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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR DETERMINATION OF ACETYL SULFISOXAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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A simple and precise RP-HPLC method was developed and validated for the determination of acetyl sulfisoxazole in blood samples. Chromatography was carried out using methanol:acetonitrile:0.01M potassium dihydrogen phosphate (25:50:25 *v/v*) as the mobile phase at a flow rate 1.2 ml/min. The analyte was monitored by using PDA detector at 260 nm. The Run time was 8 min for acetyl sulfisoxazole. The proposed method was found to have linearity in the concentration range of 2-10 μg/ml.

Key words: Acetyl sulfisoxazole, Methanol, Acetonitrile, Potassium dihydrogen phosphate.

INTRODUCTION

Sulfonamides derived from sulfanilamide (*p*-aminobenzenesulfonamide) are commonly referred to as sulfa drugs. Acetyl sulfisoxazole (O'Neil *et al* 2001), chemically named as *N*-[(4-Aminophenyl)sulfonyl]-N-(3,4-dimethyl-5-isoxazolyl)acetamide (**Figure 1**), is slightly soluble in alcohol and insoluble in water. Its melting point is 125-130°C, the molecular formula is $C_{13}H_{15}N_3O_4S$ and the molecular weight is 309.35.

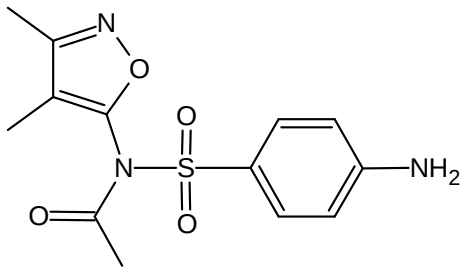

Figure 1. Structure of Acetyl sulfisoxazole

Sulfonamides are not readily biodegradable and have been detected in surface water and in secondary waste water effluents (Sharma *et al* 2006; Franek *et al* 2006; Berner and Dinh, 1992; Chien, 1988; 1989; Scott and Hollenbeck, 1991; Husson *et al* 1991; Swarbrick and Boylan, 1988; Muxlow *et al* 2001; El-Basil *et al* 1969). Most of the sulphonamides are prepared adopting Ullmann's method (Foye *et al* 2008). Literature is enriched with reports of reverse phase high performance liquid chromatographic assay to quantitated N^1-acetyl sulfisoxazole and the related manufacturing impurities such as sulfisoxazole, N^4-acetyl sulfisoxazole and N^1,N^4-diacetyl sulfisoxazole (Elrod Jr and Luka, 1982). The HPLC separations are achieved using a micro particulate octadecylsilane column with a ternary aqueous acetic acid:acetonitrile: methanol as mobile phase. Sulfonamides and erythromycin ethylsuccinate in combination in form of oral suspensions were determined using high-performance liquid chromatography and automated turbidimetry (Elrod Jr *et al* 1982).

A spectrophotometric method, involving the formation of ferric acetohydroxamate, was

earlier reported and conditions necessary for reproducible reaction were developed (Feldman and Patel, 1971). The method provides a relatively simple and rapid means by which acetyl sulfisoxazole is quantitatively determined in the presence of its main hydrolysis products, sulfisoxazole and acetic acid. Only very few HPLC methods have been reported in the literature (Chien, 1983) for the estimation of acetyl sulfisoxazole. The official assay for acetyl sulfisoxazole, the diazotization method, does not differentiate between acetyl sulfisoxazole and its main hydrolysis product, sulfisoxazole. There are no reported methods for the determination of acetyl sulfisoxazole by HPLC in human plasma. Hence the successful attempt to develop a HPLC method for the determination of acetyl sulfisoxazole in pharmaceutical formulations, is reported. Acetyl sulfisoxazole is placed official in Indian Pharmacopoeia (Indian Pharmacopoeia, 2007).

MATERIALS AND METHODS

Instrument

Chromatography was carried out using Shimadzu, Model LC-20 $AT_{VP,}$ Kromasil C-18 column, using PDA detector equipped with EMPOWER software.

Chemicals and Reagents

Acetyl sulfisoxazole was obtained as gift sample and the chemicals, potassium dihydrogen phosphate (AR grade), water (HPLC grade), acetonitrile (HPLC grade), methanol (HPLC grade) were procured from E-Merck Ltd, India.

