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Research

Analysis Of Anti T.B Drugs For Developed Rp- Hplc Method By Validation Parameters As Per Ich Guidelines.

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Abstract

This covers the separation principle, various stationary and mobile phase types, and separation-affecting variables. This article highlights the need of developing and testing such methods in addition to outlining the advantages of using RP-HPLC in industries like pharmaceutical, food, and environmental analysis. As examples of more recent advancements in RP-HPLC, new stationary and mobile phases, RP-HPLC downsizing, and hyphenated methods are also discussed. This review article provides a comprehensive tool for designing, refining, and validating RP-HPLC processes.

Keywords: Anti T.B Drugs, HPLC, Validation parameters ect.

INTRODUCTION

High performance liquid chromatography

High performance liquid chromatography is the fastest growing analytical technique for the analysis of the drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for an analysis of many drugs in both dosage forms and biological fluids. HPLC was developed in the late 1960s and 1970s. Today it is widely accepted separation technique for both sample analysis and purification in variety of areas. The successful use of liquid chromatography requires the right combination of a variety of operating conditions such as the type of,

- Column packing.
- Mobile phase and its flow rate.
- Column length and diameter.
- Column temperature and sample size.

General uses of HPLC

1. Separation of wide variety of compounds, organic, inorganic and biological compounds, polymers, chiral compounds, thermally liable compounds and small ions to macro molecules.
2. Analysis of impurities.
3. Analysis of both volatile and nonvolatile compounds.
4. Determination of neutral ionic or zwitter ionic molecules.
5. Isolation and purification of compounds.
6. Ultra trace to preparative and process scale separations.
7. Qualitative and quantitative method[6]

Method 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.

Parameters

- System suitability
- Specificity
- Method Precision
- Linearity & range
- Accuracy / Recovery studies
- Robustness

Preparation of standard and sample solution

Standard preparation

Buffer Preparation: 17.418 gm of Potassium Hydrogen Orthophosphate was mixed with 1000 ml of Mille-Q water and shaked for 15min and degassed.

Mobile phase A : Potassium Hydrogen Orthophosphate

Mobile phase B : Methanol

Chromatographic Condition

Column	: Agilent Zorbax Sb-C18, (4.6 × 250 mm, 5 µ)
Column temperature	: 30^0C
Inj. Volume	: 10 µl
Flow rate	: 1.0 ml/min
λmax	: 263 nm
Runtime	: 15

Sample preparation

Preparation of sample solution of Isoniazid and Rifampicin for trials

10 tablets were weighed accurately and finely powdered. Tablet powder of 902.55 mg equivalent to 450 mg of Rifampicin and 300 mg of Isoniazid was weighed and transferred into a 50 ml standard volumetric flask. After this 25 ml of HPLC Water (diluent) was added and sonicated for 30 minutes with intermittent shaking and cooled to room temperature. Volume was made with HPLC Water (diluent) and mixed well. 5ml of stock was pipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µg of Isoniazid per ml respectively.

The scheme of dilution was presented as follows Dilution chart:

902.55 mg ⟶ 50 ml (9000µ g/ml) [*Rifampicin*]
(6000µg/ml) [Isoniazid]

↓

5ml ⟶ 25ml (1800µg/ml) [*Rifampicin*]
(1200µg/ml) [Isoniazid]

Standard preparation Standard solution preparation

450 mg of Rifampicin (RIF) and 300 mg of Isoniazid (ISN) was accurately weighed and transferred into a 50 ml standard volumetric flask. After this 5 ml of HPLC water was added and sonicated for 30 minutes with intermittent shaking and cooled to room temperature. Volume was made with diluent and mixed well.

5ml of stock waspipetted out in to a 25ml standard volumetric flask and finally volume was made up with 25ml HPLC water (diluents). This solution was referred as Rifampicin and Isoniazid sample solution that contained 1800 µg of Rifampicin and 1200 µg of Isoniazid per ml respectively.

The scheme of standard dilution was presented as follows Dilution chart:

450 mg of RIF ⟶ 50ml (9000µg/ml) [*Rifampicin*]
(6000µg/ml) [Isoniazid]

↓

Or

300 mg of INH ⟶ 25ml
(1800µg/ml) [*RIF*]
(1200µg/ml) [ISN]

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 1: System suitability parameters and recommendations

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 HPLC system. 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.

