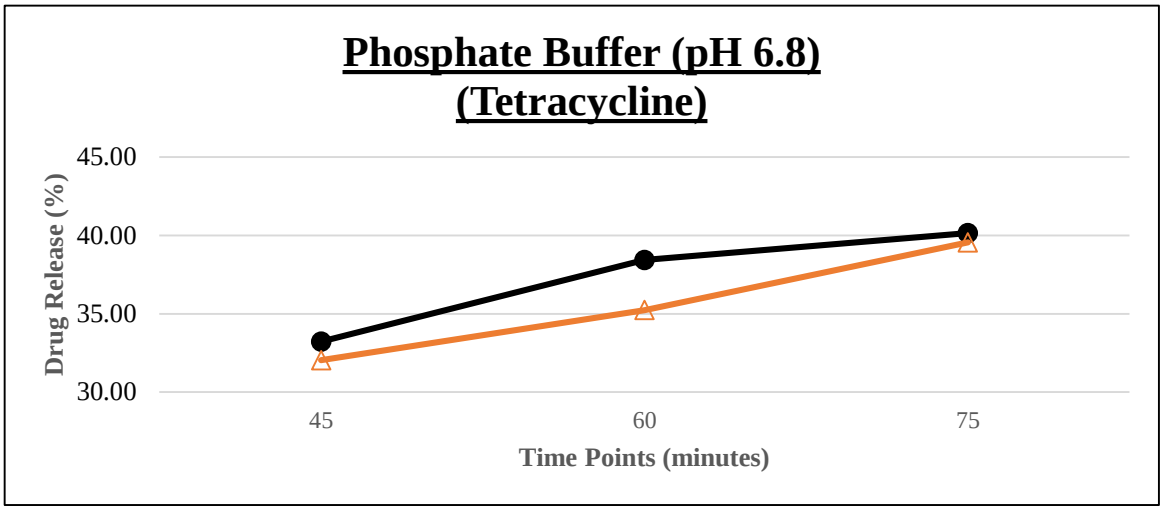

Figure-13 Graphically representation of CDP for reference and test product (pH 6.8)

A comparative dissolution study was conducted between the test product, Lume 250 mg Capsules, and the reference product, Resteclin 250 mg Capsules. The difference factor (f1) was calculated to be 2.87%, and the similarity factor (f2) was 79.27%. These values fall within the acceptable regulatory limits (f1 < 15 and f2 > 50), indicating a high degree of similarity between the dissolution profiles of the two products.
Both the test and reference formulations exhibited more than 75% drug release within 75 minutes in an acidic medium, which is a key requirement for immediate-release formulations. Additionally, the dissolution behavior was found to be comparable in acetate buffer (pH 4.5) and phosphate buffer (pH 6.8), further supporting the consistency of drug release across different physiological pH conditions.
These findings confirm that the test product meets the regulatory criteria for dissolution profile similarity, providing strong evidence of in vitro bioequivalence. As such, the data support the conclusion that Lume 250 mg Capsules perform similarly to Resteclin 250 mg Capsules, and can be considered pharmaceutically equivalent in terms of dissolution behavior.

Excipient compatibility study:
A Description of the Drug-Excipient Samples:
The following ingredients were prepared & tested for compatibility Study.

Table-04 Description of Tetracycline formulation under the compatibility study:

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
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Sr, #	RAW MATERIALS	Role of Ingredients	Percentage of Ingredients/cap.	Scale mg/capsule
1	Tetracycline HCl	**API**	93.45%	257.0*
2	Colloidal silicon dioxide	Glidant	2.90%	8.0**
3	pregelatinized starch (corn)	Lubricant	1.8%	5.000
4	stearic acid	Dissolution Enhancer	1.8%	5.000
	Empty Gelatin Capsule shell#2 (Pink/White)	To enclose medicine	----	65
Total weight of Contents/ capsule				275mg
Total weight of capsule with shell				340mg

Drug Substance-Excipient Compatibility Study:
Samples are prepared of drug substance and other ingredients as per Table#1 after one month at accelerated condition (temperature: 40°C±2°C, relative humidity 75%±5%). Drug substance compatibility is to be assessed through HPLC analysis of ingredients and drug substance in the solid state. Results of which are summarized in "Compatibility Study Report" in Table # 12.

