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RESEARCH ARTICLE

New RP-HPLC Method Development and Validation of Sulfapyridine in Pure and Tablet Dosage Forms

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ABSTRACT

The objective of this work was to develop a simple, selective, stability indicating RP-HPLC method for the determination of sulfapyridine in pure and in tablet dosage form. The proposed RP-HPLC method was validated in compliance with International Conference on Harmonization guidelines. The mobile phase consists a mixture of Acetonitrile: water: 1.0 % ortho phosphoric acid in the ratio of 70:27:3 v/v/v. Column used ODS, C_{18} RP- COLUMN (4.6mmx250mm) having 5µm particle size. The flow rate was 1.0 mL/min and detection was carried out by UV- visible spectrophotometer and was observed that the maximum absorbance (λmax) was obtained at 256nm, retention time 4.40mins. The proposed method has permitted the quantification of sulfapyridine over linearity in the range of 5-30µg/mL and its percentage recovery was found to be 99.99%. The proposed RP-HPLC method of sulfapyridine was also found to be robust and rugged as there was no significant change in the peak area, peak shape and retention time. On the basis of above facts it is concluded that "the developed RP-HPLC method was found to be easily applicable and is expected to be widely used for the routine QC analysis of sulfapyridine in the pharmaceutical industry".

KEYWORDS

Sulfapyridine, Method Development, Validation, RP-HPLC

INTRODUCTION

Sulfapyridine[1] [Figure 1] is a sulfonamide antibiotic used for the treatment of herpetiformis, benign mucous membrane pemphigoid and pyoderma gangrenosum dermatitis. Sulfapyridine is a competitive inhibitor of the bacterial enzyme dihydropteroate synthetase. This inhibited reaction is necessary in these organisms for the synthesis of folic acid by means of processing the substrate para-aminobenzoic acid (PABA) as such DNA molecules cannot be built and bacterial cell cannot undergo multiplication and die.

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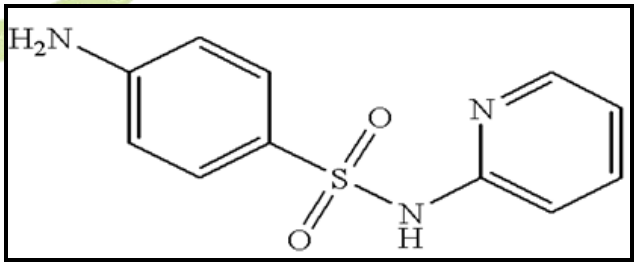

Figure 1: Structure of Sulfapyridine

It is marketed as Dagenan (manufactured by Poshchem labs. Hyderabad) available as oral dosage forms containing sulfapyridine of concentration 500mg. Its chemical name is (±)1(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). Sulfapyridine is a white crystalline powder with a molecular weight of 249.298. It is freely soluble

in DMSO, dilute mineral acids; sparingly soluble in ethanol and is practically insoluble in water. So far, to our knowledge, very analytical methods have been reported for the determination of sulfapyridine in pharmaceutical dosage forms. This prompted to develop determination of sulfapyridine in pharmaceutical dosage forms. This prompted the author to develop accurate and inexpensive RP-HPLC sensitive methods that can be considered for routine determination of sulfapyridine in pure and tablet dosage forms.

The present study describes in detail the development and validation of RP-HPLC method for the assay of sulfapyridine in pure and pharmaceutical formulations. In the present study the author attempted and developed a simple, selective stability indicating RP-HPLC method for the determination of sulfapyridine in pure and in tablet formulations as High-performance liquid chromatography (HPLC) is considered to be the most efficient technique in the field of pharmaceutical analysis because of its reliability, reproducibility and speed of analysis.

The present work describes a simple sensitive RP-HPLC method for determination of sulfapyridine in pure and in tablet dosage form. The proposed RP-HPLC method was validated in compliance with International Conference on Harmonization guidelines.

MATERIAL AND METHODS

Chemicals and Materials

The pharmaceutical grade pure sample of sulfapyridine was procured from Poshchem Laboratories limited, Hyderabad, Telangana state. Dosage formulation in the brand name of DAGENAN tablets (each 500mg tablet contains 500 mg of sulfapyridine) was purchased from local pharmacy. Acetonitrile (HPLC grade), Orthophosphoric acid (AR grade) and Dimethyl sulfoxide (DMSO) was procured from E Merck Ltd and Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system, sonicated and used.

