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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF SULPHADAIZINE IN PHARMACEUTICAL DOSAGE FORM BY HPLC

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ABSTRACT

The present work takes into account the development of Reverse Phase High Performance Liquid Chromatography (HPLC) for simultaneous method estimation and validation of sulphadiazine in pharmaceutical tablet formulation. The expected separation and peak shapes were obtained on Phenomenex 250 mm x 4.6 mm (5 µm) RP-18 column at a 40˚ C using a mobile phase consisting of orthophosphoric acid buffer (OPA) of pH 3.0 + perchloric acid ($HclO_4$): ACN (90-10) at flow rate of 1ml/min respectively. The eluent detection was carry out using UV-Visible detector at 269 nm. The retention time of sulphadiazine was 7.2 min. The method was validated for linearity, Accuracy, Precision and Robustness. Both intra-day and inter-day precision (in terms of % RSD) were lower than 2% and regression coefficient of linearity was found to be 0.9992. Specificity in terms of % RSD was found to be 0.031. This method was successfully applied for quantification of sulphadiazine in pharmaceutical formulation. The method can be employed for routine Quality Control Analysis.

INTRODUCTION

Sulphonamide are among the most widely antibiotics in the world. They have been in clinical use since 1968. These drugs are popular because they are well tolerated by patients, and they are relatively inexpensive. Sulphonamide is SO_2NH_2 functional group. The compounds which contain this functional group are called as sulphonamides. The general formula of sulphonamides R-SO_2NH_2. The term sulphonamide (sulphonamide) is also usually employed as a generic name for the derivatives of Para-amino benzene sulphonamides. Sulphonamides are derivatives of para-amino benzene sulphonamide. Sulphonamides are the first effective chemotherapeutic agents used for bacterial infection in humans. Sulphonamides have a wide range of pharmacological activities such as Oral hypoglycemic, antileprotic, anti-epileptic, anti-hypertensive, anti-bacterial, anti-protozoal, anti-fungal, anti-viral, anti-cancer, anti-inflammatory, and used as diuretic.[1,2]

EXPERIMENTAL

Materials

Pure Sulphadiazine was purchased from Yarrow Chem products, Mumbai and methanol HPLC grade, Glacial Acetic acid, Water, Acetonitrile etc were provided by JCDM college of pharmacy.

Instrument used

The following items of instruments were employed during the studies:

Table 1: Instruments used and their details

Equipment items	Manufacture
HPLC with PDA detector_LC-2010	Shimadzu corporation, Kyoto (japan)
UV-Vis spectophotometer	Shimadzu corportion, Kyoto (Japan)
Sonicator	Ultrasonics
pH meter	Mettler Toledo
Microbalance	Mettler Toledo
Micron Filters	Phenomenex (0.45µ)
Refrigenrator	Remi Elektrotechnik limited
Micro-pipette	Eppendorf

Development of HPLC method

Liquid chromatography was carried out at 40˚C temperature using Phenomenex C18, 250mm x 4.6 mm (5 µm) RP-column. The mobile phase consist of Orthophosphoric acid (2.8ml) and Perchloric acid (2ml) for 1000 ml, pH 3.0, and Acetonitrile 90:10 (v/v) which

has then vacuum filtered using a 4.5µm nylon filter in a Millipore vacuum filtration assembly and degassed prior to operating under isocratic condition at a flow rate of 1.0 ml/min. Sample injection volume was 10µl and drug were detected at single UV wavelength 269nm, with a chromatography run time of 10.01min.

Analytical Method Validation[3,4,5]

System Suitability

System suitability test was performed to confirm the reproducibility of the equipment to be used for the intended validation. The test was performed by prepared standard solution of 100 ppm and injected six times. System suitability parameter like peak asymmetry, theoretical plates, and retention periods were calculated.

Intraday and Inter-Day precision

Six replicate injections of calibration standards and controls were made at an interval of 6 h on the same day and for 7 consecutive days to evaluate intraday and inter-day accuracy and precision. The concentrations of the controls were quantitated using the linear regression line of the calibration standards. The %RSD was used to calculate inter-day precision.

Linearity

Calibration curve were constructed by plotting peak area vs. concentrations of Sulphadiazine and the regression equations were calculated. The calibration curve was plotted over the range 20% -150% of the sulphadiazine. Correlation coffiencent, v-intercept, slope of regression line and residual sum of squares were calculated from the graph.

