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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SULTIAM TABLETS (WATER AND METHANOL IN THE RATIO 85:15) BY RP-HPLC METHOD

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**ABSTRACT**

A simple, precise, rapid, specific and accurate method has been made for the estimation of Sultiam in formulation by RP – HPLC method taking water and methanol in the ratio 85:15. Standard substance was dissolved in Methanol was scanned and the spectra was recorded and the spectrum shows that $\lambda_{max}$ of Sultiam was 245 nm. Reverse phase chromatographic technique was selected by using Hypersil ODS 250 x 4.6mm, 5µm 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 µg/ ml. the correlation coefficient was found to be 0.998. By using this method, the main peak of Sultiam was eluted at 7.13 minutes. In this method the optical parameters like Correlation coefficient, Slope, Intercept, LOD and LOQ were calculated.

**Key words:** Methanol, Correlation coefficient RP-HPLC, Calibration curve, Sultian.

## INTRODUCTION

Sultiam (rINN, also known as sulthiame) is a sulfonamide and inhibitor ofthe enzyme carbonic anhy drase. It is used as an anticonvulsant. Sultiam [1] became established as a second-line drug for treatment of partial epilepsy in the 1960s and 1970s and was often used in combination with the established anti convulsant phenytoin. Chemical name of Sultiam is 4-(Tetrahydro-2H-1,2-thiazin-2-yl)benzenesulfonamide- S,S-dioxide. The molecular weight of the compound is 290.4. The molecular formula is C10H14N2O4S2. The structure of sulthiame is distinct from that of other anticonvulsants. It is a cyclic sulphonamide derivative without antimicrobial activity.

HPLC is a type of liquid chromatography [2] to quantify and analyse mixtures of chemical and synthetic compound for separating non-volatile species or thermally fragile compounds. This technique [3,4] 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. The present work was done on sultiam tablets. An analytical method was developed and validated as per ICH [5,6,7] guidelines.

## MATERIALS AND METHODS

| **Instrument** | **Made** |
|---|---|
| • Semi micro balance | Sartorius |
| • Micro balance | Sartorius |
| • pH Meter | Thermo Electron Corporation |
| • HPLC | Shimadzu |
| • HPLC | Agilent |
| • Column ODS 250x4.6mm, 5µ | Hypersil |

**Reagents and Chemicals**

- Methanol - GR-Grade

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- Water - TKA water
- Sodium hydroxide - GR-Grade
- Potassium hydroxide - GR-Grade
- Hydrogen peroxide - GR-Grade
- Hydrochloric acid - GR-Grade

**Working/Reference Standards**

- Sultiam Working Standard purity 99.7%

**Test samples**

- Sultiam tablets 50mg
- Sultiam tablets 200mg
- Placebo for Sultiam tablets 200mg.

**Filters**

- 0.45µm GHP membrane filtered (Manufactured by PALL)
- 0.45µm 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.

**Selection of chromatographic method**:

Proper selection of the method depends upon the nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, $P^{ka}$ [9] value and stability. The drug selected in the study is polar and reversed phase chromatography can be used. The reversed phase HPLC was selected for the initial separation because of its simplicity and suitability.

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.

**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.

**Justification for Method Selection**

As the product is official in monograph [10], 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-I**

**Preparation of Mobile phase**

Mix water and methanol 85:15 ratio and filter through 0.45µm nylon membrane filter and sonicate for 5 minutes.

**Diluent preparation**

Prepare the degassed mixture of water and methanol (85:15 v/v).

**Standard solution Preparation**

Weigh accurately 30 mg of Sultiam working standard and transfered 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 diluent.

**Sample solution Preparation**

20 tablets of Sultiam Weighed and crushed. Weight accurately powder equivalent to 30 mg of Sultiam, transfered 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.

**Calculation**

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

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

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. The analytical procedure shall be validated for the following parameters. System suitability, Specificity, Linearity, Range,

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Accuracy, Precision, Repeatability, Intermediate Precision, Robustness.

## RESULTS AND DISCUSSION

### Results for Analytical Method development

### Wavelength Fixation

### 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-I

### DISCUSSION

The main peak was eluted at 7.13 min with the composition of water : methanol(85:15). The peak shape ,tailing factor and theoretical plates 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 6058.The Rt value is too more, so we go to next trial.

### 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**.**

### Conclusion

From this study, it is found that the method is linear.