Equipment and Apparatus

Shimadzu (LC 8200AHT) isocratic HPLC system equipped with isocratic liquid pump and UV- Visible spectrophotometric detector was used for the analysis. The data was recorded using window based single channel software. The purity determination performed on a stainless steel column 250 mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5µm diameter (Use ODS, C18, 5µ, 250×4.6mm i.d). A downer electronic balance was used for weighing the materials.

Preparation of Mobile Phase

The mobile phase in the present assay is prepared by dissolving Acetonitrile: water: 1.0 % ortho phosphoric acid in the ratio of 70:27:3 v/v/v. This Mobile phase is filtered and degassed prior to the assay.

Preparation of Diluent

Mobile phase is used as diluent in the present assay.

Preparation and Assay of Standard Stock Solution

An accurately weighted sample of 100mg of sulfapyridine was dissolved in methanol to give standard stock solution of 100µg/mL. A series of working standard solutions of sulfapyridine in concentration range 5.0µg/mL - 25µg/mL were obtained by diluting the aliquots of stock solution with the same diluent. All the above volumetric flasks of working standard solutions were wrapped with aluminium foil and stored in the

An average of ten tablets of Dagenan tablets (each tablet contains 500 mg of sulfapyridine) were weighed and ground to fine powder. Accurately weighed powder sample equivalent to 10mg of sulfapyridine was dissolved in a 100mL volumetric flask containing 10mL of Dimethylsulfoxide (DMSO). The flask was placed in an ultrasonic bath at room dark

Procedure for Analysis of Tablet Formulation[2]

Temperature for 10 min after sonication, the solution in the flask was diluted to the mark with

diluent. A sample of 20µL of this solution was directly injected and the respective chromatogram is represented in Figure 7. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

Method Development[2-10]

A systematic study of the effect of various factors [i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters] was carried out by varying one parameter at a time and keeping all other conditions constant in developing the present RP-HPLC method for sulfapyridine. From these studies it was revealed that in the current study ODS, C18 RP-Column (4.6mmx250mm) column having 5µm particle size was used among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing.

A good symmetrical peak for sulfapyridine was obtained, when acetonitrile and water was added with 0.1% orthophosphoric acid in mobile phase. During these studies preliminary trials on mobile phase proportion were carried to provide good resolution for sulfapyridine using different compositions of mobile phase. From these trails the proportion of Acetonitrile: water: 1.0 % ortho phosphoric acid in the ratio of 70:27:3 v/v/v was finalized as it gave good symmetrical peak for sulfapyridine. The appropriate wavelength for determination of sulfapyridine was scanned by UV-visible spectrophotometer and was observed that the maximum absorbance (λmax) was obtained at 256nm. At this wavelength sulfapyridine offered high response with good linearity. The best separation with adequate resolution and symmetric peak of sulfapyridine was obtained when the injection volume was fixed to 20µL with a flow rate was set to 1.0ml/min for the mobile phase respectively. On this finalized chromatographic conditions, obtained chromatogram of sulfapyridine exhibited good peak symmetry with higher theoretical plates and elution time of 4.40min. respectively. The optimized chromatographic conditions and the representative chromatogram of sulfapyridine so obtained are represented in Table 1; and in Figure 3.

Method Validation[2-10]

System Suitability

The present HPLC system was equilibrated initially with the above said mobile phase, followed by six injections of the same standard solutions of sulfapyridine were used to evaluate the system suitability. Parameters of system suitability studies include the peak symmetry (symmetry factor), no of theoretical plates of the column, resolution, mass distribution ratio (capacity factor) and relative retention and the results of these studies were summarized in Table 2. It was observed that all the values are within the limits making the proposed method acceptable for the assay of sulfapyridine in dosage forms as reported in Table 2.

