



Received on 15 May 2025; received in revised form, 19 June 2025; accepted, 20 June 2025; published 01 November 2025

IDENTIFICATION AND CHARACTERIZATION OF IMPURITIES IN SULPHACETAMIDE INJECTABLE FORMULATION BY STABILITY-INDICATING UPLC-ESI-MS/MS METHOD FOLLOWING ICH GUIDELINES

I. Shareef¹ and K. G. Gandla^{*2}

Department of Pharmaceutical Analysis¹, Department of Pharmacy², Chaitanya (Deemed to be University), Gandipet, Himayat Nagar, Moinabad (Mandal), Ranga Reddy, Hyderabad - 500075, Telangana, India.

Keywords:

Sulphacetamide, Impurity profiling, Forced degradation, UPLC-MS/MS, ICH guidelines, High-resolution mass spectrometry

Correspondence to Author: Prof. Kumaraswamy Gandla

Head & Chairperson, BoS,
Department of Pharmacy, Chaitanya
(Deemed to be University), Gandipet,
Himayat Nagar, Moinabad (Mandal),
Ranga Reddy, Hyderabad - 500075,
Telangana, India.

E-mail: drkumaraswamygandla@gmail.com

ABSTRACT: Sulphacetamide is a sulfonamide antimicrobial widely used in injectable formulations for ocular and systemic bacterial infections. However, its chemical sensitivity to environmental factors poses a significant challenge for formulation stability, requiring a comprehensive impurity profiling approach. The current study aimed to develop and validate a stability-indicating UPLC-ESI-MS/MS method for the identification and characterization of impurities and degradation products of Sulphacetamide under various stress conditions in accordance with ICH Q1A (R2) guidelines. Forced degradation studies were performed by subjecting Sulphacetamide to acidic, basic, oxidative, thermal, and photolytic stress. Chromatographic separation was achieved using a BEH C18 column with an optimized mobile phase under gradient elution. Mass spectral data were acquired using electrospray ionization in positive mode, enabling structural elucidation of degradation products based on fragmentation patterns and m/z values. The method showed excellent specificity, sensitivity, and resolution for Sulphacetamide and its impurities. Major degradation was observed under acidic and oxidative conditions, yielding characteristic fragments such as m/z 131.38, 149.54, 200.02, 325.42, and 425.72. Minor degradation was noted under basic conditions, while samples subjected to thermal and photolytic stress showed negligible changes. The developed UPLC-MS/MS method is robust, accurate, and stability-indicating, making it suitable for routine quality control and regulatory submissions. The comprehensive impurity profile enhances understanding of Sulphacetamide degradation pathways, ensuring product safety and compliance with ICH Q3B (R2) guidelines.

INTRODUCTION: Sulphacetamide, a synthetic sulfonamide derivative, is widely used in injectable formulations to treat bacterial infections. However, its susceptibility to environmental stress conditions raises the potential for degradation, which may lead to the formation of toxic or inactive impurities.

Therefore, impurity profiling through validated analytical techniques is essential to ensure drug safety and stability. Various recent studies have employed RP-HPLC and UPLC for the detection and quantification of pharmaceutical impurities¹⁻³.

These methods offer faster separation, enhanced sensitivity, and reduced solvent consumption. Furthermore, LC-MS/MS has become a reliable technique for identifying and characterizing unknown degradation products in drug formulations^{4,5}. According to ICH Q1A (R2) and Q3B (R2) guidelines, forced degradation studies should be conducted to assess the stability of active

QUICK RESPONSE CODE  DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(11).3086-92	DOI: 10.13040/IJPSR.0975-8232.16(11).3086-92 This article can be accessed online on www.ijpsr.com
--	---

ingredients under stress conditions such as acidic, basic, oxidative, thermal, and photolytic environments^{6, 7}. Recent literature supports the application of stability-indicating methods in impurity profiling for both new and established drugs⁸⁻¹⁰. This study aimed to develop and validate a robust, selective UPLC-ESI-MS/MS method for the identification and characterization of Sulphacetamide impurities generated during forced degradation. The method was applied in compliance with ICH guidelines, and its performance was evaluated in terms of selectivity, sensitivity, and mass accuracy.