Mobile phase used

The mobile phase composition was methanol, acetonitrile and 0.01 M potassium dihydrogen phosphate (25:50:25 *v/v*). Prepared mobile phase was filtered through 0.45 μ membrane filter and sonicated. Sample solution was prepared by dissolving the drug in mobile phase and sonicated for 30 min. The mobile phase was delivered isocratically at a flow rate of 1.2 ml/min. All solutions were filtered through a 0.45 μ membrane filter before use. pH was maintained at 3.5.

Chromatographic conditions

Kromasil C-18 column (250 × 4.6 mm ID with 5 μ particle size) was maintained at ambient temperature. The injection volume was 20 μl and the total run time was 8 min. The detection was carried out at detection wavelength 260 nm. Other conditions were column length: 250 × 4.6 mm, injector type: rheodyne type injector, flow rate: 1.2 ml/min and pump pressure: 25.8 MPa.

Standard preparation

Stock solution of acetyl sulfisoxazole was prepared by dissolving accurately weighed 10 mg of drug in 10 ml methanol (final concentration - 1000 μg/ml). The prepared stock solutions were stored away from light. From the stock, standard solutions were freshly prepared during the day of analysis. From the stock solution, 0.5 mg/ml solution was prepared. Solutions in the concentration range of 0.2-1.0 mg/ml were prepared from the standard working solution.

Sample preparation

1 mg of formulation powder was taken from sulfisoxazole (1.5 mg formulation) and dissolved in 10 ml of mobile phase to prepare 0.1 mg/ml solution and injected into HPLC and chromatogram was recorded. The amount of drug present in the 1 mg formulation was calculated from linearity graph.

Mobile phase preparation

The mobile phase was prepared by mixing methanol, acetonitrile and 0.01 M Potassium dihydrogen phosphate (25:50:25 *v/v*) by ultra bath sonicator for 30 min.

Linearity and calibration

Linearity was assessed by performing single measurement at several analyte concentrations varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml. Injection was made at intervals of 13 min. The linearity was tested for the concentration ranging from 0.2 mg/ml to 1.0 mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis which was calculated by the least square regression method.

Precision

Reproducibility was performed by injecting three replicate concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions and the amount of drug were calculated in terms

of percentage relative standard deviation. The sample concentration was 0.5 mg/ml.

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre-analyzed sample formulation.

Ruggedness

Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness is also expressed in terms of percentage relative standard deviation.

Robustness

Robustness was carried out by varying two parameters from the optimized chromatographic conditions**.**

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution with that of test results those obtained from standard drug.

System suitability parameter

System suitability tests were carried out on the freshly prepared standard stock solutions of acetylsulfisoxazole and it was calculated by determining the standard deviation of the acetylsulfisoxazole standards by injecting standards in the five replicates at 6 min interval and the values were recorded, accordingly.

RESULT AND DISCUSSION

The reverse phase high performance liquid chromatography method was developed as a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, tetrahydrofuran and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, orthophosphoric acid in different volume ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then, retention time and tailing factor were calculated. Finally, methanol and acetonitrile and 0.01 M potassium dihydrogen phosphate in the volume of ratio 25:50:50 *v/v* (pH: 3.42) and kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.92 tailing. Calibration graph was found to be linear at range 0.2 mg/ml to 1.0 mg/ml. Five different concentrations of acetyl sulfisoxazole in range given above were prepared and 20 μl of each concentration was injected in HPLC (**Figure 2**). The slope (m) and intercept (c) obtained were found to be 203032.05 and 0.008202713. The correlation of coefficient (r^2) obtained was found to be 0.9998 (**Table 1**). It was observed that the concentration range showed a good relationship. The limit of detection for acetyl sulfisoxazole was found to be 15 μg/ml and the limit of quantification was found to be 40 μg/ml. It proves the sensitivity of the method. The percentage assay of acetyl sulfisoxazole in formulation was found to be 100.07% (**Table 1, Figure 4**). The relative standard deviation value obtained was below 1 which indicates the precession of the method. The validation of the proposed method was further verified by recovery studies. The data is presented in the **Table 2** and **Figure 3**. The percentage recovery was found to be 102.30% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph and was found to be 0.1113 mg/0.5 ml as shown in **Table 1** and **Figure 5**.

