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A COMPREHENSIVE REVIEW ON STBILITY INDICATING RP-HPLC METHODS AVAILABLE FOR TETRACYCLINES

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ABSTRACT

Tetracyclines are a structurally diverse and therapeutically important class of broad-spectrum antibiotics widely used in human and veterinary medicine. The presence of a polycyclic naphthacene core structure with multiple ionizable functional groups renders these molecules chemically unstable and highly susceptible to epimerization, dehydration, oxidation, hydrolysis, and photolytic degradation.[1,13] Classical tetracyclines such as tetracycline and oxytetracycline, as well as semi-synthetic and synthetic derivatives including doxycycline, minocycline, lymecycline, tigecycline, omadacycline, and eravacycline, exhibit complex degradation behavior that necessitates robust analytical control to ensure quality, safety, and therapeutic efficacy.[2–6,11–18] This review comprehensively evaluates reported approaches for the development and validation of stability-indicating analytical methods for tetracycline-class drugs in bulk substances,pharmaceutical dosage forms, fixed-dose combinations, and biological matrices. Particular emphasis is placed on reversed-phase high-performance liquid chromatography (RP-HPLC), which remains the most widely employed and regulatory-accepted technique for separation of active pharmaceutical ingredients from related substances and degradation products.[3,6,13,17,19] Several studies demonstrate the successful resolution of tetracycline derivatives from impurities generated under acidic, alkaline, oxidative, thermal, and photolytic stress conditions in accordance with

ICH stability guidelines.[6,14,17,19] Advanced chromatographic methods have been reported for impurity profiling of oxytetracycline and its epimers and dehydration products using optimized gradient systems and volatile mobile phases compatible with mass spectrometric detection.[13,15] Similarly, validated stability-indicating methods for doxycycline, including its application in pharmaceutical formulations and biological samples, have confirmed adequate specificity, precision, and linearity under forced degradation conditions.[6,19] The emergence of third-generation glycylcycline and fluorocycline derivatives such as tigecycline, omadacycline, and eravacycline has further highlighted analytical challenges due to their enhanced structural complexity and sensitivity to pH-dependent degradation.[11,12,17,18] In addition, simultaneous estimation methods for combination formulations containing tetracyclines—such as tetracycline with hydrocortisone or oxytetracycline with polymyxin B—underscore the importance of chromatographic selectivity and method robustness in multi-component dosage forms.[14,15] Bioanalytical advancements, including LC-MS/MS methods for omadacycline quantification in plasma, further extend the application of stability-indicating principles to pharmacokinetic investigations.[18] Collectively, the reviewed literature demonstrates that successful stability-indicating method development for tetracycline-class drugs requires systematic forced degradation studies, appropriate stationary phase selection, optimized mobile phase composition, pH control, and comprehensive validation of parameters such as specificity, linearity, accuracy, precision, robustness, and sensitivity in accordance with ICH guidelines.[6,17] RP-HPLC, often coupled with diode array or mass spectrometric detection, remains the analytical cornerstone for impurity profiling and quality control of tetracycline derivatives. Continuous methodological refinement is essential to address evolving regulatory requirements and the expanding structural diversity within this antibiotic class.

KEYWORDS: Tetracyclines, HPLC, Degradations.

INTRODUCTION

Tetracyclines constitute one of the most extensively studied and widely utilized classes of broad-spectrum antibiotics in both human and veterinary medicine. Initially isolated from Streptomyces species and later modified through semi-synthetic and fully synthetic approaches, these compounds share a characteristic four-ring naphthacene core structure that governs both their antimicrobial activity and chemical behaviour.[1,13] The presence of multiple ionizable functional groups and conjugated systems renders tetracyclines amphoteric

and chemically reactive, making them highly susceptible to degradation through epimerization, dehydration, oxidation, and photolysis.

