World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

STABILITY-INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR DRUGS USED IN URINARY TRACT INFECTIONS: A COMPREHENSIVE REVIEW

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DOI: https://doi.org/10.5281/zenodo.18480722

How to cite this Article: G. Haritha1*, Arunabha Mallik2, Chavana Pranaswi1, Antarakshit Datta1, Cheguri Prasanna1, Gurrampally Apphia1. (2026) Stability-Indicating Hplc Method Development And Validation For Drugs Used In Urinary Tract Infections: A Comprehensive Review. World Journal of Pharmaceutical and Life Science, 12(2), 197–201.

Article Received on 05/01/2026 **Article Revised on 25/01/2026** **Article Published on 01/02/2026**

ABSTRACT

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide and are treated with a wide range of antibacterial agents, including β-lactams, Fluoroquinolones, Sulfonamides, Nitrofurans, Fosfomycin, and Aminoglycosides. Ensuring the quality, safety, and efficacy of these drugs requires robust analytical methods capable of accurately quantifying the active pharmaceutical ingredient (API) in the presence of degradation products and impurities. Stability-indicating high-performance liquid chromatography (HPLC) methods have therefore become indispensable tools in pharmaceutical analysis and regulatory compliance. This comprehensive review critically discusses the principles and practical aspects of stability-indicating HPLC method development and validation for drugs used in UTIs. Detailed coverage is provided on drug classification, degradation behavior, chromatographic method development strategies, forced degradation studies, method validation parameters in accordance with ICH guidelines, and trends in the published literature. Current challenges and future perspectives, including the transition to UPLC and green analytical chemistry approaches, are also highlighted.

KEYWORDS: Urinary Tract Infections, Stability-Indicating Method, HPLC, Forced degradation, Method Validation, ICH guidelines.

1. INTRODUCTION

Urinary tract infections (UTIs) affect millions of individuals annually and represent a significant burden on healthcare systems. They are caused predominantly by Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Proteus species, as well as Gram-positive organisms including Enterococcus species. Pharmacological management of UTIs relies mainly on antibacterial drugs that must maintain chemical stability throughout their shelf life to ensure therapeutic efficacy and patient safety.[1]

Pharmaceutical stability is defined as the ability of a drug substance or drug product to retain its physical, chemical, microbiological, and therapeutic properties within specified limits throughout its storage and use. Drug degradation can lead to reduced potency, formation of toxic impurities, and therapeutic failure. Hence, regulatory agencies mandate the use of stability-indicating analytical methods to monitor drug stability during development, manufacturing, and post-marketing surveillance.[2]

High-performance liquid chromatography (HPLC) is the most widely employed analytical technique for stability testing due to its high resolution, sensitivity, selectivity, and versatility. A stability-indicating HPLC method is

one that accurately measures the active ingredient without interference from degradation products, impurities, excipients, or other potential components. For drugs used in UTIs, which often contain labile functional groups such as β-lactam rings, nitro groups, or heterocyclic moieties, the development of reliable stability-indicating methods is particularly critical.[3]

This review aims to provide a comprehensive and structured discussion on stability-indicating HPLC method development and validation for UTI drugs, covering theoretical considerations, experimental strategies, regulatory expectations, and future directions.

2. Drugs Used in Urinary Tract Infections: An Overview

Drugs prescribed for UTIs belong to diverse chemical and pharmacological classes, each exhibiting distinct stability and degradation characteristics.

2.1 Common Classes of UTI Drugs

β-Lactam antibiotics: Amoxicillin, ampicillin, Cefixime, ceftriaxone.

Fluoroquinolones: Ciprofloxacin, Norfloxacin, levofloxacin, Ofloxacin.

Nitrofurans: Nitrofurantoin.

Sulfonamides and combinations: Sulfamethoxazole–trimethoprim.

Phosphonic acid derivatives: Fosfomycin tromethamine.

Aminoglycosides: Gentamicin, amikacin.

