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SULTIAM TABLETS, ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC METHOD

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ABSTRACT

An accurate method has been made for the estimation of Sultiam in formulation by RP – HPLC method taking water and methanol in the ratio 60:40. The standard substance was dissolved in Methanol was scanned and the spectra was recorded and the spectrum shows that λ_{\max} of Sultiam was 245 nm. Reverse phase chromatographic technique was selected by using Hypersil ODS 250 x 4.6mm, 5 μ m column as a stationary phase with different compositions of water and methanol was selected as mobile phase for the analysis. 245 nm was selected as detection wavelength. The calibration curve was plotted using concentration against peak area. With the optimized chromatographic conditions, the drug was linear in the concentration range of 2.5 - 15 μ g/ ml. The correlation coefficient was found to be 0.998. By using this method, the main peak of Sultiam was eluted at 4.94 minutes. In this method the optical parameters like Correlation coefficient, Slope, Intercept, LOD and LOQ were calculated.

Key Words: Sultiam tablets, Carbonic anhydrase inhibitor, Methanol, RP-HPLC.

INTRODUCTION

The excipients in sultiam tablets are starch-maize, lactose, talc-purified, silica-colloidal anhydrous, gelatin, magnesium stearate, hypromellose, macrogol 4000 and titanium dioxide. Ospolot 50 mg tablets are white film-coated round tablets, debossed "50" on one side and plain on the reverse side. Ospolot 200 mg tablets are white film-coated round tablets debossed "200" on one side and scored on the reverse side. Sulthiame should not be used in patients with - known acute porphyria - hyperthyroidism or arterial hypertension. sulthiame, increase the risk of suicidal thoughts or behaviour in patients taking these drugs for any indication [1, 2].

The HPLC technique [3-7] is widely use for the separation of materials including Amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, terpenoids, pesticides, antibiotics, steroids, metals, organic species, and variety of inorganic substances. It is also use for Nano

science, molecular detection, pharmaceutical R&D, and finished doses products analysis.

MATERIALS AND METHODS

Working/Reference Standards

Sultiam Working Standard purity 99.7%

Test samples:

Sultiam tablets 50mg

Sultiam tablets 200mg

Placebo for Sultiam tablets 200mg.

Filters:

0.45 μ m GHP membrane filtered (Manufactured by PALL)

0.45 μ m NYLON membrane filtered (Manufactured by PALL)

Method development

Selection of wavelength for detection of components

Solution of sultiam was scanned in UV region [8] and spectrum was recorded. Methanol is used as a solvent and it was seen that at 245 nm the compound has very good absorbance, which can be used for the estimation of sultiam by HPLC.

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Selection of chromatographic method:

Based on the literature survey and method development data, The EUTICALS SPA API test method has been adopted with minor changes for estimation of sultiam in tablet dosage form.

From literature survey and with the knowledge of properties of selected drug, Hypersil ODS 250x4.6mm, 5µm column was chosen as stationary phase and mobile phase with different compositions of water and methanol was used [9].

Method Selection Criteria

Based on the literature survey and method development data, The EUTICALS SPA API test method has been adopted with minor changes for estimation of Sultiam in tablet dosage form for In-House Sultiam 50mg & 200 mg tablets [10].

Justification For Method Selection

As the product is official in monograph [11], but there is no official method for the analysis of sultiam in tablet dosage form. So the method of analysis directly adopted from drug master file[11,12](DMF) of Sultiam with minor changes.

Trial II**Preparation of Mobile phase**

Mix water and methanol 60:40 ratio and filter through 0.45µm nylon membrane filter and sonicate for 5 minutes.

Diluent preparation:

Prepare the degassed mixture of water and methanol (60:40 v/v).

Standard solution Preparation

Weigh accurately 30 mg of Sultiam working standard and transferred into a 100 mL volumetric flask. Add about 50 mL of diluent to dissolve it completely (sonicate if necessary) and dilute up to the mark with diluents [12].

Sample solution Preparation

20 tablets of Sultiam Weighed and crushed. Weight accurately powder equivalent to 30 mg of Sultiam, transferred into a 100mL volumetric flask and add about 50 mL of diluents to dissolve it completely and sonicate for 20 minutes with intermediate shaking, make up the volume with diluent and filtered through 0.45µm membrane filter [13].