### RANGE RESULTS

### HPLC Chromatogram of Sultiam Range

### 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 concludesthatthemethod 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 assayof 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

### HPLC Chromatogram of Sultiam at 0.8 ml flow

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 24hours and 48 hrs respectively.

### For standard solution

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

### Filter study

### Acceptance Criteria

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**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-I**

| | |
|---|---|
| Column | Hypersil ODS 250x4.6mm, 5µ |
| Flow rate | 1.0 mL/min. |
| Wave length | 245 nm |
| Injection volume | 10µL |
| Mobile Phase | Water: Methanol (85:15 v/v) |
| Runtime | 10 minutes |

**Table 2. Results for Trial-I**

| Name | Retention time(min) | Area percent | Theoretical plates (USP) | USP Tailing |
|---|---|---|---|---|
| Sultiam | 7.13 | 97 | 6058 | 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** |

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| | | |
|---|---|---|
| | **% 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 |

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| | | |
|---|---|---|
| **Avg** | **100.6** | **99.4** |
| **SD** | **0.467** | **0.496** |
| **%RSD** | **0.464** | **0.499** |

**Table 12. Changse 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 | Sample | |
|---|---|---|
| | % Assay | 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<br>24hrs sts: 6529085 | 1.00 |
| **After 48 hours** | Fresh std: 6519200<br>48hrs sts: 6517947 | 1.00 |

**Table 18. Filter study data results for sample**

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

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**Table 19. Filter study data results for Standard**

