IJPSR (2025), Volume 16, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 September 2024; received in revised form, 12 October 2024; accepted, 25 October 2024; published 01 February 2025

METHOD DEVELOPMENT AND MODEST VALIDATION OF SULFADIAZINE BY REVERSE PHASE HPLC

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Keywords:

Sulfadiazine, Antibiotic, Reverse Phase (RP) High Performance Liquid Chromatography, C18 Column, Validation, Accuracy, Precision, System suitability, Robustness

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ABSTRACT: Sulfadiazine belongs to a class of sulphonamide antibiotics. Sulfadiazine is a synthetic bacteriostatic sulphonamide that exhibits a broad spectrum of activity against gram-positive and gram-negative pathogens. The Sulfadiazine antibiotic is a member of the Sulfa drugs class. This drug is utilized for bacterial infections in the brain, ears, and urinary tract. The primary goal of the research is to develop and validate Sulfadiazine by Reverse Phase (RP) HPLC. An HPLC (Alliance, Water 2695) with a PDA detector and Avantor -ACE C18 250 mm × 4.6 mm × 5 µm column (Part Number ACE 121-2546) was used. A novel technique for the simultaneous measurement of sulfadiazine using the RP-HPLC technology was developed. The ACE C18 column (250x4.6 mm) 5µm successfully created the chromatographic conditions needed to separate sulfadiazine. The flow rate was 1 mL/min, and the mobile phase ratio of ACN: H2O was 70:30 v/v. 254 nm was the detection wavelength.

INTRODUCTION: Sulfadiazine is an antibiotic used to treat various infections caused by bacteria. Sulfadiazine works by inhibiting folic acid synthesis in bacteria by blocking the Folic Acid pathway. Sulfadiazine interferes with an enzyme called dihydropteroate synthase, which is essential for folic acid production. Sulfadiazine competes with para-aminobenzoic acid (PABA), a precursor of folic acid. By mimicking PABA, it prevents the formation of dihydrofolic acid, disrupting the folic acid pathway. Sulfadiazine doesn't kill bacteria directly but slows their growth. This allows the immune system to clear the infection more effectively.

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DOI: 10.13040/IJPSR.0975-8232.16(2).475-80

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(2).475-80

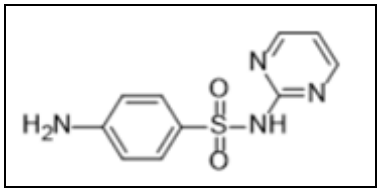

FIG. 1: MOLECULAR STRUCTURE OF SULFADIAZINE

The chemical formula of Sulfadiazine is C10H10N4O2S. Its molecular weight is 250.27 g/mol. Its IUPAC name is 4-amino-N- -2-pyrimidinyl-benzenesulfonamide. Its generic names are Sulfadiazine, Sulfapyrimidine, Pyrimal, and Debenal. It is white or slightly yellow powder. It is stable in the air and odorless. However, it gradually darkens when exposed to light.

MATERIALS AND METHODS:

Chemicals and Reagents: Sulfadiazine, Water (HPLC grade), Acetonitrile (HPLC grade), Propanol.

Instrumentation: An HPLC (Alliance, Water 2695) with a PDA detector and ACE C18 (250 mm × 4.6 mm × 5 µm) column was used and successfully created the chromatographic conditions to separate sulfadiazine analyte.

Chromatographic Condition: Mobile phase acetonitrile: water 70:30 (v/v) was used, and acetonitrile: propanol 50:50 ratio was used as diluent. The flow rate was 1.0 mL/min. UV detection 254 nm was selected as the wavelength for analysis as the drug showed good absorbance.

Preparation of Mobile Phase: A mixture of Acetonitrile 700 mL and 300 mL of water 70:30 (v/v) was prepared and degassed in an ultrasonic water bath for 5 minutes. It was then filtered through a 0.45 µ filter under vacuum filtration.

Preparation of Diluent: Acetonitrile and propanol 50:50 (v/v) prepared and degassed in an ultrasonic water bath for 5 minutes. It was then filtered through a 0.45 µ filter under vacuum filtration.

Preparation of Standard Solution: The main stock of 1000 ppm was prepared, weighed 5 mg of sulfadiazine, and transferred into a 5 ml volumetric flask, adding 5 ml of diluent.