Robustness

The ruggedness of the method was tested by the variation in different parameters, flow rate, wavelength and pH of the method. The parameters tested were variation within replicates, intraday precision, and inter-day precision

Stability of Sample Solution

In order to determine the stability of product in solution form, stability study of solution was performed by measuring the areas of sample of sulphadiazine tablet at zero hour and after1, 2, 4, 6 and 8 hours at room temperature. The variation in areas of sulphadiazine was observed and % cumulative RSD was calculated and recorded.

RESULT AND DISCUSSION

Development of HPLC method

Determination of λ max

Based on data available and literature reports, Scanning of sulphadiazine was carried out on UV spectrophotometer for determining λ max of the sulphadiazine in various conditions. Stock solution of sulphadiazine was prepared and further dilutions were made in methanol and UV scanning was performed at wavelength 200-800nm for dilutions. The λ max was found to be 269 nm

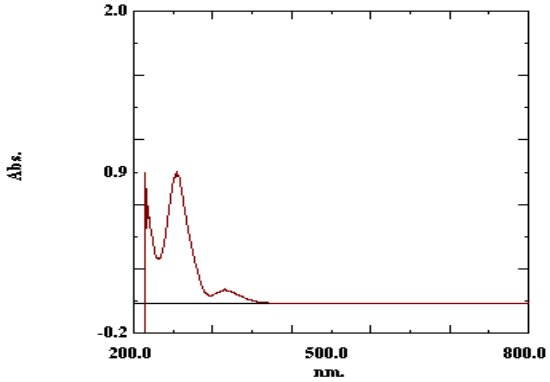

Fig. 1: Determination of λ max.

Table 2: Determination of λ max.

Sr No	Sample concentration µg/ml	Wavelength (nm)	Absorption
1	2	269	1.0
2	4	269	0.9
3	6	269	1.0
4	8	267	1.0
5	10	265	0.8

Selection of mobile phase

Table 3: Selection of mobile phase.

Trial no	Solvent system (v/v)	Flow rate(ml/min)	Time of run (minutes)
Trial 1	Phosphate buffer (KH_2PO_4) of pH 4.4: Methanol Time(min) / % of B 00.01 / 10 30 / 50 45 / 80 50 / 10 55 / 10	1.0	55.01
Trial 2	Phosphate buffer (KH_2PO_4) of pH 3.0: Methanol Time(min) / % of B 00.01 / 10 30 / 50	1.0	55.01

	45 80 50 10 55 10		
Trial 3	Sodium acetate buffer of pH 5.0: ACN Time(min) % of B 00.01 10 60 80 70 80 75 10 80 10	1.0	80
Trial 4	OPA+ TEA (pH-7.5): ACN Time(min) % of B 00.01 2 20 10 25 2 30 2	1.0	30
Trial 5	OPA+TEA (pH-5.5): Methanol Time(min) % of B 00.01 2 20 10 25 2 30 2	1.0	30
Trial 6	OPA+TEA (pH-6.0): ACN Time(min) % of B 00.01 2 20 10 25 2 30 2	1.0	30
Trial 7	Acetic acid buffer of pH-6.5: ACN Time(min) % of B 00.01 2 20 10 25 2 30 2	1.0	30
Trial 8	OPA+ACN Time(min) % of B 00.01 90 10 90	1.0	10
Trial 9	Orthophosphoric acid buffer of pH 3.0 + Perchloric acid ($HCLO_4$) : ACN Time(min) % of B 00.01 90 10 90	1.0	10

Analytical method validation

System suitability

Table 4 Result for system suitability study

Sample Name	RT(Min)	Area	USP Plate Count
Test Solution_01	7.229	1627158	1776.367
Test Solution_02	7.217	1722603	1794.773
Test Solution_03	7.201	1758238	1832.671
Test Solution_04	7.183	1724147	1892.410
Test Solution_05	7.189	1826925	1852.864
Test Solution_06	7.217	1724803	1794.771
Mean	7.206	1730646	1823.976
SD	0.015185	54617.75	36.96658
%RSD		**0.031**	

The %RSD of peak areas of Sulphadiazine and its retention time were within 2% indication the suitability of the system (table 4). These results indicate the applicability of this method to routine with no problem, its suitability being proved.

