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Development, Validation and simultaneous estimation of Sulfamethoxazole and Trimethoprim by RP-HPLC Method

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Abstract

The straightforward, precise, accurate approach has been established for such simultaneous determination of a Sulfamethoxazole as well as Trimethoprim through oral dosage form. Chromatogram has been operate via inertsil ODS C185µm (4.6 x 250mm). Mobile phase comprising phosphate - buffered as well as acetonitrile with in proportion like 30:70 has been fired up thru section at such a rate of flow like 1ml/min. Buffer in use at pH 4.6, temperature has been retained there as ambient. Optimization frequency range such as Sulfamethoxazole as well as Trimethoprim has been 249nm. Time of retention like Sulfamethoxazole as well as trimethoprim have been did find of being 2.327min as well as 4.342min. A % purification like Sulfamethoxazole as well as Trimethoprim has been did find of being 100.6% as well as 101.3% including both. a stability studies parametric such as Sulfamethoxazole as well as Trimethoprim like theoretical plates as well as tailing element have been did find of being 1.3, 5117.5.as well as 1.4, 3877.3 a resolution has been did find of being 4.1.a linear relationship research such as Sulfamethoxazole as well as Trimethoprim has been present in range of concentrations like 20 ppm-100 ppm but also 10 ppm-50 ppm as well as coefficient of correlation (r2) has been did find of being 0.9999 as well as 0.9998, % average retrieval has been did find of about 99.96% as well as 99.75%, % RSD such as reproducibility was 0.31 as well as 0.38, % RSD such as medium precision has been 0.12 but also 0.15 including both. A precision research was accurate, robust as well as reproducible. LOD valuation has been 0.48 as well as 0.55, but also LOQ valuation has been 0.95 but also 1.10 including both.

Keywords: Sulfamethaxazole and Trimethoprim, RP-HPLC

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1. Introduction

HPLC is indeed a very delicate analytical tool most generally uses it for quantity and quality analyze like pharmaceutical drugs. Its basic concept benefit like HPLC in comparison to conventional column chromatography has been enhanced resolution of a differentiated material, quicker detachment period as well as the improving the accuracy, precision as well as sensitivity8. Normal phase chromatography through normal phase method, a stationary phase (e.g. silica gel) seems to be polar throughout evolution as well as the mobile phase would be polar and non - polar inside this particular method, non-polar substances move quicker and thus are solubilized initial. (1) This because fewer affinity among solvent but also stationary phase. Polar molecules have been maintained such as longer duration with in column because of more affinity toward the stationary phase as well as require more time to also be eluted through column. That's not beneficial through pharmaceutical applications because most of the opioid particles polar through nature as well as requires a lot of time to also be solubilized as well as intercepted. (2) Thus this particular method isn't really broadly used during pharmacist. Reversed - phase chromatography (RP-HPLC) a stationary phase has been non-polar mostly a hydrocarbon as well as the mobile phase is comparatively polar such like water, methanol as well as acetonitrile. Through RPC a soluble compounds have been solubilized with in sequence of their own lowering polarities. All those are able to prepare along going to treat a surface like silanol group with just an organochlorosilane solvent. (3,4)

High performance liquid chromatography relies on it method like adsorbent, portion, ion exchange as well as size separation, depending on type like stationary phase

utilised. High performance liquid chromatography includes a solid stationary phase, normally stuffed inside of the stainless- structural steel, as well as a liquid mobile phase. Detachment of a elements of such a solution outcomes through the variation with in relative dispersion ratio analysis of a solvent between both the 2 stages.

A most of such HPLC detachment is completed to reversed phase separation; possibly around 90%.through reversed phase separation organic compounds have been kept separate based on degree like hydrophilic/hydrophobic. (5) There will be a similarity between both the degree like lipophilicity as well as retention with in column. That's the record like mobile phase parametric impacting retention as well as detachment through reversed phase. Eluent arrange through normal phase hplc demonstrates that such polar solvent solubilize subsequently and after that non - polar solvents lypophilic ones. (6)

2. Materials and Methods

Reagents

Along with triple-distilled water, all the components used in this experiment were of analytical quality. Potassium Dihydrogen Orthophosphate, Di Potassium Hydrogen Orthophosphate, Acetonitrile, Methanol, Dimethyl formamaide, Ortho phosphoric acid, were purchased from S.D. Fine Chem Ltd. Sulfamethoxazole and trimethoprim were purchased form JRS Labs.

3. Analytical Method Validation:

3.1 Specificity

A system suitability such as specificisty has been conducted to determine if there is the certain involvement of the any alloying elements through retention time like analysis peak. (7,8)

Sulfamethoxazole and Trimethoprim Identification

The sample and standard solutions prepared are injected in to the HPLC system.

Acceptance criteria:

Chromatogram of sample as well as standard Retention time should be identical/ nearer

Interference of Blank

Without API only diluent is injected (i.e. mobile phase).

Acceptance criteria:

The chromatogram of Diluent neither show any peaks that should interfere with sample peaks

Interference of Placebo:

The excipients of the tablet without API, the solution prepared is injected

Procedure:

Each sample is injected in an order by placing them in a auto sampler injector. Then three chromatogram are over layered to check if there any interference with the analyte peak.

Acceptance criteria:

The chromatogram of Diluent should not show any peaks that should interfere with sample peaks.

3.2 Linearity

Preparation of stock solution:

Sulfamethoxazole:

20 mg like Sulfamethoxazole working standard has been weighed accurately as well as transmitted it into a 10 ml fresh clean measuring cylinder as well as about 2 ml of solvent has been got to add. Then that is ultrasonication of about disperse this totally but also decided to make volume up to mark also with solvent. (9) Much farther 25.0ml from above standard solutions has been burette it in to a 100 ml volumetric flask and also has been dissolved up to the mark as for solvent.

Trimethoprim:

4mg like trimethoprim working standard has been weighed accurately as well as transmitted into the 10ml fresh clean volumetric flask or about 2ml of dissolution medium has been got to add. So it is pretreated of about disperse totally but also decided to make volume up to mark also with solvent. Much farther 25.0ml from above standard solutions has been burette it in to a 100ml measuring cylinder and also was dissolved up to mark as for solvent.

Preparation of Level–I (20ppm of Sulfamethoxazole & 10ppm of Trimethoprim):

0.4ml of Sulfamethoxazole stock solution and 1ml of Trimethoprim standard solution was taken within 10ml of volumetric flask and diluted up to mark as or solvent.

Preparation of Level – II (40ppm of Sulfamethoxazole & 20ppm of Trimethoprim):

0.8ml of Sulfamethoxazole stock solution and 2ml of Trimethoprim stock solution has taken 10ml of volumetric flask as well as diluted up on to mark as for solvent.

Preparation of Level – III (60ppm of Sulfamethoxazole & 30ppm of Trimethoprim):

1.2ml of Sulfamethoxazole stock solution and 3ml of Trimethoprim standard solution was begun to take within 10ml of volumetric flask and diluted up in to mark as for solvent.

Preparation of Level – IV (80ppm of Sulfamethoxazole & 40ppm of Trimethoprim):

1.6ml of Sulfamethoxazole stock solution and 4ml of Trimethoprim standard solution was begun to take within 10ml of volumetric flask as well as diluted up on to mark as for solvent.

Preparation of Level – V (100ppm of Sulfamethoxazole & 50ppm of Trimethoprim):

2ml of Sulfamethoxazole stock solution and 5ml of Trimethoprim Standard solution has begun to take within 10ml of volumetric flask as well as dissolved up on to mark as for solvent.

Procedure:

Every stage has been infused into in the chromatographic system as well as the peak shape has been evaluated. a graph like peak shape vs concentration (on x-axis concentration and on y-axis peak area) has been obtained by plotting as well as the coefficient of correlation has been measured. (10)

Acceptance criteria

Correlation coefficient should be more than 0.999 (r^2>0.999).

3.3 Range

Based over precision, linear relationship as well as accurateness statistics this can be conclusively proved that such assay procedure has been precise, linear but also accurate with in range like 20µg-100µg as well as 10µg- 50µg of Sulfamethoxazole but also Trimethoprim respectively . (11,12)

3.4 Accuracy

Preparation of standard stock solution (Sulfamethoxazole and Trimethoprim):

Subsequently measure 20 mg of Sulfamethoxazole as well as 4mg like Trimethoprim working standard have been transmitted it in to a 10ml of fresh dry volumetric flasks.approximately 2ml like dissolution medium is added as well as sonicated of about disintegrate this entirely as well as made volume up to the mark as for Diluent. Further 25ml of above solution was pipetted it in to a 100ml measuring cylinder as well as solubilized up to mark as for solvent. (13)

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh 10mg of Sulfamethoxazole and 2mg of Trimethoprim working standard have been did weigh as well as transmitted it in to a 10ml of fresh dry measuring cylinder and around 2ml of diluents has been got to add but also sonicated of about disperse this entirely but also made volume up to the markas for Diluent. Much farther 25ml of an above Sulfamethoxazole as well as Trimethoprim have been pipetted it in to a 100ml measuring cylinder as well as dilution up to the mark as for solvent.

For preparation of 100% solution (With respect to target Assay concentration):

Appropriately measure 20mg of Sulfamethoxazole as well as 4mg of trimethoprim operating standard have been transmitted it in to a 10ml of fresh clean volumetric flasks. Most of 2ml of diluent has been got to add but also ultrasonication of about disperse this totally as well as created volume up to the mark as for Diluent. Further 25ml of the above standard solutions was pipetted it in to a 100ml measuring cylinder as well as prepared by diluting up to the mark thus for diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Appropriately weigh 30mg of Sulfamethoxazole as well as 6mg trimethoprim of operating standard have been did weigh but also transmitted it in to a 10ml of fresh clean measuring cylinder and also about 2ml of diluent has been added but also ultrasonication of about disperse this entirely as well as decided to make volume up to mark also with diluent. Much farther 25ml of the above Sulfamethoxazole and trimethoprim solution have been pipetted it in to a 100ml measuring cylinder as well as prepared by diluting up to the mark as for diluents.

Procedure:

A standard solution, accuracy -50%, accuracy -100% as well as accuracy -150% solutions have been infused. The quantity did find as well as amount given such as Sulfamethoxazole as well as trimethoprim and the individual retrieval as well as average retrieval values were determined.

Acceptance criteria:

Accuracy % mean recovery should be between 98 - 102%

3.5 Precision

Repeatability

Preparation of standard Stock solution:

Appropriately weigh 10mg like Sulfamethoxazole as well as 2mg of trimethoprim working standard have been did weigh as well as transmitted it in to a 10ml of fresh dry measuring cylinder and also about 2ml of solvent has been got to add as well as sonicated of about disperse this totally as well as created volume up to mark with solvent. Further 25ml of the above Sulfamethoxazole as well as trimethoprim were pipetted it in to a 100ml measuring cylinder as well as solubilized up to mark as for solvent. (14)

Procedure:

A standard sample is injected such as 5 times as well as the regions over all 5 infusions through HPLC have been evaluated. A % rsd again for region of 5 replicate infusions has been did find to be within prescribed limit.