Calculation

Determine the amount of % sultiam in sultiam tablets according to the following formula.

$$\% \text{ Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{100} \times \frac{100}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{Avg. wt}}{\text{LA}} \times 100$$

Where,

AT = Average area of Sultiam in sample solution.

AS =Average area of the five replicate injections of Sultiam in standard solution.

WS= Weight of Sultiam in standard solution in mg

WT= Weight of sample in sample solution in mg

P = Potency of the Sultiam standard on as is basis

Avg. wt = average weight of tablet

LA = Label amount of Sultiam tablets.

Validation of Developed Method and Forced Degradation Studies of Sultiam Tablets By RP-HPLC

The validation describes the procedure for validation of assay method of sultiam 50 mg & 200 mg tablets by HPLC as per ICH Guidelines [14-16].

RESULTS AND DISCUSSION**Results for Analytical Method development****Discussion**

Solution of sultiam was scanned in UV region and spectrum was recorded. Methanol is used as a solvent and it was seen that at 245 nm, the compound has very good absorbance, which can be used for the estimation of sultiam by HPLC method.

Method Development Trials**Trial-II****Discussion**

The main peak was eluted at 4.94 min with the composition of water : methanol(60:40 v/v). The peak shape ,tailing factor of sultiam peak was found to be satisfactory. The tailing factor of sultiam peak was found to be 1.37. The theoretical plates for sultiam peak was found to be 5796.

Results for Analytical Method Validation**Diluent****System suitability results****Acceptance criteria**

The % RSD of area of Sultiam in replicate injections of standard solution should not be more than 2.0. The tailing factor of Sultiam peak should not be more than 2.0. The theoretical plates of Sultiam peak should be more than 2000. The above results reveal that the system is suitable for analysis.

Linearity Results

The linearity response of the Sultiam was determined and found to be linear at the concentration levels shown in the following table and was found to be meeting the acceptance criteria.

Result

The Correlation Coefficient for Sultiam is 1. From this study, it is found that the method is linear.

RANGE RESULTS**Results**

The % RSD for the individual recoveries of each level and mean recovery are not more than 2.0 %. The % recovery at each level and mean recovery are between 98.0% to 102.0%. This study concludes that the method is accurate in the range of 25% to 150% of working concentration.

ACCURACY

The % RSD for the individual recoveries of each level and mean recovery are not more than 2.0 %. The % recovery at each level and mean recovery are between 98.0% to 102.0%.

PRECISION**Repeatability (Method Precision)****Acceptance Criteria**

The % RSD for the assay of Sultiam for six replicate samples should be less than 2.0 %.

Result

The % RSD of Assay from six test preparations for 50mg and 200mg is 0.806 and 0.846. The study concludes that the test results obtained by this method are repeatable and the method is found to be precise.

Intermediate precision:(Analyst-II)**Results**

The % RSD for the assay of Sultiam for six replicate samples for 50mg and 200mg is 0.464 and 0.499. The cumulative % RSD for Assay of Sultiam for six sample preparations intermediate precision along with six sample preparations of repeatability study for 50mg and 200mg is 0.819 and 0.663. The study proves that the

method is rugged for the variabilities like two different instrument, different columns, two different analysts on two different days.

RESULTS FOR ROBUSTNESS**Changes in chromatographic conditions**

The method remains unaffected due to deliberate changes to the analytical method.

Solution Stability**Acceptance Criteria****For sample solution**

The assay value shall not differ from the initial value by more than 2.0 %.

For standard solution

The assay similarity factor should be between 0.98 to 1.02.

Observations**Result****For sample solution**

The % Assay differs by 1.10% & 1.00% from initial value after 24 hours and 48 hrs respectively.

For standard solution

The similarity factor from the initial value after 24 hours & 48 hours is 1.00 and 1.00. It is concluded from the above result that the test and standard solutions are stable at room temperature up to 48 hours.

Filter study**Acceptance Criteria****For sample solution**

The assay value shall not differ from the centrifuged sample to filtered samples by more than 2.0 %.

For standard solution

The similarity factor should be between 0.98 to 1.02.