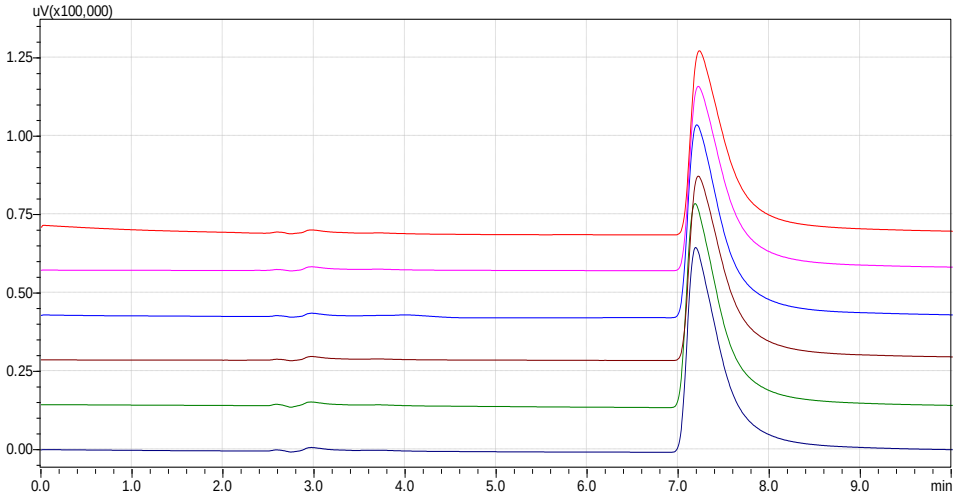

Fig. 2: A typical chromatogram of System Suitability.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. Repeatability was evaluated in terms of % RSD. The low % RSD value indicating that the method has good precision. Thus, showing that the equipment used for the study worked correctly for the developed analytical method and was being highly repetitive.

Table 5: Intra-day precision.

Name	RT (Min)	Area	Plate count
Test Solution_01	7.134	1922447.6	1876.367
Test Solution_02	7.179	1958238.2	1894.234
Test Solution_03	7.124	1927158.3	1732.671
Test Solution_04	7.229	2048243.5	1792.410
Test Solution_05	7.201	2024147.3	1794.771
Test Solution_06	7.220	1832443.7	1867.132
Mean	**7.181167**	**1952113.1**	1867.132
SD	0.04018	71052.85105	57.2974359
%RSD		**0.036**	

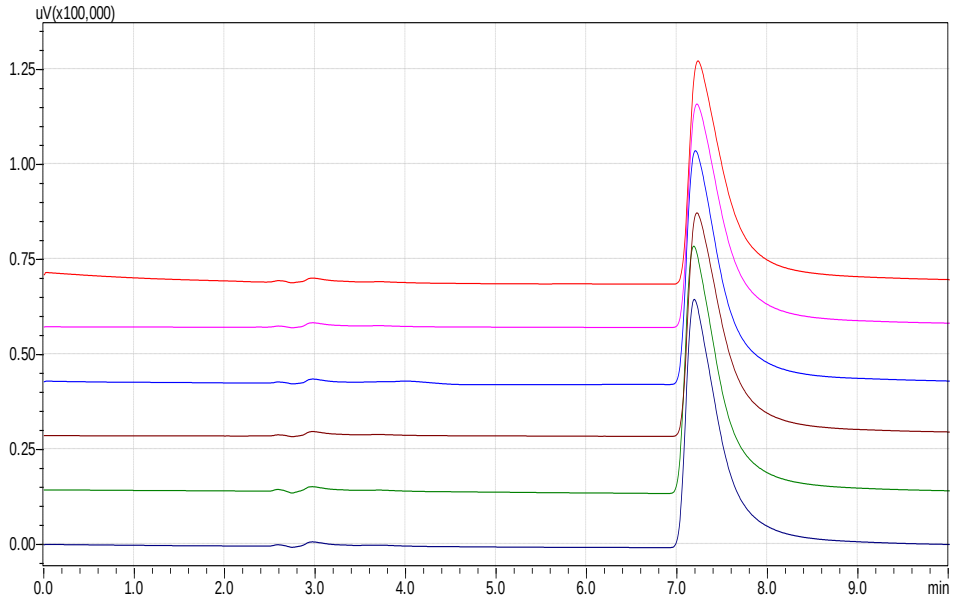

Fig. 3: A typical chromatogram of Intraday Precision.

Table 6: Intermediate precision results.