Acceptance criteria:

A % rsd again for region of 5 standard infusions outcomes should be no more than 2.

Intermediate Precision (Ruggedness)

To evaluate its transitional precision (also referred to as ruggedness) of a technique, precision has been characterized by different days through using different make column of the same measurements.

Preparation of standard stock solution:

Appropriately weigh 20 mg of Sulfamethoxazole as well as 4mg of trimethoprim operating standard have been transmitted it in to a 10ml of fresh clean volumetric flasks. most of 2ml of diluent is added as well as sonicated of about disperse this entirely but also decided to make volume up to mark also with diluent. Much farther 25ml of above solution was pipetted it in to a 100ml measuring cylinder but also prepared by diluting up to mark as for Diluent.

Procedure:

A standard sample has been infused such as five times as well as a region with every five infusions evaluated through hplc. Its %rsd for such region of 5 simulate infusions has been did find is within the prescribed limit.

Acceptance criteria:

A % RSD for such region of 5 specimen infusions outcomes should be no more than 2%.

3.6 Robustness

As a part of its robustness, premeditated transition with in flow rate, mobile phase component has been decided to make to assess its affect upon that technique. (15,16)

The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 500ppm of Sulfamethoxazole and 100ppm of Trimethoprim was prepared and analyzed using the varied flow rates along with method flow rate. The organic composition in the mobile phase was varied from 65% to75%.

Preparation of Standard stock solution:

Appropriately weigh 20 mg of Sulfamethoxazole as well as 4mg of trimethoprim operating standard have been transmitted it in to a 10ml of fresh clean volumetric flasks. most of 2ml of diluent is added as well as sonicated of about disperse this entirely but also decided to make volume up to mark also with Diluent. Much farther 25ml of above solution was pipetted it in to a 100ml measuring cylinder but also prepared by diluting up to mark as for Diluent.

Acceptance criteria: The method should unaffected for the slightest changes

3.7 LOD

Preparation of stock solution (100ppm):

Appropriately weigh 20 mg of Sulfamethoxazole as well as 4mg of trimethoprim operating standard have been transmitted it in to a 10ml of fresh clean volumetric flasks. most of 2ml of diluent is added as well as sonicated of about disperse this entirely but also decided to make volume up to mark also with Diluent. Much farther 25ml of above solution was pipetted it in to a 100ml measuring cylinder but also prepared by diluting up to mark as for Diluent.

1ml of stock solution was acquired through 10ml of volumetric flask as well as diluted up to mark as for Diluent.

Acceptance criteria: Not more than 3

3.8 LOQ

Preparation of stock solution (100ppm):

Appropriately weigh 20 mg of Sulfamethoxazole as well as 4mg of trimethoprim operating standard have been transmitted it in to a 10ml of fresh clean volumetric flasks. most of 2ml of diluent is added as well as sonicated of about disperse this entirely but also decided to make volume up to mark also with Diluent. (17) Much farther 25ml of above solution was pipetted it in to a 100ml measuring cylinder but also prepared by diluting up to mark as for Diluent.

Acceptance criteria:

No more than 10

4. Results and Discussion

Wavelength Detection

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual land mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Sulfamethoxazole and Trimethoprim was obtained and the isobestic point of Sulfamethoxazole and Trimethoprim showed absorbance's maxima at 249 nm [Figure 1].

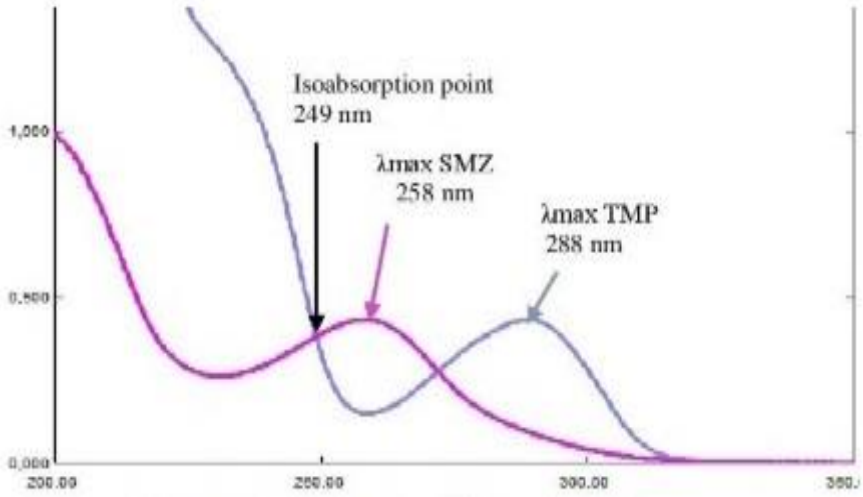

Figure 1. Overlapping spectrum of Sulfamethoxazole and Trimethoprim Isoabsorption Point 249nm

5. Validation Results:

5.1 Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak [Table 1 &

2]. The study was performed by injecting blank [Figure 2 & 5].

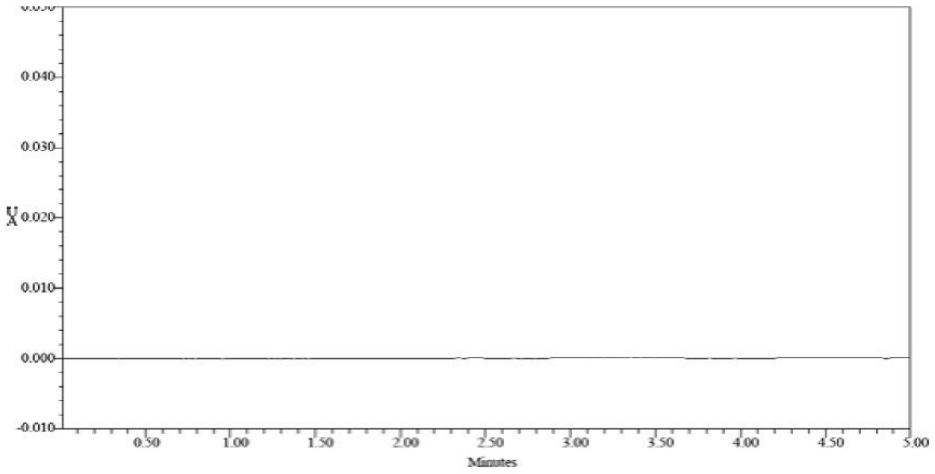

Figure 2. Chromatogram of blank Injection

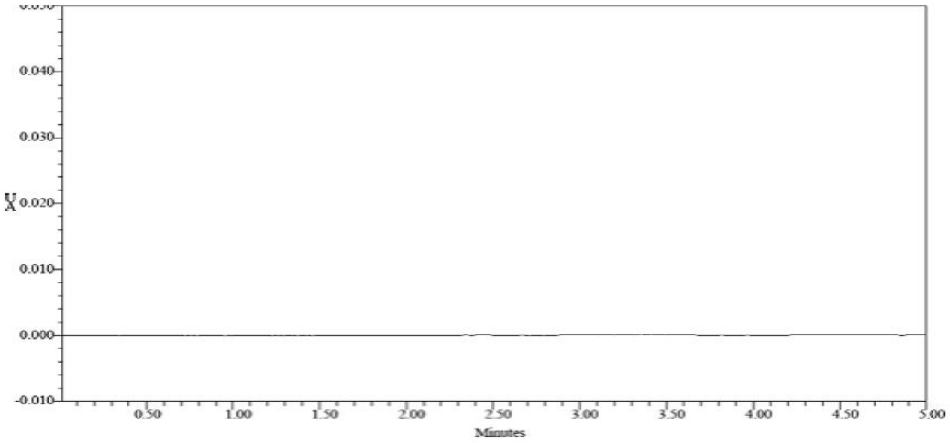

Figure 3. Chromatogram of Placebo Injection

Table 1. Details of specificity standard injection

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Sulfamethoxazole	2.237	7913799	394185	5117.5	1.3	
2	Trimethoprim	4.342	1853381	162758	3877.3	1.4	4.1

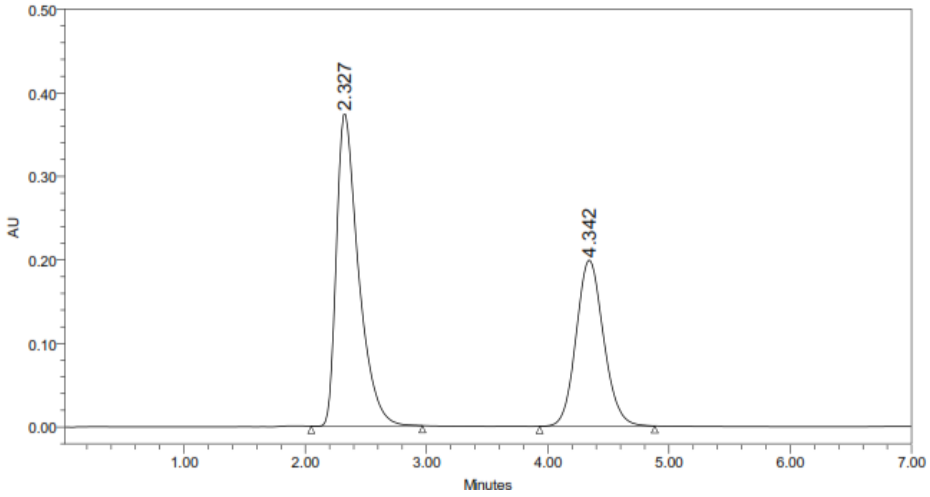

Figure 4. Chromatogram of Specificity Standard injection

Table 2. Details of specificity sample injection

S.No.	Peak name	Rt	Area	Height	USP Plate	USP	USP
1	Sulfamethoxazole	2.326	7726354	376488	5225	1.60	
2	Trimethoprim	4.344	1722571	158418	3823	1.11	4.3

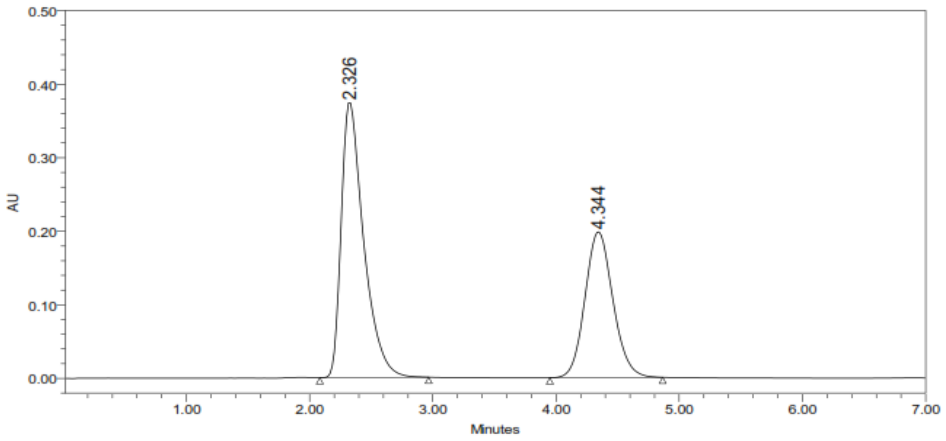

Figure 5. Chromatogram of Sample Injection

5.2 Linearity

The linearity study was performed for the concentration of 20ppm to100ppm and 10ppm to 50ppm level. Each level was injected into chromatographic system [Figure 6 & 10]. The area of each level was used for calculation of correlation coefficient [Table 3 & 4].