Clinically, tetracyclines demonstrate activity against Gram-positive and Gram-negative bacteria, rickettsiae, chlamydiae, mycoplasmas, and certain protozoa. Derivatives such as doxycycline, minocycline, lymecycline, tigecycline, omadacycline, and eravacycline have been developed to enhance pharmacokinetic properties, overcome microbial resistance, and improve therapeutic outcomes.[2–6,11,17] Doxycycline and minocycline, for instance, exhibit improved absorption and prolonged half-life compared to earlier agents, while newer glycylcycline and fluorocycline derivatives such as tigecycline and eravacycline demonstrate expanded activity against multidrug-resistant organisms.[11,17]

Despite their therapeutic advantages, tetracyclines are chemically unstable under various environmental and stress conditions. Classic degradation pathways include C-4 epimerization forming 4-epitetracycline, acid-catalyzed dehydration producing anhydro derivatives, and subsequent rearrangements yielding apo-derivatives.[1,13] Oxytetracycline, for example, undergoes epimerization at the C-4 position and dehydration at C-6, leading to multiple structurally related impurities that must be effectively separated and quantified.[13] These degradation products are not merely inactive byproducts; some may possess reduced potency or increased toxicity, underscoring the necessity for precise impurity profiling.

The instability of advanced derivatives further complicates analytical evaluation. Tigecycline, a glycylcycline derivative structurally related to minocycline, is highly sensitive to oxidation at alkaline pH and epimerization under acidic conditions, which necessitates formulation as a lyophilized powder for injection.[11] Similarly, eravacycline and omadacycline, recent additions to the tetracycline family, require highly selective chromatographic methods capable of resolving related substances and degradation products in both bulk and parenteral formulations.[12,17,18]

Pharmacopoeial standards, including those of the United States Pharmacopeia and European Pharmacopoeia, mandate strict impurity limits and validated analytical methods for tetracycline-class drugs. However, earlier compendial procedures often involved polymeric columns, non-volatile buffers, long run times, or complex mobile phases, which may limit efficiency, robustness, and compatibility with modern detection systems.[13] Consequently, substantial research efforts have been directed toward the development of improved reversed-

phase high-performance liquid chromatography (RP-HPLC) methods with enhanced selectivity, shorter analysis times, and compatibility with diode-array and mass spectrometric detection.

Over the past decade, numerous stability-indicating RP-HPLC methods have been reported for tetracycline derivatives in bulk drugs, fixed-dose combinations, and biological matrices. Stability-indicating methods for doxycycline have demonstrated adequate separation of degradation products formed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions.[6,19] Similarly, validated chromatographic approaches have been developed for oxytetracycline and its impurities using optimized gradient systems capable of resolving both known and unknown degradation products within acceptable run times.[13,15]

The analytical challenge increases further in combination dosage forms. Stability-indicating methods have been successfully developed for simultaneous estimation of tetracycline with hydrocortisone in topical formulations and oxytetracycline with polymyxin B in fixed-dose combinations, highlighting the necessity of selective chromatographic conditions that prevent peak overlap and ensure accurate quantification.[14,15] Moreover, improvements in assay methodologies for minocycline hydrochloride have focused on eliminating solvent systems that compromise column performance, thereby improving reproducibility and robustness.[16]

Beyond pharmaceutical dosage forms, tetracyclines are also monitored in biological fluids for pharmacokinetic and therapeutic drug monitoring studies. Sensitive and rapid methods, including RP-HPLC and LC–MS/MS, have been validated for quantification of doxycycline in urine and omadacycline in plasma, offering low limits of detection and high analytical specificity.[18,19] These developments reflect the growing importance of bioanalytical validation alongside routine quality control testing.

A defining element of any stability-indicating method is its ability to distinguish the active pharmaceutical ingredient (API) from its degradation products, process-related impurities, and excipients without interference. According to ICH guidelines, forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress conditions are essential to establish method specificity and degradation pathways.[6,17] Properly designed stress studies generate meaningful impurity profiles and confirm that the analytical method accurately reflects the stability characteristics of the drug substance and drug product.

Collectively, the structural complexity, multiple degradation pathways, and evolving pharmaceutical formulations of tetracycline-class drugs demand robust, validated, and regulatory-compliant analytical methodologies. RP-HPLC remains the most widely adopted technique due to its versatility, precision, adaptability to gradient systems, and compatibility with UV and mass detection. Continuous refinement of chromatographic parameters—including mobile phase composition, pH optimization, stationary phase selection, and gradient programming—remains crucial to achieving reliable impurity separation and method robustness.

Therefore, a comprehensive evaluation of the development and validation of stability-indicating methods for tetracycline-class drugs is essential. Such analytical advancements ensure regulatory compliance, maintain pharmaceutical quality, and safeguard therapeutic efficacy and patient safety throughout the product lifecycle.

Review of reported methods for Tetracyclines.