Name	RT (Min)	Area	%Area	Height
Test Solution_01	7.113	1865975.2	100	58701
Test Solution_02	7.179	1879443.0	100	58479
Test Solution_03	7.219	1844805.5	100	60432
Test Solution_04	7.239	1859740.0	100	59049
Test Solution_05	7.173	1892197.8	100	57826
Test Solution_06	7.174	1892194.1	100	58483
Mean	**7.1846**	**1868432.3**		
SD	0.04344	162884.06		
%RSD		**0.008**		

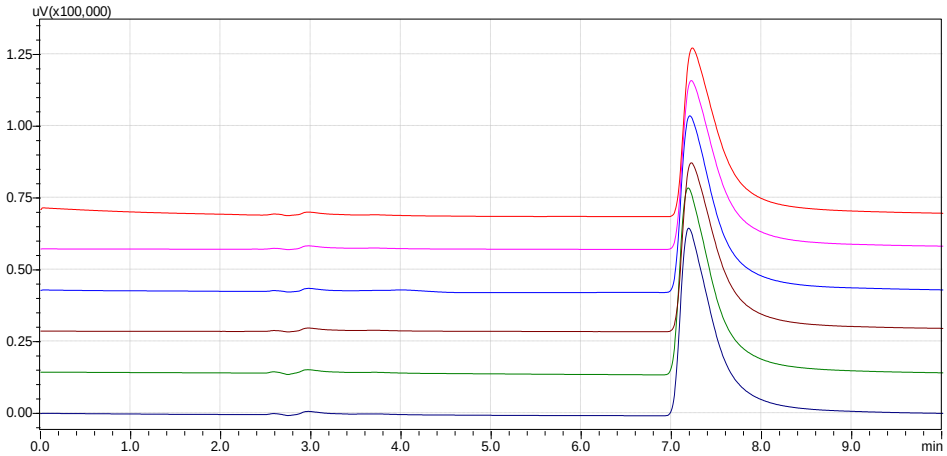

Fig. 4: Overall results for precision studies.

Linearity

The calibration curve was constructed by plotting response factor against the concentration of drug. sulphadiazine exhibited linearity of the concentration range of 40-150µg/mL injected and chromatograms were recorded. The linearity was observed by linear regression analysis. Before injecting the concentrations, the mobile phase was run through the system for 30 minutes for column equilibration.

Linear calibration curve of Sulphadiazine

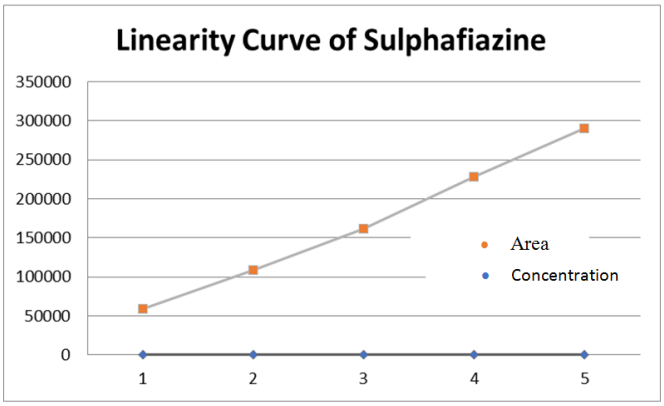

Fig. 5: Linear calibration curve of Sulphadiazine.

Table 7: Linearity data of Sulphadiazine.

Sr.no	Concentration (µg/ml)	Area	RT(Min)	Peak height
1	40	59446.9	7.215	21540
2	80	108172.0	7.248	37952
3	100	162260.3	7.217	58701
4	120	228562.7	7.545	89072
5	150	290885.5	7.061	112849

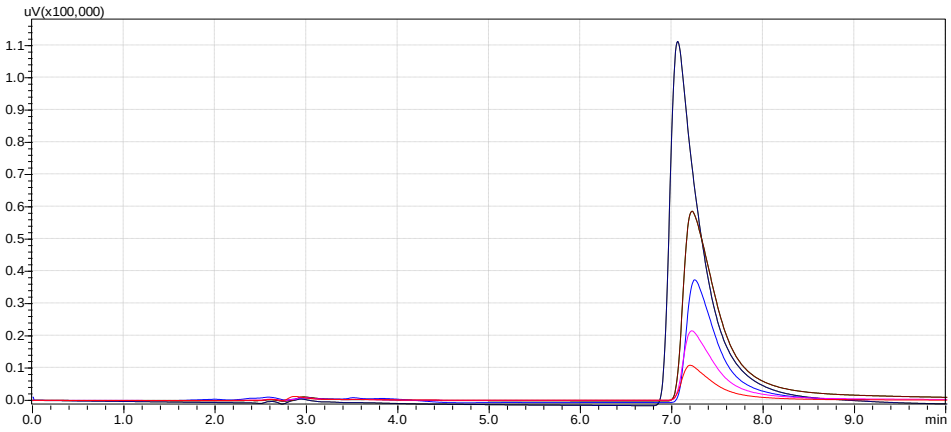

Fig. 6: Overlay chromatogram of linearity.