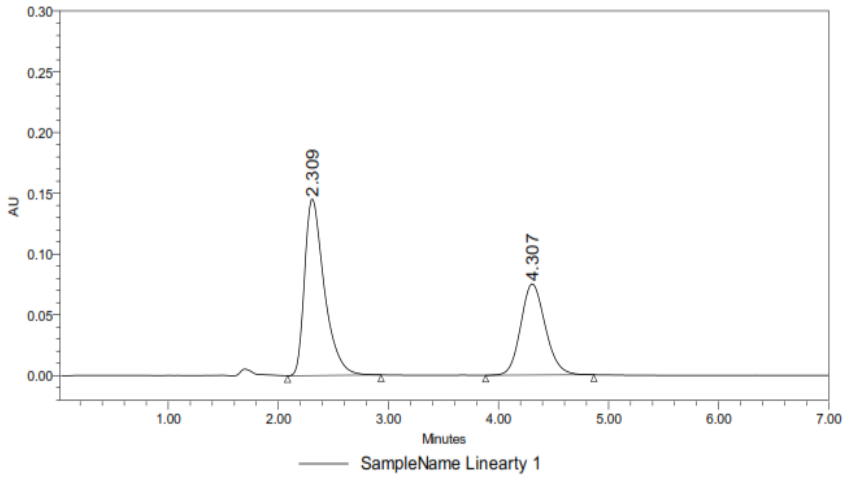

Figure 6. Chromatogram for Linearity Inj Level-1

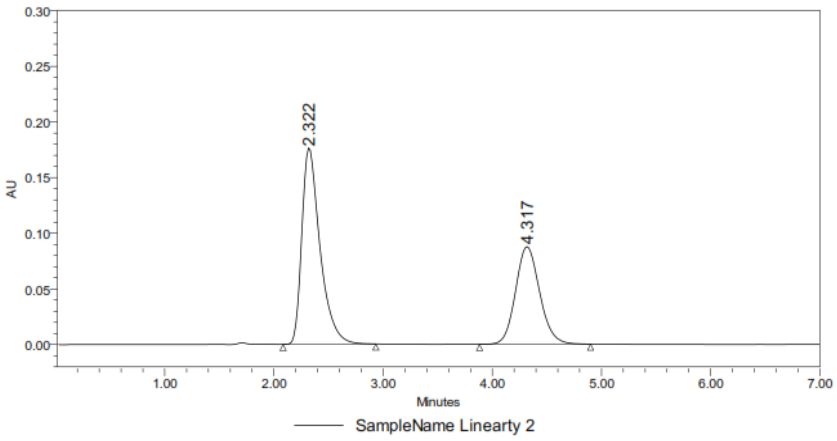

Figure 7. Chromatogram for Linearity Inj Level-II

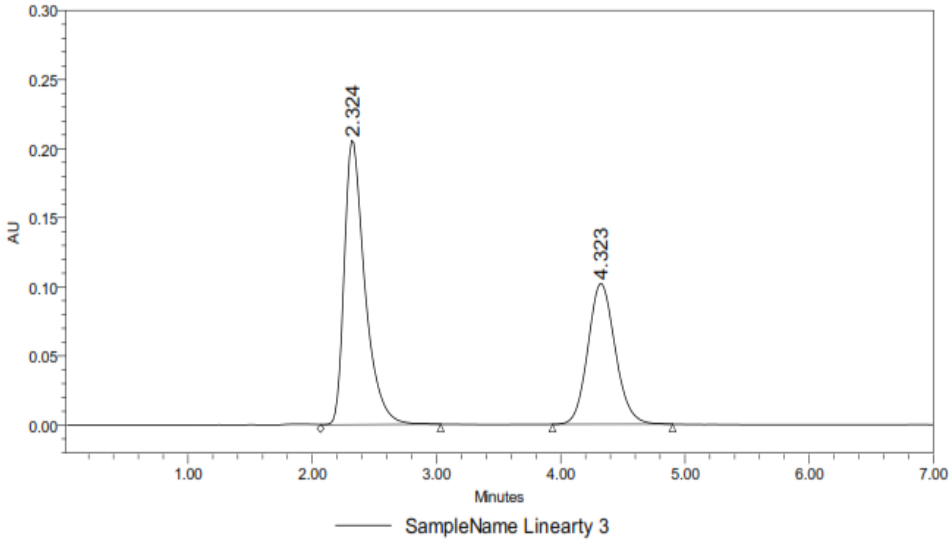

Figure 8. Chromatogram for Linearity Inj Level-III

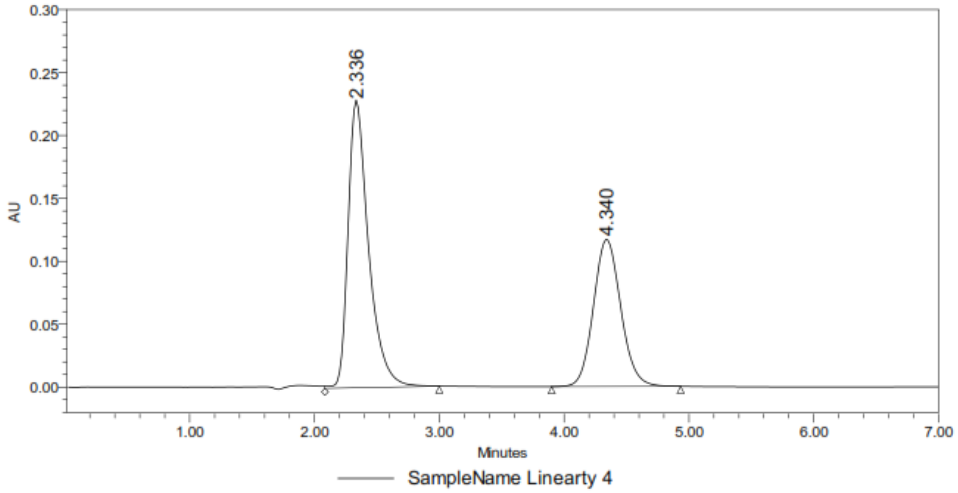

Figure 9. Chromatogram for Linearity Inj Level-IV

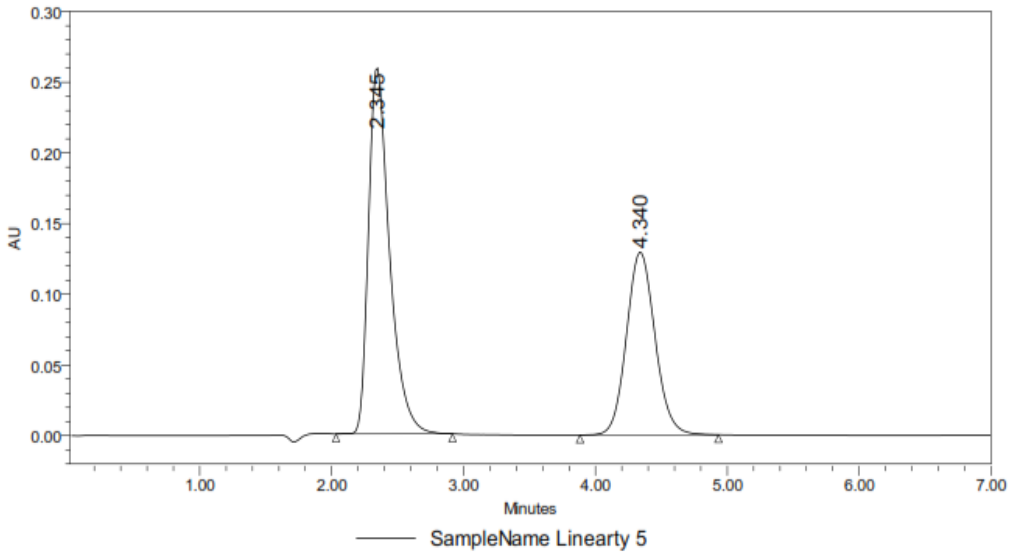

Figure 10. Chromatogram for Linearity Inj Level-V

Linearity Results

Table 3. Linearity results of Sulfamethoxazole

S.NO	SAMPLE NAME	CONCENTRATION	RT	AREA	HEIGHT
1	Linearity 1	20 ppm	2.309	1810101	145867
2	Linearity 2	40 ppm	2.322	2044873	176895
3	Linearity 3	60 ppm	2.324	2367122	206674
4	Linearity 4	80 ppm	2.336	2102248	228475
5	Linearity 5	100 ppm	2.345	2869772	259345

Table 4. Linearity results of Trimethoprim

S.NO	SAMPLE NAME	CONCENTRATION	RT	AREA	HEIGHT
1	Linearity 1	10 ppm	4.304	1164173	74586
2	Linearity 2	20 ppm	4.323	1342555	87689
3	Linearity 3	30 ppm	4.214	1556824	101999
4	Linearity 4	40 ppm	4.524	1774565	117084
5	Linearity 5	50 ppm	4.340	1956421	129409

5.3 Range

The linearity study was performed for concentration range of 20ppm-100ppm and 10ppm–50ppm of Sulfamethoxazole and Trimethoprim. The correlation coefficient was found to be 0.9999 and 0.9998.

5.4 Accuracy

The accuracy study was performed for 50%, 100% and 150 % for of Sulfamethoxazole and Trimethoprim. Each level was injected in triplicate in to chromatographic system [Figure 11 to 19]. The area of each level was used for calculation of % recovery [Table 5 & 6].