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

**Fig 1. UV spectrum of Sultiam**

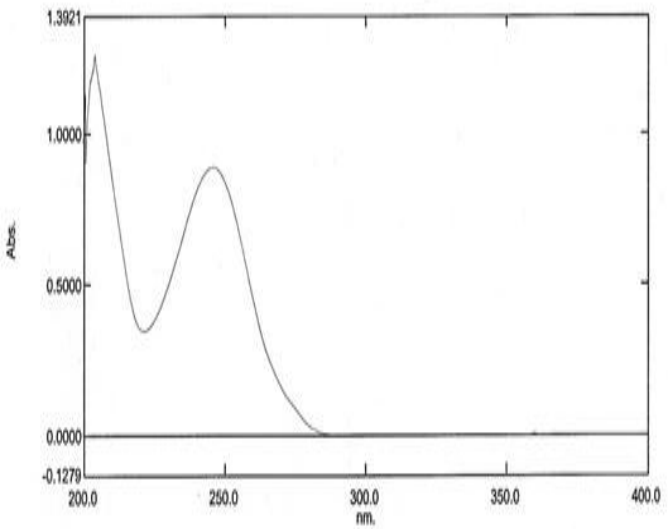


**Fig 2. HPLC Chromatogram of Trial-I**

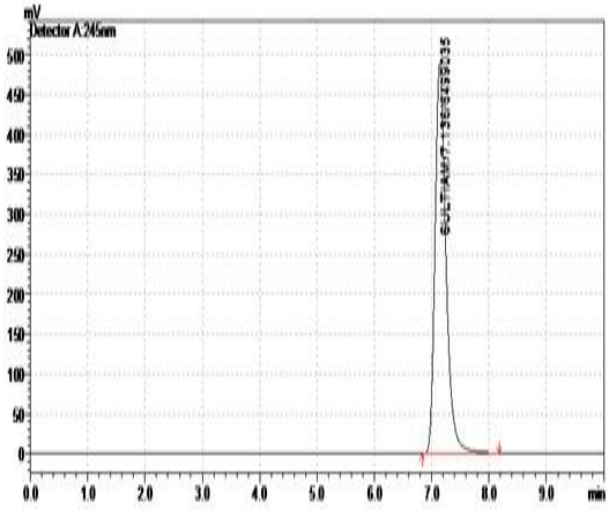


**Fig 3. HPLC Chromatogram of Diluent**

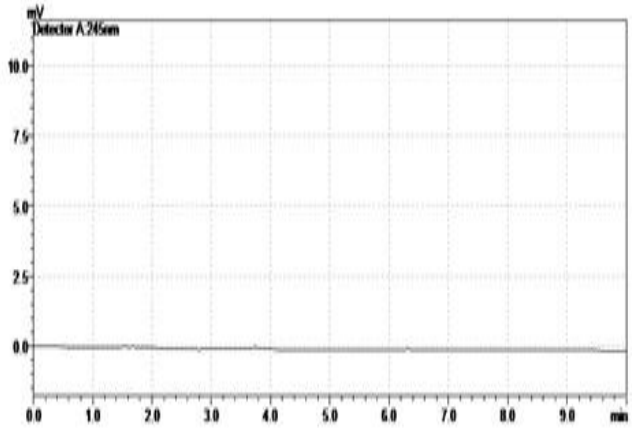


**Fig 4. HPLC Chromatogram of system suitability**

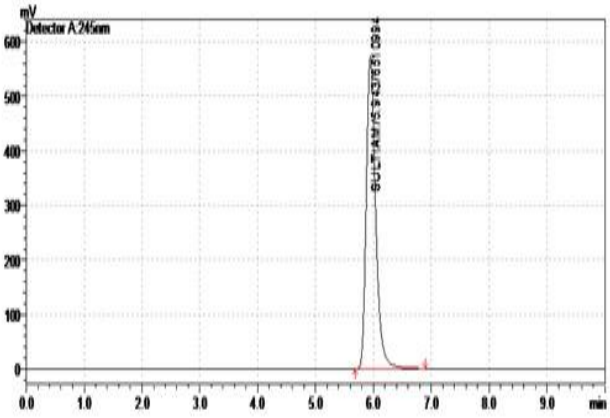


**Fig 5. HPLC Chromatogram of Sultiam Linearity**

**Fig 6. HPLC Chromatogram of Sultiam Range**

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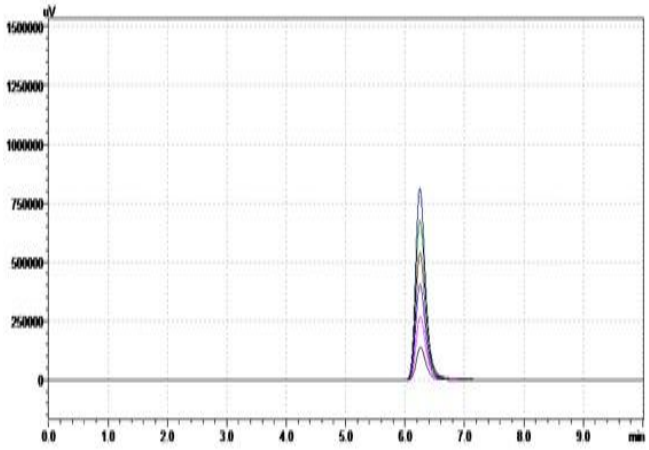