Robustness

Table 8. Change in flow rate of the developed method

Flow rate (ml/min)	Area	RT (min)	Concentration µg/ml
0.9	313370.9	7.835	100
1.0	282033.9	7.065	100
1.1	264170.8	6.471	100

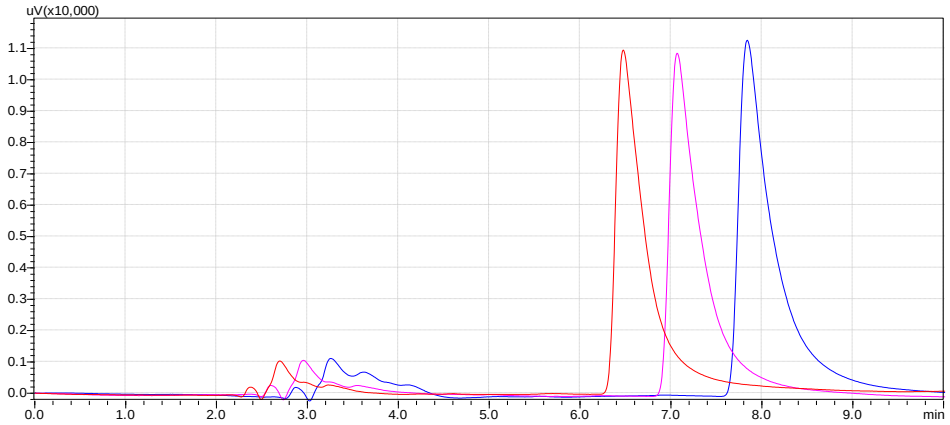

Fig. 7: Change in flow rate.

Table 9. Change in pH of the developed method

pH	Area	RT (min)	Concentration µg/ml
2.9	1986564.8	6.708	100
3.0	1826925.1	7.189	100
3.1	1991562.8	7.365	100

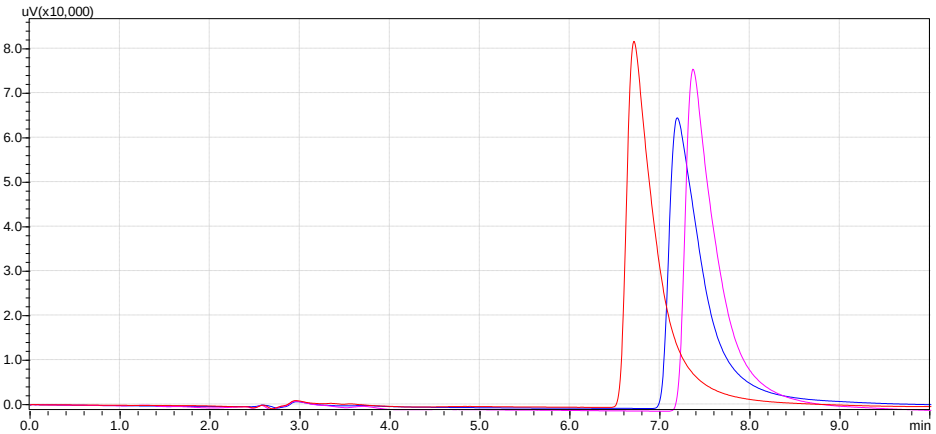

Fig. 8: Change in pH.

Table 10: Change in wavelength of the developed method.

