World Journal of
Pharmacy and Medical Science

ISSN: 3049-3501
Impact Factor: 5.157
CODEN (USA): WJPMAA

Review Article
2026 Volume 2, Issue 8
Page No.: 31-36

IMPURITY PROFILING, STRUCTURAL REACTIVITY AND STABILITY CHALLENGES OF FOSFOMYCIN: A CRITICAL REVIEW WITH EMPHASIS ON EPOXIDE INTEGRITY AS A QUALITY DETERMINANT

Pooja Deshpande[1], Dinesh Dantkale[2], Mahesh Mali[2], Santosh Rawool[2], Sharad Rode*[2]

[1]Director, SRS Pharmaceuticals Pvt. Ltd (Research Centre), Mumbai, Maharashtra, India.
[2]SRS Pharmaceuticals Pvt. Ltd (Research Centre), Mumbai, Maharashtra, India.

Article Received: 27 May 2026 Article Review: 17 June 2026 Article Accepted: 08 July 2026

***Corresponding Author: Sharad Rode**
SRS Pharmaceuticals Pvt. Ltd (Research Centre), Mumbai, Maharashtra, India.
DOI: https://doi.org/10.5281/zenodo.21721072

How to cite this Article: Pooja Deshpande, Dinesh Dantkale, Mahesh Mali, Santosh Rawool, Sharad Rode (2026). IMPURITY PROFILING, STRUCTURAL REACTIVITY AND STABILITY CHALLENGES OF FOSFOMYCIN: A CRITICAL REVIEW WITH EMPHASIS ON EPOXIDE INTEGRITY AS A QUALITY DETERMINANT. World Journal of Pharmacy and Medical Science, 2(8): 31-36.

ABSTRACT
Fosfomycin is a structurally unique phosphonic acid antibiotic widely used for the treatment of urinary tract infections (UTIs), particularly those caused by multidrug-resistant (MDR) pathogens. Unlike conventional β-lactam or glycopeptide antibiotics, fosfomycin exerts bactericidal action through irreversible inhibition of MurA enzyme ((UDP-N-acetylglucosamine enolpyruvyl transferase) via its strained epoxide ring. While its small molecular size and high aqueous solubility offer formulation advantages, the molecule's inherent chemical reactivity presents significant stability and impurity-related challenges. The integrity of the epoxide moiety is directly linked to therapeutic activity, making impurity profiling a critical quality attribute (CQA).

KEYWORDS: Fosfomycin, Epoxide degradation, Impurity profiling, MurA inhibition, Phosphonic acid antibiotics, Stability, Forced degradation.

1. INTRODUCTION

Increasing resistance to essential antibiotics worldwide and shortage of new antibiotic molecules is serious concern and causes threat to global health. Therefore, there is unmet medical need to look for alternative treatment options for multidrug resistant pathogens.

Fosfomycin is choice of antibiotic candidate and alternative treatments for multidrug resistant pathogens.[1]

Fosfomycin was first discovered in Spain in 1969 from cultures of the Streptomyces species. Fosfomycin is a broad-spectrum bactericidal epoxide antibiotic used for acute uncomplicated urinary tract infections (UTIs). It is commercially available in two salt forms such as Fosfomycin trometamol (oral sachet, single-dose therapy) under trade name MONUROL and Fosfomycin disodium (intravenous formulation for systemic infections) under trade name CONTEPO.

It has a broad antibacterial spectrum of activity against a wide range of bacteria, including gram-negative micro-organisms and some gram-positive bacteria, such as *staphylococci*. It is especially valued for its activity against multidrug-resistant organisms, including extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Staphylococcus aureus*. Its mechanism of action is by targeting bacteria with mucopeptide synthesis by inhibiting phosphoenolpyruvate transferase, the first enzyme involved in the synthesis of peptidoglycan.

This review discusses fosfomycin chemistry, stereochemical considerations, physicochemical behaviour, degradation pathways, synthesis and characterization of major degradation impurities, and their impact on safety, efficacy, and regulatory compliance. Special emphasis is placed on epoxide ring-opening impurities and methanol-derived O-methoxy derivatives. The review highlights the need for stringent chiral control, moisture-protective packaging, and

advanced analytical strategies to ensure product quality.[2-3]

2. CHEMICAL STRUCTURE AND STEREOCHEMISTRY

2.1 Chemical Identity

Fosfomycin is chemically designated as (1R,2S)-epoxypropyl phosphonic acid, with the molecular formula $C_3H_7O_4P$ and a molecular weight of 138.06 g/mol (free acid form). It belongs to the class of phosphonic acid antibiotics, a relatively rare structural category among antibacterial agents.