Preparation of Standard Working Solutions (100% Solution): Taken 200 µL of standard solution was diluted with 2 ml of mobile phase and was mixed well.

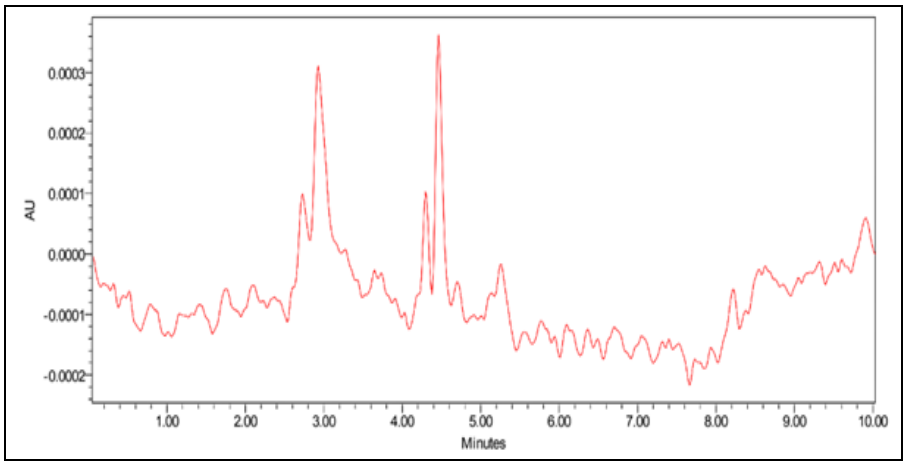

FIG. 2: METHOD CHROMATOGRAM REPRESENTING BLANK SOLUTION

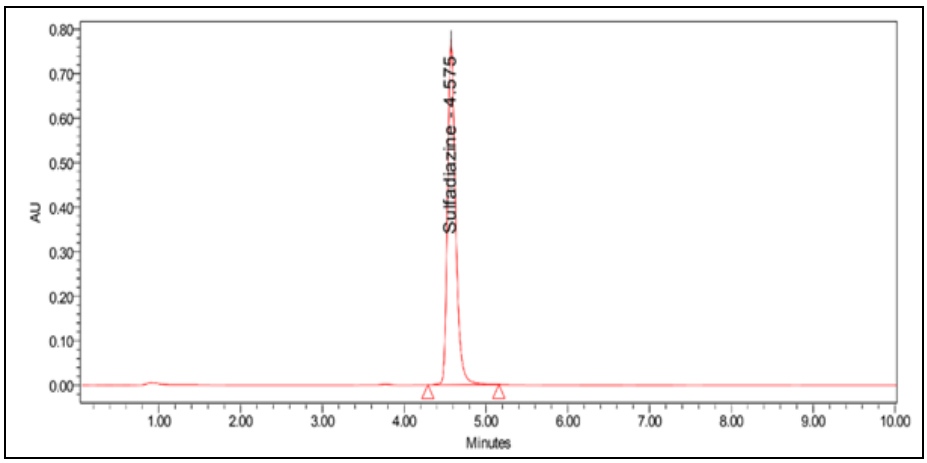

FIG. 3: METHOD CHROMATOGRAM REPRESENTING SULFADIAZINE RT AT 4.57 MIN

Method Validation Report:

System Suitability: A system suitability test was conducted to ensure that the equipment would be reproducible for the required validation. A 100-ppm prepared standard solution was used for the test and injected five times. Calculations was made for the system suitability parameters such as peak asymmetry, theoretical plates, and retention durations.

The results obtained for the system suitability are based on ICH Q2 (R2) guidelines. The %RSD, plate count, and tailing factor were found to be ≤2, ≥2000, and ≤2, respectively **Table 1.**

TABLE 1: SYSTEM SUITABILITY TEST FOR SULFADIAZINE

System Suitability Data						
Replicate	**Retention Time (min)**	**Area count**	**%Area**	**Height (UV)**	**USP Tailing**	**USP Plate Count**
1	4.57	5160172	100	769130	1.26	11181
2	4.57	5223580	100	769237	1.28	10841
3	4.57	5197388	100	769518	1.27	11108
4	4.57	5217422	100	762763	1.3	11024
5	4.57	5197921	100	760854	1.28	10827

Average	**%RSD**	**Coefficient Value**	**RSD**
5199297	24771.37	0.5	0.5

Linearity: Peak area *vs.* Sulphadiazine concentrations were plotted to create a calibration curve and the regression equations are computed. The calibration curve was plotted for the 50%-150% range of sulphadiazine. From the graph, the correlation coefficient, residual sum of squares, v-intercept, and slope of the regression line were determined. The R2 value for linearity concentration ranges of Sulfadiazine from 50-150ppm was found to be 0.999 **Table 2 and Fig. 9**, which is in the range as per ICH guidelines.