Excipient Selection:
Selection of the excipient is based on the results of compatibility studies and/or formulation.
The selection of excipient grade and supplier is to be based on previous formulation experience and knowledge about excipients that have been used successfully in approved products.
The API is compatible with many of the common tablet excipients as seen from excipient compatibility results, following pharmacopeial excipients were chosen for product development respective of their functions:

Excipients Selection:
All excipients used in the formulation were selected based on compatibility studies and found to be compatible which is pharmacopeial and previously used in different oral dosage form.
Figures 7, 8, and 9 illustrate key aspects of the development of Lume 250 mg Capsules, which contain Tetracycline HCl (equivalent to 250 mg of Tetracycline base). The formulation was designed with a focus on achieving consistent drug performance, stability, and patient compliance. A well-structured formulation approach ensured optimal selection of excipients, hard capsule fill properties, and manufacturing parameters. This strategic development process aimed to produce a robust and reproducible dosage form that meets regulatory and quality standards.
The figures support the formulation design by showing data from validated analytical methods used to ensure the quality and uniformity of the final product. This ensures that every batch of Lume 250 mg Capsules consistently meets the required specifications throughout its shelf life. Together, the formulation strategy and analytical validation demonstrate a scientifically sound development process, contributing to the

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http://amresearchreview.com/index.php/Journal/about
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http://amresearchreview.com/index.php/Journal/about

product's overall safety, efficacy, and quality. These figures collectively highlight the technical and regulatory diligence involved in bringing Lume 250 mg Capsules to a stable and market-ready pharmaceutical product.

Table-11 Excipients compatibility study results After one month storage at accelerated conditions:

Excipient Compatibility Study After One Month (Temperature: 40°C±2°C, relative humidity 75%±5%).									
Sr. #	**Composition**	**Theoretical Contents**	**Weight (mg)**		**Concentration (mg/mL)**		**Area**		**%Age Of Contents**
			STD.	SPL.	STD.	SPL.	Ave. STD.	SPL.	
1.	Standard API	100%	250	250	0.25	0.25	2182577.6	2187253	100.03
2.	Standard API + Aerosil 200	100%	250	250 + 8.0	0.25	0.25	2182577.6	2158951	98.74
3.	Standard API+ Starch	100%	250	250 + 3.0	0.25	0.25	2182577.6	2150625	98.36
4.	Standard API + Setric Acid	100%	250	250 + 3.0	0.25	0.25	2182577.6	2165903	99.05
5.	Standard API + Capsule Shell	100%	250	250 + 65	0.25	0.25	2182577.6	2165903	99.05

The chosen excipients in the solid oral formulation (Tetracycline HCl, equivalent to 250 mg Tetracycline Base) were found to be compatible, with no adverse interactions observed under one month of accelerated conditions (40°C ± 2°C / 75% RH ± 5%).

Analytical Method Validation Report:

The analytical method for Lume 250 mg Capsules was verified in accordance with ICH Guideline Q2 (R1), November 2005, confirming its suitability for routine quality control. System suitability parameters—including %RSD (0.14%), tailing factor (1.63), and theoretical plates (5620)—met all acceptance criteria, demonstrating system precision and chromatographic efficiency The method showed excellent specificity, with no interference observed from placebo or blank preparations at the retention time of the active ingredient in both standard and sample solutions. Accuracy was confirmed through recovery studies, with results ranging from 99.70% to 101.25%, well within acceptable limits. Robustness testing showed that the method remained stable over 4 hours, even under slight pH variations, with consistent chromatographic results. The method also demonstrated high precision, with repeatability and intermediate precision values of 0.25% and 0.44% RSD, respectively. Additionally, the method satisfied

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criteria for linearity and range, further supporting its reliability. Overall, the method was proven to be accurate, precise, specific, robust, and suitable for its intended analytical purpose.

Stability Study (Accelerated Conditions):
A formulation stability study of Lume 250 mg Capsules (Filled in ALU-ALU Foil having 7 hard capsule/ Blister) conducted under accelerated conditions (40°C ± 2°C / 75% RH ± 5%) up to six month yielded satisfactory results, indicating physical and chemical studies.
The chosen excipients in the formulation (Tetracycline HCL, equivalent to 250mg Tetracycline Base) were found to be compatible, with no adverse interactions observed under one month of accelerated conditions (40°C ± 2°C / 75% RH ± 5%).

Discussion:
The development of Lume 250mg Capsules, containing Tetracycline HCl (equivalent to 250 mg Tetracycline base), demonstrated robust formulation design supported by Comprehensive analytical and stability data. All selected excipients were pharmacopeial and proven compatible through pre-formulation and accelerated compatibility studies, ensuring stability and safety of the product. Comparative dissolution testing revealed high similarity to the reference product, Resteclin 250 mg, with an f1 value of 2.87% and f2 value of 79.27%, confirming dissolution equivalence across various media and supporting in vitro bioequivalence Analytical method verification, performed in accordance with ICH Q2(R1), confirmed system suitability, specificity, accuracy (recovery 99.70%–101.25%), precision (repeatability and intermediate %RSD < 0.5%), robustness, and linearity—ensuring the method's reliability for routine quality control. Stability studies under ICH-recommended accelerated conditions (40°C ± 2°C / 75% RH ± 5%) over six months demonstrated that the formulation remained physically and chemically stable, with no significant changes or interactions observed, especially when packaged in ALU-ALU blister packs. Collectively, these results validate the formulation's quality, safety, and regulatory compliance, positioning it for successful market approval.