Range and Linearity

In the present study the linearity studies were carried out at concentration levels corresponding to 10µg/mL, 15µg/mL, 20µg/mL, 25µg/mL and 30µg/mL test solution of sulfapyridine were prepared separately and 20µL of each concentration was injected into the above said HPLC system and the response was read and the corresponding chromatograms at each concentration level were recorded Figure 4 (1-5). From these chromatograms a calibration curve was constructed by plotting the peak areas of the drug versus concentration of sulfapyridine Figure 5.

The linear regression equation for the calibration curve of sulfapyridine was found to be Y= 33379x - 4241.3 with a coefficient of regression r^2=0.9992 respectively. The chromatograms of sulfapyridine obtained during linearity study were shown in Figure 4(1-5) and the calibrated results of sulfapyridine were tabulated in Table 2, 3 respectively.

The obtained response was linear which apparently revealed the capability of proposed

RP-HPLC method to reproduce/repeat the results within the linear range, consistently meeting the standard norms for method validation (ICH guidelines).

Limits of Detection and Quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The calculated LOD and LOQ values were 0.840 and 2.80µg/ml, respectively indicating the adequate sensitivity of the method for sulfapyridine in pure and formulations.

Precision

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The precision of the present method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in Table 4.

Accuracy (Recovery Studies)

The accuracy of the proposed method was established by carrying out recovery experiments through standard addition technique. For this purpose, pure sulfapyridine was spiked to the pre analyzed formulation.

The total amount of sulfapyridine was once again found by the proposed method. The recovery of the sulfapyridine was calculated and the results are presented in Table 5.

These results showed that the common excipients present in tablets did not interfere in the assay of sulfapyridine by the proposed method revealing that the proposed method was found to be accurate. The validated chromatograms and the results of accuracy given in Figure 6(a-c) and in Table 5.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010AHT) and Water‘s Breeze HPLC by different operators. Robustness of the method was determined by making slight changes in the chromatographic conditions (i.e, Change in flow rate and column temperature) and the results are given in Table 6, 7. The developed RP-HPLC method was found to be robust and rugged as the peak area of sulfapyridine was not apparently affected by small variation in the chromatographic conditions.

Determination of Sulfapyridine in Formulations

The validated RP-HPLC method was applied to the determination of sulfapyridine in tablets [DAGENAN; 500mg]. 20µL of sample solution of sulfapyridine was injected into the injector of liquid chromatograph system. The retention time was found to be 4.51min. for sulfapyridine. The amount of sulfapyridine present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Figure 7; and in Table 8 respectively.

