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ESTIMATION FOR METHOD DEVELOPMENT AND VALIDATION OF RIFAMPICIN IN ORALDOSAGE FORM BY RP- HPLC

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ABSTRACT

A Reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Rifampicin in tablet formulation. Good chromatographic separation was achieved isocratically using a Prontosil C18 column (250 x 4.6mm, 3µm) and mobile phase consisting of acetonitrile: 0.02M sodium dihydrogen phosphate buffer (60:40) with 1.5ml of Triethyl Amine , adjusted to pH 6.5 with Orthophosphoric acid, at flow rate 1ml/min. The first method of these three drugs which involves absorbance measurement at 211nm The retention time of Rifampicin and was found to be 2.38min, 2.747min and 3.660min respectively. Linearity was obtained in the range of 8-38 µg/ml,18-53 µg/ml and 32-116 µg/ml respectively. The correlation coefficient for calibration curve of all three peaks was found to be 0.9999.

Keywords: Rifampicin, RP-HPLC, Validation , Prontosil C18 column etc.

Introduction:

The pharmaceutical analysis defined as “the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of a given sample of a medicine, the detection and estimation of impurities, which may be present in drug substance (or) given sample of medicine”.The substance may be a single compound or a mixture of compounds and may be in the form a tablet, pill, capsule, ampoule, liquid, mixture or an ointment. The quality control tests involve methods which embrace chemicals, physio – chemical, instrumental, microbiological (or) biological procedures. The pharmaceutical analysis deals with the subject of determining the composition of material in terms of the elements or compound (drug) present in the system.

2. METHOD VALIDATION

VALIDATION

According to ICH guidelines method validation can be defined as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".Such validated analytical method for qualitative and quantitative testing of the drug molecule assume greater importance when they are employed to generate quality and safety compliance data during development, pre-formulation studies and post approval of drug products.

The ICH of Technical Requirements for the Registration of Pharmaceutical for human use has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics

Parameters Used for Assay Validation

The validation of the assay procedure was carried out using the following parameters.

1) Parameters:

1.1 System suitability

1.2 Specificity

1.3 Method Precision

1.4 Linearity & range

1.5 Accuracy / Recovery studies

1.6 Robustness

SYSTEM SUITABILITY

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, HPLC method and the procedure should be capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole. System suitability parameters and recommendations were shown in the table no.3

- Table no. 3 System suitability parameters andrecommendations

S.No	Parameters	Recommendations
1	Theoretical plates (N)	>2000
2	Tailing factor (T)	≤ 2
3	Resolution (Rs)	> 2 between peak of interest and the closest eluting potential Interference
4	Repeatability	RSD ≤ 1% for N ≥5 is desirable
5	Capacity factor (k^1)	> 2.0
6	Relative retention	Not essential as long as the resolution is stated

Procedure:

- A standard solution was prepared by using Isoniazid and Rifampicin working standards as per test method and was injected six times into the HPLCsystem.
- The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections for Isoniazid and Rifampicin retention times and peak areas. Resulted chromatogram was shown in the chromatogram fig.no.3.

Fig no.3 Chromatogram of standard 1

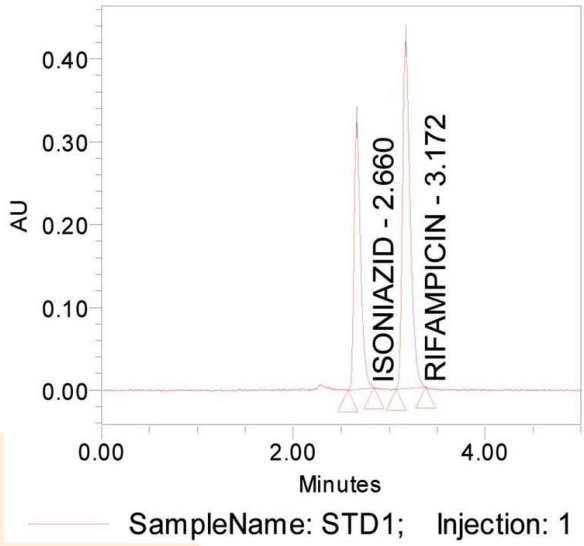


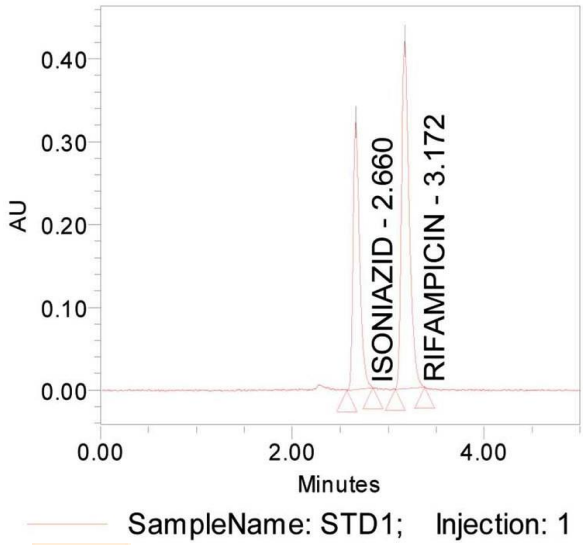
Table no.4 Data for system suitability of ISONIAZID Name: ISONIAZID

	Sample Nam	Inj	Nam e	RT	Area	U SPResolution	U SPTailing	U SPPlateCoun
1	STD 1	1	ISONIAZI	2 .660	1518803		1 .469	7755
Mean					1518803			
%RSD								

Table no.5 Data for system suitability of RIFAMPICIN Name: RIFAMPICIN

	Sample Na m	Inj	Nam e	RT	Area	U SPResolutio	U SPTailing	U SPPlateCount
1	STD 1	1	RIFAMPICI	3 .172	2348101		1 .412	7613
Mean					2348101			
%RSD								