Table 1. Chromatographic conditions for Trial-II

Column	Hypersil ODS 250x4.6mm, 5 μ
Flow rate	1.0 mL/min.
Wave length	245 nm
Injection volume	10 μ L
Mobile Phase	Water: Methanol (60:40 v/v)
Runtime	10 minutes

Table 2. Results for Trial-II

Name	Retention time(min)	Area percent	Theoretical plates (USP)	USP Tailing
Sultiam	4.967	101	5796	1.37

Table 3. Results of Chromatogram of System suitability

Parameter	Sultiam
Tailing Factor	1.33

%RSD of area	0.041
Theoretical plates	7211
Retention Time	5.943

Table 4. Different levels of Linearity solutions and Areas

Linearity Level	Volume of Stock solution (mL)	Final dilution (mL)	Conc. (in ppm)	Response (mean area)
Level 1 (25%)	2.5	50	75	1618205
Level 2 (50%)	5.0	50	150	3203326
Level 3 (75%)	7.5	50	225	4822620
Level 4 (100%)	10.0	50	300	6394527
Level 5 (125%)	12.5	50	375	8053488
Level 6 (150%)	15.0	50	450	9674189
Y – intercept				21742.64
Slope				-12515.41
Correlation Coefficient				1

Table 5. Recovery data Results(25%)

Recovery level I (25%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	74.62	73.50	98.5
2	74.42	74.05	99.5
3	74.47	74.05	99.4
Mean			99.1
SD			0.551
% RSD			0.56

Table 6. Recovery data Results (50%)

Recovery level II (50%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	149.49	148.40	99.3
2	149.24	148.49	99.5
3	148.97	148.57	99.7
Mean			99.5
SD			0.200
% RSD			0.20

Table 7. Recovery data Results(100%)

Recovery level III (100%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	305.72	300.41	98.3
2	304.44	301.49	99.0
3	301.56	301.25	99.0
Mean			98.8
SD			0.404
% RSD			0.41

Table 8. Recovery data Results(150%)

Recovery level IV (150%)			
Analysis no.	Quantity added in ppm	Quantity recovered in ppm	% Recovery
1	446.05	443.67	99.5
2	447.54	444.35	99.3
3	448.13	444.41	99.2
Mean			99.3
SD			0.153
% RSD			0.15

Table 9. Summary of Recovery data Results

Recovery Level	% Mean Recovery
Recovery level -25%	99.1
Recovery level -50%	99.5
Recovery level -100%	98.8
Recovery level-150%	99.3
Mean Recovery	99.2
SD	0.299
% RSD	0.30

Table 10. Repeatability: (Analyst-I)

Sample. No	% Assay For 50mg	% Assay For 200mg
Sample Preparation-1	100.5	98.9
Sample Preparation-2	101.3	98.8
Sample Preparation-3	102.4	99.4
Sample Preparation-4	102.5	98.4
Sample Preparation-5	102.1	100.7
Sample Preparation-6	101.0	99.9
Avg	101.6	99.4
SD	0.819	0.841
%RSD	0.806	0.846

Table 11. Intermediate precision Results

Sample. No	% Assay For 50mg	% Assay For 200mg
Sample Preparation-1	99.9	99.9
Sample Preparation-2	100.3	99.9
Sample Preparation-3	100.5	99.8
Sample Preparation-4	100.9	99.0
Sample Preparation-5	101.0	99.0
Sample Preparation-6	101.1	98.9
Avg	100.6	99.4
SD	0.467	0.496
%RSD	0.464	0.499

Table 12. Change in mobile phase flow rate

Robustness Criteria	%RSD	Tailing factor	Theoretical plates
Change in mobile phase flow rate by - 0.2ml/ minutes(Flow rate =0.8 ml/minutes)	0.028	1.38	6586
Actual mobile phase flow rate 1ml/minutes	0.107	1.33	7211
Change in mobile phase flow rate by +0.2ml / minutes(Flow rate =1.2 ml/minutes)	0.017	1.34	6000

Table 13. Changes in wavelength

Robustness Criteria	%RSD	Tailing factor	Theoretical plates
Change in wavelength by-2nm (243nm)	0.199	1.36	6390
Actual wavelength 245nm	0.107	1.33	7211
Change in wavelength by+2nm(247)	0.025	1.35	6413