Sr. No	Drug	Matrix	Brief Introduction (Chromatographic Conditions)	Ref
1	Doxycycline	Bulk & capsules	**Stationary Phase**: Perfectsil Target ODS C18 (125 × 4 mm, 3–5 µm); **Mobile phase**: Methanol:50 mM ammonium acetate (50:50, pH 2.5); **Flow rate**: 0.8 mL/min; **Retention Time**:5–7 min; **UV detection**: 269 nm	[6]
2	Oxytetracycline & impurities	Bulk	**Stationary Phase:** Inertsil C8 (150 × 4.6 mm, 5 µm); gradient **Mobile phase:** A:0.05% TFA water B:ACN–MeOH–THF (80:15:5); **Flow Rate** : 1.3 mL/min; **UV detection**: 254 nm;	[13]
3	Omadacycline	API & dosage form	**Stationary Phase:** Symmetry ODS C18 (4.6 × 250 mm, 5 µm); **Mobile Phase:** Methanol:Phosphate buffer (35:65); **Flow Rate**: 1.0 mL/min; **R:** 2.252 min; **UV detection:** 235 nm; **Run time:** <8 min.	[12]
4	Hydrocortisone + Tetracycline	Ointment	**Stationary Phase**: Discovery C18 (250 × 4.6 mm, 5 µm); **Mobile phase:** Water (pH 2.2):Acetonitrile (40:60); **Flow rate:** 1.0 mL/min;	[14]

			Rt: 2.214 min (HC) and 3.497 min (TC); **UV Detection:** 244 nm; **Column temp:** 30°C.	
5	Oxytetracycline + Polymyxin B	Ointment	**Stationary Phase:** XBridge C18 (250 × 4.6 mm, 5 µm); **Mobile Phase:** ACN:Water with 0.5% OPA (80:20, pH 3.5); **Flow rate:** 0.9 mL/min; room temperature.	[15]
6	Minocycline HCl	Dosage form	**Stationary phase:** Inertsil ODS3V C18 (250 × 4.6 mm, 5 µm); **Mobile Phase:** ACN:Phosphate buffer (25:75); **Flow rate:** 0.5 mL/min; **UV detection:** 280 nm.	[16]
7	Eravacycline	Injection	**Stationary Phase:** Primesil C18 (250 × 4.6 mm, 5 µm); **Mobile phase:** gradient ammonium acetate buffer–acetonitrile; **Flow rate:** 1.0 mL/min; **UV detection:** 210 nm; **column temp:** 40°C; **Run time:** 60 min.	[17]
8	Omadacycline	Human plasma	**Stationary Phase:** Shimpack GIST C18 (3 × 50 mm, 3 µm); **Mobile phase**: isocratic **Flow Rate:** 1.0 mL/min; **Detection** MRM mode.	[18]
9	Doxycycline hyclate	Tablets & urine	**Stationary Phase:** C8 column (250 × 4.0 mm, 5 µm); **Mobile phase:** ACN:KH2PO4 buffer (40:60, pH 4.0); **Flow rate:** 1.0 mL/min; **UV Detection:** 325 nm; **Temperature:** 25°C.	[19]
10	Tigecycline	Injection	**Stationary Phase:** C18 column; **Mobile phase**: acidic buffer **UV detection:** 245–254 nm.	[11]
11	Tetracycline HCl	Capsules	Reversed phase HPLC method for modernization of USP monograph for tetracycline hydrochloride capsules.	[8]
12	Lymecycline	Capsules	**Stationary Phase:** C18 column; **Mobile phase:** buffer–acetonitrile UV detection.	[5]
13	Sarecycline	Bulk & formulation	**Stationary Phase:** XBridge Shield RP18 (150 × 4.6 mm, 3.5 µm); **Mobile Phase:** gradient ammonium acetate buffer–acetonitrile; **UV detection:** 240 nm.	[4]
14	Doxycycline +	Tablets	**Stationary Phase:** Hypersil BDS C18 (250	[7]