Fig no.3Chromatogram of standard 1



Tableno.4 Data for system suitability of ISONIAZID Name: ISONIAZID

	Sample Name	Inj	Nam e	RT	Area	U SPResolution	U SPTailing	U SPPlateCount
1	STD 1	1	ISONIAZID	2 .660	1518803		1 .469	7755
Mean					1518803			
%RSD								

Tableno.5 Data for system suitability of RIFAMPICIN Name:RIFAMPICIN

	Sample Name	Inj	Nam e	RT	Area	U SPResolution	USPTailing	USPPlateCount
1	STD 1	1	RIFAMPICIN	3 .172	2348101		1 .412	7613
Mean					2348101			
%RSD								

Fig no.4Chromatograms of standard 2

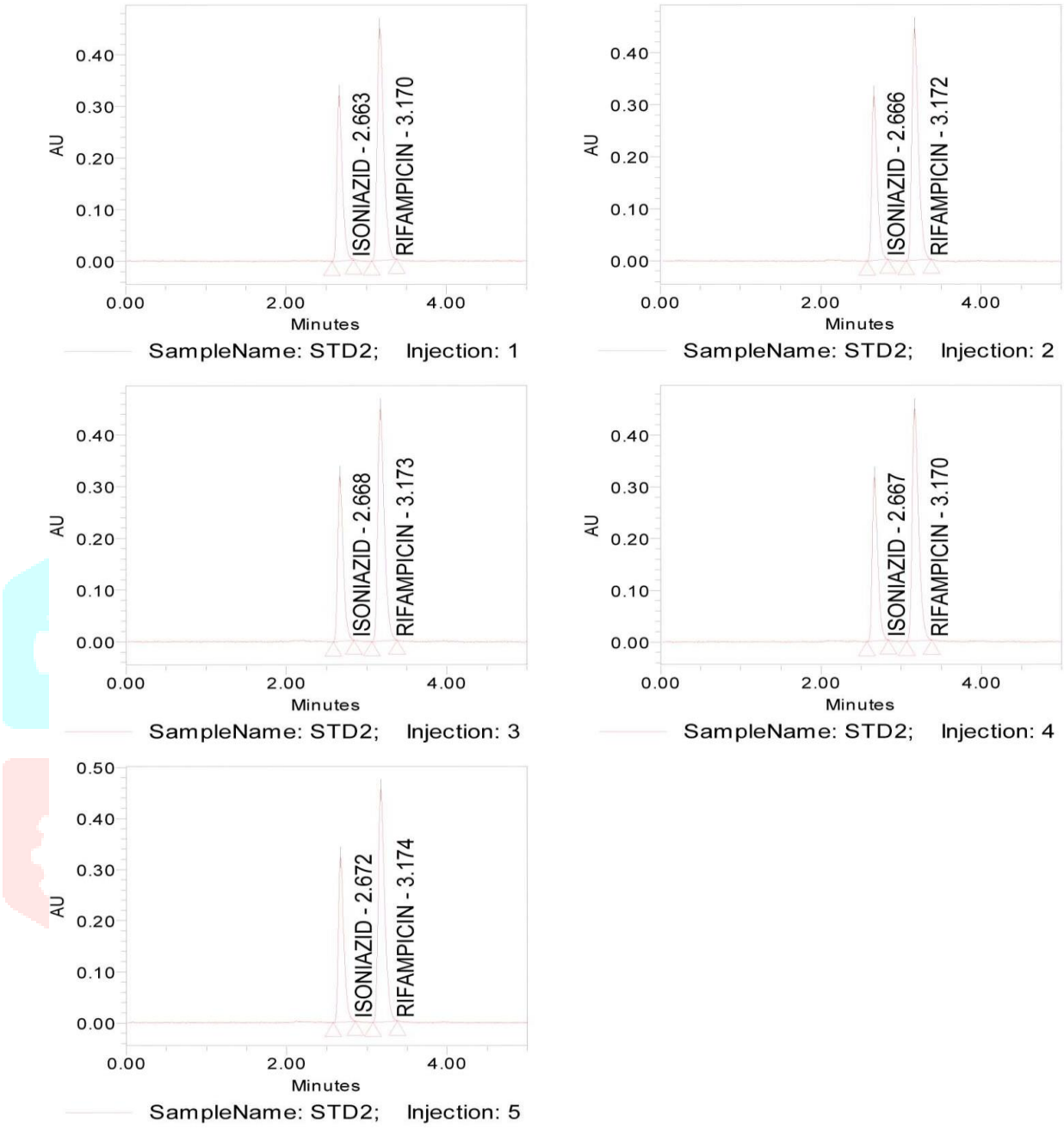


Table no.6 Results of system suitability (ISONIAZID) Name: ISONIAZID

	Sample	Inj	Nam e	RT	Area	USPResolutio	USP Tailing	USPPlateCount
1	STD 2	1	ISONIAZI	2 .663	151632		1 .436	7479
2	STD 2	2	ISONIAZI	2 .666	150283		1 .426	7316
3	STD 2	3	ISONIAZI	2 .668	151642		1 .428	7288
4	STD 2	4	ISONIAZI	2 .667	150767		1 .424	7576
5	STD 2	5	ISONIAZI	2 .672	152012		1 .448	7392
Mean					151267			
%RSD					0 .5			

Table no.7 Results of system suitability (RIFAMPICIN)

	Sample Name	Inj	Nam e	RT	Area	USPResolution	U SPTailing	U SPPlateCo unt
1	STD 2	1	RIFAMPICI	3 .170	2518297		1 .420	7539
2	STD 2	2	RIFAMPICI	3 .172	2514902		1 .407	7382
3	STD 2	3	RIFAMPICI	3 .173	2535682		1 .402	7460
4	STD 2	4	RIFAMPICI	3 .170	2520334		1 .428	7586
5	STD 2	5	RIFAMPICI	3 .174	2528250		1 .390	7441
Mean					2523493			
%RSD					0 .3			

Name: RIFAMPICIN

SPECIFICITY

Specificity is the ability to assess unequivocally of an analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Blank, standard, placebo, all known related compounds, spiked sample, sample solutions were prepared and injected into the chromatographic system for identification and interference with the Isoniazid and Rifampicin peaks.