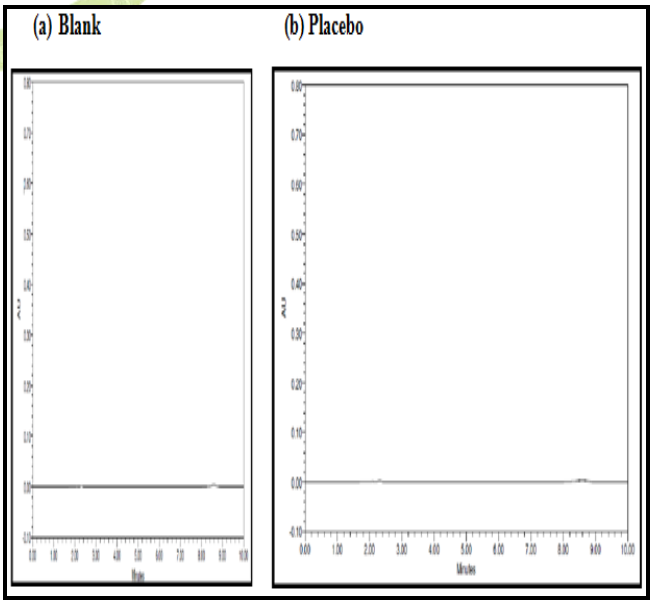

Figure 2: (a) & (b) Chromatogram for the Blank & Placebo

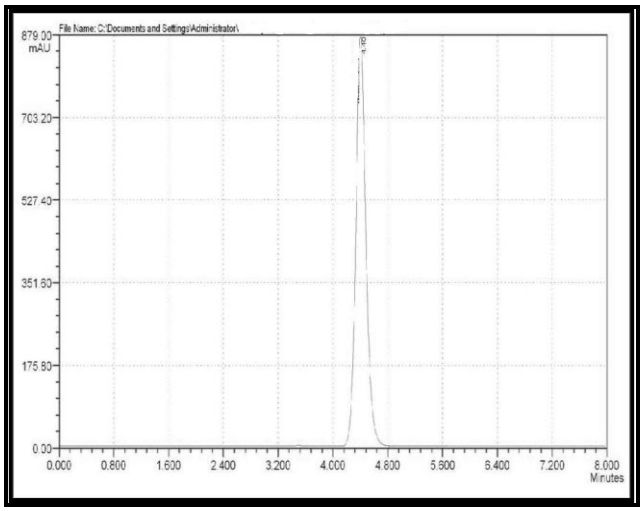

Figure 3: Validative Chromatogram of Sulfapyridine

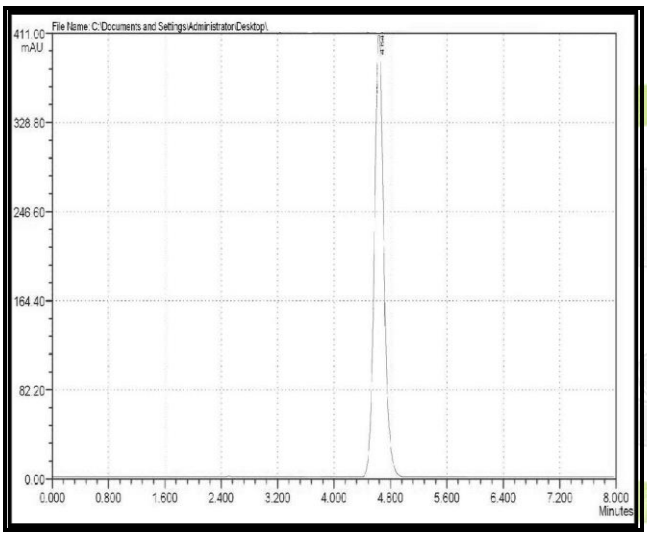

Figure 4.1: Linearity Chromatogram of Sulfapyridine (10µg/mL)

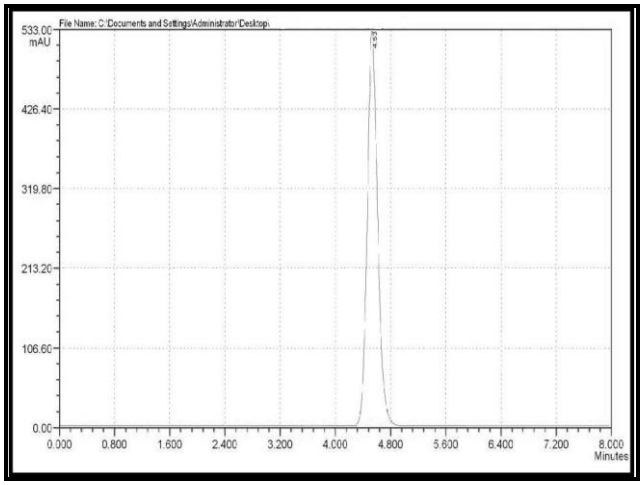

Figure 4.2: Linearity Chromatogram of Sulfapyridine (15.0µg/mL)

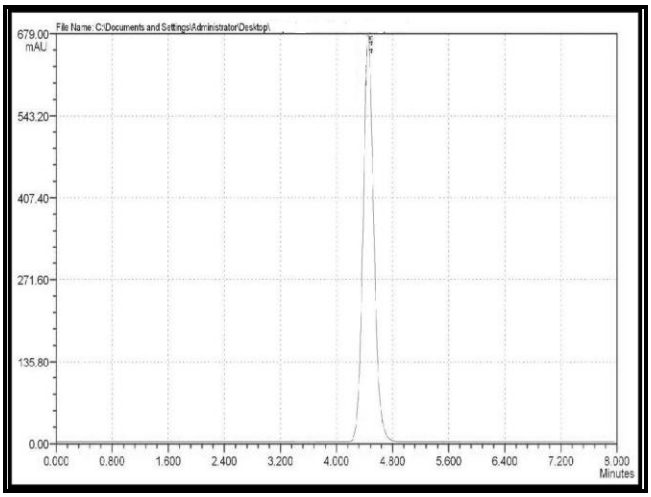

Figure 4.3: Linearity Chromatogram of Sulfapyridine (20.0µg/mL)