Table 14. Changes in column temperature

Robustness Criteria	%RSD	Tailing factor	Theoretical plates
Change in column temperature by -5°C (20°C)	0.033	1.34	6275
Actual column temperature (25°C)	0.107	1.33	7211
Change in column temperature by +5°C (30°C)	0.067	1.37	6458

Table 15. Changes in mobile phase organic phase

Robustness Criteria	%RSD	Tailing factor	Theoretical plates
Change in mobile phase organic phase by -10% (270ml)	0.029	1.36	6889
Actual organic phase (300ml)	0.107	1.33	7211
Change in mobile phase organic phase by -10% (330ml)	0.040	1.37	5796

Table 16. Solution stability -sample preparations

Preparation	% Assay	Sample Difference
Initial	98.9	NA
After 24 hours	100.0	1.10
After 48 hours	99.9	1.00

Table 17. Solution stability -standard preparations

Preparation	Standard	
	Standard area	Similarity factor
Initial	6502984	NA
After 24 hours	Fresh std:6529379 24hrs sts: 6529085	1.00
After 48 hours	Fresh std: 6519200 48hrs sts: 6517947	1.00

Table 18. Filter study data results for sample

Sample No.	% Assay for Sultiam	Difference
Centrifuged	99.9	NA
0.45 μ GHP filtered	99.9	0.0
0.45 μ Nylon filtered	99.8	0.1

Table 19. Filter study data results for Standard

Sample No.	Standard area for Sultiam	Similarity factor
Centrifuged	6502984	NA
0.45 μ GHP filtered	6518601	1.00
0.45 μ Nylon filtered	6512257	1.00

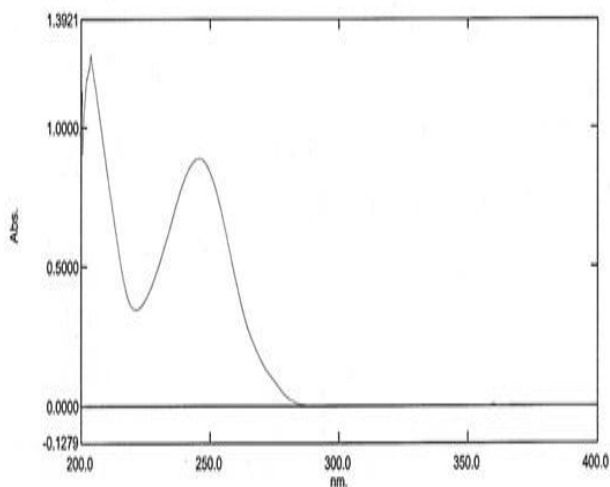
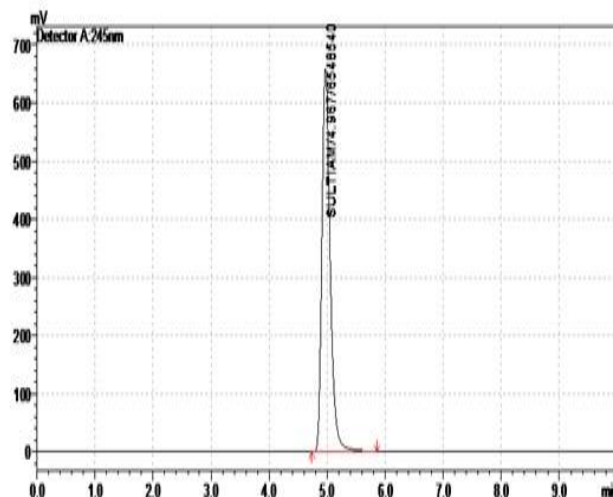
Fig 1. UV spectrum of Sultiam**Fig 2. HPLC Chromatogram of Trial-II**