**Fig 7. HPLC Chromatogram of Sultiam 0.8 ml flow**

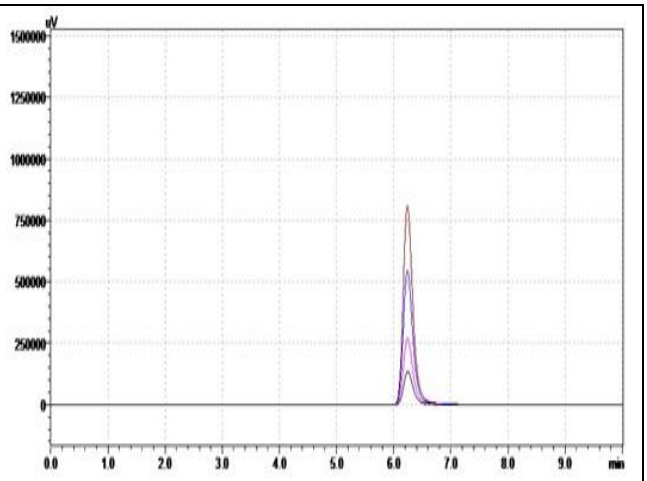


**Fig 8. HPLC Chromatogram of Sultiam 1.2 ml flow**

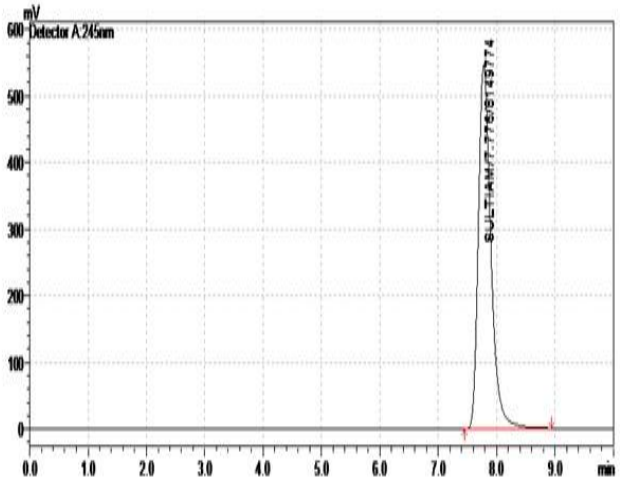


**Fig 9. HPLC Chromatogram of Sultiam 243 nm**

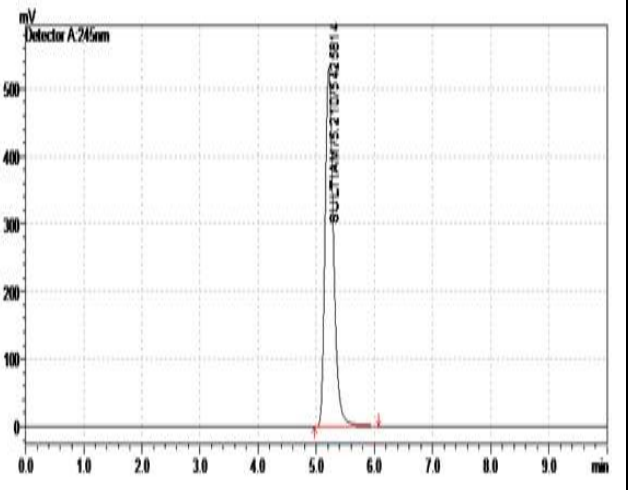


**Fig 10. HPLC Chromatogram of Sultiam 247 nm**

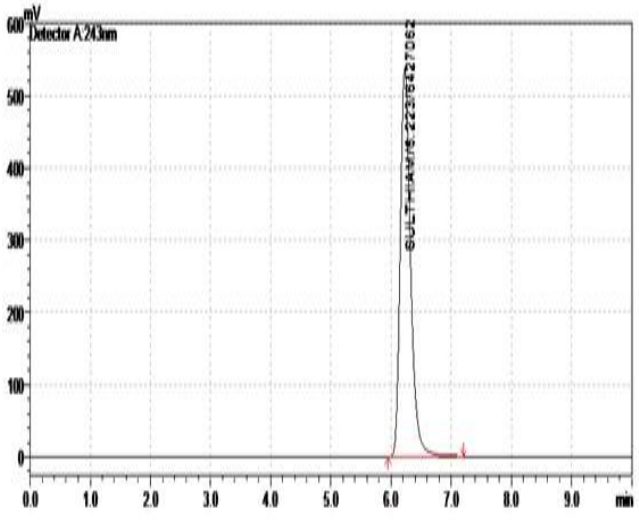


**Fig 11. HPLC Chromatogram of Sultiam Low column oven temperature**

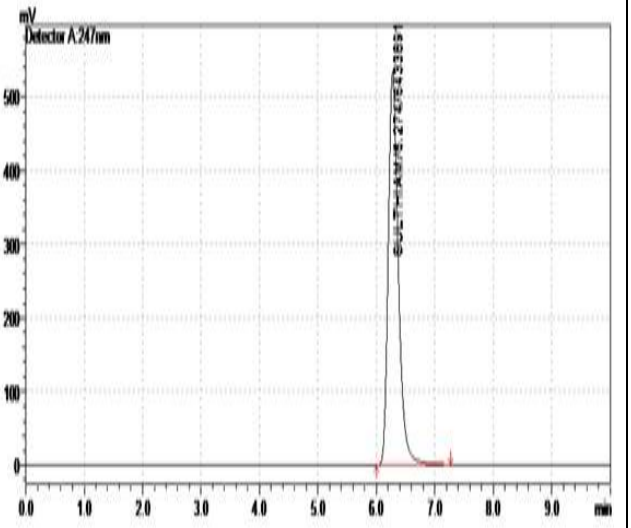


**Fig 12. HPLC Chromatogram of Sultiam High column oven temperature**

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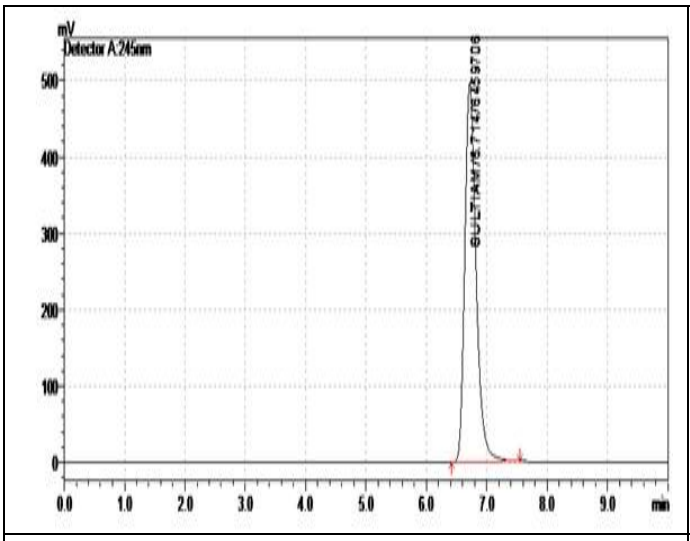