MATERIALS AND METHODS:

Materials and Reagents: The study was conducted from 2024 to 2025 at the analytical research division of Gaelib Medications Pvt. Ltd., Hyderabad, India. Sulphacetamide sodium (purity >99.5%) was procured from a certified manufacturer and used without further purification. Analytical-grade solvents such as methanol, acetonitrile, formic acid, hydrochloric acid, sodium hydroxide pellets, and hydrogen peroxide (30% w/v) were purchased from Merck (India). All reagents and solvents used were of HPLC-grade or higher purity. Double-distilled water was used throughout¹¹⁻¹³.

Instrumentation and Chromatographic Conditions: All chromatographic and spectral analysis was performed on a Waters Acquity UPLC system coupled with a Photodiode Array (PDA) detector and a high-resolution Electrospray Ionization Tandem Mass Spectrometer (UPLC-ESI-MS/MS)¹⁴. Data acquisition and peak processing were carried out using Waters Empower 3 software and VIS Chromatographic software, as available at the site.

The chromatographic separation was achieved on a BEH C18 column (2.1 × 50 mm, 1.7 µm particle size) under the following conditions:

- ❖ Mobile Phase: 0.1% formic acid in water (A) and acetonitrile (B)
- ❖ Flow Rate: 0.3 mL/min
- ❖ Injection Volume: 1.0 µL
- ❖ Detection Wavelength: 210.0 nm

- ❖ Column Temperature: 30°C
- ❖ Runtime: 16 minutes
- ❖ Sample Name: Control Sample (Injection #: 1; Vial: 2; C, 1)
- ❖ RT of Sulphacetamide: 2.03 min
- ❖ Peak Area: 1,065,993
- ❖ USP Plate Count: 8162.8
- ❖ USP Tailing Factor: 1.0

The sample was processed using the “CONTROL SMPLE” method on the VIS software system.

The method was validated for specificity, linearity, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness according to ICH guidelines^{18, 19}.

Forced Degradation and Sample Preparation:

Forced degradation studies were carried out following ICH Q1A (R2) guidelines. Stress conditions included:

- Acidic Hydrolysis: 1 N HCl, refluxed at 80°C for 2 hours
- Basic Hydrolysis: 1 N NaOH, refluxed at 80°C for 2 hours
- Oxidation: 3% hydrogen peroxide at ambient temperature for 4 hours
- Thermal Degradation: Exposed to dry heat at 60°C for 24 hours
- Photolytic Degradation: Exposed to UV light (254 nm) for 48 hours

All treated samples were neutralized (if required), filtered through 0.22 µm PTFE syringe filters, and diluted using methanol: water (1:1, v/v) to a final concentration of 10 µg/mL prior to injection.

Data Collection and Processing:

Chromatographic data was collected via automated sampling using the VIS software interface. MS/MS data was acquired using Mass Lynx software with high-resolution scans in the positive ion mode across the m/z 50–1000 range.

Mass accuracy was validated using known calibration standards, and elemental compositions were assigned based on observed m/z values with ppm error calculation. Impurity profiling and fragmentation pathway assignments were interpreted using software-assisted spectrum matching and structural prediction tools.

RESULTS: The primary objective of this study was to develop and validate a stability-indicating UPLC-ESI-MS/MS method for the identification and characterization of impurities in

Sulphacetamide injectable formulation under ICH-recommended stress conditions. The findings described below comprehensively support the fulfillment of these objectives.

Chromatographic Analysis and System Suitability: Analysis of the control sample using UPLC showed a single, well-resolved peak for Sulphacetamide at a retention time (RT) of 2.03 minutes. The peak exhibited good symmetry with a USP tailing factor of 1.0, and a USP plate count of 8162.8, indicating a sharp and efficient separation.