Fig 3. HPLC Chromatogram of Diluent

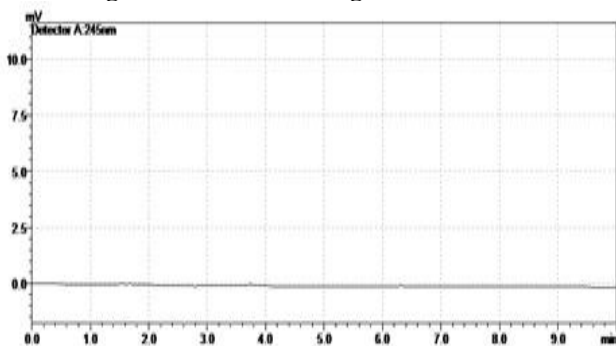


Fig 4. HPLC Chromatogram of system suitability

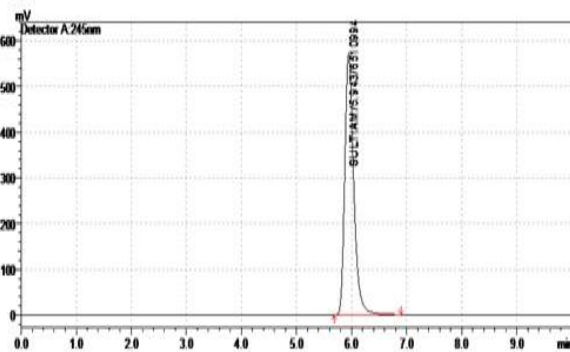


Fig 5. HPLC Chromatogram of Sultiam Linearity

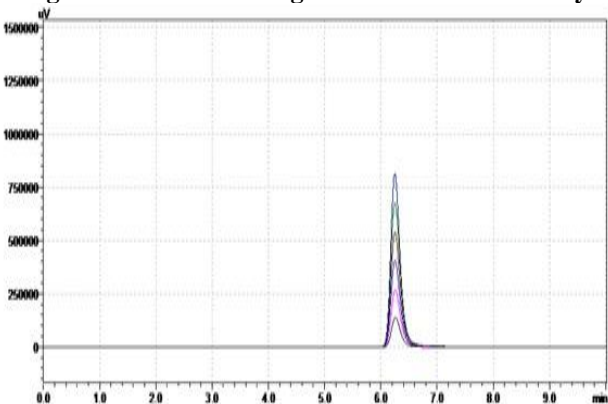


Fig 6. HPLC Chromatogram of Sultiam Range

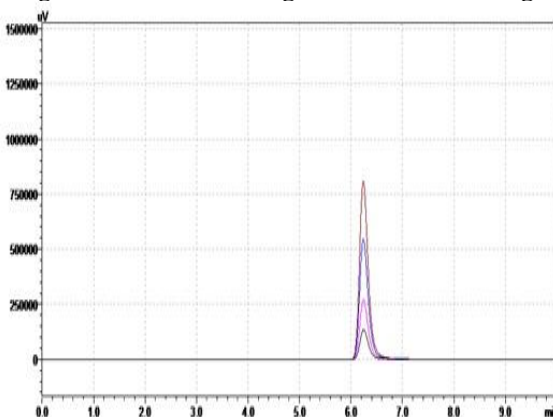


Fig 7. HPLC Chromatogram of Sultiam 0.8 ml flow

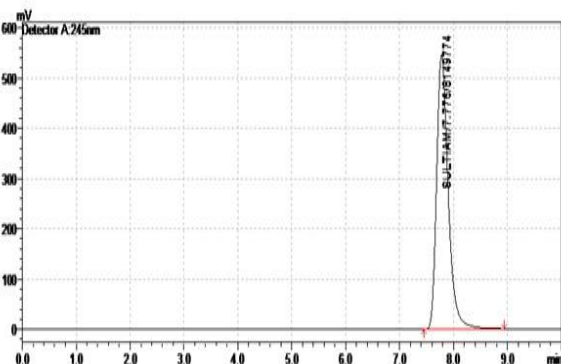


Fig 8. HPLC Chromatogram of Sultiam 1.2 ml flow

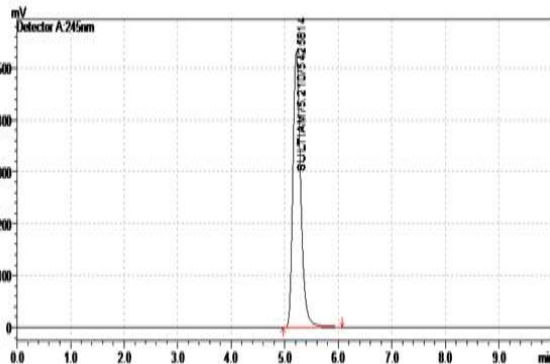