**Fig 13. HPLC Chromatogram of Sultiam organic phase - 10% (270ml)**

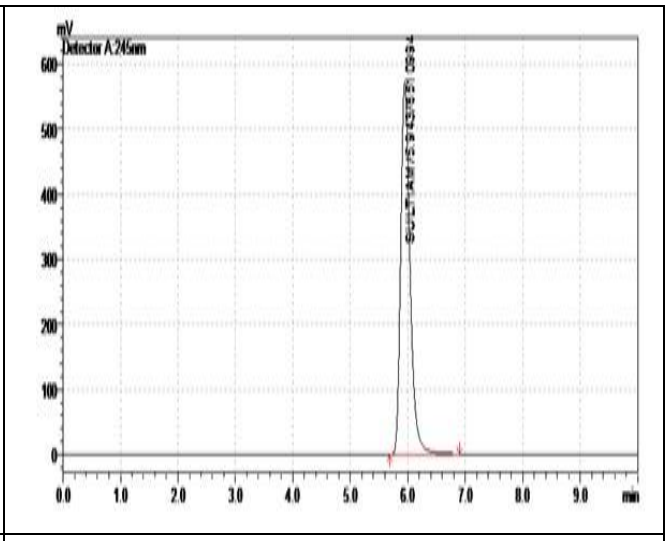
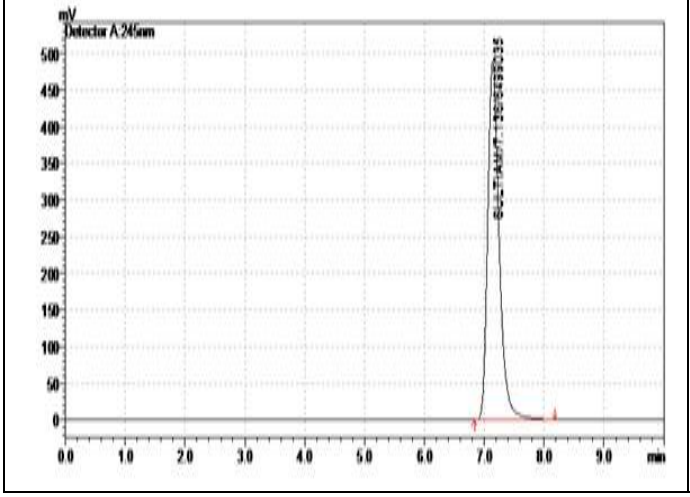
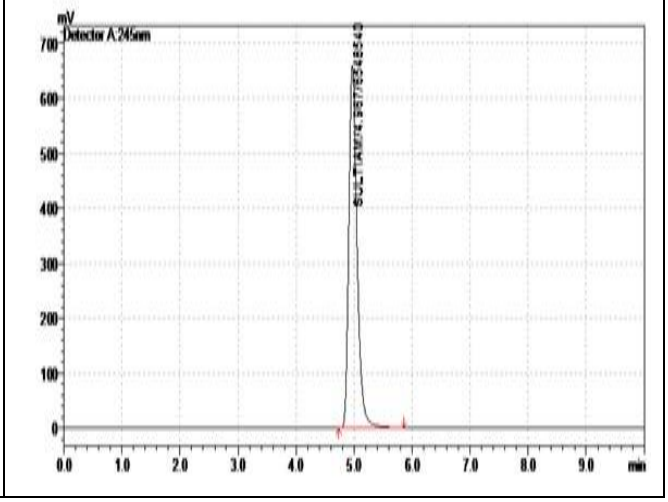



**Fig 14. HPLC Chromatogram of Sultiam organic phase +10% (330ml)**




## 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 85:15 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.

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.

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