Conclusion/Summary:
The Lume 250 mg Capsule exhibits in vitro bioequivalence study as per WHO Annex-3 section 2.2.4 Bioequivalence and dissolution studies; with the reference product (Resteclin 250 mg) as demonstrated by f1/f2 values and dissolution profiles across multiple pH conditions. The formulation is physically and chemically stable, with all excipients confirmed compatible under accelerated stability testing. The analytical method used for assay and quality control of the product is validated and verified, meeting all predefined acceptance criteria for specificity, accuracy, precision, robustness, linearity, range and system suitability. Therefore, the product is considered pharmaceutically equivalent and analytically reliable, after six months of stability according ICH guideline Q1A(R2) studies meet the chemically and physically specifications.

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References:

Shanmuganathan, D., et al., Teratogenicity of tetracyclines. World J Pharm Res, 2025. **14**(6): p. 737-749.

Chopra, I. and M. Roberts, Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews, 2001. **65**(2): p. 232-260.

Nelson, M.L. and S.B. Levy, The history of the tetracyclines. Annals of the New York Academy of Sciences, 2011. **1241**(1): p. 17-32.

Demain, A.L., Antibiotics: natural products essential to human health. Medicinal research reviews, 2009. **29**(6): p. 821-842.

Wiseman, L.R., et al., Meropenem: a review of its antibacterial activity, pharmacokinetic properties and clinical efficacy. Drugs, 1995. **50**(1): p. 73-101.

Leyden, J.J., Therapy for acne vulgaris. New England Journal of Medicine, 1997. **336**(16): p. 1156-1162.

Stachelek, M., et al., Overcoming bacterial resistance to antibiotics: The urgent need–a review. Annals of Animal Science, 2021. **21**(1): p. 63-87.

Fukuda, Y., New approaches to overcoming bacterial resistance. Drugs Future, 2009. **34**(2): p. 127-136.

Galluccio, G., et al., Advances in the pathogenesis and treatment of rosacea: a phenotype-based therapeutic approach. Cosmetics, 2024. **11**(1): p. 11.

Thiboutot, D., et al., New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. Journal of the American Academy of Dermatology, 2009. **60**(5): p. S1-S50.

Agwuh, K.N. and A. MacGowan, Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. Journal of Antimicrobial Chemotherapy, 2006. **58**(2): p. 256-265.

Chukwudi, C.U., rRNA binding sites and the molecular mechanism of action of the tetracyclines. Antimicrobial agents and chemotherapy, 2016. **60**(8): p. 4433-4441.

Schreiner, A. and A. Digrams, Pharmacokinetics of Tetracycline and doxycycline in serum and suction blister fluid. Chemotherapy, 1985. **31**(4): p. 261-265.

Abuelella, K.E., et al., PoLumer-Based Biomaterials for Wound Healing: Advances in Natural, Synthetic and Hybrid Biodegradable PoLumers for Scar Reduction and Skin Regeneration. Bulletin of Pharmaceutical Sciences Assiut University, 2025. **48**(1): p. 135-174.

Christiansen, C.S., et al., Acne management in Norway: general practitioner and dermatologist prescriptions (2012-2019), a nationwide overview. BJGP open, 2025.

Merk, K., In-vivo-Porphyrinproduktion von Propionibacterium acnes unter systemischer Aknetherapie mit Isotretinoin und Minozyklin. 2004, lmu.

Leyden, J.J., A review of the use of combination therapies for the treatment of acne vulgaris. Journal of the American Academy of Dermatology, 2003. **49**(3): p. S200-S210.

Zouboulis, C.C., et al., European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. Journal of the European Academy of Dermatology

Online ISSN 3007-3197
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and Venereology, 2015. **29**(4): p. 619-644.

Marasca, C., et al., The pharmacology of antibiotic therapy in hidradenitis suppurativa. Expert review of clinical Pharmacology, 2020. **13**(5): p. 521-530.

Drucker, A.M., et al., Association between antihypertensive medications and risk of skin cancer in people older than 65 years: a population-based study. Cmaj, 2021. **193**(15): p. E508-E516.

Ak, M., A comprehensive review of acne vulgaris. J. Clin. Pharm, 2019. **1**(1): p. 17-45.

Lavande, J.P., S.K. Bais, and M. Patil, A COMPREHENSIVE REVIEW ON ACNE VULGARIS.

Kim, Y.-S. and H.S. Kim, Tetracyclines revisited: tetracyclines in the field of dermatology. Dermatology, 2024. **240**(5-6): p. 844-858.