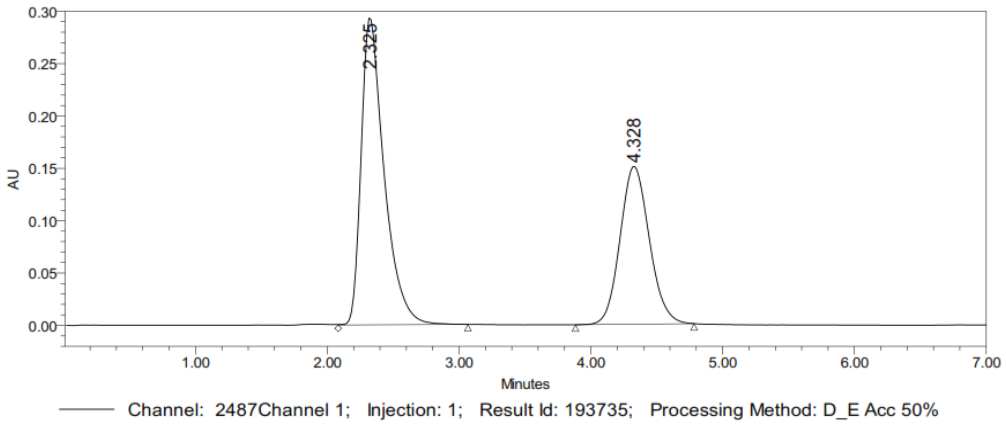

Figure 11. Chromatogram showing accuracy 50% injection-1

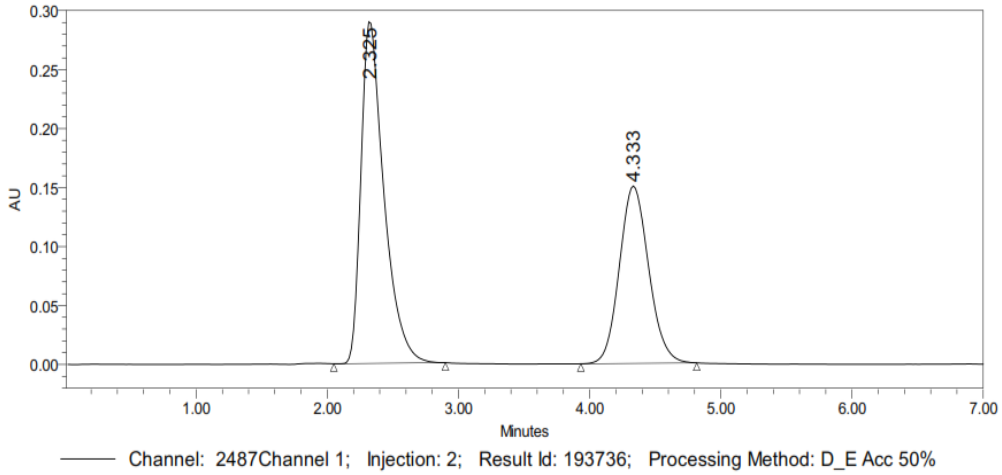

Figure 12. Chromatogram showing accuracy 50% injection-2

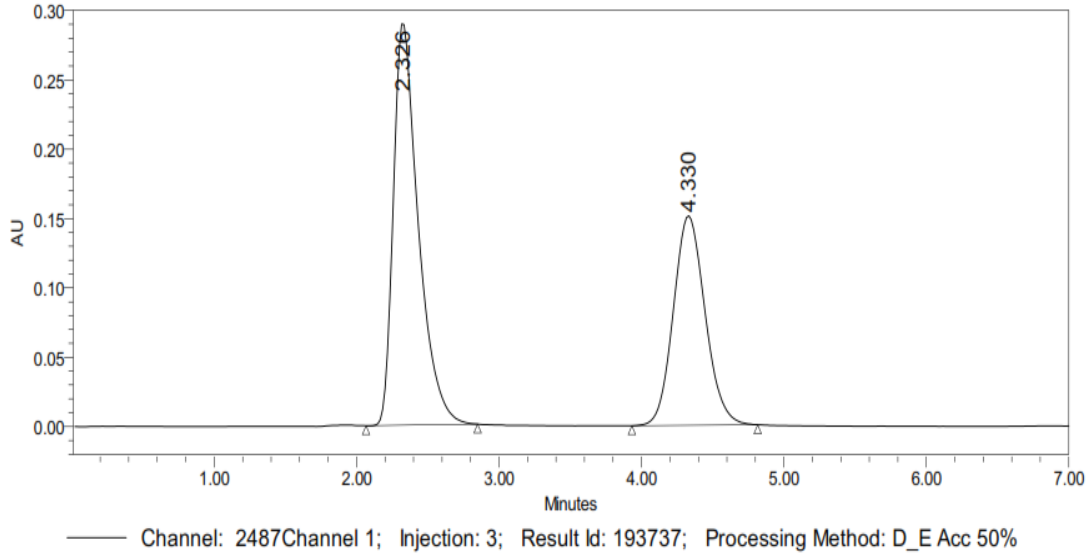

Figure 13. Chromatogram showing accuracy 50% injection-3

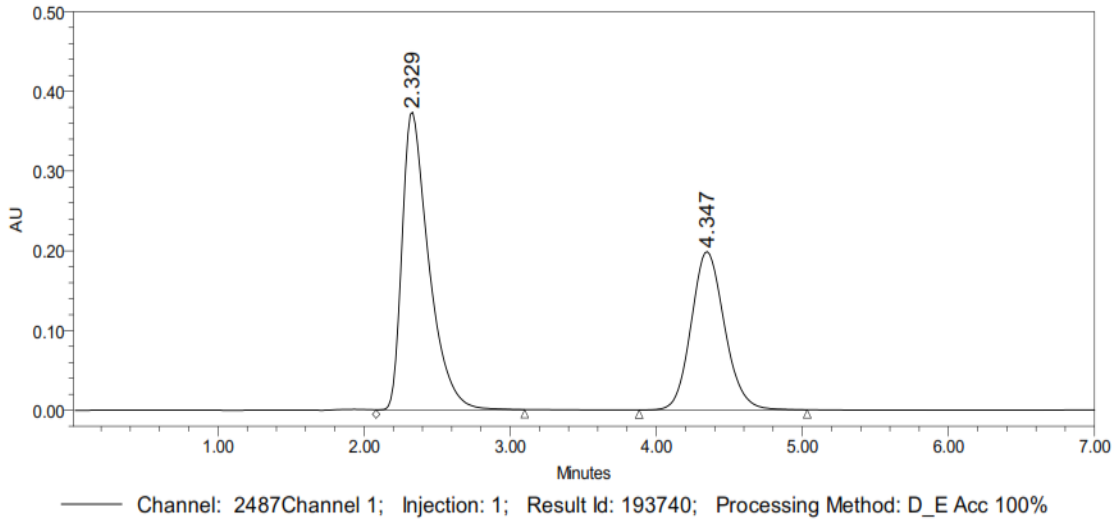

Figure 14. Chromatogram showing accuracy 100% injection-1

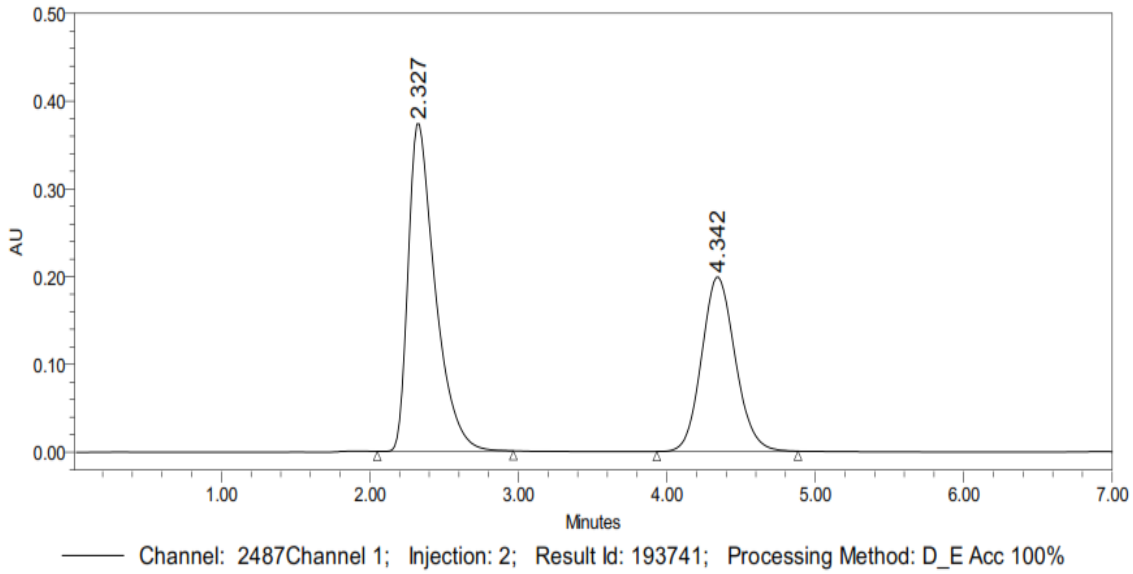

Figure 15. Chromatogram showing accuracy 100% injection-2

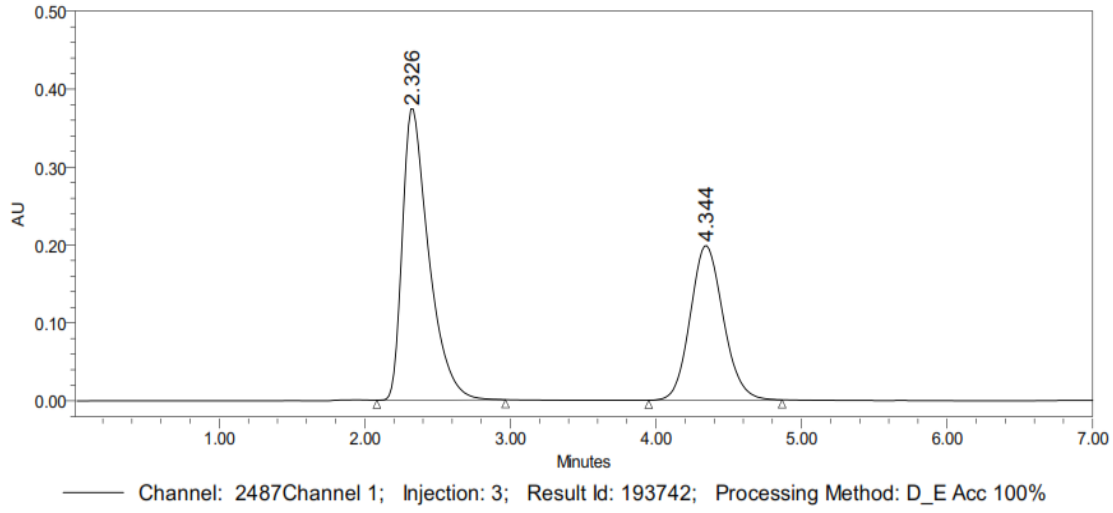

Figure 16. Chromatogram showing accuracy 100% injection -3

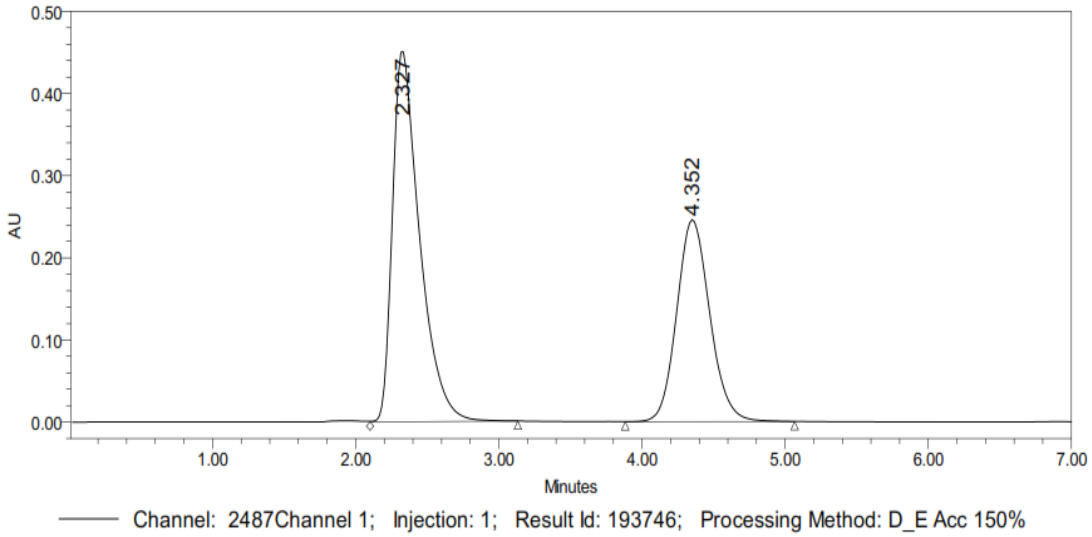

Figure 17. Chromatogram showing accuracy 150% injection -1

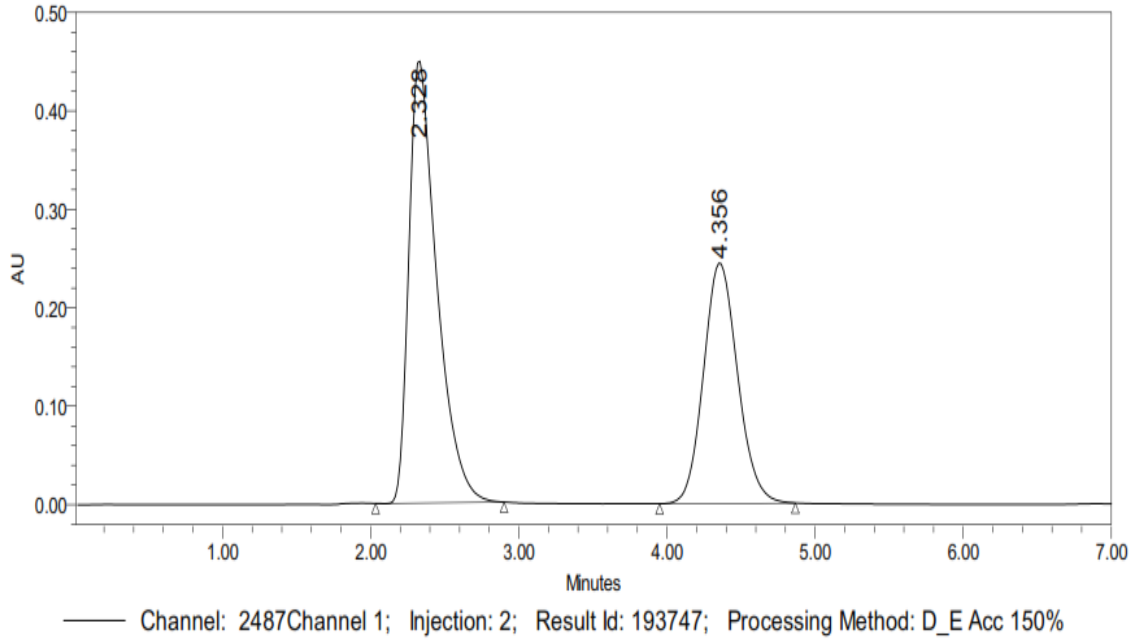

Figure 18. Chromatogram showing accuracy 150% injection-2

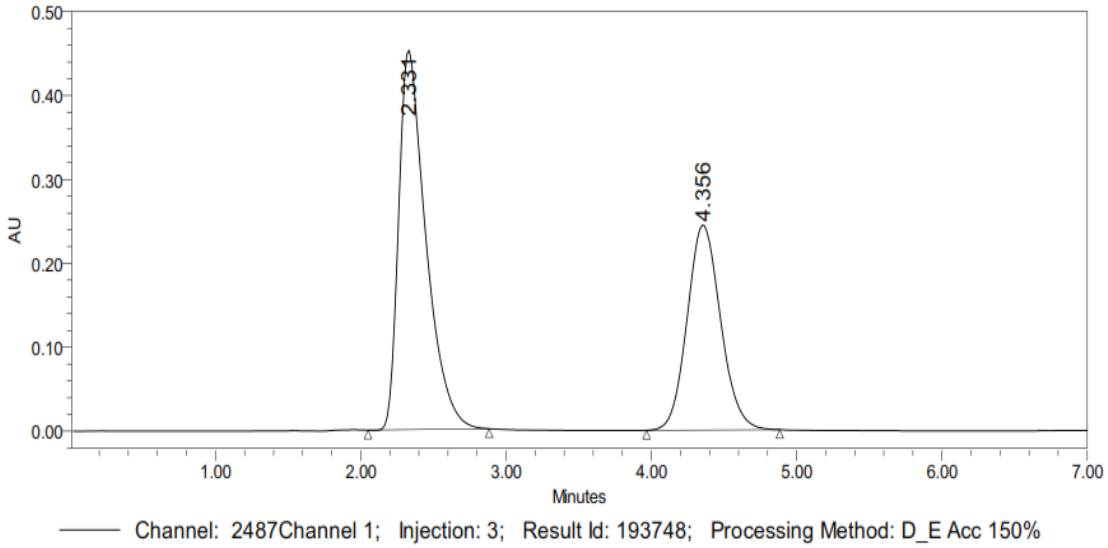

Figure 19. Chromatogram showing accuracy 150% injection-3

The accuracy results for Sulfamethoxazole

Table 5. Accuracy results of Sulfamethoxazole

S.NO	%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
1	50%	3966896	10	9.9	99.9%	100%
		3984578	10	9.7	99.7%	
		3899645	10	10.4	100.4%	
2	100%	7899459	20	19.94	99.94%	99.98%
		7903685	20	20.1	100.1%	
		7910234	20	19.9	99.9%	
3	150%	10775823	30	29.8	99.8%	99.9%
		10629846	30	29.9	99.9%	
		10753698	30	30	100%	

The accuracy results for Trimethoprim

Table 6. Accuracy results of Trimethoprim

S.NO	%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
1	50%	910623	5	4.8	98 %	100 %
		909752	5	4.9	99 %	
		916691	5	5.3	100.3 %	
2	100%	1793761	10	9.7	97 %	100 %
		1799786	10	10.3	103 %	
		1853381	10	10	100 %	
3	150%	2780075	15	14.96	99.6%	99.26%
		2764329	15	14.8	98%	
		2770142	15	15.2	100.2%	

5.5 Precision

Repeatability:

The precision study was performed for five injections of Sulfamethoxazole and Trimethoprim. Each standard injection was injected in to chromatographic system [Figure 20 to 24]. The area of each Standard injection was used for calculation of % RSD [Table 7 &8].