Placebo Interference:

A study to establish the interference of placebo was conducted. Sample preparation of placebo was done as that of test sample preparation of assay method. Chromatogram of placebo did not show any additional peaks. This indicated that the excipients used in the formulation did not interfere in the assay of Isoniazid and Rifampicin tablets. Resulted chromatograms were shown below.

Blank Interference:

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method and are shown below.

Fig no.5

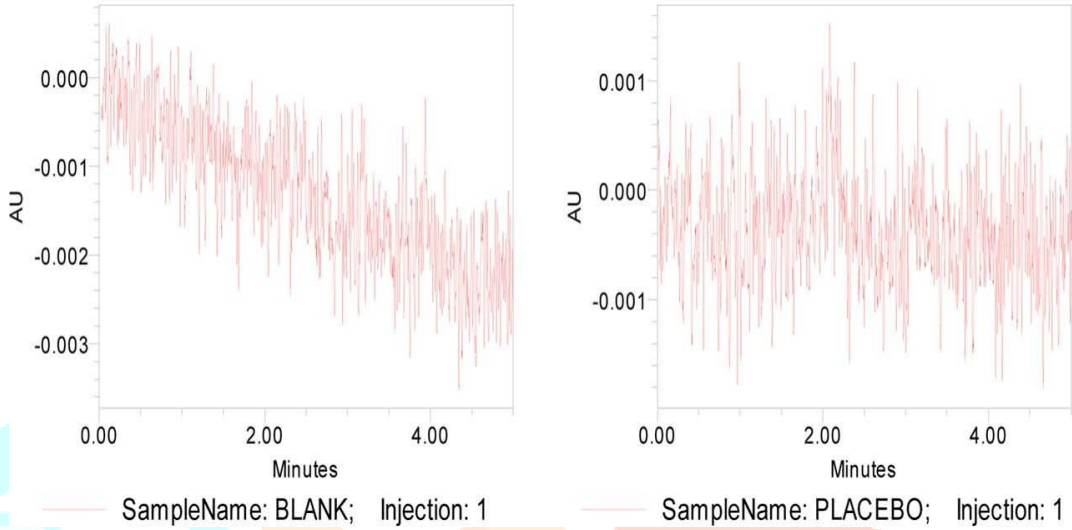


Table no.8 Component Summary Table for ISONIAZID

	Sample Name	Inj	Name	RT	Area
1	Blank	1	ISONIAZID	2.600	
2	Placebo	1	ISONIAZID	2.600	

Table no.9 Component Summary Table for RIFAMPICIN

	Sample Name	Inj	Name	RT	Area
1	Blank	1	RIFAMPICIN	3.100	
2	Placebo	1	RIFAMPICIN	3.100	

Precision:

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as %RSD for the statistically significant number of samples.

Method Precision:

Six sample preparations were prepared individually using single batch of Isoniazid and Rifampicin tablets (1/32 mg) as per test method and injected each solution. Resulted chromatogram was shown in the fig. no. 6. And data was shown in below table10.