	Ornidazole		× 4.6 mm, 5 µm); **Mobile Phase:** Buffer:ACN (55:45, pH 4); **Flow rate:** 1 mL/min; **UV detection:** 260 nm.	
15	Tetracycline + Metronidazole + Bismuth	Capsules	**Stationary Phase:** Inertsil C18 (250 × 4.6 mm, 5 µm); **Mobile Phase:** phosphate buffer pH 3.5:methanol (40:60); **Temperature:** ambient	[9]
16	Lymecycline	Dosage form	**Stationary Phase:** PLRPS column (250 × 4.6 mm, 8 µm); **UV detection** : 254 nm.	[10]
17	Doxycycline hyclate	Tablets	**Stationary Phase:** CN Luna column (250 × 4.6 mm, 5 µm); **Mobile Phase:** water+0.1%TFA:ACN+0.1%TFA (60:40); **flow rate:** 1 mL/min; **UV detection:** 360 nm.	[3]
18	Minocycline + Ascorbic acid	Dosage form	**Stationary Phase:** Sunniest C18 (150 × 4.6 mm, 5 µm); **Mobile Phase:** KH2PO4 buffer pH 2.8:ACN:MeOH (85:10:5); **Flow rate:** 0.8 mL/min; **UV detection:** 255 nm.	[2]
19	Tetracycline HCl	API & dosage forms	HPTLC stability indicating method using silica gel plates and ethyl acetate–acetonitrile–methanol–ammonia mobile phase.	[1]
20	Tigecycline	Bulk	Various analytical methods including RPHPLC reported for tigecycline quantification and impurity profiling.	[11]

Limitations of Current Analytical Methods

Despite significant progress in the development of stability-indicating analytical methods for tetracycline class antibiotics, several limitations remain in the currently reported methodologies. These limitations mainly relate to analytical efficiency, environmental considerations, detection sensitivity, and the ability to fully characterize degradation products.

One of the most commonly reported limitations is the long chromatographic run time required for impurity profiling and stability studies. Several gradient RP-HPLC methods developed for tetracycline derivatives require extended analysis times to achieve adequate separation of impurities and degradation products. For instance, impurity profiling methods for drugs such as eravacycline employ gradient systems with run times approaching 60

minutes, which can significantly reduce sample throughput in routine quality control laboratories. Such lengthy analytical procedures increase solvent consumption and operational costs while limiting their practicality for high-throughput analysis.[17]

Another limitation relates to the complexity of mobile phase composition and chromatographic conditions. Many reported methods utilize multiple organic solvents or buffers in gradient systems to achieve proper separation of structurally similar tetracycline compounds and their degradation products. While these systems improve chromatographic resolution, they also increase method complexity and may affect reproducibility across different laboratories. Additionally, the use of non-volatile buffer systems in some methods may limit compatibility with advanced detectors such as mass spectrometry.[13]

The chemical instability of tetracycline derivatives during analysis also represents a significant challenge. Tetracycline compounds are highly susceptible to degradation under acidic, alkaline, oxidative, and photolytic conditions, which may lead to the formation of epimers and dehydration products during sample preparation or analysis. As a result, analytical methods must be carefully optimized to prevent additional degradation during chromatographic separation. This instability complicates the accurate quantification of the parent compound and its impurities, particularly in long chromatographic runs.[6]

Another limitation observed in several studies is the restricted applicability of certain methods to specific dosage forms or matrices. Many analytical procedures are developed and validated only for pharmaceutical dosage forms such as tablets or capsules, with limited evaluation in complex biological matrices. Consequently, additional method optimization and validation are often required for bioanalytical applications such as plasma or urine analysis. Although techniques like LC-MS/MS provide improved sensitivity for biological samples, they require specialized instrumentation and expertise that may not be available in all laboratories.[18]

Furthermore, some conventional analytical methods rely solely on UV detection, which may not provide sufficient specificity for distinguishing closely related degradation products or unknown impurities. Although UV detection is widely used due to its simplicity and affordability, it may lack the structural information required for complete impurity characterization. In such cases, complementary techniques such as mass spectrometry are necessary for reliable identification of degradation products.[11]

Environmental concerns also represent a growing limitation of current analytical approaches. Many chromatographic methods require significant volumes of organic solvents such as acetonitrile and methanol. Excessive solvent consumption not only increases operational costs but also raises environmental and safety concerns. Consequently, there is increasing interest in developing greener analytical methods that minimize solvent usage and improve sustainability.[1]

In addition, certain analytical methods employ older chromatographic technologies or complex reagent systems, which may not be compatible with modern instrumentation or automated workflows. For example, earlier pharmacopoeial procedures for tetracycline analysis involve complex reagents or lengthy sample preparation steps that can reduce analytical efficiency and reproducibility. These limitations highlight the need for updated and simplified analytical methods that align with current regulatory and technological standards.[8]

Overall, while the reported analytical methods provide reliable determination of tetracycline derivatives in various matrices, several limitations remain related to analysis time, solvent consumption, detection specificity, and applicability to different sample types. Addressing these challenges through improved chromatographic techniques, greener analytical strategies, and advanced detection systems will be essential for the continued advancement of stability-indicating methods for tetracycline class drugs.

