

## Research Article



## RP-HPLC Method Development and Validation for Simultaneous Estimation of Isoniazid and Rifapentine in Synthetic Mixture

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### ABSTRACT

Isoniazid and Rifapentine are used in combination (ratio 1:1) as tablet dosage form for the treatment of *Mycobacterium tuberculosis*. Objective of present work was to develop simple, precise, accurate and rapid chromatographic method for simultaneous estimation of both drugs using RP-HPLC. Developed method was validated as per ICH Q2(R1) guideline for quantitative determination of Isoniazid and Rifapentine in Synthetic mixture. RP-HPLC was developed using the mobile phase [Acetonitrile: Phosphate buffer pH 4 (85:15) pH-6 adjusted with 1% TEA], Flow rate 1 ml/min and detected at 262 nm. The separation was carried out on shim-pack solar C18 (250mm × 4.6 mm, 5µm). The linearity for ISO and RFP were between 5-25µg/ml with co-relation co-efficient 0.9979 and 0.9975 respectively. Limit of detection for ISO and RFP were found to be 0.20110 and 0.18813 µg/ml and limit of quantification for ISO and RFP were found to be 0.60941 µg/ml and 0.57012 µg/ml respectively. Recovery of both drugs were found in range of 98-102%. Further % R.S.D. was found to be less than 2% for Repeatability, Intraday and Interday Precision study. The % assay was found to be 99.60 % and 99.50 % for ISO and RFP respectively. The developed RP-HPLC method was found to be accurate, precise and robust and can be applicable to marketed formulation.

**Keywords:** Isoniazid, Rifapentine, RP-HPLC, Quantitative determination.

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### INTRODUCTION

Isoniazid (ISO) is Chemically Pyridine-4-carbohydrazide anti-tuberculosis it inhibits the formation of the mycobacterial cell wall. Rifapentine (RFP) is chemically (7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-26-[[[1E)-[[[4-Cyclopentyl piperazin-1-yl] imino] methyl]-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-6,23-dioxo-8,30-dioxo-24-azatetracyclo [23.3.1.1.1[5].0[6]] triacont a1,3,5(28), 9,19,21,25(29),26-octaen-13-yl]-acetate. It is higher bacteriostatic and bactericidal activities especially against intracellular bacteria growing in human monocyte-derived macrophages. Rifapentine inhibits DNA-dependent RNA polymerase in susceptible strains of *M. tuberculosis*. Rifapentine acts via the inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death<sup>1-4</sup>. ISO is official in IP, JP, EP, USP and RFP is not official in any Pharmacopoeia. From Literature review for estimation of Isoniazid: UV, HPLC, HPTLC, UPLC method were reported for individually and along with other drug and for rifapentine only UV and HPLC methods reported on

individually<sup>5-20</sup>. After conducting literature survey on analytical method development and validation for simultaneous estimation of Isoniazid and Rifapentine till date there has not been reported any analytical method.

### MATERIALS AND METHODS

#### Instrumentation

The HPLC analysis was carried out using SHIMADZU LC-2010 CHT, Auto sampler Column: Shim-pack solar C18 (250mm × 4.6mm, 5µm) Detector, UV Detector Software, LC Solution version 1.25 for separation.

#### Chemical and Reagent

Isoniazid (ISO) and Rifapentine (RFP) were given as gift sample from Macleods pharmaceutical Private Limited Kachigam daman. Acetonitrile HPLC Grade (Rankem chemicals), HPLC Grade water (Rankem chemicals), HPLC Methanol (Rankem chemicals), OPA (HPLC grade Fisher Scientific), Glacial Acetic Acid (HPLC grade, Thomas Baker, Mumbai) and Triethylamine (HPLC grade, Thomas Baker, Mumbai) were used in the research work.

#### Chromatographic condition

Stationary Phase: Shim-pack solar C18 (250 × 4.6 mm, 5µm)

Mobile phase: Acetonitrile: Phosphate buffer pH 4 (85:15 %v/v) pH-6 adjusted with 1% TEA

Flow rate: 1.0 ml/min

Wavelength: 262 nm



### Preparation of stock solutions

10 mg of ISO and 10 mg of RFP was weighed and transferred into different two 10 ml volumetric flask it was dissolved in methanol and volume was made up to the mark with HPLC grade methanol to give a solution containing 1000 µg/ml.