Fig no.6Chromatograms for sample

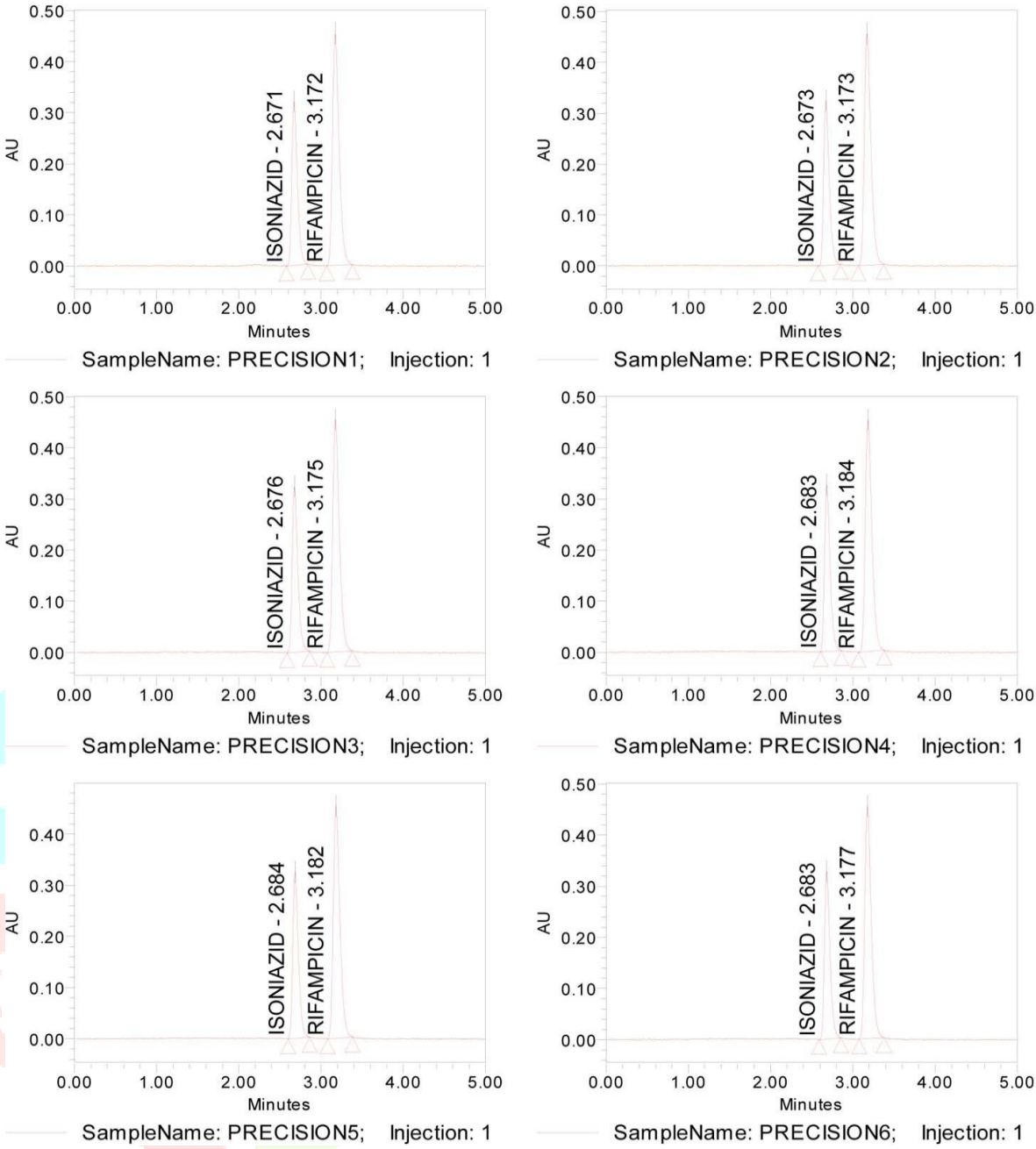


Table no.10 Data for precision (ISONIAZID)

Name: ISONIAZID

	SampleNam e	In	Nam e	RT	Area
1	PRECISION 1	1	ISONIAZID	2 .671	1519920
2	PRECISION 2	1	ISONIAZID	2 .673	1516120
3	PRECISION 3	1	ISONIAZID	2 .676	1511693
4	PRECISION 4	1	ISONIAZID	2 .683	1518392
5	PRECISION 5	1	ISONIAZID	2 .684	1514151
6	PRECISION 6	1	ISONIAZID	2 .683	1511595

Tableno.11 Data for precision (RIFAMPICIN)

Name: RIFAMPICIN

	SampleNam e	Inj	Nam e	RT	Area
1	PRECISION 1	1	RIFAMPICIN	3 .172	2525898
2	PRECISION 2	1	RIFAMPICIN	3 .173	2527812
3	PRECISION 3	1	RIFAMPICIN	3 .175	2527001
4	PRECISION 4	1	RIFAMPICIN	3 .184	2529333
5	PRECISION 5	1	RIFAMPICIN	3 .182	2520997
6	PRECISION 6	1	RIFAMPICIN	3 .177	2521813

Table no.12 Calculated data for repeatability of Isoniazid andRifampicin

S.No	Sample Weigh t	Sample Area-1	Sample Area- 2	% Ass ay	% Assay
1	902.55	1519920	2525898	100	100
2	902.55	1516120	2527812	99	100
3	902.55	1511693	2527001	99	100
4	902.55	1518392	2529333	99	100
5	902.55	1514151	2520997	99	100
6	902.55	1511592	2521813	99	100
Average Assay				99	100
STD				0.23	0.13
%RSD				0.23	0.13

Acceptance criteria:

The % RSD of individual Isoniazid and Rifampicintablet from the six units should be not more than 2.0%.

All assay values should be within the 90.0 % - 110.0 % of label claim.

LINEARITY AND RANGE

Linearity

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of an analyte in the sample.

Range

Range of an analytical procedure was the interval between the upper and lower concentration (amount) of an analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has suitable level of precision, accuracy and linearity.

Standard solutions of Isoniazid and Rifampicin at concentration levels from 50 % to 150 % of standard solution were injected into HPLC system. The linearity graph was plotted from 50 % to 150

Acceptance criteria

a. The correlation coefficient (r^2) must be NLT0.999.
b. The RSD of replicate injections for lower and upper level concentrations should not be more than2.0 %.