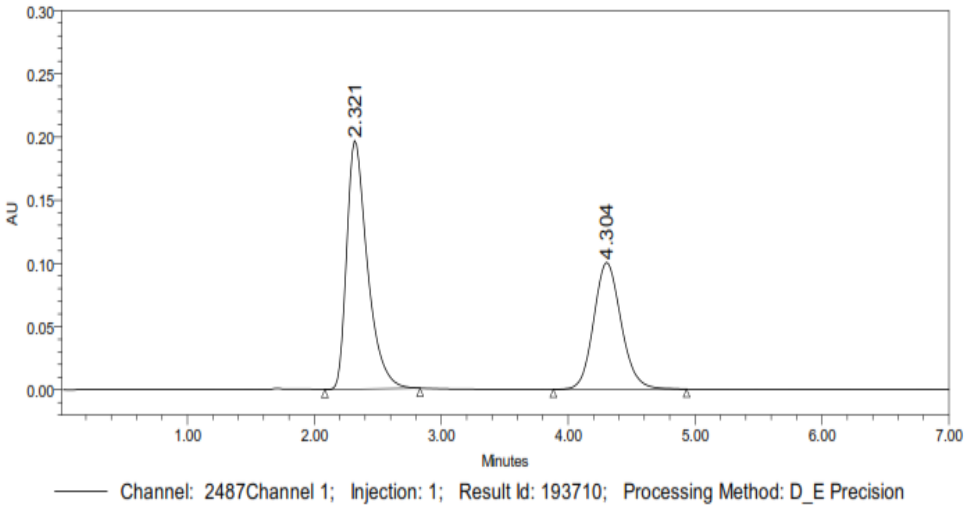

Figure 20. Chromatogram of Standard Inj-1

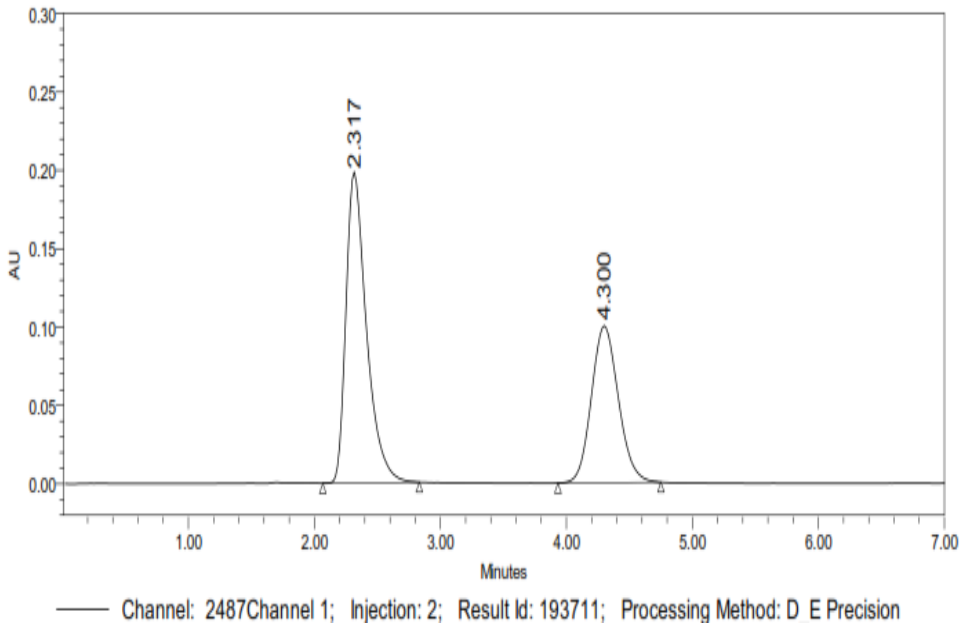

Figure 21. Chromatogram of Standard Inj-2

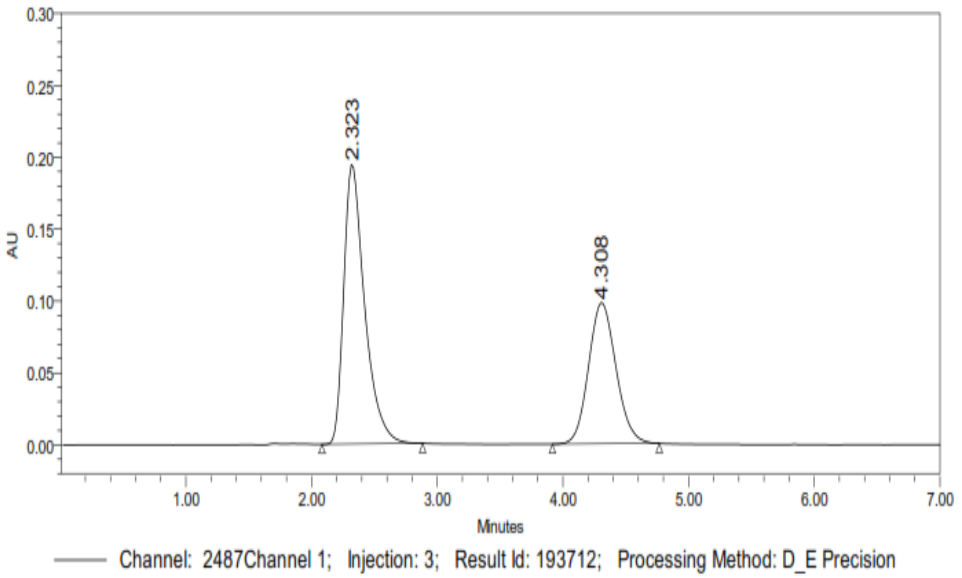

Figure 22. Chromatogram of Standard Inj-3

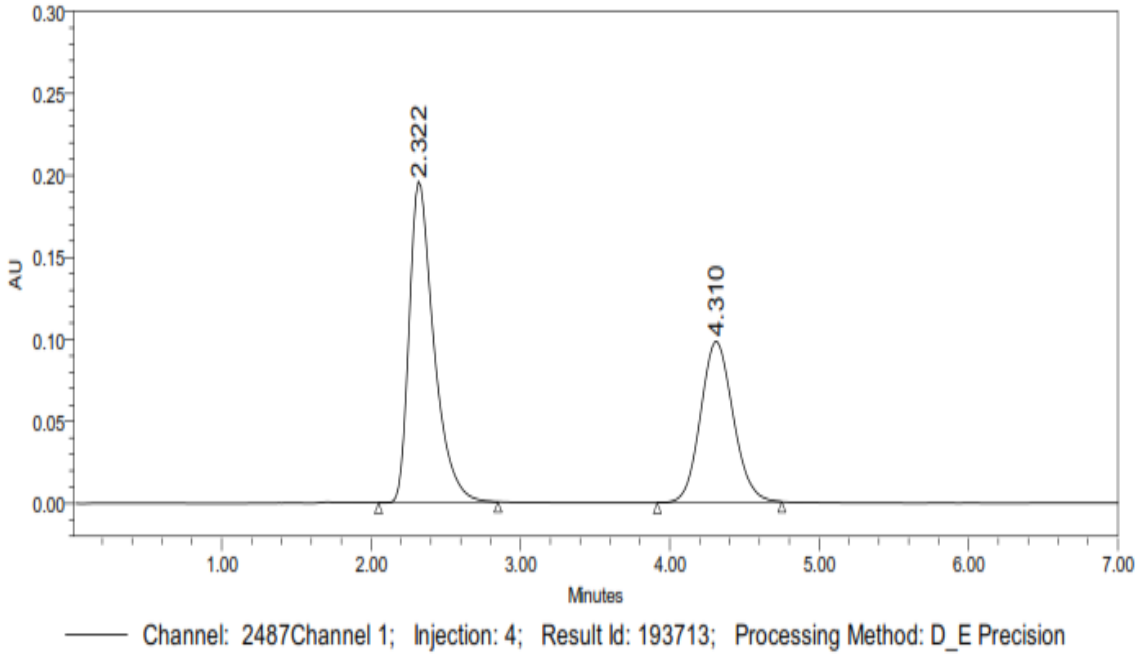

Figure 23. Chromatogram of Standard Inj-4

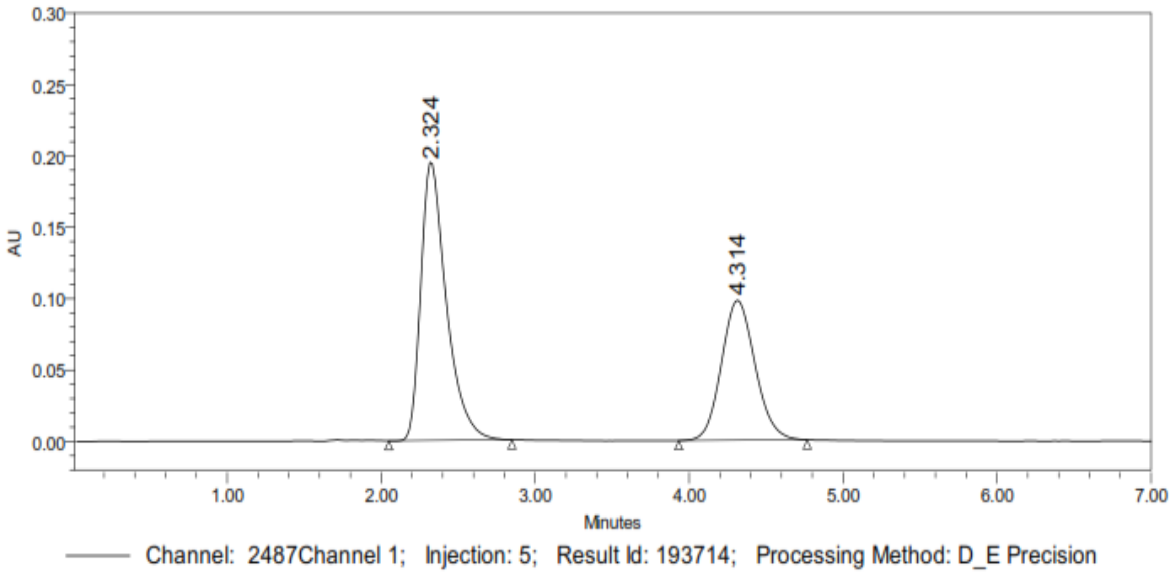

Figure 24. Chromatogram of Standard Inj-5

Repeatability results

Table 7. The Repeatability results of Sulfamethoxazole

S.NO	Name	RT	Area	Height
1	Sulfamethoxazole	2.320	2265419	196958
2	Sulfamethoxazole	2.341	2204588	197584
3	Sulfamethoxazole	2.356	2247569	195874
4	Sulfamethoxazole	2.344	2258741	194583
5	Sulfamethoxazole	2.325	2258967	194587
Mean			2255501	
Std.dev			6545.5	
%RSD			0.31	

Table 8. The Repeatability results of Trimethoprim

S.NO	Name	RT	Area	Height
1	Trimethoprim	4.302	1401475	100274
2	Trimethoprim	4.305	1401345	100078
3	Trimethoprim	4.325	1402415	98425
4	Trimethoprim	4.315	1404775	98165
5	Trimethoprim	4.312	1408614	98154
Mean			1491354	
Std.dev			5882.5	
%RSD			0.38	

5.6 Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of Sulfamethoxazole and Trimethoprim**.** Each standard injection was injected into chromatographic system [Figure 25 to 29]. The area of each standard injection was used for calculation of % RSD [Table 9 to 10].

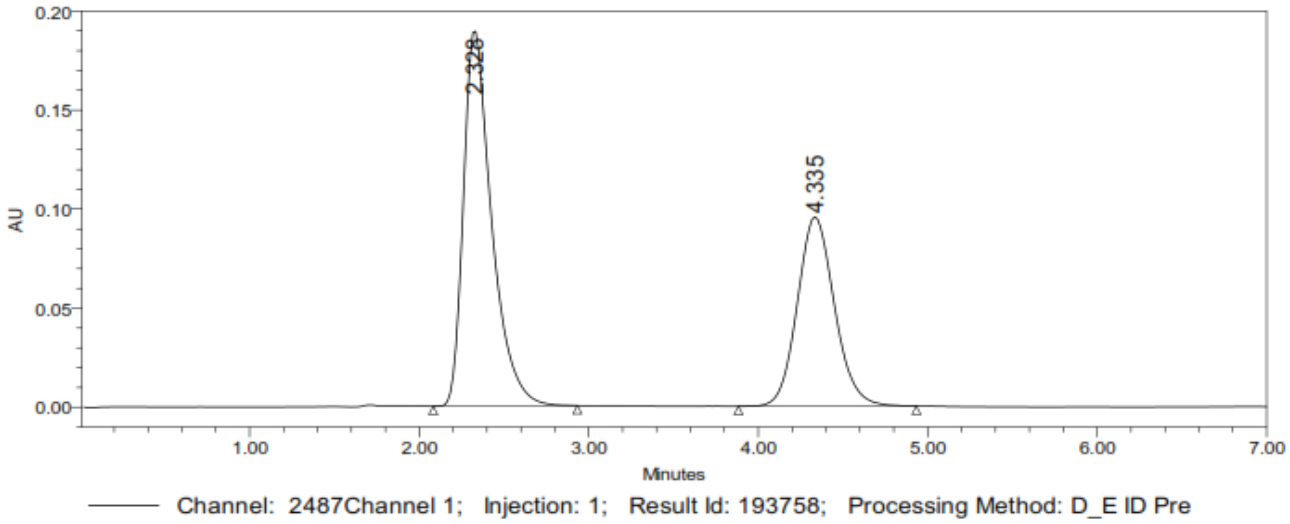

Figure 25. Chromatogram of Standard Inj-1 (ID Precision)

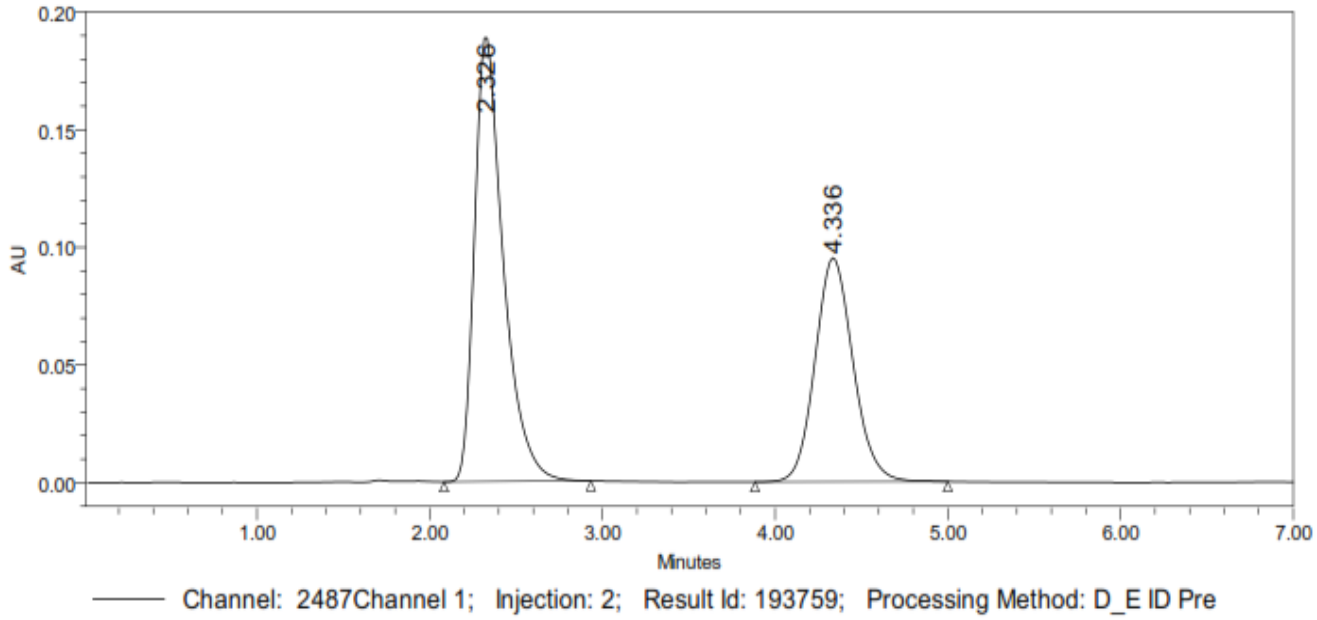

Figure 26. Chromatogram of Standard Inj-2(ID Precision)