CONCLUSION

The RP-high performance liquid chromatographic method developed and validated for the analysis of acetyl sulfisoxazole from their formulations was found to be accurate and precise. Thus, the proposed HPLC method is better as compared with the other methods available in the literature for the analysis and assay of acetyl sulfisoxazole. The serum studies adds further scope to the present investigation. This method can be successfully applied for the routine quality control analysis of acetyl sulfisoxazole formulations.

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Table 1. Optical characterization of acetyl sulfisoxazole

Parameters	Acetyl sulfisoxazole
Linearity range(mg/ml)	0.2-1.0
Correlation coefficient (r)	0.9998
Slope (m)	203032.05
Intercept (c)	0.008202713
Limit of detection (LOD; μg/ml)	15
Limit of Quantification (LOQ; μg/ml)	40
Tailing factor	1.65
Retention time (min)	3.748
Theoretical plates	10429
(%) R.S.D	0.1043
(%) Accuracy	102.30
(%) Assay	100.07
Serum (0.5 mg/ml)	0.1113

Table 2. Recovery data of acetyl sulfisoxazole

Pharmaceutical formulation (Brand name)	Labelled amount (mg)	Percentage assay	Percentage recovery
Sulfisoxazole	1.5 mg	100.07	102.30

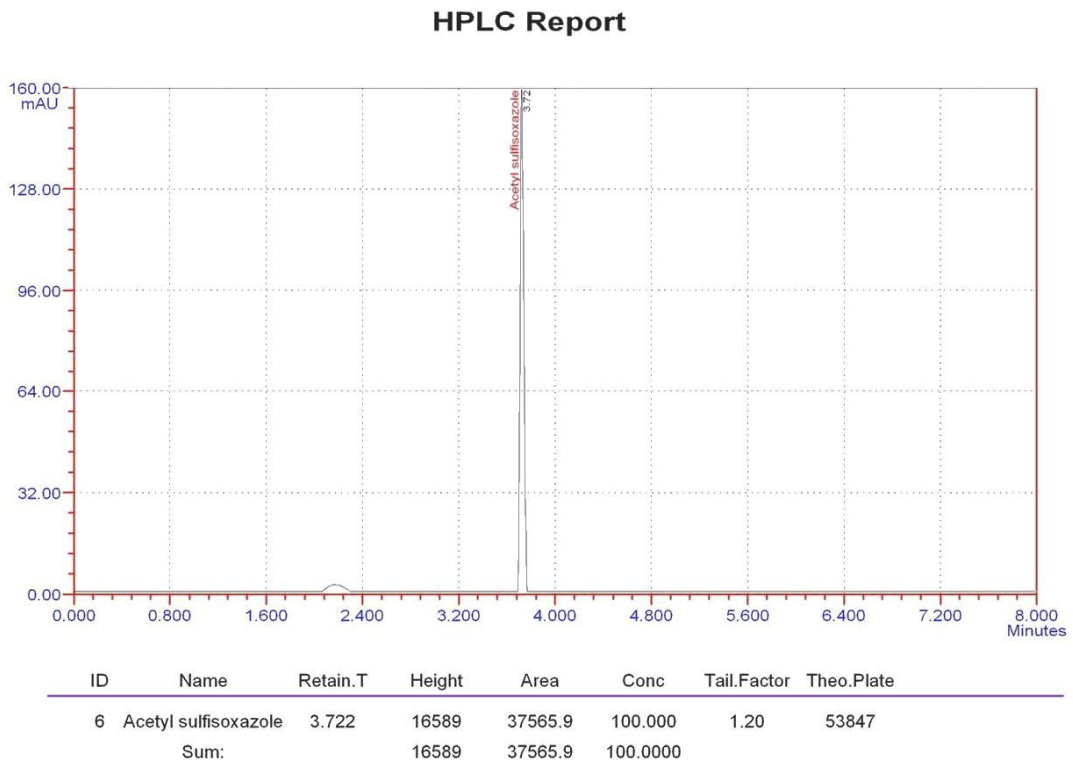
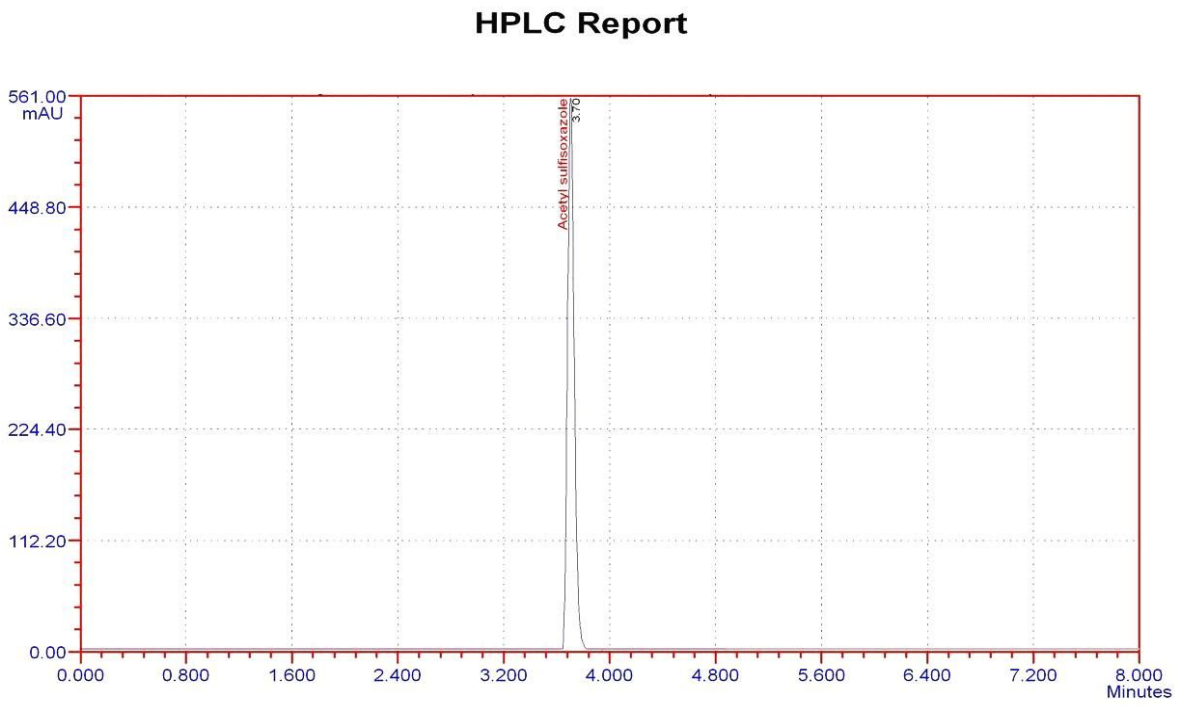
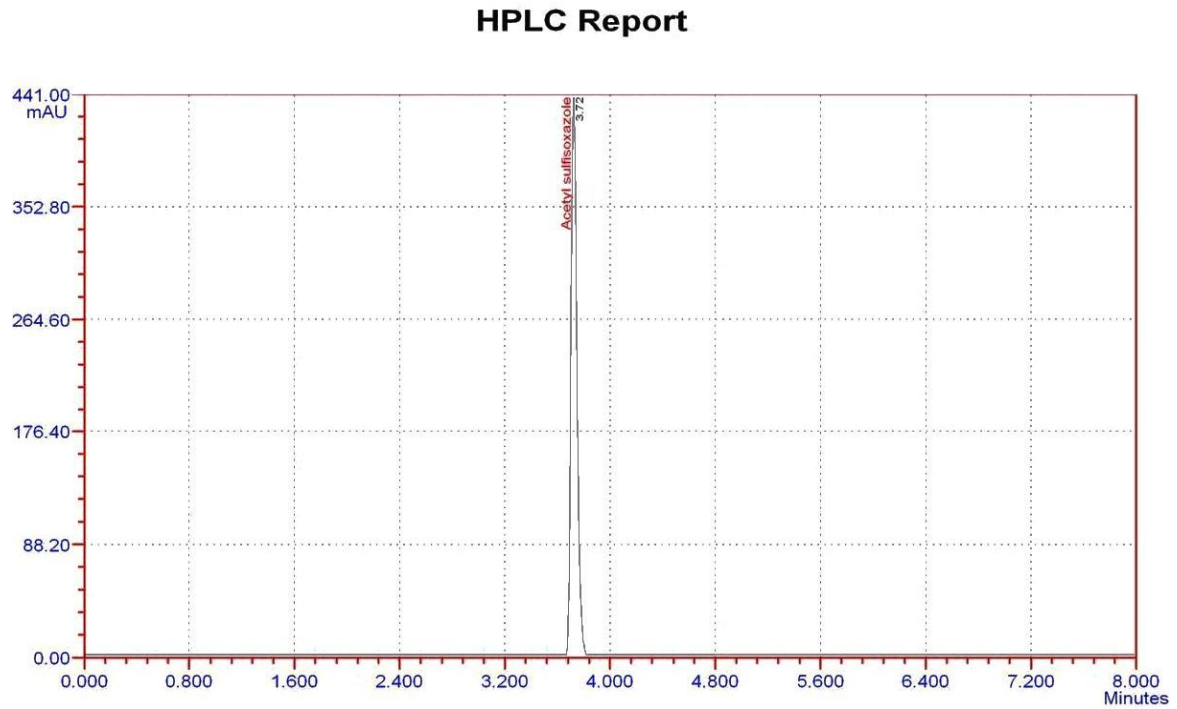