Fig no.7Chromatograms for linearity

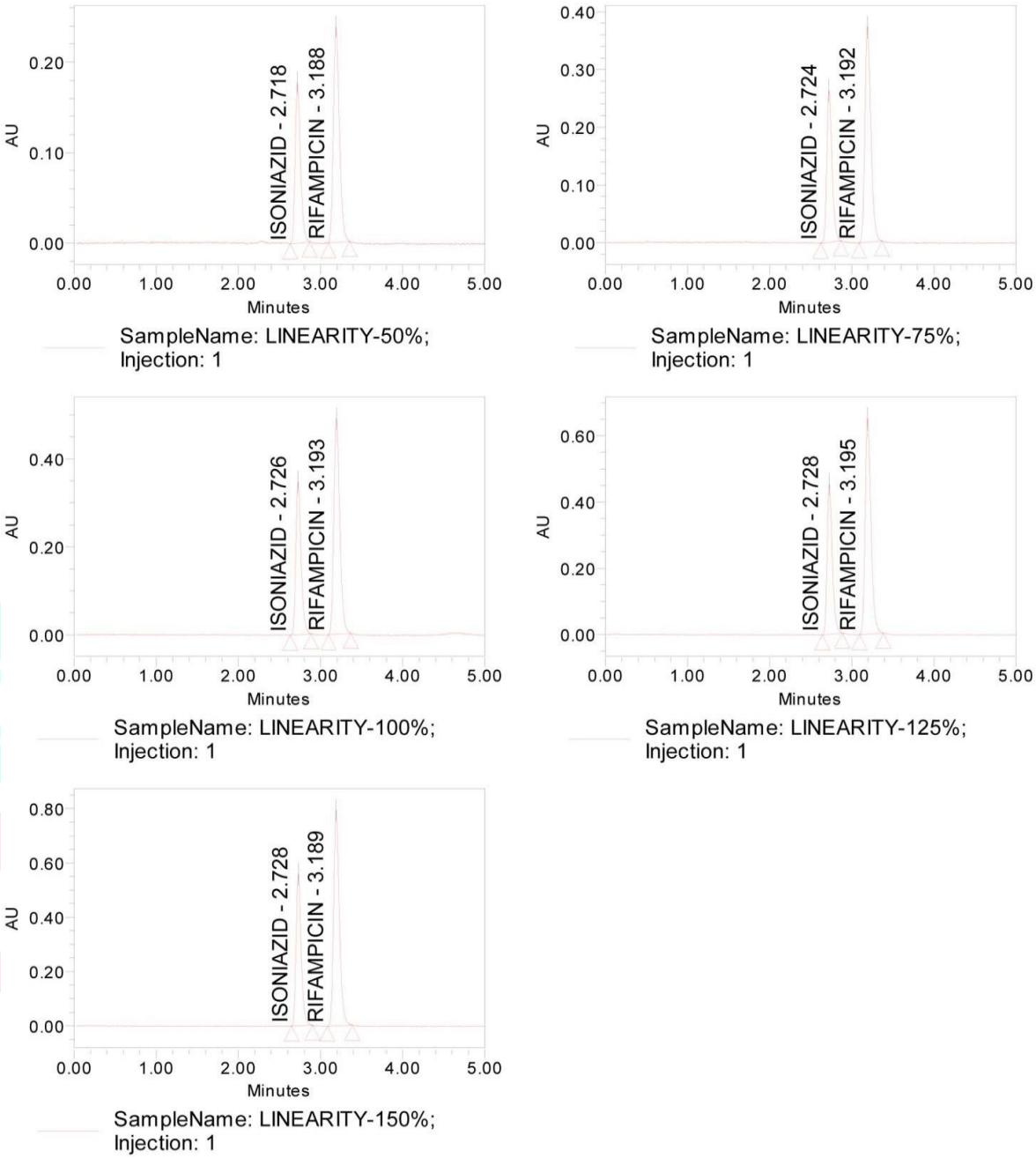
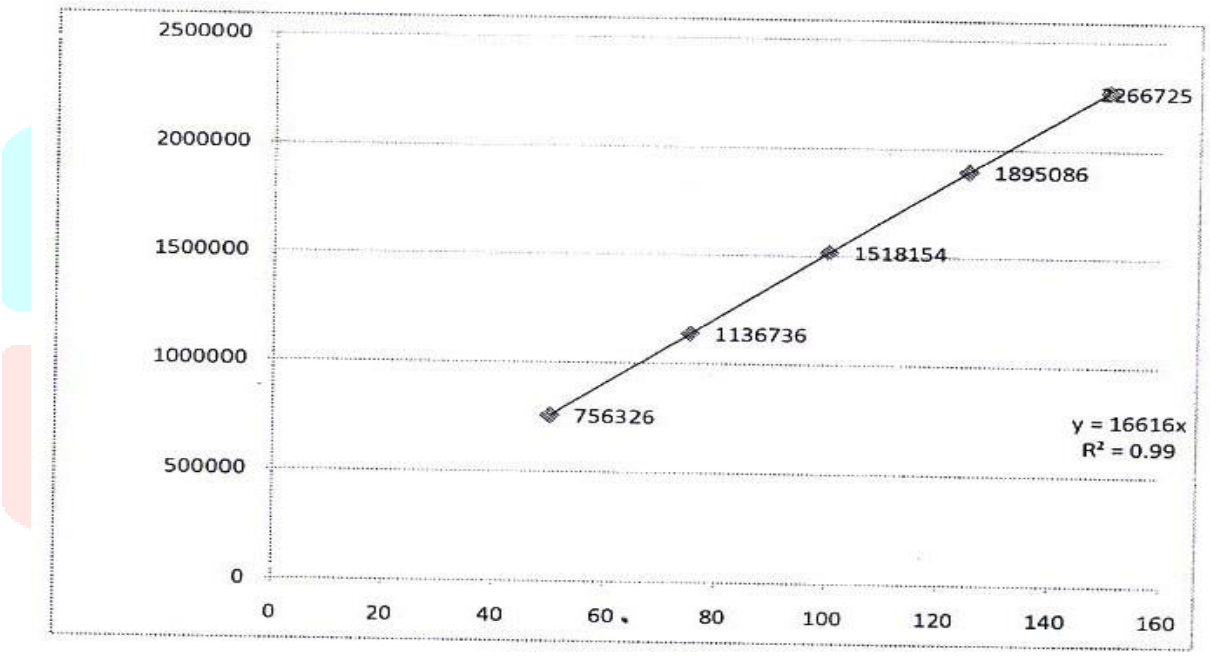


Table no.13 Data for linearity (ISONIAZID)

Name: ISONIAZID

	Sample Nam e	In	Nam e	RT	Area
1	LINEARITY-5 0 %	1	ISONIAZID	2 .718	756326
2	LINEARITY-7 5 %	1	ISONIAZID	2 .724	1136736
3	LINEARITY-1 00 %	1	ISONIAZID	2 .726	1518154
4	LINEARITY-1 25 %	1	ISONIAZID	2 .728	1895086
5	LINEARITY-1 50 %	1	ISONIAZID	2 .728	2266725