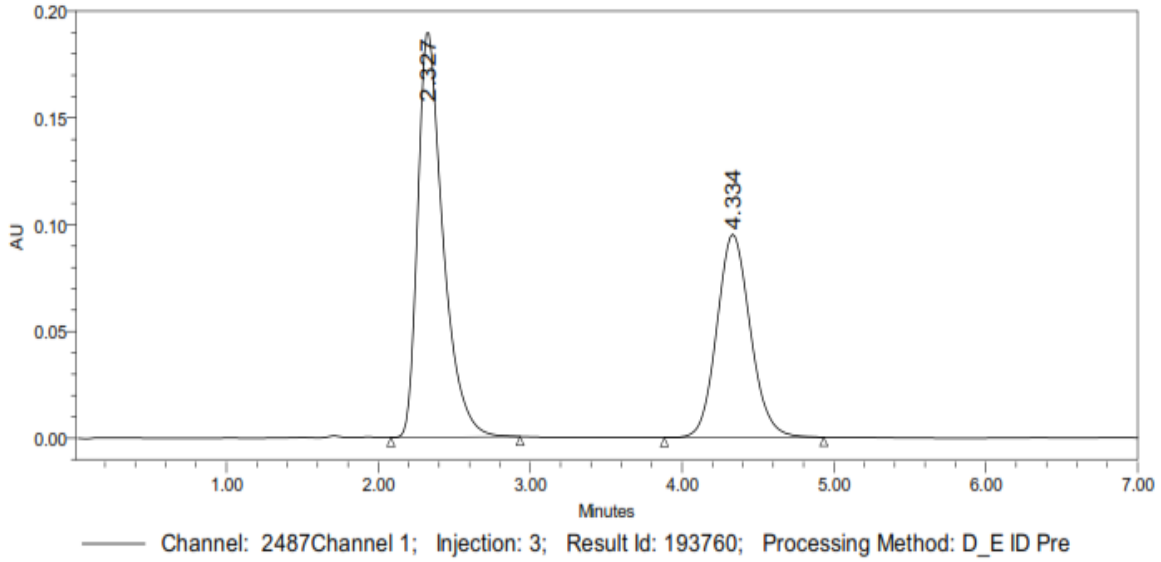

Figure 27. ChromatogramofStandardInj-3 (ID Precision)

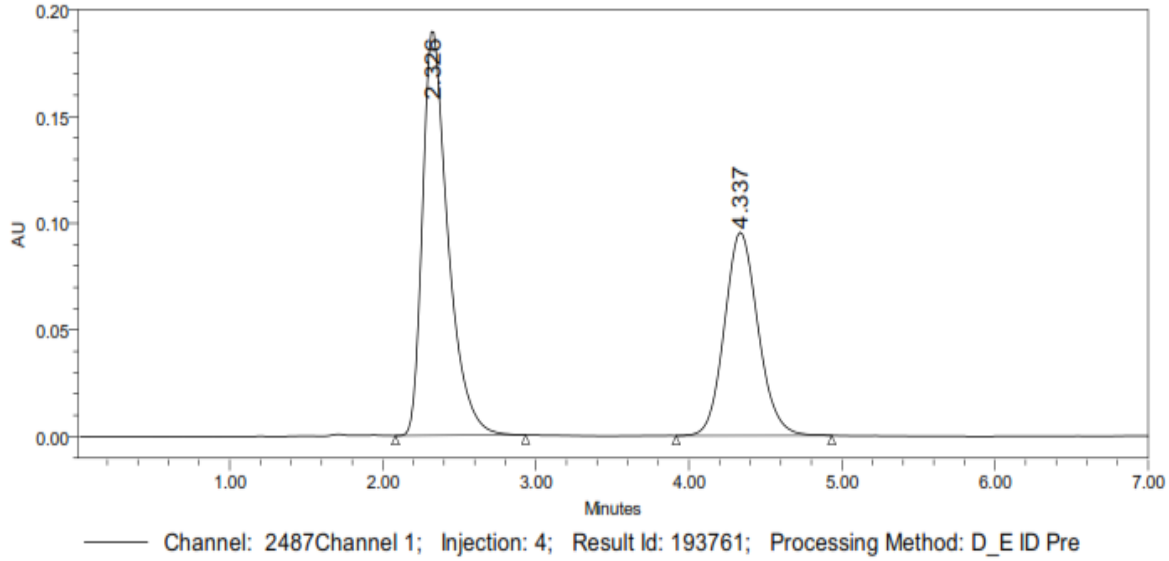

Figure 28. Chromatogram of Standard Inj-4 (ID Precision)

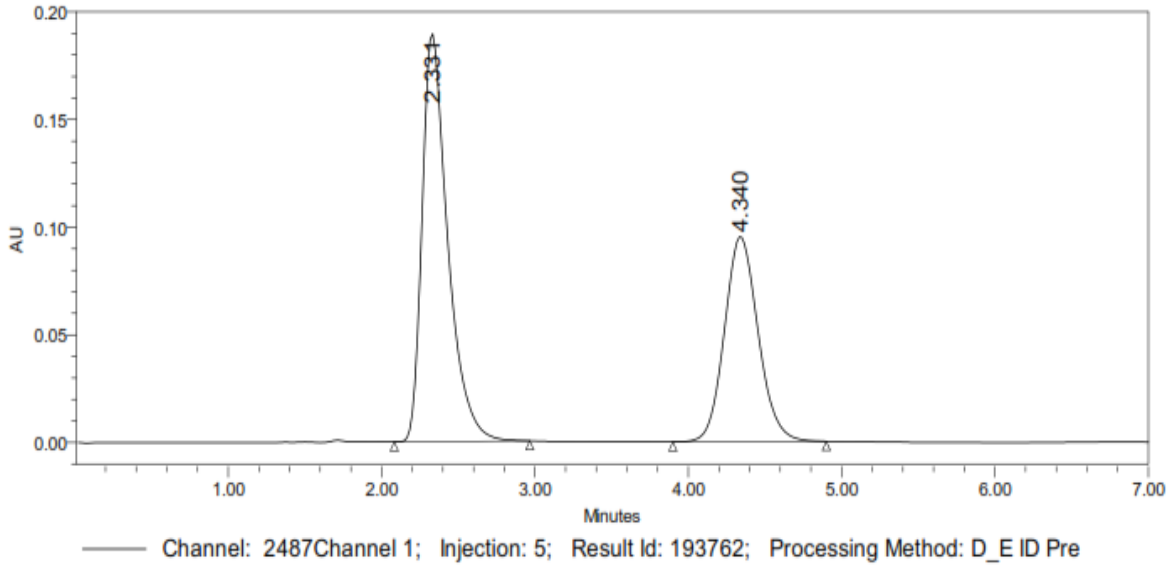

Figure 29. Chromatogram of Standard Inj-5 (ID Precision)

Ruggedness results

Table 9. The Ruggedness results of Sulfamethoxazole

S.NO	Name	RT	Area	Height
1	Sulfamethoxazole	2.325	2165419	186958
2	Sulfamethoxazole	2.315	2104588	187584
3	Sulfamethoxazole	2.356	2147569	185874
4	Sulfamethoxazole	2.325	2158741	184583
5	Sulfamethoxazole	2.331	218967	184587
Mean			219546	
Std.dev			2109	
%RSD			0.12	

Table 10. The Ruggedness results of Trimethoprim

S.NO	Name	RT	Area	Height
1	Trimethoprim	4.302	1401475	95623
2	Trimethoprim	4.305	1401342	95152
3	Trimethoprim	4.325	1402412	95168
4	Trimethoprim	4.315	1404773	95163
5	Trimethoprim	4.312	1408612	95153
Mean			1455258	
Std.dev			2345.5	
%RSD			0.15	

5.7 Robustness

As a part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A) Flow Rate of Sulfamethoxazole and Trimethoprim

The robustness was performed for the flow rate variations from 0.8 ml/min to 1.2ml/min. Standard solution 60µg/ml of Sulfamethoxazole & 30µg/ml of Trimethoprim was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method [Figure 30 to 31].

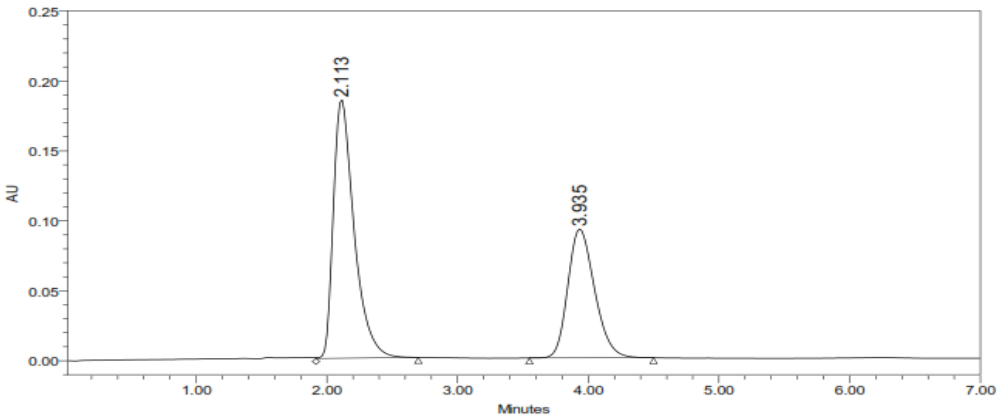

Figure 30. Chromatogram for Robustness more flow

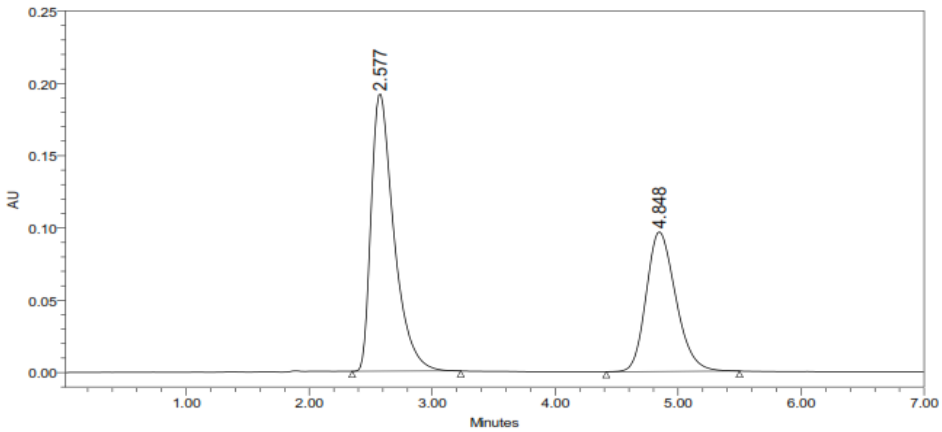

Figure 31. Chromatogram for Robustness less flow

The results are summarized

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly [Table 11 & 12]. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min.