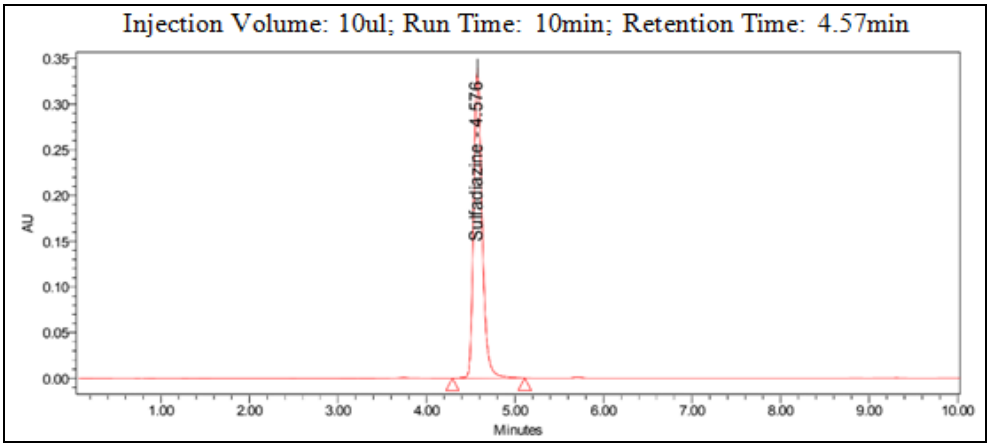

FIG. 4: LINEARITY DETERMINATION OF 50% STANDARD

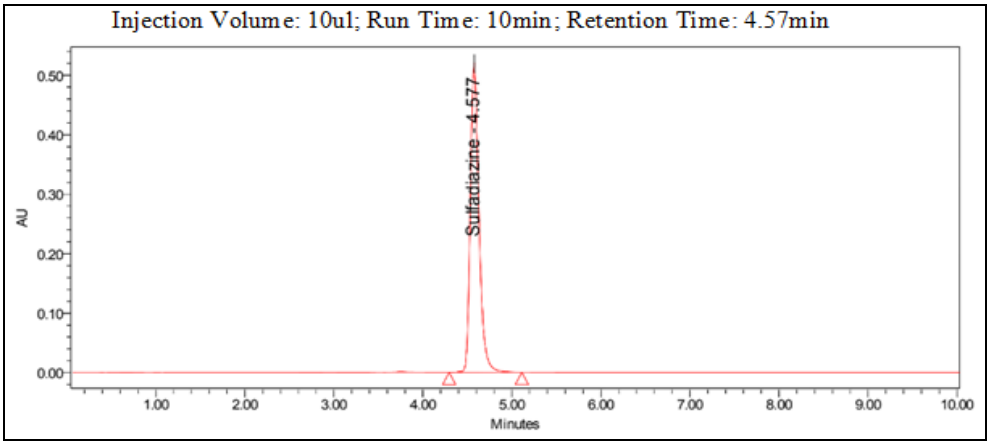

FIG. 5: LINEARITY DETERMINATION OF 80% STANDARD

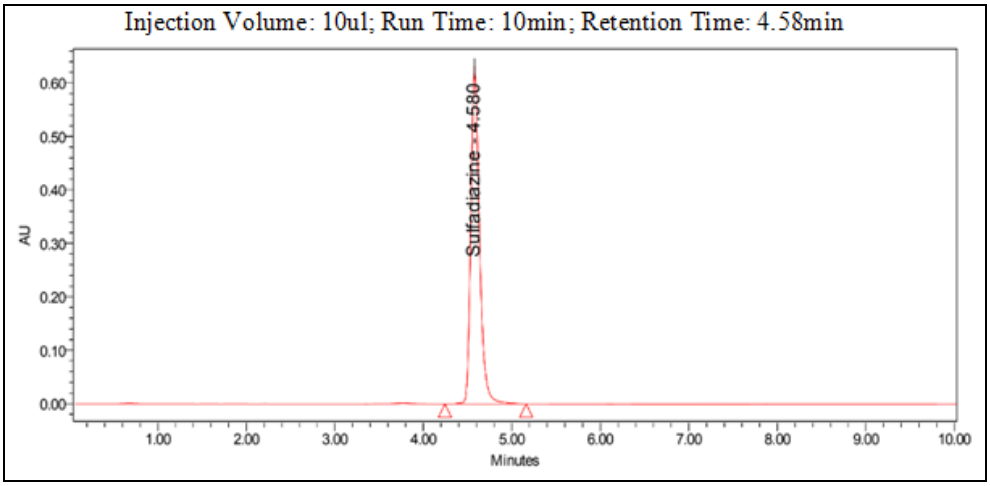

FIG. 6: LINEARITY DETERMINATION OF 100% STANDARD

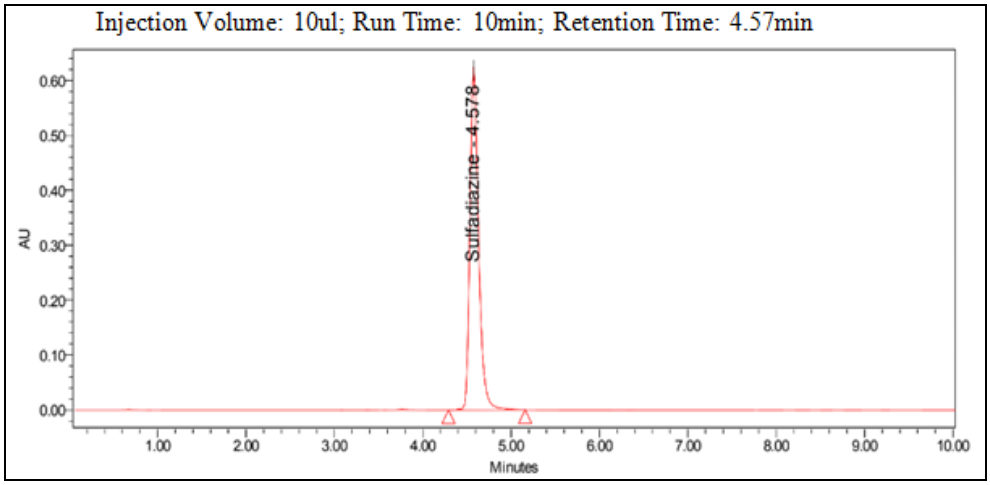

FIG. 7: LINEARITY DETERMINATION OF 120% STANDARD

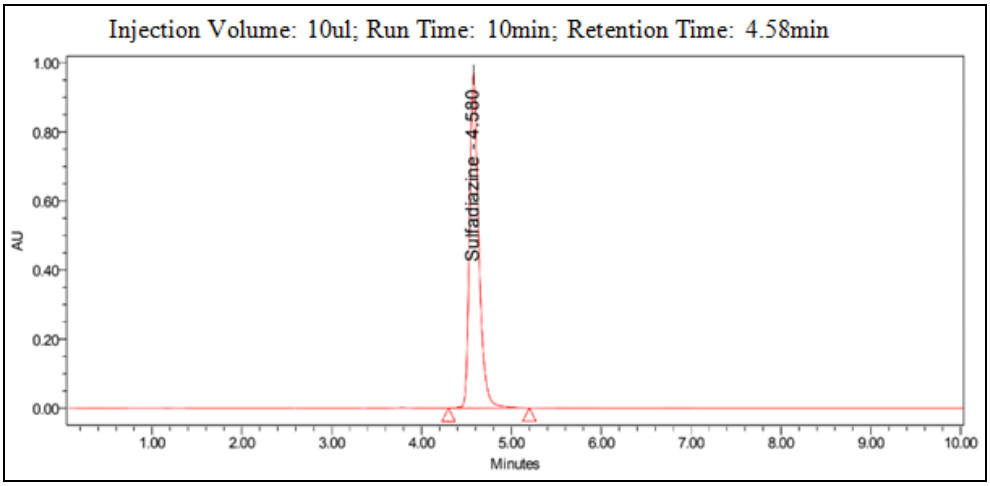

FIG. 8: LINEARITY DETERMINATION OF 150% STANDARD

TABLE 2: LINEARITY TEST FOR SULFADIAZINE

Level (%)	Area	Retention Time	%Area	Height	Intensity
50	548030	2.96	100	53595	0.215
80	821889	2.98	100	76615	0.301
100	945876	3.01	100	81860	0.314
120	1164751	3.03	100	97839	0.390
150	1448431	3.01	100	115188	0.460

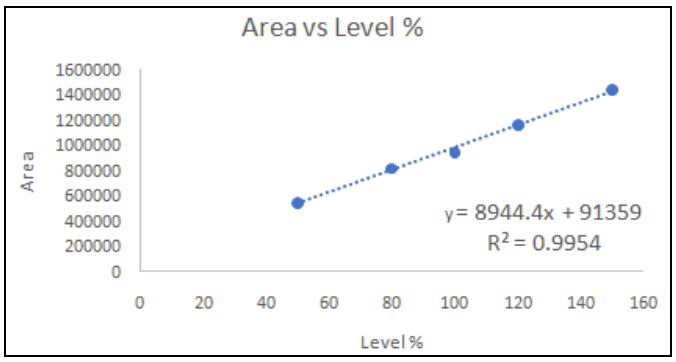

FIG. 9: AREA VS % LEVEL GRAPH FOR DETERMINATION OF LINEARITY

Accuracy: The standard levels of 80 and 120% were spiked in triplicate, and the standard 100% was spiked six times. Retention time, area, average, standard deviation, and % RSD was found.