Figno.8 Calibration curve for Isoniazid

Table no.14 Calculated data for linearity (ISONIAZID)

ISONIAZID		
Conc%	Area	Concentration (µg/ml)
50	756326	600
75	1136736	900.00
100	1518154	1200.00
125	1895086	1500
150	2266725	1800

Table no.15 Data for linearity (RIFAMPICIN)

Name: RIFAMPICIN

	Sample Nam e	Inj	Nam e	RT	Area
1	LINEARITY-5 0 %	1	RIFAMPICIN	3 .188	1260352
2	LINEARITY-7 5 %	1	RIFAMPICIN	3 .192	1897143
3	LINEARITY-1 00 %	1	RIFAMPICIN	3 .193	2525789
4	LINEARITY-1 25 %	1	RIFAMPICIN	3 .195	3154260
5	LINEARITY-1 50 %	1	RIFAMPICIN	3 .189	3784968

Figno.9 Calibration curve for Rifampicin

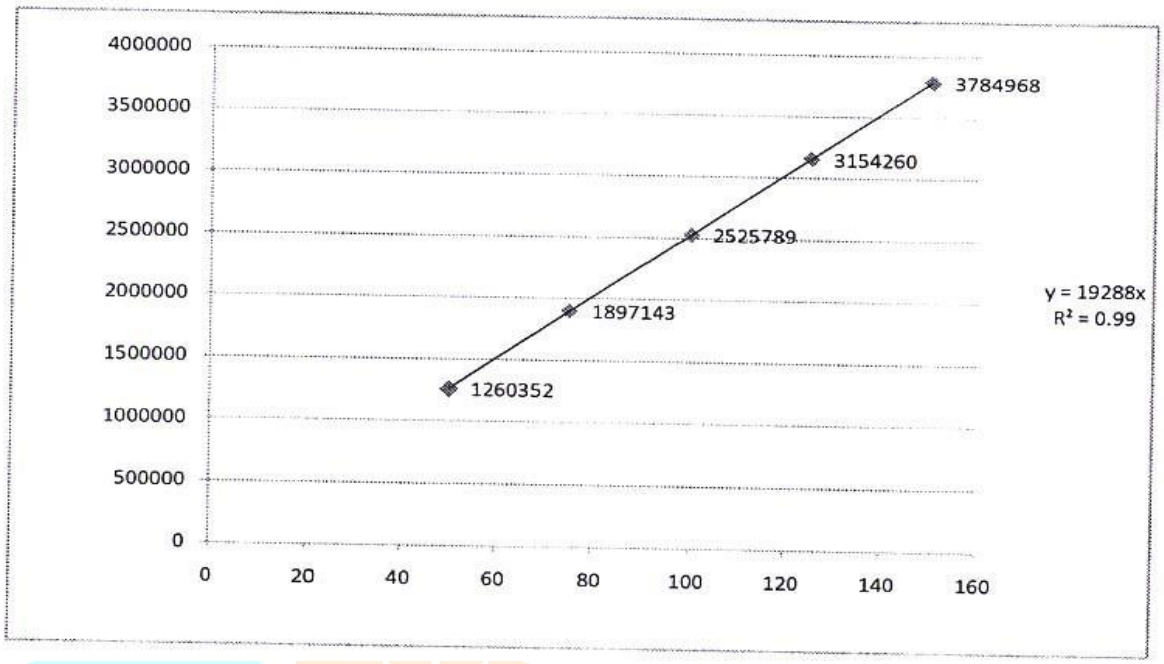


Table no.16 Calculated data for linearity (RIFAMPICIN)

RIFAMPICIN		
Conc%	Area	Concentration (µg/ml)
50	1260352	900
75	1897143	1350
100	2525789	1800
125	3154260	2250
150	3784968	2700.00

METHOD ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the values which is accepted either as a conventional true value or an accepted reference value for the observed value.