Wavelength	Area	RT (Min)	Concentrations µg/ml
267	298254.8	7.060	100
269	162715.6	7.229	100
271	282033.9	7.065	100

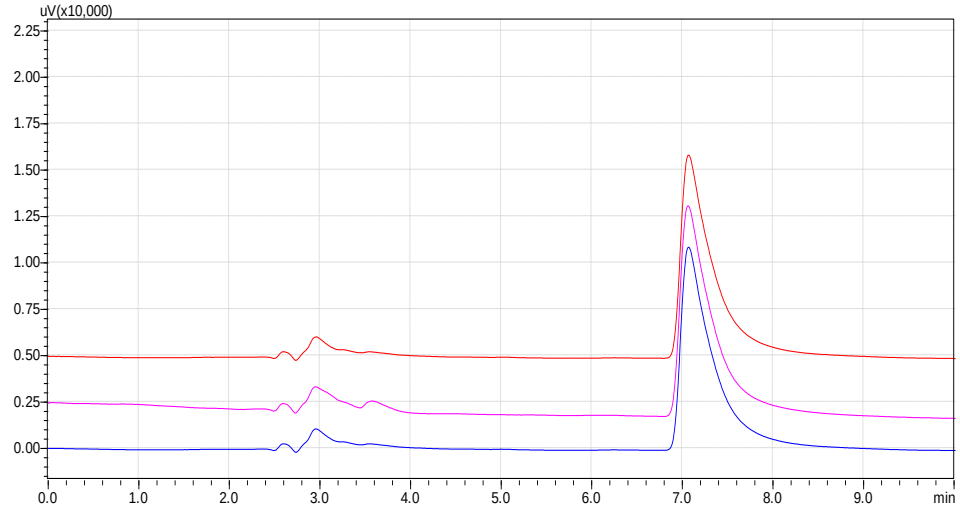

Fig. 9: Change in Wavelength.

Stability of analytical solution

Table 11: Result of stability of solution.

Time of sampling (hours)	Area	Height
Initial	162758.6	58479
01	172260.3	58701
02	175823.8	61432
04	182414.8	65049
06	182692.5	65268
08	172260.3	58701

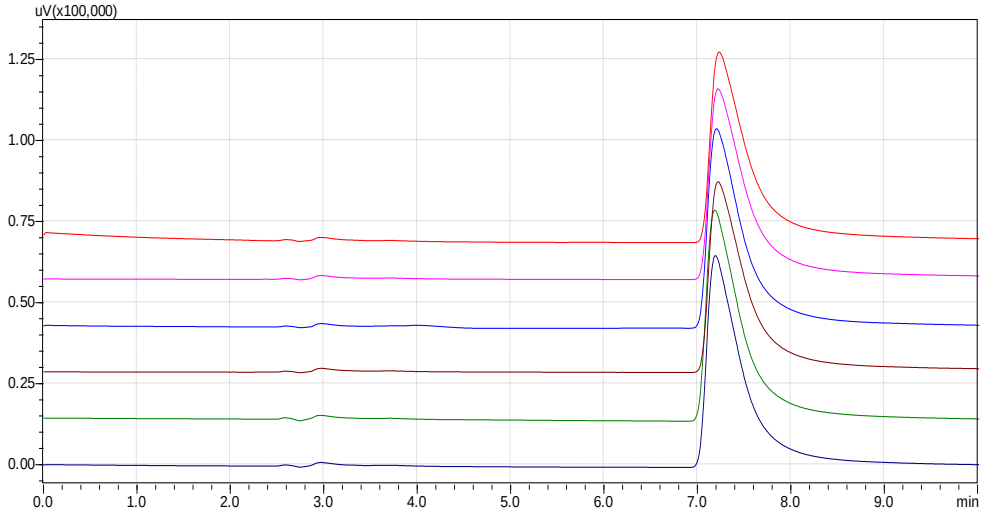

Fig. 10: A typical chromatogram of solution stability.

As an evident from table 15 there is slight variation in areas of test solution of Sulphadiazine tablet with time. After 8 hours the cumulative % RSD value is 1.57% for Sulphadiazine, which is well within the acceptance criteria that are less than 2.0 %. Therefore, it can be established that the product in solution form is stable for at least 8 h.

CONCLUSION

The purposed method is simple, accurate and precise and selective for the estimation of Sulphadiazine in tablet

dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in a good agreement with their respective label claims. Hence, the development method can be easily and conveniently adopted for routine analysis of Sulphadiazine in tablet dosage form.

REFERENCES

1. Sulfonamide (slideshare.net) classification of sulphonamide review article - Bing
2. Sulfadiazine | C10H10N4O2S - PubChem (nih.gov)
3. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, October 1994, Geneva.
4. Rao G, Goyal A. An Overview on Analytical Method Development and Validation by Using HPLC. The Pharmaceutical and Chemical Journal, 2016; 3(2): 280-289.
5. Zir Chrom®-EZ & Zir Chrom®-MS; Method Development Guide; Column Use Tip; www.zirchrom.com, 2014; 22: 1-28.