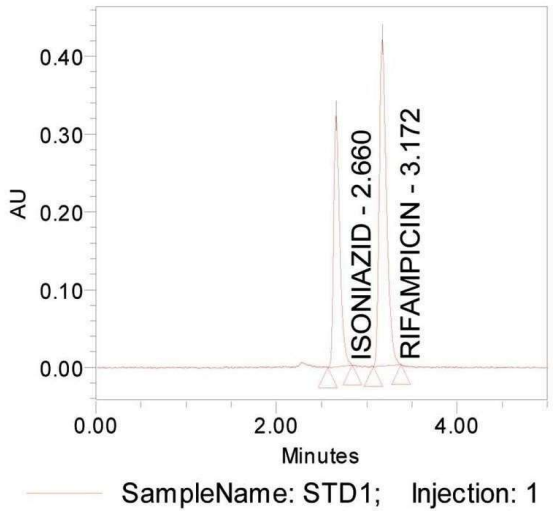

Fig 1: Chromatogram of standard 1

Table 2: Data for system suitability of ISONIAZID Name: ISONIAZID

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

Table 3: 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								

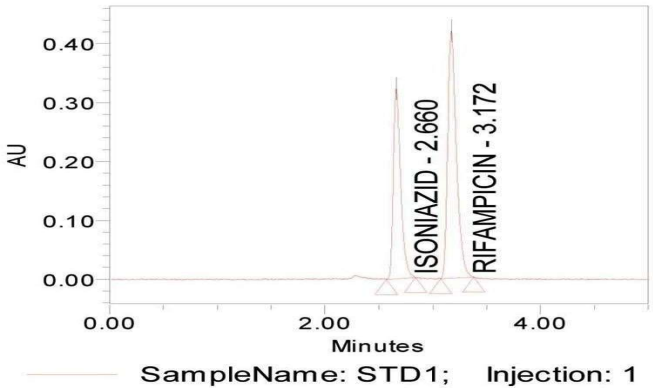

Fig 2: Chromatogram of standard 1

Table 4: Data for system suitability of ISONIAZID Name: ISONIAZID

	Sample Name	Inj	Nam e	RT	Area	USP Resolution	USP Tailing	USP Plate Count
1	STD 1	1	ISONIAZID	2 .660	1518803		1 .469	7755
Mean					1518803			
%RSD								

Table 5: Data for system suitability of RIFAMPICIN Name: RIFAMPICIN

	Sample Name	Inj	Nam e	RT	Area	USP Resolution	USP Tailing	USP PlateCount
1	STD 1	1	RIFAMPICIN	3 .172	2348101		1 .412	7613
Mean					2348101			
%RSD								

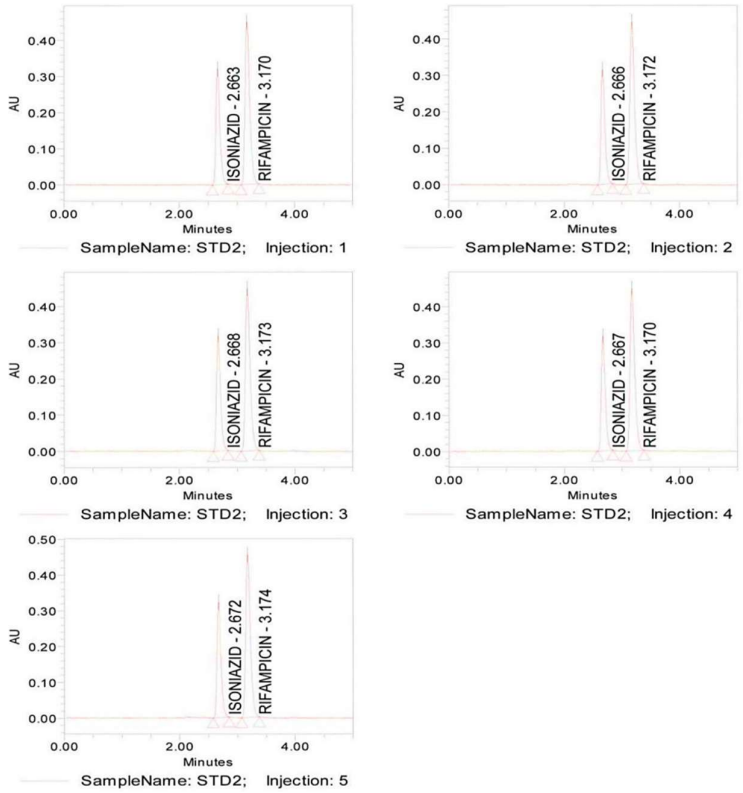

Fig 3: Chromatograms of standard 2

Table 6: Results of system suitability (ISONIAZID) Name: ISONIAZID

	Sample	Inj	Nam e	RT	Area	USPResolutio	USP Tailing	USPPlateCo unt
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 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			

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

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.