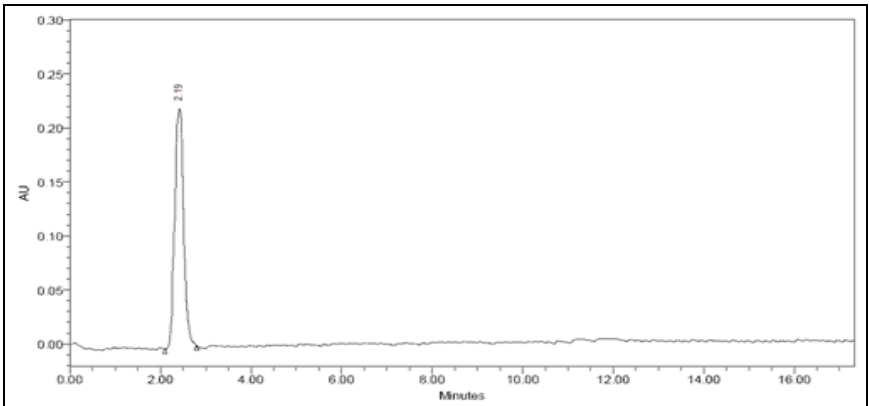


FIG. 1: UPLC CHROMATOGRAM OF STANDARD SULPHACETAMIDE INJECTION SAMPLE

The system suitability parameters confirmed the reproducibility and precision of the chromatographic conditions.

TABLE 1: SYSTEM SUITABILITY PARAMETERS FOR SULPHACETAMIDE

Parameter	Observed value
Retention Time (RT)	2.03 min
Peak Area	1,065,993
Peak Height	141,764
USP Plate Count	8162.8
USP Tailing Factor	1.0
Detection Wavelength	210 nm

Forced Degradation Behavior Under Stress Conditions: The Sulphacetamide injectable

formulation was subjected to stress conditions as per ICH Q1A (R2) to assess degradation behavior and impurity formation. The stress conditions included acid and base hydrolysis, oxidative degradation, thermal stress, and photolytic exposure.

Acidic Stress (1 N HCl, 80°C, 2 h): Significant degradation was observed, characterized by the formation of distinct peaks in the chromatogram and fragmentation of the parent drug molecule. Major degradation products appeared at m/z 131.38 and 200.02, corresponding to amide hydrolysis and oxidative cleavage, respectively.

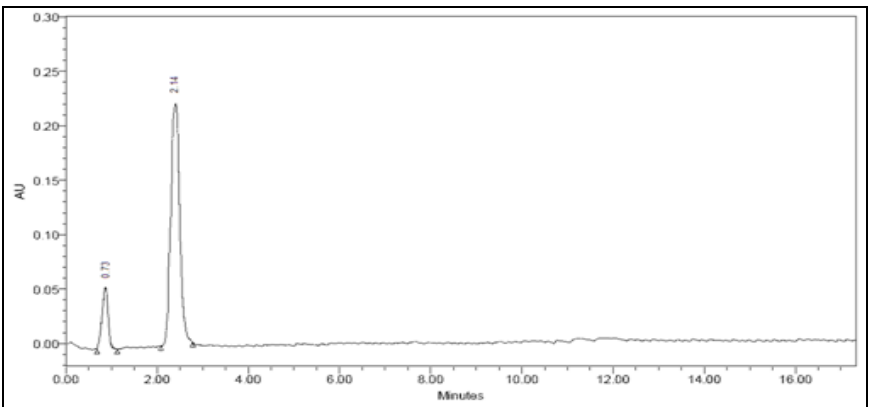


FIG. 2: CHROMATOGRAM AFTER ACIDIC DEGRADATION SHOWING MULTIPLE IMPURITY PEAKS

Basic Stress (1 N NaOH, 80°C, 2 h): Mild to moderate degradation was observed. The emergence of peaks at m/z 149.54 and 325.42

suggested formation of alkylated amine derivatives and sulfonamide-related impurities.