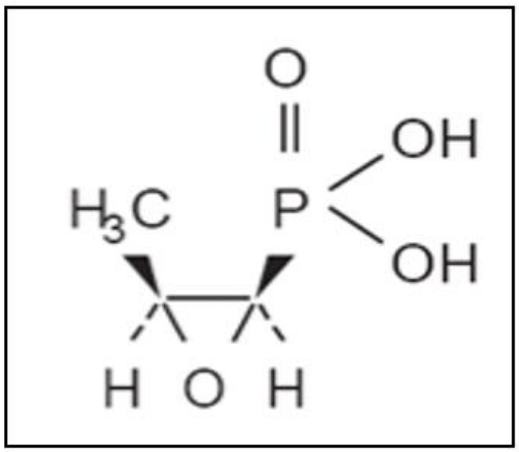

Figure 1: Chemical structure of Fosfomycin.

Structurally, fosfomycin is composed of two critical functional moieties such as a highly strained three-membered epoxide ring and a strongly polar phosphonic acid group (-$PO(OH)_2$). Despite its small molecular size, this dual functional architecture imparts both potent antibacterial activity and inherent chemical instability.

The epoxide ring is responsible for the molecule's electrophilic reactivity and mechanism-based covalent inhibition of bacterial enzymes. In contrast, the phosphonic acid group contributes to high aqueous solubility and ionic interaction with the biological target.

However, this same structural arrangement renders the molecule susceptible to hydrolytic and nucleophilic degradation, explaining its sensitivity to moisture and pH variations. Thus, fosfomycin represents a unique case in which structural simplicity coexists with complex stability behaviour. For pharmaceutical use, fosfomycin is converted to salts that are sufficiently stable during storage and form aqueous solutions that have a physiologically tolerable pH value. The European Pharmacopoeia describes three fosfomycin salts. These are the monohydrate of the calcium salt of fosfomycin (CAS 26016-98-8), the disodium salt of fosfomycin (CAS 26016-99-9), and trometamol-fosfomycin (CAS 78964-85-9).[4-6]

2.2 Stereochemistry and optical purity

Fosfomycin contains two chiral centres, located at carbon positions C-1 and C-2. The biologically active form corresponds exclusively to the (1R,2S) stereochemical configuration. Any deviation from this configuration significantly reduces antibacterial potency.

Stereochemical integrity is of paramount importance because the molecule's interaction with the MurA enzyme (UDP-N-acetylglucosamine enolpyruvyl transferase) is highly stereospecific. Incorrect stereochemistry alters the spatial orientation of the epoxide ring, thereby reducing its ability to undergo nucleophilic attack by the active-site cysteine residue of MurA.

Under strongly alkaline conditions, racemization may occur, leading to the formation of less active or inactive stereoisomers. The enantiomer (+)-cis-(1S, 2R)-epoxypropylphosphonic acid is common byproduct formed during synthesis of fosfomycin. As per European pharmacopoeia the limit for this enantiomeric impurity is of maximum 1.0 %. Consequently, optical purity must be carefully monitored during active pharmaceutical ingredient (API) manufacturing using validated chiral high performance liquid chromatography (HPLC) methods. From a regulatory and quality perspective, stereochemical control constitutes a critical quality attribute (CQA) for Fosfomycin.[7-8]

2.3 Functional group significance and reactivity

2.3.1 Epoxide ring

The epoxide ring is the pharmacophoric core of Fosfomycin. Due to its three-membered cyclic structure, it possesses significant ring strain, which increases its electrophilic character. This strained configuration enables the molecule to form a covalent bond with the MurA enzyme, irreversibly inhibiting the first committed step in bacterial peptidoglycan biosynthesis.

However, this beneficial reactivity also introduces chemical vulnerability. The epoxide ring is highly susceptible to:

- Acid and base catalyzed hydrolysis
- Nucleophilic attack by solvents (e.g., water, alcohols)
- Moisture-induced degradation during storage

When the epoxide ring undergoes opening, the molecule loses its ability to covalently bind MurA enzyme, resulting in complete or substantial loss of antibacterial activity. Therefore, preservation of epoxide integrity is directly linked to therapeutic efficacy.