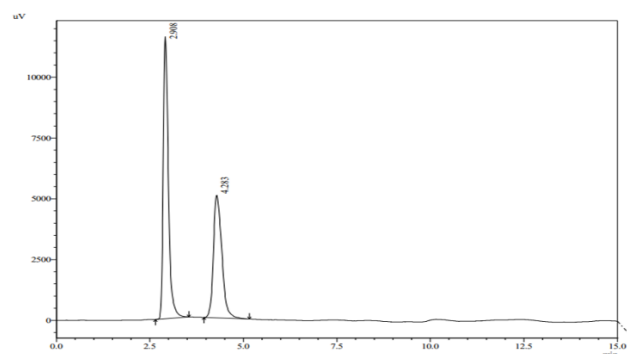
### Preparation of working solution:

Aliquot of 1ml from above both standard stock solution was pipette out into 10 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing 100 µg/ml. from above solution 5-25 µg/ml range of both ISO and RFP working stock solution of precisely measured it (0.5, 1, 1.5, 2, 2.5 mL) separately and transfer both of drug in 10 ml volumetric flask and mitigated with methanol in HPLC method used HPLC grade methanol.

### Method Development

The mobile phase consisting of Acetonitrile, Methanol and Water in varying proportions and change in pH was tried but it doesn't show satisfactory separation. The Final ratio of optimized mobile phase was Acetonitrile: Phosphate buffer pH 4 (85:15 %v/v) pH 6 adjusted with 1% TEA which was found to give good separation for the peaks of Isoniazid with retention time 2.908 min and Rifapentine 4.283 min, as shown in the Figure.1. In addition to this, UV

spectra of individual drugs were recorded at the wavelength range from 200 to 400 nm and 262 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.



**Figure 1:** Chromatogram of ISO and RFP in Acetonitrile : Phosphate buffer pH 4 (85:15%v/v) pH6 with 1% TEA

### Method Validation

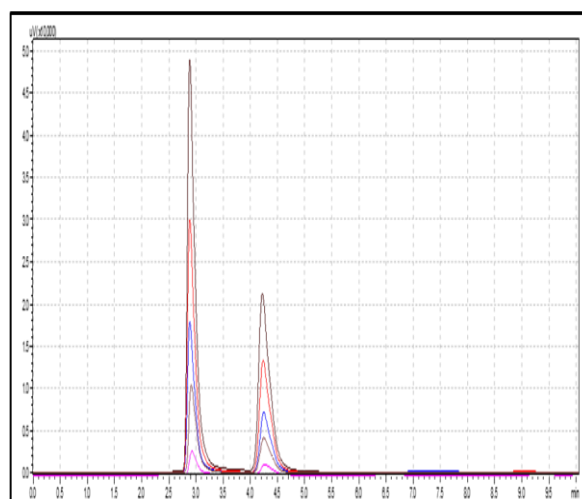
The method was validated in accordance with ICH guidelines. System suitability System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as retention time (Rt), number of theoretical plates (N), asymmetry factor, resolution factor were determined. The results are shown in table No 1.

**Table No. 1 System suitability**

Drug	Parameters	Mean ±S.D. (n=5)	%RSD
ISO	Retention time	2.914 ± 0.00922	0.3164
	Theoretical plate	14312.16 ± 124.47	0.8697
	Tailing factor	1.505 ± 0.0104	0.6968
RFP	Retention time	4.2333 ± 0.0294	0.6954
	Theoretical plate	11642 ± 113.43	0.9743
	Tailing factor	1.505 ± 0.0116	0.8971
	Resolution	4.1303 ± 0.03706	0.8972

### Linearity

The linearity study was carried out for both drugs at five different concentration levels. The linearity of ISO and RFP was in the range 5-25 µg/ml . chromatogram show in Fig 2 and data are depicted in table no. 2. The plots of peak area versus the respective concentration were found to be linear with regression coefficient ( $R^2 = 0.9960$ ) for Isoniazid show in Fig 3 and ( $R^2 = 0.9951$ ) For RFP show in fig 4.