TABLE 3: ACCURACY TEST FOR 80% (ACN: H_2O)

Rt	Area
2.97	906854
2.97	911300
2.97	911738

Average	Standard Deviation	%RSD
909964	2702.228	0.296

TABLE 4: ACCURACY TEST FOR 100% (ACN: H_2O)

Retention Time	Area
2.99	1083182
3.01	1078160
2.90	1079196
3.05	1074062
3.03	1077240
3.02	1076339

Average	Standard Deviation	%RSD
1078030	3070.771	0.284

TABLE 5: ACCURACY TEST FOR 120% (ACN: H_2O)

Retention Time	Area
3.03	1332580
3.03	1333969
3.03	1337203

Average	Standard Deviation	%RSD
1334584	2372.067	0.1777

Precision: Standard and sample were injected 6 times respectively. Retention time, area, average, standard deviation and %RSD were found.

TABLE 6: PRECISION TEST FOR 100% STD SULFADIAZINE

Retention Time	Area
3	1070548
3	1063675
3	1067256
3	1070924
3	1077175
3	1064364

Average	Standard Deviation	%RSD
1068990	5016.329	0.469

Robustness: The study assessed the robustness of Sulfadiazine against fluctuations in flow rate from 0.4 mL/min to 0.6 mL/min and mobile phase ratio from more organic phase to less organic phase ratio. The flow rate variance greatly impacted the procedure.

		System Suitability Results	
S. no.	Change in organic composition in mobile phase	USP Plate Count	USP Tailing
1	10% less	6069	1.13
2	Actual	11108	1.28
3	10% more	448	1.26

CONCLUSION: The current RP HPLC method was inexpensive for the analysis of sulfadiazine. It was accurate, precise, sensitive, specific, robust, and repeatable. ACE C18 column (4.6×250mm) 5µ was used, 1 mL/min was the flow rate, 70:30 (v/v) acetonitrile: water was used as mobile phase and 254 nm detection wavelength was used.

ACKNOWLEDGMENT: We acknowledge GITAM School of Pharmacy (deemed to be University) and RIKI GLOBAL for providing us all the necessary resources for the article.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Srikanth G, Phanindra VS, Sultana S, Vadayar KP, Akshitha GL and Kumar KN: Method development and modest validation of sulfadiazine by reverse phase HPLC. Int J Pharm Sci & Res 2025; 16(2): 475-80. doi: 10.13040/IJPSR.0975-8232.16(2).475-80.