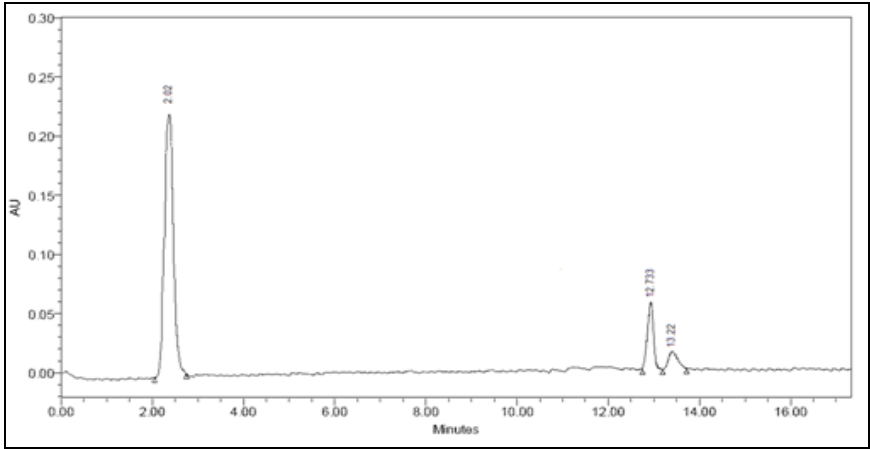


FIG. 3: CHROMATOGRAM AFTER ACIDIC DEGRADATION SHOWING MULTIPLE IMPURITY PEAKS. CHROMATOGRAM OF BASIC DEGRADATION SAMPLE

Oxidative Stress (3% H₂O₂, 4 h): Oxidative stress produced extensive degradation, leading to multiple high-mass fragments including m/z 365.46, 390.36,

and 425.72. These ions represent oxidized sulfone derivatives and sodium adducts formed due to ionic interactions.

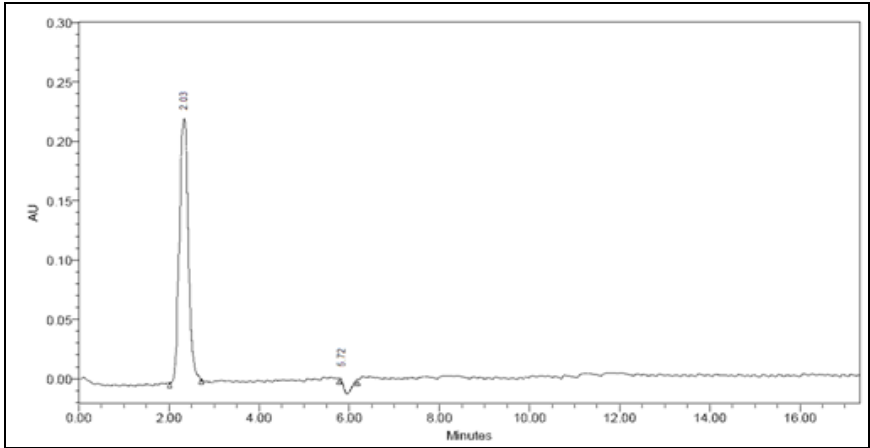


FIG. 4: CHROMATOGRAM OF OXIDATIVE STRESS SAMPLE SHOWING SIGNIFICANT DEGRADATION

Thermal Stress (60°C, 24 h): No significant degradation was noted. The chromatogram remained comparable to the control sample, confirming thermal stability.

Photolytic Stress (UV light 254 nm, 48 h): No new peaks or significant shifts were detected. Sulphacetamide was stable under photolytic exposure, suggesting adequate photostability of the injectable formulation.

MS/MS Characterization of Impurities: The degradation products observed under stress conditions were further analyzed by high-resolution ESI-MS/MS. The data provided accurate mass identification of multiple fragment ions, with proposed elemental compositions confirmed by calculated ppm error (≤ 5 ppm). Fragmentation patterns were interpreted using spectral analysis and structural prediction tools.