Drugs used in the management of urinary tract infections (UTIs) encompass a broad spectrum of antibacterial agents with diverse chemical structures, mechanisms of action, and stability profiles. The analytical behavior of these drugs during stability studies is strongly influenced by their functional groups, pKa values, solubility characteristics, and susceptibility to environmental stress conditions.[4-6]

2.2 Classification of UTI Drugs and Chemical Characteristics

Table 1: Classification of UTI Drugs and Chemical Characteristics.

Drug Class	Representative Drugs	Key Functional Groups	Analytical Stability Concerns
β-Lactam antibiotics	Amoxicillin, Ampicillin, Cefixime, Ceftriaxone	β-lactam ring, amide	Highly susceptible to acid/base hydrolysis
Fluoroquinolones	Ciprofloxacin, Norfloxacin, Levofloxacin, Ofloxacin	Carboxylic acid, fluorine, piperazine	Photodegradation, oxidation
Nitrofurans	Nitrofurantoin	Nitro group, imide	Oxidative and hydrolytic degradation
Sulfonamides	Sulfamethoxazole	Sulfonamide linkage	Hydrolysis, photolysis
Combination drugs	Trimethoprim–Sulfamethoxazole	Diaminopyrimidine, sulfonamide	Differential degradation rates
Phosphonic acid derivatives	Fosfomycin tromethamine	Epoxide, Phosphonic acid	Hydrolytic instability
Aminoglycosides	Gentamicin, Amikacin	Multiple hydroxyl and amino groups	Poor UV chromophore, derivatization often required

2.3 Analytical Challenges for UTI Drugs

UTI drugs present unique analytical challenges due to:

- Structural diversity leading to wide polarity range
- Presence of multiple ionizable functional groups
- Formation of closely eluting degradation products
- Low UV absorbance for certain classes (e.g., aminoglycosides)

Therefore, stability-indicating HPLC methods must be carefully optimized to ensure adequate resolution and sensitivity.[7]

2.4 Stability Concerns

Many UTI drugs are susceptible to hydrolysis, oxidation, photolysis, and thermal degradation. For example, β-lactam antibiotics undergo rapid hydrolysis under acidic and alkaline conditions, while fluoroquinolones are sensitive to light and oxidative stress. These characteristics necessitate carefully optimized chromatographic conditions to separate the API from its degradation products.[8]

3. Concept of Stability-Indicating HPLC Methods

A stability-indicating method is an analytical procedure that is specific and selective for the API and can detect changes in drug purity and potency due to degradation. According to ICH guidelines, such methods should effectively resolve the API peak from all degradation products and impurities, ensuring accurate quantification during stability studies.[9]

Key features of stability-indicating HPLC methods include:

- Adequate resolution between API and degradation peaks
- Peak purity confirmation using diode array or mass detection
- Robustness under small deliberate variations in method parameters

4. HPLC METHOD DEVELOPMENT FOR UTI DRUGS

Method development is a systematic process aimed at achieving optimal separation, sensitivity, and reproducibility.[10]

4.1 Selection of Stationary Phase

Reverse-phase HPLC (RP-HPLC) is most commonly used. C18 columns are preferred due to their broad applicability and strong hydrophobic interactions. In some cases, C8 or phenyl columns may be selected to improve selectivity for aromatic or moderately polar compounds.[11]

4.2 Mobile Phase Selection

The mobile phase typically consists of a buffer combined with an organic modifier such as acetonitrile or methanol. Buffer pH is selected based on the pKa of the drug to control ionization and improve peak shape. For UTI drugs, phosphate or acetate buffers in the pH range of 2.5–7.0 are frequently employed.[12]

4.3 Detection Techniques

UV–Visible detection is widely used due to its simplicity and cost-effectiveness. Many UTI drugs exhibit strong UV absorbance between 210–280 nm. Diode array detectors (DAD) provide additional spectral information for peak purity assessment.[13]

4.4 Optimization Parameters

Critical parameters optimized during method development include:

- Flow rate
- Column temperature
- Mobile phase composition and gradient profile
- Injection volume

5. Stability and Forced Degradation Studies

Stability studies evaluate how the quality of a drug substance or product varies with time under the influence of environmental factors such as temperature, humidity, and light. Forced degradation studies, also known as stress testing, are a critical component of stability-indicating method development.[14-15]

Forced degradation studies are integral to establishing the stability-indicating nature of an HPLC method. These studies intentionally degrade the drug under stress conditions to generate potential degradation products.[16]

5.1 Role of Stability Studies

Stability studies are conducted to:

- ✓ Establish retest periods and shelf life
- ✓ Identify degradation products and pathways
- ✓ Support formulation development
- ✓ Demonstrate the stability-indicating capability of analytical methods.[17]

5.2 Purpose of Forced Degradation

- ✓ To identify degradation pathways
- ✓ To demonstrate method specificity
- ✓ To support formulation development and packaging selection

5.3 Stress Conditions

Common stress conditions include:
Acidic hydrolysis: Using dilute HCl
Alkaline hydrolysis: Using NaOH or KOH
Oxidative degradation: Using hydrogen peroxide
Thermal degradation: Elevated temperatures
Photolytic degradation: Exposure to UV or visible light
For UTI drugs, hydrolytic and oxidative stresses are particularly relevant due to the presence of labile functional groups.[18]

5.4 Forced Degradation Conditions Applied to UTI Drugs

Table-2: Forced Degradation Conditions Applied to UTI Drugs.

Stress Condition	Typical Reagents / Conditions	Observed Effects on UTI Drugs
Acidic hydrolysis	0.1–1 N HCl	Rapid degradation of β-lactams
Alkaline hydrolysis	0.1–1 N NaOH	Ring opening in β-lactams and Fosfomycin
Oxidative stress	3–30% H_2O_2	Significant degradation of nitrofurantoin and fluoroquinolones
Thermal stress	60–80°C	Gradual degradation of most UTI drugs
Photolytic stress	UV/Visible light	High sensitivity of fluoroquinolones and sulfonamides

Stress conditions are optimized to achieve 5–20% degradation, ensuring meaningful evaluation without complete drug decomposition.

6. METHOD VALIDATION ACCORDING TO ICH GUIDELINES

Validation confirms that the developed method is suitable for its intended purpose. ICH Q2(R1) outlines key validation parameters.[19-21]

6.1 Specificity
The ability to unequivocally assess the API in the presence of impurities, degradation products, and excipients.

6.2 Linearity and Range
Demonstration of a direct proportional relationship between concentration and response over a specified range, typically 80–120% of the test concentration.

6.3 Accuracy
Closeness of agreement between the true value and the value found, usually assessed by recovery studies.[22]

6.4 Precision
Includes repeatability (intra-day) and intermediate precision (inter-day, analyst-to-analyst).

6.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)
Determines the sensitivity of the method for detecting low levels of the analyte.

6.6 Robustness
Evaluation of the effect of small deliberate changes in method parameters such as pH, flow rate, and mobile phase composition.[23]

7. Literature Review (Stability-Indicating HPLC Methods for UTI Drugs)
Numerous studies have reported stability-indicating HPLC methods for UTI drugs. Most methods employ RP-HPLC with C18 columns and UV detection. Forced degradation studies consistently demonstrate significant degradation under hydrolytic and oxidative conditions for β-lactams and Nitrofurans, while fluoroquinolones show notable photodegradation.

Recent literature trends indicate increasing use of gradient elution, shorter column lengths, and smaller particle sizes to improve resolution and reduce analysis time. The application of DAD and LC–MS for degradation product identification is also gaining prominence.[24]

A substantial body of literature reports validated stability-indicating HPLC methods for drugs used in UTIs. Most studies employ reverse-phase HPLC with UV or diode-array detection, while recent studies increasingly incorporate LC–MS for degradation product characterization.