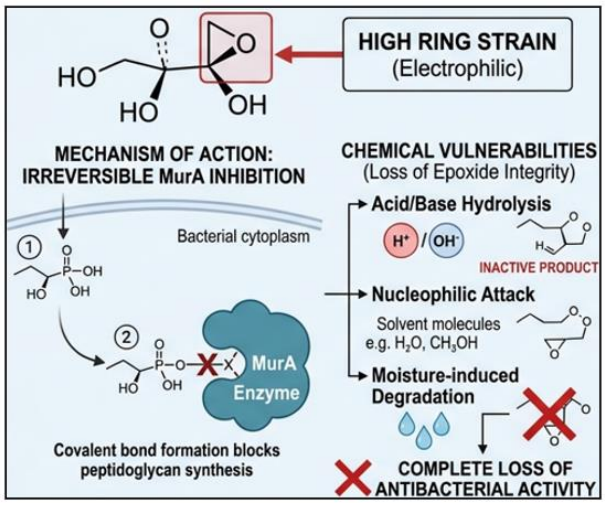

Figure 2: Epoxide Ring – Pharmacophoric Core and Instability.

2.3.2 Phosphonic acid group

The phosphonic acid group is highly polar and ionizable, with a pKa of approximately 2–3. This functionality contributes significantly to the drug's physicochemical behavior.

The presence of the phosphonate moiety:

- Enhances aqueous solubility
- Facilitates ionic interactions with the MurA enzyme
- Reduces lipophilicity
- Imparts hygroscopic characteristics

While high solubility is advantageous for oral formulations (e.g., Fosfomycin trometamol), the hygroscopic nature of the phosphonic acid group increases sensitivity to environmental moisture, thereby accelerating degradation processes.[9-10]

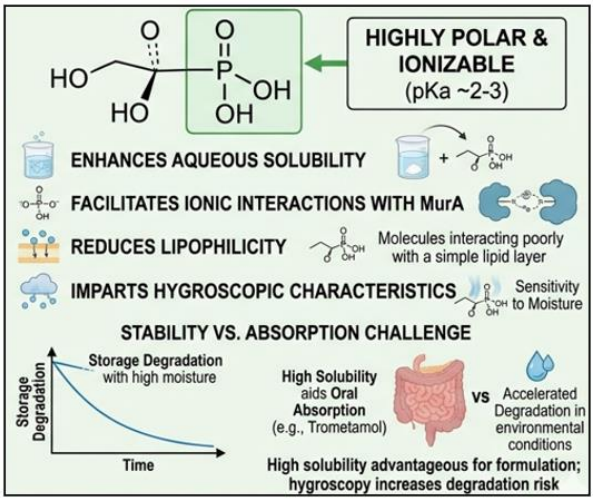

Figure 3: Phosphonic Acid Group – Physicochemical properties and Stability.

2.4 Physicochemical properties

Fosfomycin is typically obtained as a white crystalline powder that is freely soluble in water due to its ionic phosphonate group. It demonstrates relative stability under neutral pH conditions but becomes increasingly unstable in strongly acidic or alkaline environments.

A notable analytical challenge arises from its weak UV chromophore, with a maximum absorption wavelength (λmax) around 210–220 nm. Because it lacks an extended conjugated system, UV detection sensitivity is limited, particularly at low concentrations.

Due to its high polarity:

- Lipophilicity is minimal.
- Retention on conventional C18 reversed-phase columns is weak.
- Alternative analytical techniques such as derivatization, Evaporative Light Scattering Detection (ELSD) or
- Liquid chromatography-Mass spectrometry (LC-MS) may be required.

These physicochemical characteristics significantly influence analytical method development and validation.[4-6]

3. IMPURITY PROFILE OF FOSFOMYCIN

Impurities in fosfomycin may originate from either synthetic processes or degradation pathways. Given the molecule's structural reactivity, impurity profiling is a critical aspect of quality control.

3.1 Process-related impurities:

During synthesis, several process-related impurities may be generated. These commonly include:

- Phosphorous acid, originating from phosphorus-based intermediates
- Epichlorohydrin, used in epoxide formation
- Residual or unreacted starting materials

Such impurities must be identified, quantified, and controlled in accordance with ICH Q3A guidelines for impurities in new drug substances. Appropriate purification and process optimization are necessary to ensure compliance with regulatory limits.