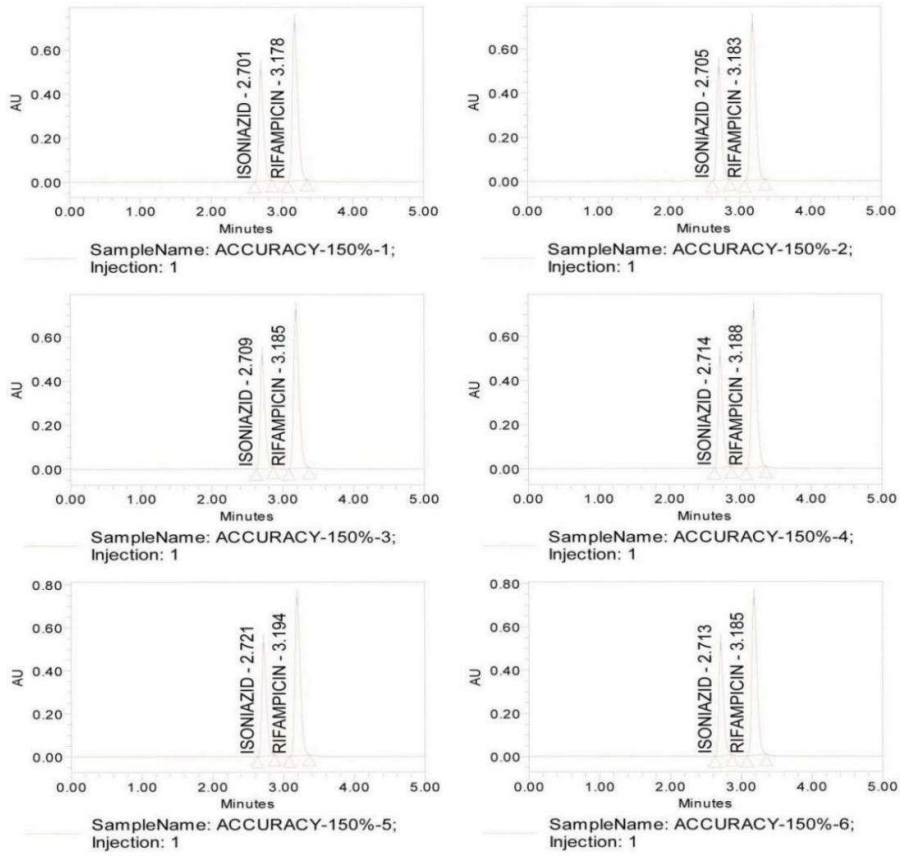

Fig 4: Chromatograms for sample of 50% concentration

Table 8: Data for accuracy of 50% concentration of Isoniazid 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 9: Data for accuracy of 50% concentration of Rifampicin
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

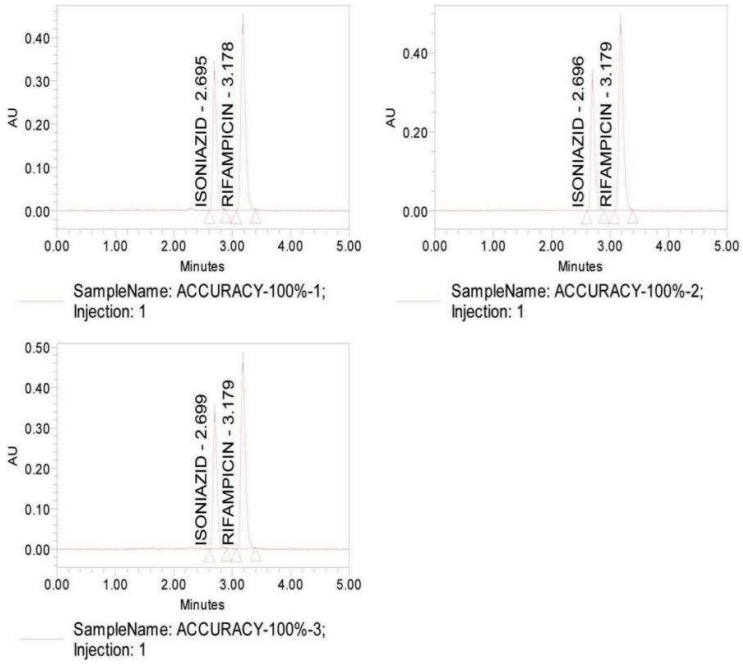

Fig 11: Chromatograms for sample of 100% concentration

Table 10: Data for accuracy of 100% concentration of Isoniazid
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 11: Data for accuracy of 100% concentration of Rifampicin
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