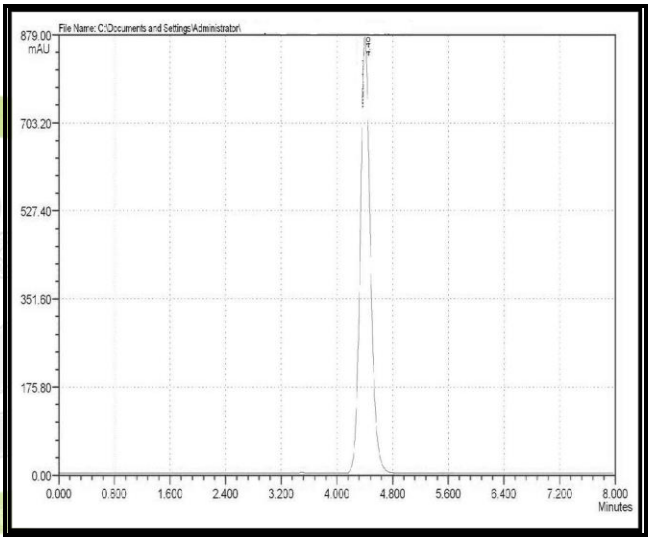

Figure 4.4: Linearity Chromatogram of Sulfapyridine (25.0µg/mL)

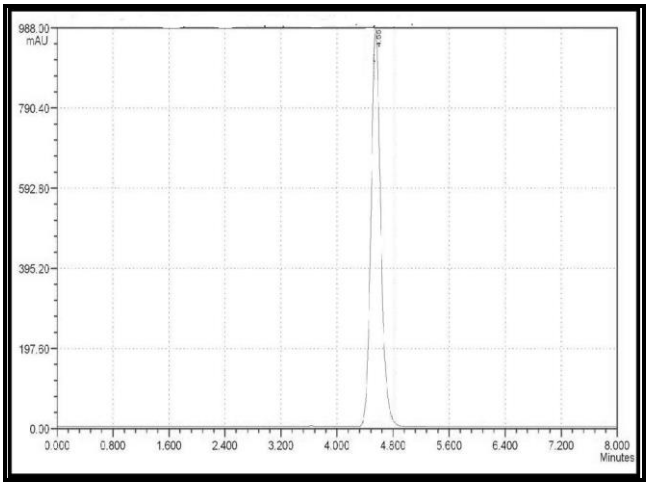

Figure 4.5: Linearity Chromatogram of Sulfapyridine (30.0µg/mL)