7.1 Summary of Published Stability-Indicating HPLC Methods
Table-3: Summary of Published Stability-Indicating HPLC Methods.

Drug	Column	Mobile Phase	Detection	Stress Conditions	Reference
Ciprofloxacin	C18	Phosphate buffer: ACN	UV (278 nm)	Acid, base, oxidative, light	Ref. 11
Norfloxacin	C18	Buffer: MeOH	UV (275 nm)	Hydrolytic, oxidative	Ref. 12
Nitrofurantoin	C18	Buffer: ACN	UV (265 nm)	Oxidative, thermal	Ref. 13
Sulfamethoxazole	C18	Buffer: ACN	UV (254 nm)	Acid, base, light	Ref. 14
Trimethoprim–SMX	C18	Gradient elution	UV (230 nm)	Multiple stresses	Ref. 15
Amoxicillin	C18	Buffer: MeOH	UV (220 nm)	Acid/base hydrolysis	Ref. 16
Cefixime	C18	Buffer: ACN	UV (254 nm)	Hydrolytic, oxidative	Ref. 17
Fosfomycin	HILIC	ACN: Buffer	RI/UV	Hydrolytic	Ref. 18

These studies consistently demonstrate adequate resolution between APIs and degradation products, confirming the suitability of RP-HPLC for routine stability testing.

8. Regulatory Perspectives
Regulatory agencies such as the US FDA, EMA, and CDSCO require validated stability-indicating methods for product approval and lifecycle management. Compliance with ICH guidelines ensures global acceptability of analytical data. For UTI drugs, stability data generated using validated HPLC methods are critical for establishing shelf life and storage conditions.[25]

9. Future Perspectives
Future research is expected to focus on:

- Transition from conventional HPLC to UPLC for higher throughput
- Green analytical chemistry approaches using eco-friendly solvents
- Hyphenated techniques (LC–MS/MS) for comprehensive impurity profiling
- Use of quality-by-design (QbD) principles in method development

These advancements will enhance method efficiency, sustainability, and regulatory compliance.

10. DISCUSSION
The reviewed literature highlights that reverse-phase HPLC remains the analytical technique of choice for stability-indicating analysis of UTI drugs due to its robustness, accessibility, and regulatory acceptance. β-lactam antibiotics consistently show extensive hydrolytic degradation, necessitating careful pH control during analysis. Fluoroquinolones and sulfonamides exhibit pronounced photolytic and oxidative degradation,

emphasizing the importance of light protection and antioxidant evaluation during formulation.[26]

The majority of reported methods comply with ICH validation requirements, demonstrating acceptable linearity, accuracy, precision, and robustness. However, challenges remain in the analysis of highly polar drugs such as Fosfomycin and aminoglycosides, where alternative chromatographic modes or derivatization techniques are required. Recent advancements such as UPLC, quality-by-design (QbD)-based method development, and hyphenated techniques (LC–MS/MS) offer enhanced resolution, reduced analysis time, and improved degradation profiling.[27-30]

11. CONCLUSION

This comprehensive review underscores the critical role of stability-indicating HPLC method development and validation in ensuring the quality, safety, and efficacy of drugs used in urinary tract infections. A thorough understanding of drug chemistry, degradation behavior, and regulatory expectations is essential for developing reliable analytical methods. Forced degradation studies, combined with systematic method validation, confirm the specificity and robustness of these methods. Future research should focus on greener analytical approaches, wider application of QbD principles, and advanced detection techniques to address current analytical challenges and meet evolving regulatory demands.

Stability‑indicating HPLC methods play a vital role in ensuring the quality and safety of drugs used in urinary tract infections. Comprehensive method development, supported by forced degradation studies and rigorous validation, is essential to meet regulatory expectations. The reviewed literature highlights the robustness and versatility of RP‑HPLC for UTI drugs, while emerging trends point toward faster, greener, and more informative analytical techniques. Continued innovation in this field will further strengthen pharmaceutical quality control and patient safety.

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