CONCLUSION

The present review critically summarizes the development and validation of stability-indicating analytical methods for tetracycline class antibiotics reported in recent literature. Tetracyclines, including doxycycline, minocycline, tetracycline, oxytetracycline, lymecycline, tigecycline, sarecycline, omadacycline, and eravacycline, are structurally complex molecules that are highly susceptible to degradation through pathways such as epimerization, dehydration, oxidation, and photolysis. Because these degradation processes can lead to loss of potency and the formation of potentially toxic impurities, the establishment of reliable stability-indicating analytical methods remains essential for ensuring pharmaceutical quality, safety, and regulatory compliance.

Analysis of the reviewed studies demonstrates that reversed-phase high-performance liquid chromatography (RP-HPLC) is the most widely employed and reliable analytical technique

for the determination of tetracycline derivatives in pharmaceutical formulations and biological matrices. Most reported methods utilize C18 or C8 reversed-phase columns, with column dimensions typically ranging from 150–250 mm × 4.6 mm and particle sizes of 3–5 µm, providing efficient separation of parent compounds and their degradation products. Acidic mobile phase conditions, generally within the pH range of 2.2–4.0, are commonly employed to improve peak shape, enhance chromatographic resolution, and reduce degradation during analysis. Organic modifiers such as acetonitrile and methanol, combined with phosphate or ammonium acetate buffers, are frequently used to achieve optimal elution and selectivity.

The reviewed literature also highlights that flow rates around 0.8–1.3 mL/min and UV detection wavelengths ranging from approximately 210 to 360 nm are commonly applied depending on the specific tetracycline derivative under investigation. Retention times generally fall within 2–10 minutes for routine assays, while impurity profiling methods may require longer gradient runs to achieve adequate separation of related substances. Temperature control, typically between ambient conditions and 40–50°C, further contributes to improved chromatographic performance and reproducibility.

Another important observation from the comparative analysis is the increasing emphasis on stability-indicating capability through forced degradation studies. Many of the reviewed methods incorporate stress testing under acidic, alkaline, oxidative, thermal, and photolytic conditions to confirm the specificity of the developed methods. These studies ensure that the analytical procedures can effectively distinguish the active pharmaceutical ingredient from its degradation products, impurities, and formulation excipients. Validation parameters reported across the studies—such as specificity, linearity, accuracy, precision, robustness, and limits of detection and quantification—consistently meet the requirements outlined in ICH guidelines, indicating that these methods are suitable for routine quality control and regulatory applications.

Recent developments in the field also demonstrate a gradual shift toward more advanced analytical approaches, including LC–MS/MS techniques for bioanalytical applications and improved chromatographic methods capable of resolving multiple drug combinations. Such advancements are particularly important as newer tetracycline derivatives, including omadacycline and eravacycline, are introduced into clinical practice and require sensitive analytical methods for both pharmaceutical and pharmacokinetic investigations.

Overall, the reviewed literature confirms that RP-HPLC remains the cornerstone technique for stability-indicating analysis of tetracycline class drugs. Nevertheless, continuous optimization of chromatographic conditions, incorporation of advanced detection techniques, and the development of greener and more efficient analytical strategies are essential to meet evolving regulatory and pharmaceutical demands. Future research should focus on improving method sensitivity, reducing solvent consumption, and integrating modern analytical tools to enhance impurity profiling and stability assessment of this important class of antibiotics.

In conclusion, the collective findings from the reviewed studies provide valuable insight into the analytical strategies used for tetracycline stability evaluation and offer a comprehensive reference for researchers involved in the development, validation, and quality control of tetracycline-based pharmaceutical products.