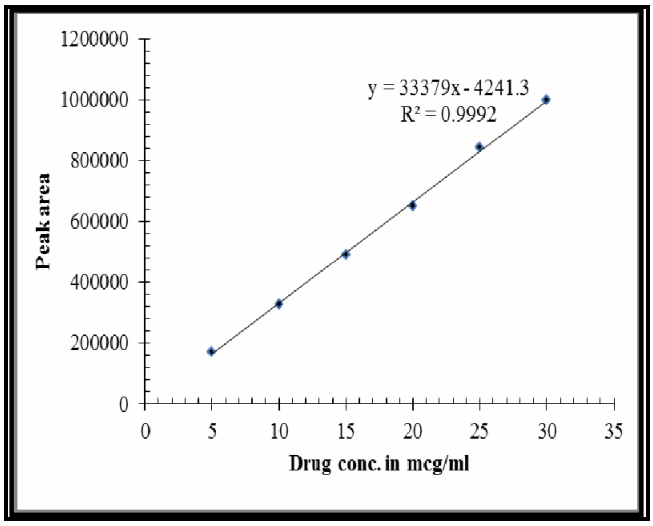

Figure 5: Calibration Curve oF Sulfapyridine

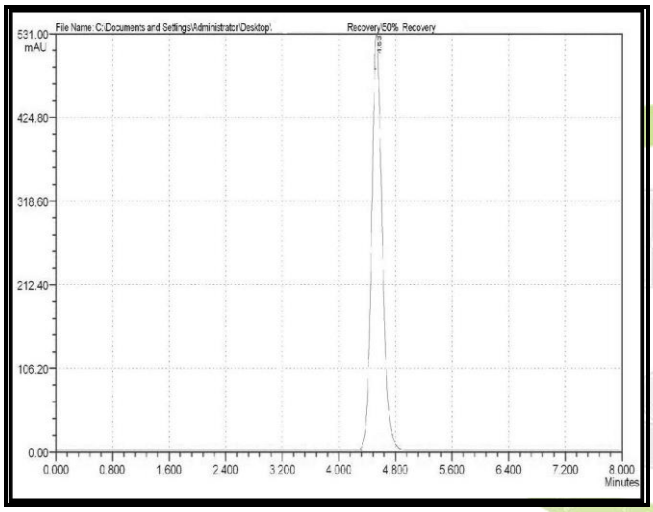

Figure 6(a): Accuracy Chromatogram for Sulfapyridine (50%)

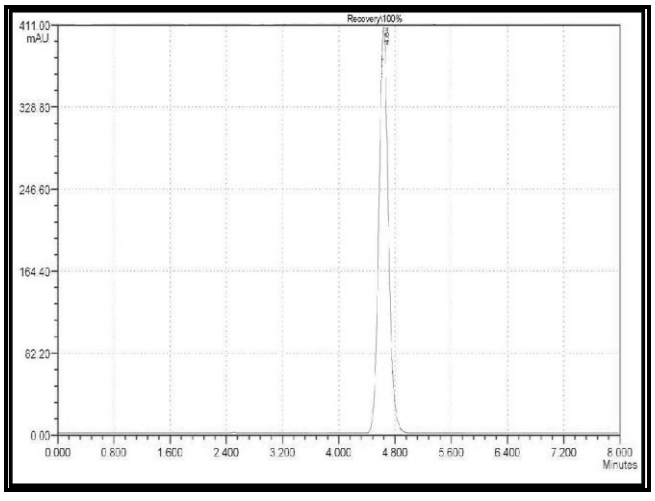

Figure 6(b): Accuracy Chromatogram for Sulfapyridine (100%)

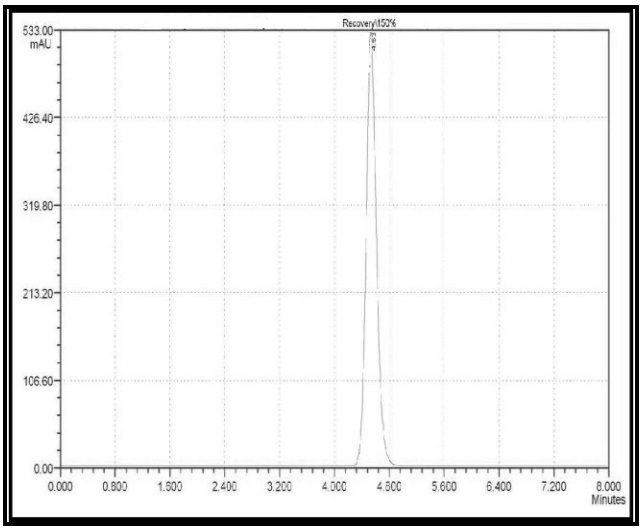

Figure 6(c): Accuracy Chromatogram for Sulfapyridine (150%)

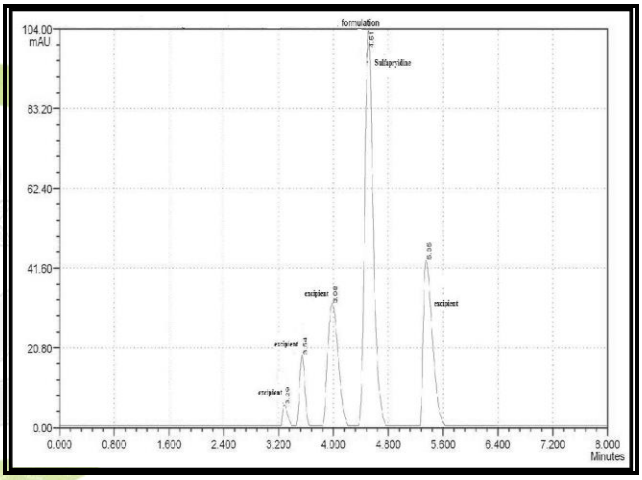

Figure 7: Validative Chromatogram of sulfapyridine in Formulation

Table 1: Optimized Chromatographic Conditions

Chromatographic Parameters	Peak HPLC
Elution	Isocratic
Mobile phase	Acetonitrile: water 1.0% ortho phosphoric acid (70:27:3 v/v/v)
Column	ODS C-18 RP (4.6 mm i.d x 250 mm)
Flow rate	1.0 min/ Ml
Detection	UV at 256nm