3.2 Degradation impurities

Because of the epoxide ring's reactivity, degradation occurs predominantly via ring-opening mechanisms, especially under conditions of moisture, heat, or extreme pH.

3.3 Epoxide ring - opened diol (Impurity A)

The most frequently observed degradation product is 1,2-dihydroxypropyl phosphonic acid, commonly referred to as Impurity A.

This impurity forms through hydrolytic opening of the epoxide ring, typically under:

- Elevated temperature
- High humidity (40°C / 75% RH)
- Acidic or basic stress conditions

Impurity A lacks the strained epoxide ring required for MurA inhibition and therefore exhibits no antibacterial activity. It is considered the primary stability-indicating impurity.

3.4 Impurity B

Impurity B results from nucleophilic attack on the epoxide ring under forced degradation conditions. It involves formation of an amino-diol derivative and represents advanced degradation under stress environments.

3.5 Impurity C

Impurity C, identified as 2-amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogen phosphate, likely forms via rearrangement or secondary transformation reactions during degradation.

3.6 Impurity D

Impurity D is a more complex phosphonic acid-linked dimeric structure. It forms under prolonged stress conditions and represents a late-stage degradation product involving intermolecular reactions.

3.7 O-Methoxy impurity:

When methanol is present as a solvent during stress testing or analytical procedures, it can act as a nucleophile and attack the epoxide ring. This results in the formation of a methoxy-alcohol derivative known as the O-methoxy impurity. This impurity underscores the importance of solvent selection during forced degradation studies and highlights the potential for artifact formation during analytical method development.[11-13]

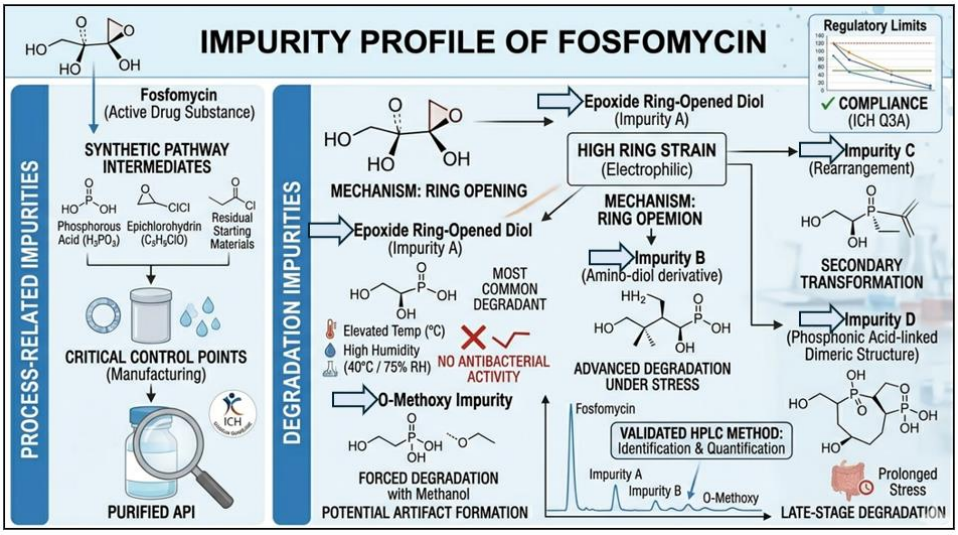

Figure 4: Impurity Profile of Fosfomycin.

3.8 Synthesis and isolation of degradation impurities

Major degradation impurities can be intentionally generated through forced degradation studies, including:

- Thermal stress
- Acidic or basic hydrolysis
- Controlled moisture exposure

Isolation and purification of these impurities are critical for structural confirmation and toxicological qualification. Techniques commonly employed include:

- Resin-based desalting to remove inorganic residues
- Preparative high performance liquid chromatography (HPLC) purification
- Lyophilization to obtain stable solid forms

Subsequent characterization using Nuclear Magnetic Resonance (NMR), Mass spectrometry (MS), and Infrared spectroscopy (IR) confirms structural identity.[14-15]

3.9 Impact of impurities

3.9.1 Impact on therapeutic efficacy

The antibacterial activity of fosfomycin is entirely dependent on the integrity of the epoxide ring. Ring-opened impurities are incapable of forming the covalent bond with MurA enzyme and therefore lack antibacterial efficacy.