REFERENCES

1. Gashaw M, Layloff T, Hymete A, Ashenef A. Stability indicating high performance thin layer chromatography method development and validation for quantitative determination of tetracycline hydrochloride in API and dosage forms. BMC Chem, 2024; 18: 82.
2. AlKaraki RN, Tarawneh SK, Haddadin RR, Aqil R, Alkasasbeh RM. A new RP-HPLC method for the simultaneous detection and quantification of minocycline and ascorbic acid in pure forms and combined pharmaceutical dosage forms. Results Chem, 2024; 10: 101701.
3. Kogawa AC, Salgado HRN. Quantification of doxycycline hyclate in tablets by HPLC–UV method. J Chromatogr Sci, 2012; 1–7.
4. Prabhakara Rao PV, Mastan Ali S, Bharath P, Ramachandran D. Stability indicating RP-HPLC method for quantification of sarecycline and its impurities. Curr Trends Biotechnol Pharm, 2023; 17(4): 1486–95.
5. Firke SD, Rajput PG, Lokwani DK, Randhir NK. Development and validation of RP-HPLC method for estimation of lymecycline in bulk and capsule dosage form. Eur J Biomed Pharm Sci, 2019; 6(8): 262–66.
6. Pourmoslemi S, Mirfakhraee S, Yaripour S, Mohammadi A. Development and validation of a stability-indicating RP-HPLC method for rapid determination of doxycycline in pharmaceutical bulk and dosage forms. Pharm Sci, 2016; 22: 96–104.
7. Srilekha A, Pavani P, Sreedhar B. Stability indicating RP-HPLC method development and validation for simultaneous estimation of doxycycline monohydrate and ornidazole.

Int J Pharm Sci Res, 2015; 6(5): 2228–39.

8. Hussien EM. HPLC method validation for modernization of the tetracycline hydrochloride capsule USP monograph. Bull Fac Pharm Cairo Univ, 2014; 52: 239–44.
9. Prathap B, Gobinath M, Haribaskar V, et al. Stability indicating RP-HPLC method development and validation for simultaneous estimation of bismuth subcitrate, tetracycline, and metronidazole. Asian J Pharm, 2018; 12(4 Suppl): S1310–S1316.
10. Rao TN, Patrudu TB, Srinivasa Rao T, Parvathamma T. Simple and sensitive validated RP-HPLC-UV method for the determination of lymecycline in pharmaceutical dosage forms. Int J Pharm Sci Res, 2012; 3(7): 2301–5.
11. Rakholiya B, Shah P, Patel Y, Patel G, Patel S, Patel A. A review on analytical methods for tigecycline estimation from its bulk and dosage form. J AOAC Int, 2023; 106(6): 1689–95.
12. Raveendra Babu G, Deepthi S, Priyanka GB, Priya TS, Dhachinamoorthi D. New development and validation of reverse phase-HPLC determination for omadacycline in API and marketed dosage form. Int J Res Pharm Chem, 2022; 12(4): 445–49.
13. Kahsay G, Shraima F, Villatte P, Rotger J, Cassus-Coussère C, Van Schepdael A, et al. Development and validation of a reversed phase liquid chromatographic method for analysis of oxytetracycline and related impurities. J Pharm Biomed Anal, 2013; 75: 199–206.
14. Varaganti MS, Chengalva P, Gundala A. Novel stability-indicating HPLC assay method for simultaneous estimation of hydrocortisone and tetracycline. Asian J Pharm Clin Res, 2019; 12(10).
15. Chaudhary T, Kurmi BD, Singh D. Convenient estimation of oxytetracycline and polymyxin B by a novel HPLC method: development and validation. Explor Drug Sci, 2023; 1: 6–17.
16. Agarwal A, Singhvi IJ, Gupta SK, Gupta KC, Thakkar M. Method development and validation for assay of minocycline hydrochloride in dosage forms by RP-HPLC. Int J Chem Sci, 2012; 10(4): 2191–98.
17. Venkata Prabhakara Rao P, Mastan Ali S, Bharath P, Ramachandran D. Stability indicating RP-HPLC method for quantification of eravacycline and its impurities. J Chem Health Risks, 2023; 13(6): 1967–79.
18. Koppuravuri NP, Vijaya Lakshmi A. Development and validation of a sensitive HPLC-MS method for quantification of omadacycline in plasma. Drug Invent Today, 2020; 14(2): 14–18.

19. Ramesh PJ, Basavaiah K, Tharpa K, Vinay KB, Revanasiddappa HD. Development and validation of RP-HPLC method for determination of doxycycline hyclate in spiked human urine and pharmaceuticals. J Pre-Clin Clin Res, 2010; 4.