Injection volume	20µl
Temperature	Ambient
Retention time	4.40 minutes
Run time	6 minutes
Area	842341 Mau
Theoretical plates	3978
Tailing factor	1.83

Table 2: System Suitability Parameters

Parameters	**Sulfapyridine**
Retention time	4.40
USP Plate count	3678
USP Tailing	1.32

Table 3: Linearity, LOD and LOQ Data for Sulfapyridine

Concentration (µg/ml)	**Area (mAU)**
5	169489
10	328976
15	490643
20	650078
25	842341
30	997862
Regression equation; Intercept (a)	33379
Slope (b)	-42413
Correlation coefficient	0.9992
Standard deviation on intercept (Sa)	9352.07624
Standard deviation on slope (Sb)	480.2782987
LOD	0.840 LOQ 2.80
LOQ	2.80

Table 4: Results of Method Precision

S No.	**Name**	**Area**
1	Injection-1	997862
2	Injection-2	987145
3	Injection-3	997714
4	Injection-4	980056
5	Injection-5	995612
6	Injection-6	1006781
*Avg		994195
Std Dev		9336.588
% RSD		0.939
Average of six determinations		

Table 5: Recovery Studies of the Proposed RP-HPLC Method

Level	**Sulfa-pyridine in Tablet** (µg/ml)	**Pure Drug Added** (µg/ml)	**Drug Found*** **(**µg/ml)	**% Recovery**
50%	10	5	14.99	99.93
100%	10	10	19.98	99.99
150%	10	15	24.96	99.84

All the values are the averages of three determinations

Table 6: Evaluation Data of Ruggedness Study

No of Injections	Ruggedness	
	Analyst -1	Analyst-2
	Area	Area
Injection-1	997862	1E+06
Injection-2	987145	987145
Injection-3	997714	980981
Injection-4	980056	989456
Injection-5	995612	996653
Injection-6	1006781	996753
*AVG	994195	993975
STDEV	9336.588	11022.9
%RSD	0.939	1.108

All the values are the averages of three determinations

Table 7: Sulfapyridine Evaluation Data of Robustness

Robust conditions		RT	Peak Area
Flow rate	0.8 ml/min	4.65	855645
	1.2 ml/min	4.60	857880
Temp.	40^0C	4.68	846987
	45^0C	4.67	856745

Table 8: Result Analysis of Tablet Containing Sulfapyridine

Ph'ceutical Formulation	Amount of Sulfapyridine		% Recovery
	Labelled (mg)	Found* (mg)	
DAGENAN	500	499.95	99.99%

*Average of three determinations

CONCLUSION

The RP-HPLC method developed by the author for the analysis of sulfapyridine in pure and dosage formulations was found to be precise and accurate, as revealed by the statistical data of analysis. The analytical assay of sulfapyridine was completed within 5.0min revealing the rapidity of the proposed RP-HPLC method. The linearity results showed that an excellent correlation existed between peak area and concentration of sulfapyridine was within the concentration range tested. The values of slope and intercept of the calibration graph indicated the high reproducibility of the proposed method. High values of correlation coefficient validated the linearity of the calibration plot and obedience to Beer's laws. Furthermore, the low values of LOD and LOQ indicated that the developed RP-HPLC method can be employed over a wide concentration range for linearity. The proposed RP-HPLC method of sulfapyridine was also found to be robust and rugged as there was no significant change in the peak area, peak shape and retention time. On the basis of above facts it is concluded that **"**the developed RP-HPLC method was found to be easily applicable and is expected to be widely used for the routine QC analysis of sulfapyridine in the pharmaceutical industry".

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