As degradation increases:

- Potency decreases
- Assay values decline
- Clinical effectiveness may be compromised

A direct correlation exists between impurity levels and antimicrobial performance.

3.9.2 Impact on stability

Fosfomycin degradation accelerates under:

- High temperature and humidity (40°C / 75% RH)
- Long-term storage without moisture protection
- Inappropriate packaging systems

These conditions lead to:

- Increased formation of ring-opened impurities
- Reduced shelf life
- Progressive assay loss

Therefore, moisture-protective packaging is essential.

3.9.3 Regulatory impact

Impurities in fosfomycin are considered:

- Critical Quality Attributes (CQAs)
- Stability-indicating parameters
- Potential safety risks under ICH Q3A and Q3B

Epoxide-related degradation products are categorized as high risk due to:

- Mechanism-based loss of pharmacological activity
- Structural modification of the API
- Potential unknown toxicological implications.[9, 11-15]

4. ANALYTICAL CHALLENGES

The analytical evaluation of fosfomycin presents several challenges due to:

- High polarity
- Weak UV absorption
- Poor retention on conventional C18 columns

Advanced analytical strategies may include:

- Ion-pair chromatography
- Hydrophilic interaction chromatography (HILIC)
- LC-MS/MS
- Derivatization approaches
- Chiral HPLC for stereochemical assessment

Method development must ensure specificity toward ring-opened degradation products.[16-17]

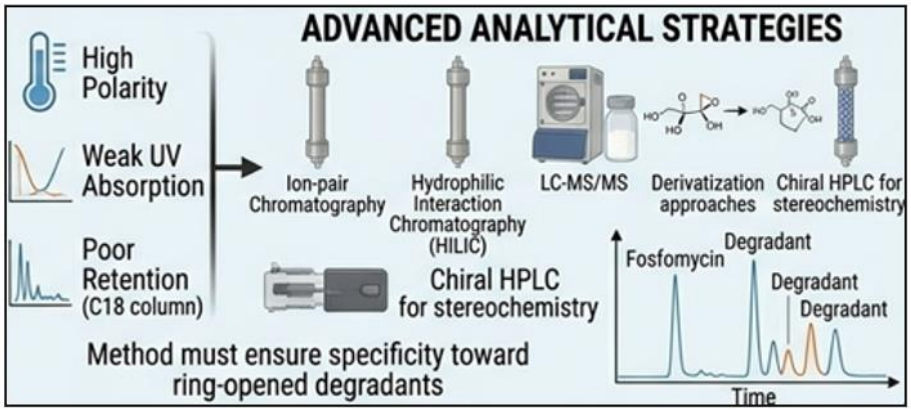

Figure 5: Fosfomycin Analytical Challenges.

5. PACKAGING AND FORMULATION CONSIDERATIONS

To preserve epoxide integrity and minimize degradation:

- Aluminium–aluminium blister packs or HDPE containers with desiccant are recommended.
- Residual moisture must be tightly controlled.
- Alkaline excipients should be avoided.
- Methanol should not be used in analytical stress studies unless specifically evaluating methoxy impurity formation.

Proper packaging and formulation design are essential to ensure long-term stability and therapeutic reliability.

Formulation excipients containing reducing sugars and aldehyde groups should be avoided while formulation development of Fosfomycin tromethamine oral formulation because causing undesirable colour formation in the formulation. Additionally, the presence of sucrose in the formulations of cutting-edge technology restricts their administration to those who are diabetic.

Hence there is need to develop oral fosfomycin formulation with an excipient system that is compatible with the active principle which do not pose any pharmacological and biopharmaceutical risk.[18-19]