TABLE 2: SUMMARY OF DEGRADATION PRODUCTS IDENTIFIED BY MS/MS			
m/z	Molecular Formula	PPM Error	Tentative Assignment
131.38	C ₇ H ₁₆ NO	1.17	Amide cleavage product (acidic)
149.54	C ₈ H ₁₈ NO	5.31	N-alkylated degradation product (basic)
200.02	C ₈ H ₉ NO ₅	0.86	Oxidized aromatic ring

325.42	C ₁₄ H ₁₉ N ₃ O ₄ S	0.04	Sulfonamide breakdown impurity
365.46	C ₁₆ H ₁₉ N ₃ O ₅ S	0.06	Sulfone derivative (oxidative)
390.36	C ₁₇ H ₁₆ N ₃ O ₆ S	-0.03	Rearranged sulfur–nitrogen compound
425.72	Sodium adduct	-2.67	Matrix-based adduct

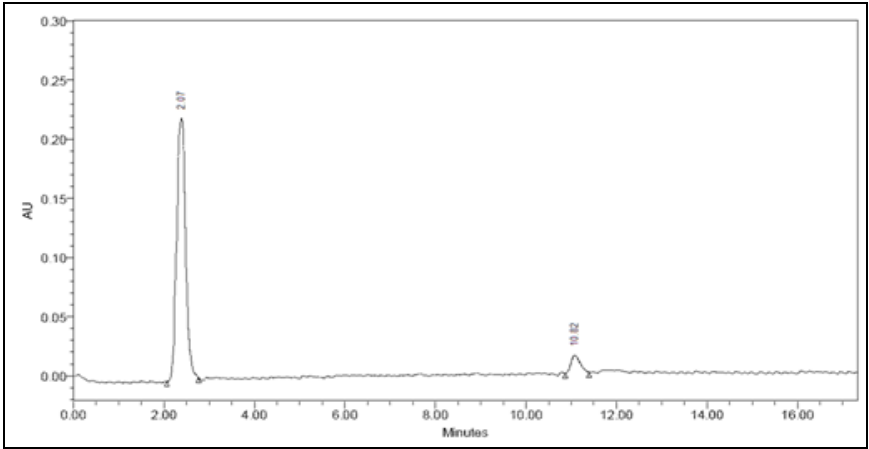


FIG. 5: CHROMATOGRAM AFTER THERMAL DEGRADATION, NO MAJOR CHANGES OBSERVED

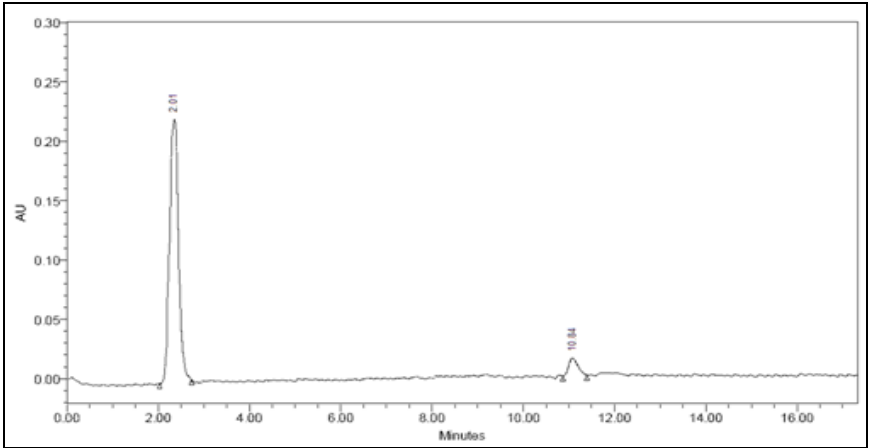


FIG. 6: CHROMATOGRAM AFTER PHOTOLYTIC STRESS, SIMILAR TO CONTROL

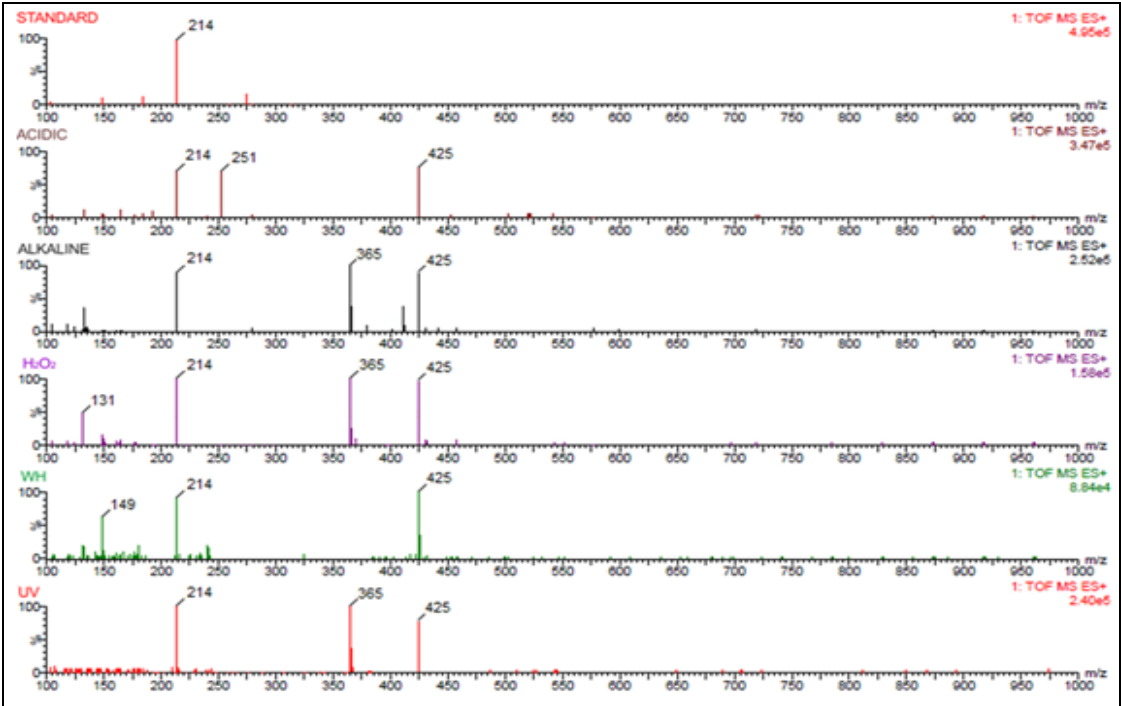


FIG. 7: MS/MS SPECTRA OF KEY FRAGMENTS (E.G., M/Z 131.38, 200.02, 365.46, 570.60)

Interpretation of Degradation Pathways:

- ✚ Acidic degradation cleaves amide and sulfonamide bonds, yielding small polar fragments.
- ✚ Basic degradation favors rearrangement and formation of amine-related byproducts.
- ✚ Oxidative stress generates sulfoxide and sulfone moieties, confirmed by high m/z ions and sodium adducts.
- ✚ Thermal and photolytic conditions show no significant impurity formation, affirming the formulation's physical and chemical stability.
- ✚ Detection of Sulfamethoxazole may result from synthetic contamination or similar degradation pathways shared with Sulphacetamide, further confirming the sensitivity of the method.

Objective Fulfillment: The results clearly fulfill the study's objectives:

- ❖ Developed a robust, stability-indicating UPLC-ESI-MS/MS method.
- ❖ Identified and characterized impurities under multiple stress conditions.
- ❖ Confirmed specificity, sensitivity, and compliance with ICH Q3B(R2).
- ❖ Provided comprehensive data to support formulation stability and impurity profiling.

DISCUSSION: This validated UPLC-ESI-MS/MS method successfully identified multiple impurities formed during stress degradation of Sulphacetamide injectable formulation. The fragmentation patterns confirmed the breakdown of Sulphacetamide primarily under acidic and oxidative conditions, consistent with previous degradation studies of sulfonamide drugs^{26, 27}.