Fig 9. HPLC Chromatogram of Sultiam 243 nm

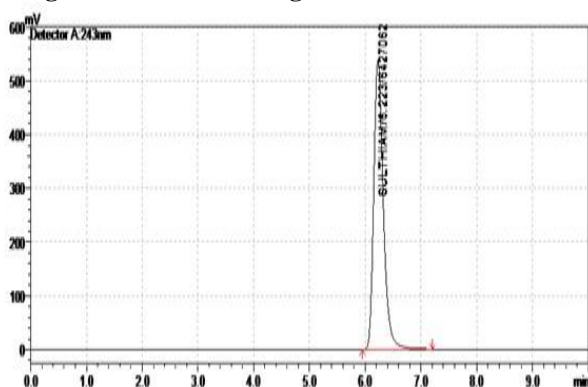


Fig 10. HPLC Chromatogram of Sultiam 247 nm

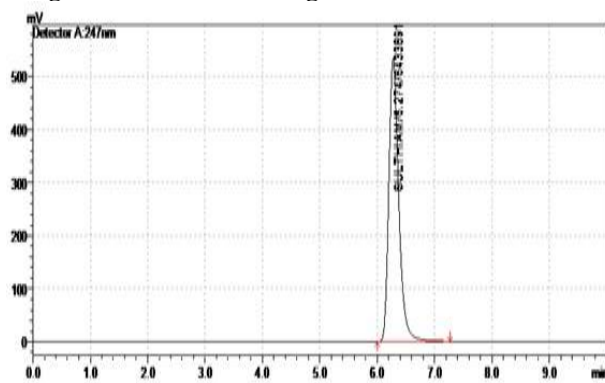


Fig 11. HPLC Chromatogram of Sultiam Low column oven temperature

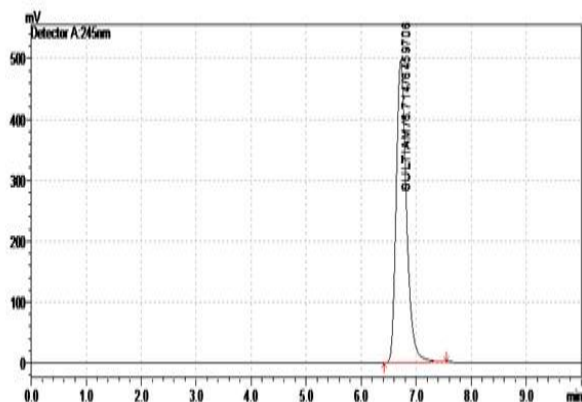


Fig 12. HPLC Chromatogram of Sultiam High column oven temperature

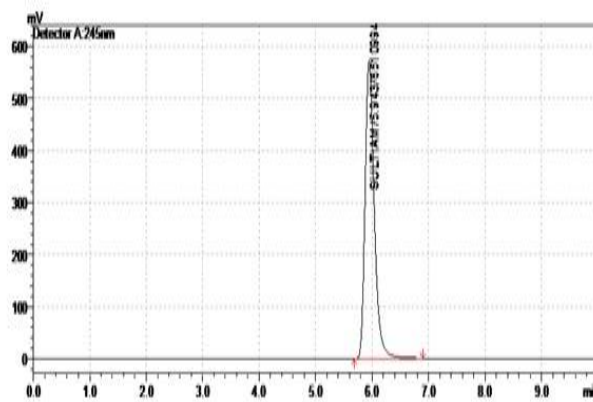


Fig 13. HPLC Chromatogram of Sultiam organic phase - 10% (270ml)

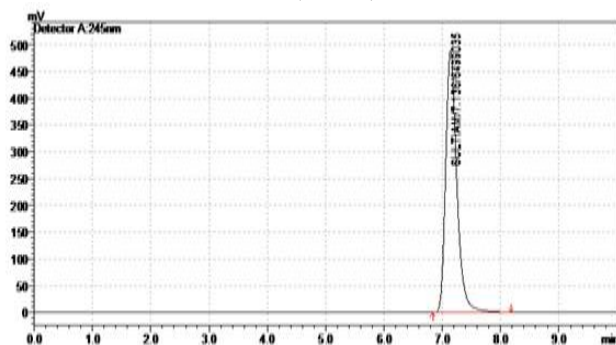
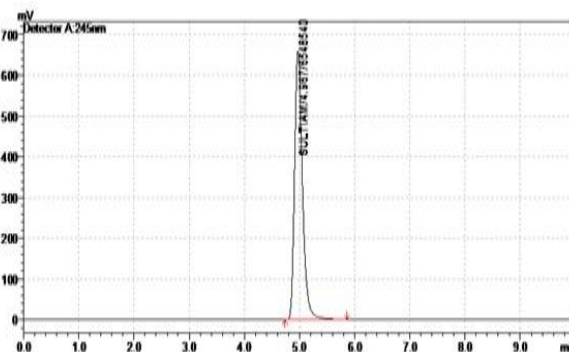