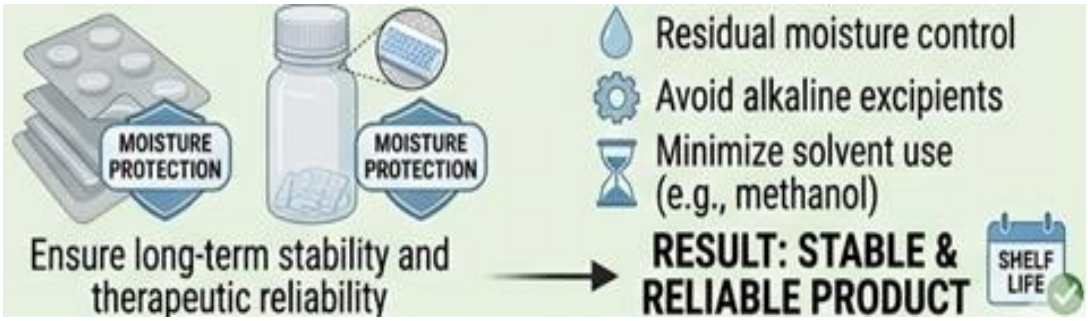

Figure 6: Fosfomycin packaging and formulation considerations.

6. CONCLUSION

Fosfomycin represents a chemically simple yet pharmacologically powerful antibiotic whose therapeutic efficacy is intrinsically linked to the integrity of its strained epoxide ring. The molecule's hygroscopic nature and susceptibility to nucleophilic attack make impurity control a central aspect of quality assurance. Degradation pathways predominantly involve epoxide ring-opening reactions, leading to inactive diol and solvent-derived impurities. Therefore, epoxide preservation is not merely a stability concern but a pharmacodynamic necessity. Robust impurity profiling, stereochemical control, and moisture-protective strategies are indispensable for ensuring product safety, efficacy, and regulatory compliance.

7. REFERENCES

1. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MA. Antimicrobial Resistance: A growing serious threat for global public health. Healthcare (Basel), 2023; 11(13): 1946.
2. Kamerling IMC et al. Fosfomycin: Pharmacological, clinical and future perspectives. Antibiotics (Basel), 2017; 6(4): 24.
3. Raz R. Fosfomycin: an old - new antibiotic. Clinical Microbiology and Infection, 2012; 18(1): 4-7.
4. BenchChem Technical Support Team. Fosfomycin: A technical guide to its chemical structure, phosphonic acid group, and mechanism of action, 2026; 1-8.
5. Fosfomycin. National Library of Medicine – Pubchem, https://pubchem.ncbi.nlm.nih.gov/compound/Fosfomycin
6. Drug bank. Fosfomycin. https://go.drugbank.com/drugs/DB00828
7. Cao Y, Peng Q, Li S, Deng Z, Gao J. The intriguing biology and chemistry of fosfomycin: the only marketed phosphonate antibiotic. RSC Advances - The Royal Society of Chemistry, 2019; 9: 42204-42218.
8. Perales A, Martinez RM, Fayos J, Von CLC, Fernandez M. The absolute configuration of active and inactive fosfomycin. Acta Crystallographica, Section B: Structural Crystallography and Crystal Chemistry, 1982; B38(10): 2763-4.
9. Garcia AC, Blazquez J, Rojas AR. Molecular mechanisms and clinical impact of acquired and intrinsic fosfomycin resistance. Antibiotics, 2013; 2: 217-236.
10. Hashemian SM, Farhadi Z, Farhadi T. Fosfomycin: the characteristics, activity, and use in critical care. Therapeutics and Clinical Risk Management, 2019; 15: 525–530.
11. Nagaraju PT, Tekuru M. Review article on impurity profiling. International Journal of Pharmacy and Pharmaceutical Science, 2025; 7(2): 307-312.
12. Fosfomycin & Its Impurities. SynZeal. https://www.synzeal.com/en/fosfomycin-impurities.
13. fosfomycin and its Impurities. Pharmaffiliates. https://www.pharmaffiliates.com/en/parentapi/fosfomycin-impurities
14. Castaldi G, Giordano C. Europe Patent Publication EP0251324A2, 1992.
15. Divasto R, Marino M. US Patent US12480920, 2025.
16. Haipeng J, Hong C, Nian C, Jing Z, Xiulian J. Quantitative ^{31}P-NMR spectroscopy for the determination of fosfomycin and impurity A in pharmaceutical products of fosfomycin sodium or calcium. Magnetic Resonance in Chemistry, 2015; 53(6): 454-459.
17. Liu H, Liu C. Impurity profiling of fosfomycin by capillary zone electrophoresis. Zhongguo Kangshengsu Zazhi, 2011; 36(10): 765-771.
18. Sebastian Vogt S. US Patent Publication US20150283291A1, 2015.
19. Josep MS, Jose R, Jose MC. European Patent EP1747781B1, 2010.