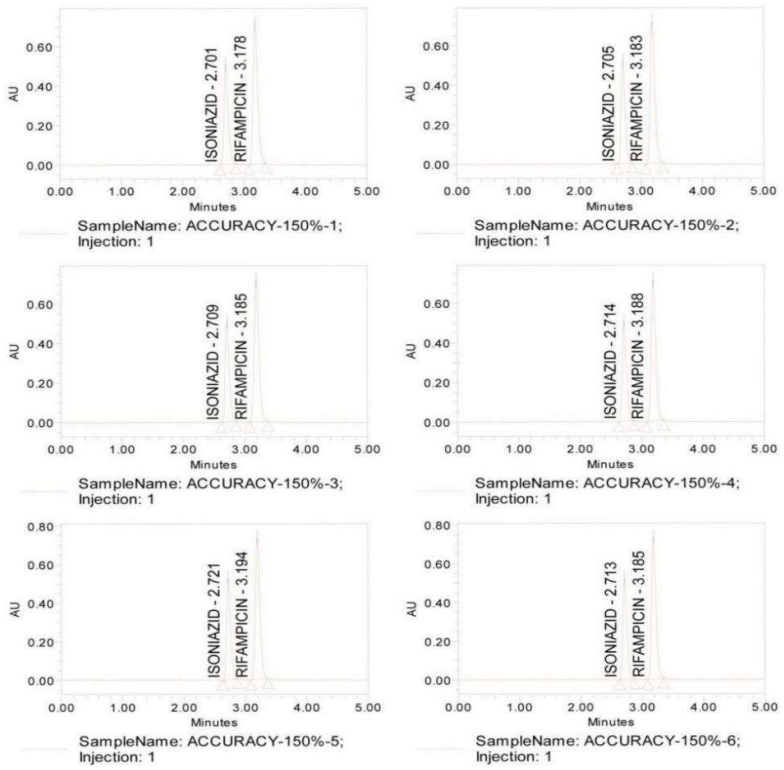

Fig 12: Chromatograms for sample of 150% concentration

Table 12: Data for accuracy of 150% concentration of Isoniazid
Name: ISONIAZID

	SampleNam e	Inj	Nam e	RT	Area
1	ACCURACY-1 50 %-1	1	ISONIAZID	2 .701	2260654
2	ACCURACY-1 50 %-2	1	ISONIAZID	2 .705	2262479
3	ACCURACY-1 50 %-3	1	ISONIAZID	2 .709	2260325
4	ACCURACY-1 50 %-4	1	ISONIAZID	2 .714	2262513
5	ACCURACY-1 50 %-5	1	ISONIAZID	2 .721	2263234
6	ACCURACY-1 50 %-6	1	ISONIAZID	2 .713	2266385

Table 13: Data for accuracy of 150% concentration of Rifampicin
Name: RIFAMPICIN

	SampleNam e	Inj	Nam e	RT	Area
1	ACCU RACY-1 50 %-1	1	ISONIAZID	2 .701	2260654
2	ACCU RACY-1 50 %-2	1	ISONIAZID	2 .705	2262479
3	ACCU RACY-1 50 %-3	1	ISONIAZID	2 .709	2260325
4	ACCU RACY-1 50 %-4	1	ISONIAZID	2 .714	2262513
5	ACCU RACY-1 50 %-5	1	ISONIAZID	2 .721	2263234
6	ACCU RACY-1 50 %-6	1	ISONIAZID	2 .713	2266385

Table 14: Results of Accuracy study (ISONIAZID)

ISONIAZID						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**%`Recovery**	**% Mean**
50%	451.28	756728	594.007	594.91	100	
50%	451.28	756907	594.007	594.05	100	
50%	451.28	756975	594.007	594.10	100	100
50%	451.28	756326	594.007	594.59	100	

50%	451.28	756274	594.007	594.55	100	
100%	902.55	1512475	1188.000	1189.04	100	
100%	902.55	1518251	1188.000	1193.58	100	100
100%	902.55	1512296	1188.000	1188.90	100	
150%	1353.83	2260654	1782.007	1777.23	100	
150%	1353.83	2262479	1782.007	1778.66	100	100
150%	1353.83	2260325	1782.00	1776.9	100	
150%	1353.83	2262513	1782.007	1778.69	100	
150%	1353.83	2263234	1782.007	1779.26	100	
150%	1353.83	2266385	1782.007	1781.74	100	

Table 15: Results of Accuracy study (RIFAMPICIN)

RIFAMPICIN				
Sample Area	**µg/ml added**	**µg/ml found**	**% Recove ry**	**% Mean**
1265170	891.010	899.73	101	
1262461	891.010	897.81	101	
1261719	891.010	897.28	101	
1263056	891.010	898.23	101	101
1268196	891.010	901.89	101	
1262096	891.010	897.55	101	
2523741.00	1782.000	1795.78	101	101
2525279.00	1782.000	1795.87	101	
2528251.00	1782.000	1797.98	101	
3782160	2673.010	2689.71	101	
3785672	2673.010	2692.21	101	
3782793	2673.010	269016	101	101
3785575	2673.010	2692.14	101	
3788145	2673.010	2693.97	101	
3789803	2673.010	2695.15	101	

Table 16: Validation parameters and acceptance criteria for INH and RIF

S. No	**Validation parameters**	**Specification**	**Results**	
		System suitability	Isoniazid	Rifampicin
1	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%	100	101

		to 103%		
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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