Fig no.10 Chromatograms for sample of 50% concentration

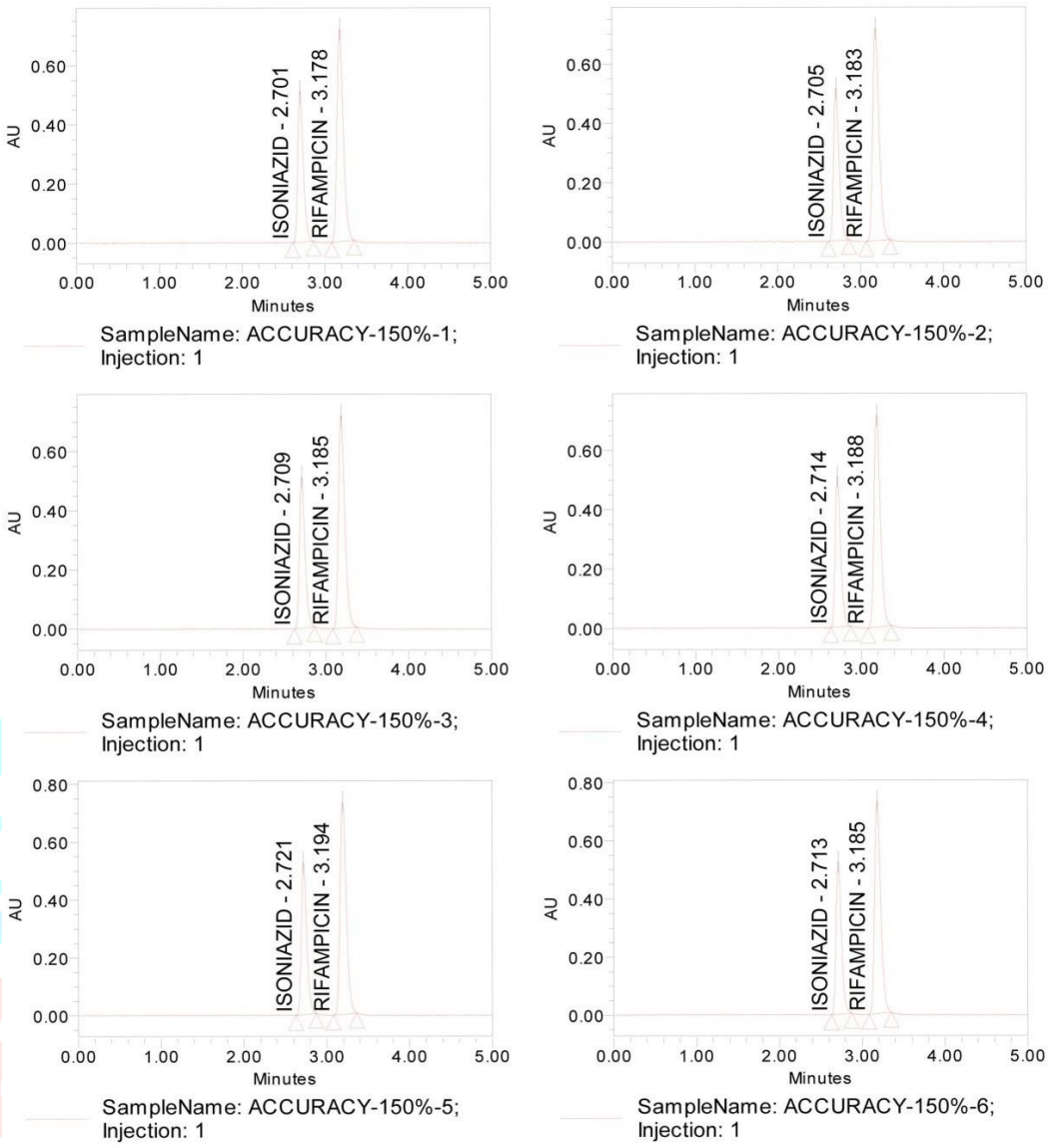


Table no.17 Data for accuracy of 50% concentration ofIsoniazid Name:ISONIAZID

	SampleNam e	Inj	Nam e	RT	Area
1	ACCURACY-5 0 %-1	1	ISONIAZID	2 .688	756728
2	ACCURACY-5 0 %-2	1	ISONIAZID	2 .687	756907
3	ACCURACY-5 0 %-3	1	ISONIAZID	2 .687	756975
4	ACCURACY-5 0 %-4	1	ISONIAZID	2 .696	756326
5	ACCURACY-5 0 %-5	1	ISONIAZID	2 .699	756274
6	ACCURACY-5 0 %-6	1	ISONIAZID	2 .695	756141

Table no.18 Data for accuracy of 50% concentration ofRifampicin

Name: RIFAMPICIN

	SampleNam e	Inj	Nam e	RT	Area
1	ACCURACY-5 0 %-1	1	RIFAMPICIN	3 .183	1265170
2	ACCURACY-5 0 %-2	1	RIFAMPICIN	3 .180	1262461
3	ACCURACY-5 0 %-3	1	RIFAMPICIN	3 .178	1261719
4	ACCURACY-5 0 %-4	1	RIFAMPICIN	3 .187	1263056
5	ACCURACY-5 0 %-5	1	RIFAMPICIN	3 .187	1268196
6	ACCURACY-5 0 %-6	1	RIFAMPICIN	3 .182	1262096