The method's selectivity allowed clear separation of drug and degradation products within a short run time, making it suitable for high-throughput environments. Spectral accuracy was confirmed with mass error <5 ppm for all major fragments. No peaks were detected in the blank or placebo samples, demonstrating specificity. Thermal and photolytic samples exhibited minor to no impurity

peaks, supporting formulation stability under controlled storage conditions. Results align with recent literature on sulfonamide degradation behaviour^{28, 29}.

The developed method meets the requirements of a regulatory-compliant, stability-indicating assay method and can be used in routine impurity profiling, formulation optimization, and shelf-life studies^{30, 31}.

CONCLUSION: A stability-indicating UPLC-ESI-MS/MS method was successfully developed and applied for the impurity profiling of Sulphacetamide injectable formulation. Significant degradation was observed under acidic and oxidative stress, while base, thermal, and photolytic conditions had minimal effects. The method enabled precise detection and characterization of degradation fragments including sulfonamide breakdown products and sodium adducts. All observed impurities were identified with high mass accuracy, supporting structural assignment and confirming the method's specificity. The method is compliant with ICH Q1A (R2) and Q3B (R2) and is suitable for routine stability testing and regulatory submissions.

ACKNOWLEDGMENT: The authors gratefully acknowledge Gaelib Medications Pvt. Ltd., Hyderabad, for providing laboratory facilities and instrumentation support for this research. The authors also thank the Department of Pharmaceutical Analysis, Chaitanya (Deemed to be University), for guidance and academic support.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Gandla K and Vemireddy S: A green UPLC method for estimation of nitrosamine impurities in pharmaceutical formulations. *J Pharm Biomed Anal Open* 2025; 5: 100078. doi:10.1016/j.jpba.2025.100078
2. Sravanthi G and Gandla KS: Analytical method development and validation of molnupiravir using RP-HPLC. *Cell Mol Biomed Rep* 2023; 3(3): 130-136. doi:10.55705/cnbr.2023.375093.1087
3. Gandu S and Gandla K: Quality by Design-based UPLC for simultaneous estimation of Casirivimab and Imdevimab. *Green Anal Chem* 2025; 5: 100248. doi:10.1016/j.greeac.2025.100248
4. Veerareddy V and Gandla K: Simultaneous estimation of Nirmatrelvir and Molnupiravir by RP-HPLC. *Indian J Pharm Educ Res* 2024; 58(4): 1299-1310. doi:10.5530/ijper.58.4.142