Fig 14. HPLC Chromatogram of Sultiam organic phase +10% (330ml)



SUMMARY AND CONCLUSION

Present investigation was performed for the determination of Sultiam in tablet dosage forms using chromatographic methods with mobile phase as Water and Methanol in the ratio 60: 40 respectively. Literature survey reveals that there are no specific RP-HPLC methods available for the determination of Sultiam in tablet dosage forms. Henceforth we planned to develop a precise, accurate, less time consuming and with low solvent cost RP-HPLC method, was developed and validated as per the ICH guidelines. The assay method for Sultiam in tablet dosage form was developed and validated as per ICH guidelines. The results were found to be within the acceptance limit.

REFERENCES

1. Snyder LR, Kirkland JJ and Glajch LJ. Practical HPLC Method Development. *John Wiley and Sons*, 1997, 98-102.
2. Bauman, John Wiley and Sons. Absorption spectroscopy, Chapter 9, 1962, 23-27.
3. David WG. Pharmaceutical Analysis. Edinburgh: Churchill Livingstone Harcourt publishers, 2000, 237-252.
4. Validation of Analytical Procedures. Methodology, ICH Harmonized Tripartite Guidelines, 1996, 1-8.
5. Christopher RM, Thomas RW. Development and validation of analytical methods, Noida: Reed Elsevier India Private Limited, 2009, 1-67.
6. Frank AS. Hand book of Instrumental Techniques for Analytical Chemistry, Pearson edition, 2004, 147-159.
7. Nash RA, Watcher AH. Validation of analytical methods process in Pharmaceutical process validation. 3rd, 2003, 507-524.

The validated method was found to be simple, specific, precise, accurate, Robust and Rugged for the estimation of Sultiam in tablet dosage form. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy, suitable for chemist-to-chemist and day-to-day for routine analysis as well as for stability analysis.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No Interest

8. Martin W, Dieter B. Carbonic anhydrase inhibitor Sulthiame reduces intracellular pH and Epileptiform activity of Hippocampal CA3 Neurons. *Journal of Epilepsia*, 43, 2002, 469-474.
9. Ashutosh Kar. *Pharmaceutical Drug Analysis*. 2nd ed. New Delhi: New age International (p) Ltd, Publishers, 2005, 293-311.
10. Jeffery GH, Bassett J, Mendham J, Denny KC. *Vogel's Text Book of Quantitative Chemical Analysis*. 5th ed. England: Longman scientific & technical, 8(11), 1988, 645-677.
11. Sharma YR. *Elementary organic spectroscopy, Principles and Chemical Applications*, S.Chand & Company Pvt. Ltd., 7361, Ram nagar, New Delhi- 110055, Fifth revised Edition, 2013, 11-63.
12. Lough WJ, Wainer IW. *High Performance Liquid Chromatography: fundamental principles & practice*. Glasgow (UK): Blackie Academic & Professional, 1995, 2-28.
13. Desman. Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry. *Journal of Pharma Biomedical Analysis*, 996, 867-869.
14. Khoscorur GA, et al. Simple and rapid HPLC methods for simultaneous determination of multiple antiepileptic drugs in human serum. *Journal of Chromatography*, 53, 2001, 345-349.
15. Berry DJ, Clarke LA, Vallins G E. Determination of sulthiame, tetrahydro-2-p-sulphamoyl-phenyl-2h-1,2-thiazine-1,1-dioxide, in plasma at therapeutic concentrations, using high-performance liquid chromatography. *Journal of Chromatography*, 171, 1979, 241-242.
16. Validation of Analytical Procedures. *Methodology, ICH Harmonized Tripartite Guidelines*, 1996, 1-8.