Fig no.11Chromatograms for sample of 100% concentration

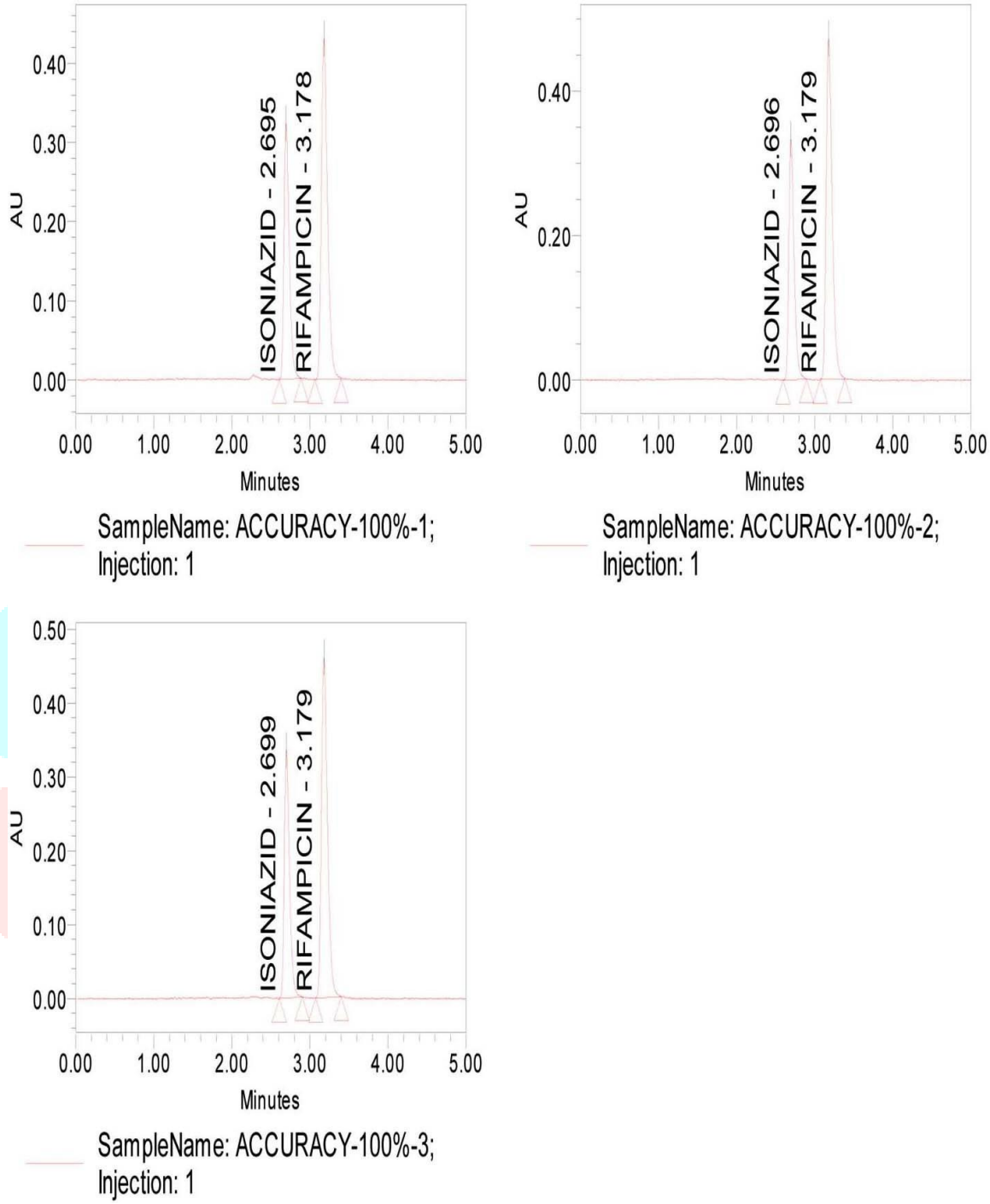


Table no.19 Data for accuracy of 100% concentration ofIsoniazid

Name: ISONIAZID

	Sample Name	Inj	Name	RT	Area
1	ACCURACY – 100% -1	1	ISONIAZID	2.695	1512475
2	ACCURACY – 100% -2	1	ISONIAZID	2.696	1518251
3	ACCURACY – 100% -3	1	ISONIAZID	2.699	1512296

Table no.20 Data for accuracy of 100% concentration ofRifampicin

Name: RIFAMPICIN

	Sample Name	Inj	Name	RT	Area
1	ACCURACY – 100% -1	1	RIFAMPICIN	3.178	2523741
2	ACCURACY – 100% -2	1	RIFAMPICIN	3.179	2525279
3	ACCURACY – 100% -3	1	RIFAMPICIN	3.179	2528251

RESULTS AND DISCUSSION

Analytical method development and method validation was performed for RP-HPLC method for the Isoniazid and Rifampicin in tablet formulation as per ICH norms for the following parameters: system suitability, linearity and precision (repeatability), intermediate precision (ruggedness), specificity and accuracy. The summary of results obtained in analytical method development and validation were tabulated in table no.26.

VALIDATION SUMMARY REPORT

The observations and results obtained for each of the parameters like system suitability, linearity, precision (repeatability), specificity, accuracy and robustness lies well within the acceptance criteria. So the developed method was simple, specific, linear, precise, and accurate and robustness could be extensively used for the Isoniazid and Rifampicin in tablet formulation system.

Table no. 26Validation parameters and acceptance criteria for INH and RIF

S. No	Validation parameters	Specification	Results	
1	System suitability		Isoniazid	Rifampicin
	Retention time	Not applicable	2.660	3.172
	Tailing	NMT 2	1.469	1.412
	Resolution	NLT 2		3.697
	Theoretical plates	NLT 2500	7755	7613
	Similarity factor	0-98 to 1.02	0.99	0.99
	%RSD	NMT 2.0%	0.5	0.3
2	Specificity	There is no peak in blank at the Rt of analyte	Nil	Nil
		There is no peak in placebo at the Rt of analyte	Nil	Nil
3	Precision	The value should be between 97% to 103%	100	100
			99	100
			99	100
			99	100
			99	100
			99	100
		The %RSD of six replicate assay results NMT 2.0%	0.23	0.13
4	Accuracy (50%)	The value should be between 97% to 103%	100	101
	Accuracy (100%)	The value should be between 97% to 103%	100	101
	Accuracy (150%)	The value should be between 97% to 103%	100	101
5	Linearity	Correlation coefficient NLT 0.999	0.998	0.997
6	LOD	Not applicable	2.88 µg/ml	2.77 µg/ml
7	LOQ	Not applicable	9.58 µg/ml	9.22 µg/ml
8	Range	Not applicable	600µg to 1800 µg/ml	900µ g to 2700 µg/ml
9	Robustness(Flow-1)			
	Tailing	NMT 2	1.421	1.378
	Resolution	NMT 2	Nil	3.596
	Theoretical plates	NLT 2500	7399	7386
	Robustness(Flow-2)			
	Tailing	NMT 2	1.398	1.410
	Resolution	NMT 2	Nil	3.578
	Theoretical plates	NLT 2500	7247	7350
	Robustness(Temp-1)			
	Tailing	NMT 2	1.393	1.374

	Resolution	NMT 2	Nil	3.590
	Theoretical plates	NLT 2500	7510	7233
	Robustness(Temp-2)			
	Tailing	NMT 2	1.411	1.364
	Resolution	NMT 2	Nil	3.601
	Theoretical plates	NLT 2500	7515	7300

CONCLUSION

From the results obtained, it was observed that the developed method was proven to be specific, precise, linear, accurate, rugged and robust and is suitable for its intended purpose. So the above work performed gives documented evidence that the analytical method for the Isoniazid and Rifampicin by RP-HPLC in tablet dosage forms will consistently analyze these drugs quantitatively and could be used for routine analysis.

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