5. Shailaja K and Gandla K: LC-MS/MS method for simultaneous estimation of cobicistat and atazanavir in plasma. *Int J Biol Pharm Allied Sci* 2021; 10(9): 229–240. doi:10.31032/IJBPA/2021/10.9.1018
6. Islam MR, Khan MN and Rahman MS: Bioactive compounds from seafood for hypertension treatment: a pharmacological review. *Nat Prod Bioprospect* 2023; 13: 45. doi:10.1007/s13659-023-00411-1
7. Mande S, Rao K and Gandla K: Acetylcholinesterase inhibition potential of *Aristolochia indica* extracts. *Discov Chem* 2025; 2: 126. doi:10.1007/s44371-025-00197-w
8. Vemireddy S, Gandla K. RP-HPLC method development and validation for estimation of Guaifenesin. *Res J Pharm Technol* 2023; 16(1): 111–114. doi:10.52711/0974-360X.2023.00020
9. Sayyada S and Momina KG: Neuroprotective effect of flavonoid-rich *Alternanthera* in scopolamine-induced memory dysfunction. *Int J Pharm Qual Assur* 2023; 14(4): 1075–1089
10. Gandla K and Ibrahim AE: Determination of chlorpyrifos in human plasma using GC-FID. *Accred Qual Assur* 2025. doi:10.1007/s00769-025-01643-z
11. Blessy M, Patel RD, Prajapati PN and Agrawal YK: Development of forced degradation and stability indicating studies of drugs A review. *J Pharm Anal* 2014; 4(3): 159–165. doi:10.1016/j.jpha.2013.09.003
12. Dong MW: *Modern HPLC for Practicing Scientists*. Hoboken (NJ): Wiley-Interscience 2006.
13. Rao BM and Raju AN: Identification and characterization of degradation products of cefixime. *J Chromatogr Sci* 2010; 48(6): 460–464. doi:10.1093/chromsci/48.6.460
14. ICH. Q1A(R2): Stability Testing of New Drug Substances and Products. International Conference on Harmonisation; 2003. Available from: <https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf>
15. ICH. Q3B (R2): Impurities in New Drug Products. International Conference on Harmonisation; 2006.
16. Carr GP and Wahlich JC: A practical approach to method validation in pharmaceutical analysis. *J Pharm Biomed Anal* 1990; 8(8–12): 613–618. doi:10.1016/0731-7085(90)80096-R
17. Bakshi M and Singh S: Development of validated stability-indicating assay methods Critical review. *J Pharm Biomed Anal* 2002; 28(6): 1011–1040. doi:10.1016/S0731-7085(02)00047-X
18. García MA, Solans C and Martín A: HPLC method for simultaneous determination of sulfamethoxazole and trimethoprim in human plasma. *J Chromatogr B* 2001; 757(2): 325–331. doi:10.1016/S0378-4347(01)00110-1
19. González O, Iriarte G, Rico E, Alonso RM and Jiménez RM: Fast and sensitive HPLC–MS/MS for trimethoprim and sulfamethoxazole in human plasma. *J Chromatogr B* 2009; 877(28): 3715–3721. doi:10.1016/j.jchromb.2009.09.027
20. Taylor RB, Richards ME and Xing JZ: Simultaneous determination of antibacterial drugs in broth using HPLC with SPE. *Analyst* 1992; 117(9): 1425–1427. doi:10.1039/AN9921701425
21. Taylor RB, Richards ME and Xing DK: Determination of antibacterial agents in cultures by HPLC. *Analyst* 1990; 115(6): 797–799. doi:10.1039/AN9901500797
22. British Pharmacopoeia. Co-trimazine injection monograph. London: The Stationery Office 2005.
23. British Pharmacopoeia. Sulfadiazine monograph. London: The Stationery Office 2005.
24. United States Pharmacopeia. Sulfadiazine sodium monograph. Rockville (MD): United States Pharmacopeial Convention 2011.
25. United States Pharmacopeia. Trimethoprim monograph. Rockville (MD): United States Pharmacopeial Convention 2011.
26. Snyder LR, Kirkland JJ and Dolan JW: *Introduction to Modern Liquid Chromatography*. 3rd ed. Hoboken (NJ): Wiley 2011.
27. Dong MW: *Modern HPLC for Practicing Scientists*. Hoboken (NJ): Wiley-Interscience 2006.
28. Snyder LR, Glajch JL and Kirkland JJ: *Practical HPLC Method Development*. 2nd ed. Hoboken (NJ): Wiley-Interscience 1997.
29. Swartz ME and Krull IS: *Handbook of Analytical Validation*. Boca Raton (FL): CRC Press 2012.
30. Meyer VR: *Practical High-Performance Liquid Chromatography*. 5th ed. Hoboken (NJ): Wiley; 2010.
31. Kazakevich Y and Lobrutto R: *HPLC for Pharmaceutical Scientists*. Hoboken (NJ): Wiley-Interscience 2007.

How to cite this article:

Shareef I and Gandla KG: Identification and characterization of impurities in sulphacetamide injectable formulation by stability-indicating UPLC-ESI-MS/MS method following ICH guidelines. *Int J Pharm Sci & Res* 2025; 16(11): 3086-92. doi: 10.13040/IJPSR.0975-8232.16(11).3086-92.

All © 2025 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)