Figure 2. Chromatogram of acetyl sulfisoxazole (Standard)



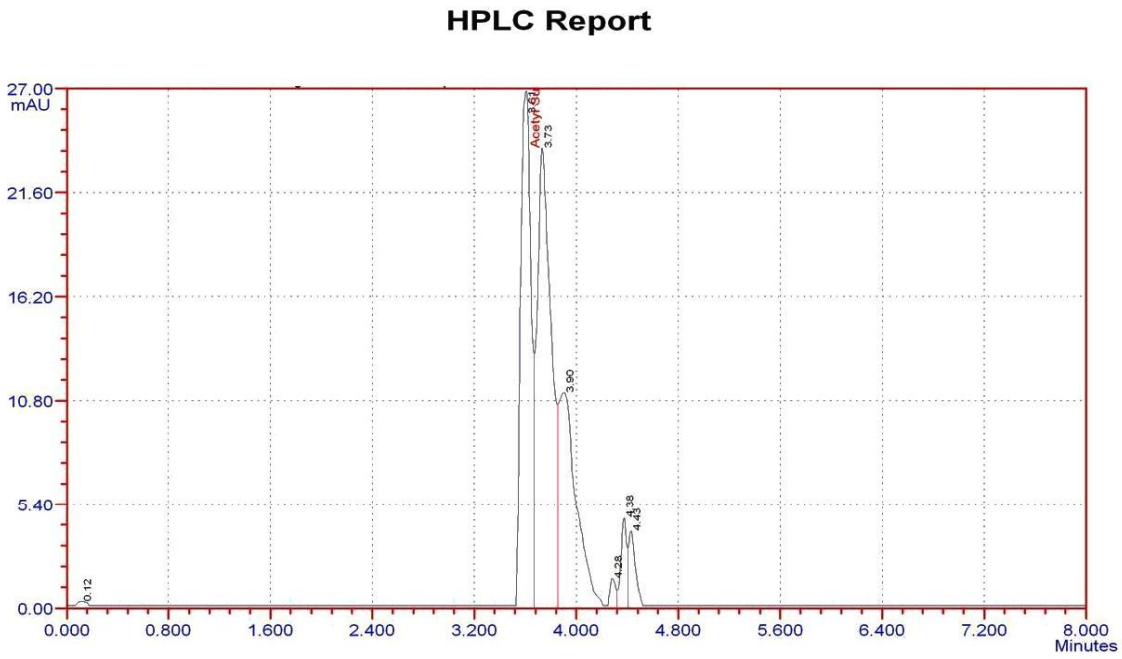
ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
6	Acetyl sulfisoxazole	3.705	56250	201268.1	100.000	1.43	21369
	Sum:		56250	201268.1	100.0000		

Figure 3. Chromatogram of acetyl sulfisoxazole (Accuracy)



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
6	Acetyl sulfisoxazole	3.724	44640	160024.4	100.000	1.15	21506
	Sum:		44640	160024.4	100.0000		

Figure 4. Chromatogram of acetyl sulfisoxazole (Formulation assay)



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.119	56	516.0	0.688	1.30	3
2		3.607	3084	18932.4	25.233	0.80	6881
3	Acetyl Sulfisoxazole	3.732	2784	22606.9	30.130	1.45	4210
4		3.904	1517	20086.1	26.770	3.70	1732
5		4.284	556	2525.6	3.366	0.87	17724
6		4.375	872	3416.5	4.553	0.76	24855
7		4.429	804	6557.4	8.740	7.27	5876
8		8.275	52	390.6	0.521	0.94	24191
	Sum:		9725	75031.4	100.0000		

Figure 5. Chromatogram of acetyl sulfisoxazole (Serum)

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