Table 11. Robustness System suitability results for Sulfamethoxazole (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	4953	1.56
2	1.0	5117.5	1.3
3	1.2	5032	1.6

Table 12. System suitability results For Trimethoprim (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	38143	1.5
2	1.0	3887.3	1.4
3	1.2	3754	1.51

*****Results for actual flow (1.0ml/min) have been considered from Assay standard.

Mobile Phase:

The Organic composition in the Mobile phase was varied from70% to 60%. Standard solution 300µg/ml of Sulfamethoxazole & 3µg/ml of Trimethoprim was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method [Figure 32 and 33].

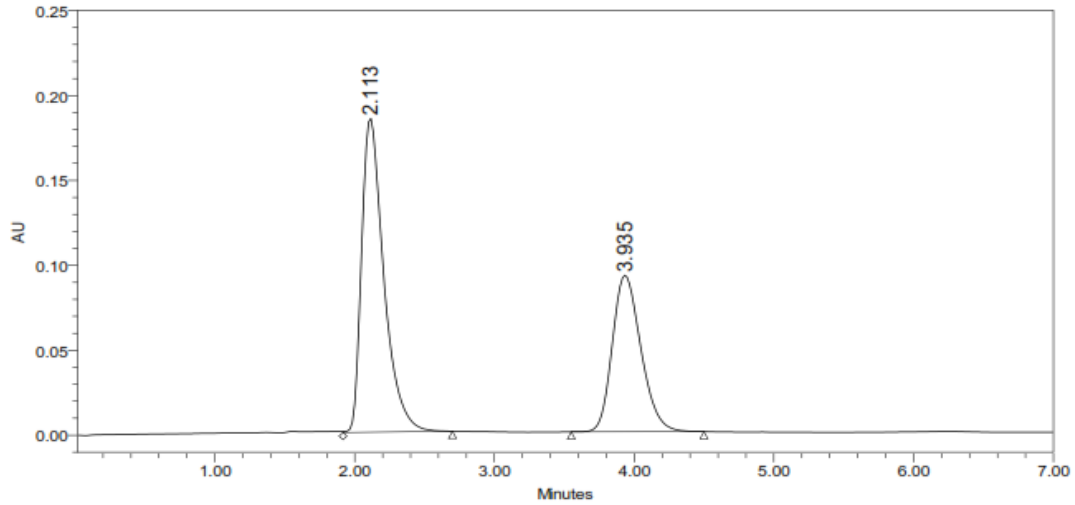

Figure 32. Chromatogram for Robustness more organic

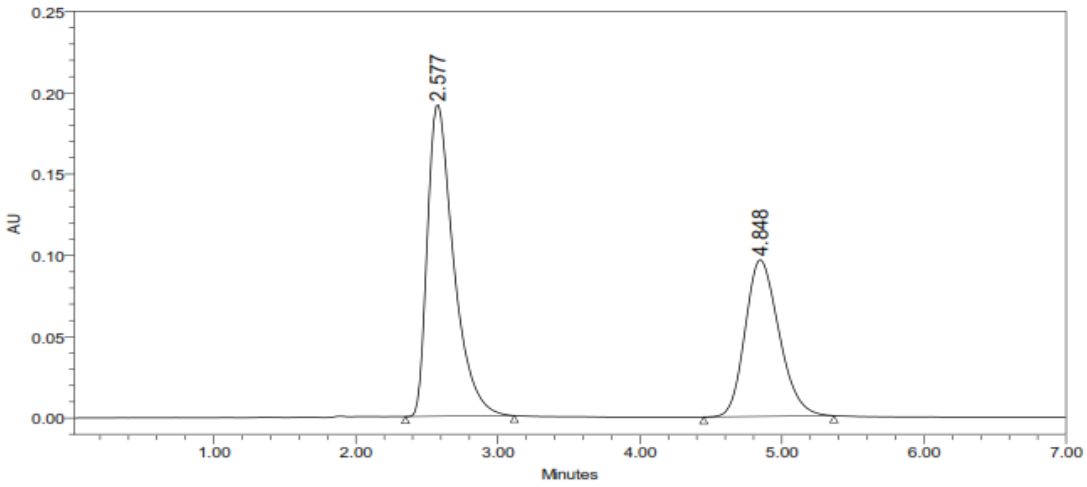

Figure 33. Chromatogram for Robustness less organic

The results are summarized on evaluation of the above results; it can be concluded that the variation in 10% organic composition in the mobile phase affected the method significantly [Table 13 & 14]. Hence it indicates

that the method is robust even by change in mobile phase ±10

Table 13. Robustness System suitability results for Sulfamethoxazole (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	5083.3	1.56
2	Actual	5117.5	1.3
3	10%More	5109.2	1.6

Results for actual Mobile phase composition (55:45Buffer: ACN) have been considered from Accuracy standard.

Table 14. Robustness System suitability results forTrimethoprim (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	3748.5	1.6
2	Actual	3877.3	1.4
3	10%More	3848.0	1.5

5.8 Limit of Detection

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD accordingtothe formula. The standard deviation of the response can be determined based onthe standard deviation of y-intercepts of regression lines.

Formula:$\mathrm{LOD} = 3.3 \times \frac{\sigma}{s}$

Where

σ: Standard deviation (SD)

S: Slope

Acceptance Criteria:

S/N Ratio value should not be more than 3 for LOD solution.

5.9 Quantization Limit

Calculation of Trimethoprim S/N Ratio:

Average Baseline Noise obtained from Blank : 46µV

Signal Obtained from LOQ solution : 51µV

S/N= 51/46=1.10

Calculation of Sulfamethoxazole S/N Ratio:

Average Baseline Noise obtained from Blank: 49µV

Signal Obtained from LOQ solution: 47µV

S/N= 47/49=0.95

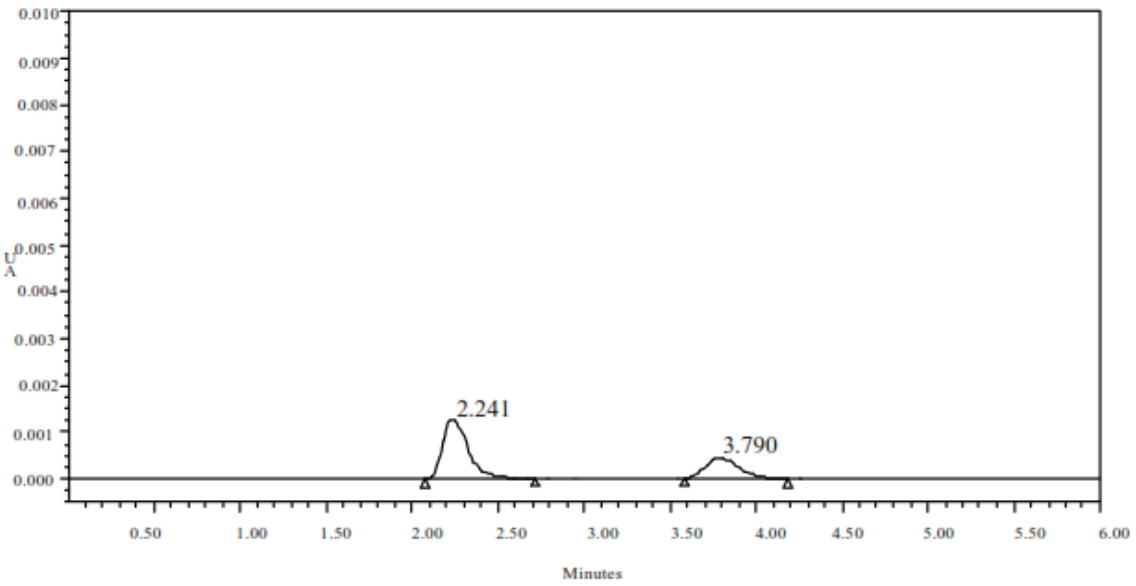

Figure 34. Chromatogram for Limit of Detection

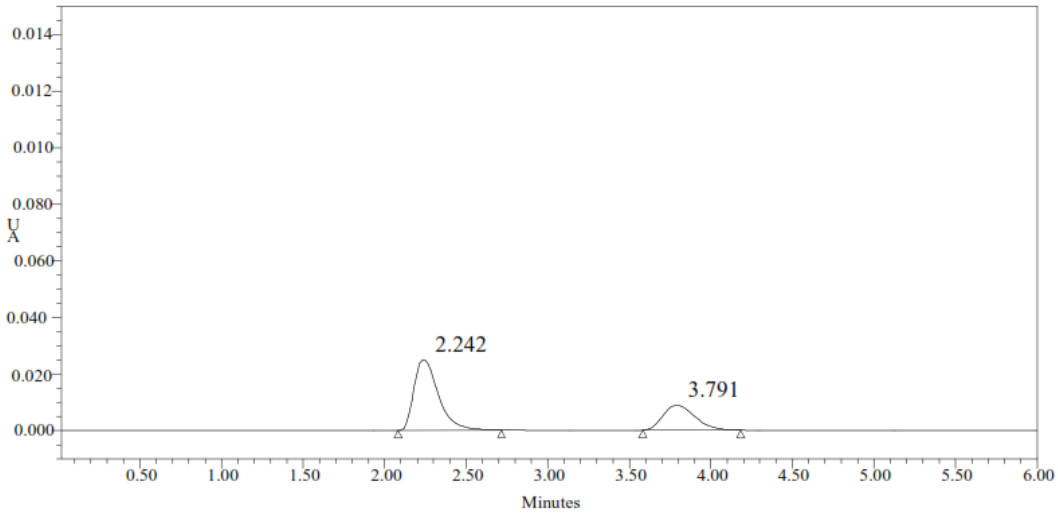

Figure 35. Chromatogram for limit of Quantification

6. Conclusion

The established hplc technique had been ascertained and that it was did find of being simplicity, precise, sensitive and accurate again for simultaneous determination like sulfamethaxazole as well as trimethoprim its pure state even in its pharmaceutical dosage forms. Hence, this system can successfully but also comfortably accept such as regular quality control assessment like sulfamethaxazole as well as trimethoprim pure and also its pharmaceutical formulations.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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