**Figure 2:** Overly chromatogram of ISO (5-25µg/ml) and RFP (5-25µg/ml)

**Table 2:** Linearity data of ISO and RFP

Drugs	Concentration (µg/ml)	Mean peak area ±S.D.	%RSD
ISO	5	29450±124.3061	0.4220
	10	111791.8±870.7759	0.7789
	15	199455.2±895.8023	0.4491
	20	312116.6±1162.959	0.3126
	25	414842.6±1647.976	0.3972
RFP	5	29128±87.5602	0.3005
	10	79473±317.545	0.3995
	15	152720±838.508	0.5490
	20	226564±860.657	0.3798
	25	301920±1560.49	0.5168

**Precision**

Precision data shown in table no.3

**Repeatability**

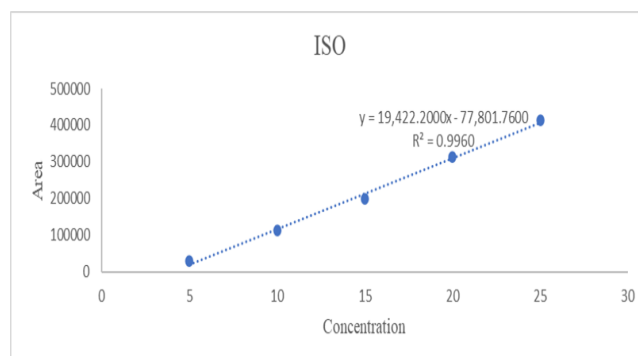
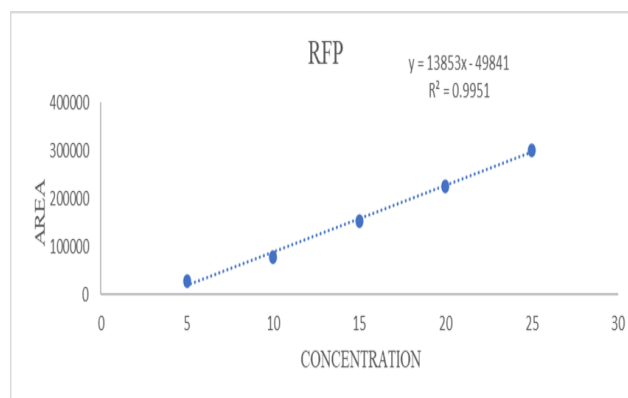
Repeatability of the developed method was assessed by analyzing samples from the same batch 6 times with standard solutions containing concentrations 15 µg/ml for both ISO and RFP and % R.S.D. as calculated.

**Intraday precision**

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 10, 15, 20 µg/ml for ISO and RFP. Solutions were analyzed thrice (n=3) on the same day within short interval of time and % R.S.D. was calculated.

**Interday precision**

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 10, 15, 20 µg/ml for both ISO and RFP. Solutions were analyzed thrice (n=3) on the three different day and % R.S.D. was calculated.

**Figure 3:** Calibration curve of ISO**Figure 4:** Calibration curve of RFP**Table 3:** Precision data for ISO and RFP

Repeatability data for ISO and RFP			
Drugs	Concentration (µg/ml)	Mean peak Area ±S.D.(n=5)	%R.S.D
ISO	15	199433 ± 511.8814	0.2566
RFP	15	152093 ± 379.0633	0.2492
Intraday precision for ISO and RFP			
ISO	10	111554 ± 696.07	0.6239
	15	198874 ± 948.78	0.4770
	20	311500 ± 1054.76	0.3386
RFP	10	79488 ± 177.01	0.2226
	15	152239 ± 536.53	0.3524
	20	226113 ± 698.90	0.3091
Interday precision for ISO and RFP			
ISO	10	111854 ± 941.07	0.8413
	15	199134 ± 1120.71	0.5627
	20	312400 ± 1821.43	0.5830
RFP	10	79035 ± 503.062	0.6365
	15	152939 ± 937.846	0.6132
	20	225906 ± 1008.5	0.4464

**Accuracy**

For accuracy study data from nine determinations over three concentrations at 80%, 100% and 120% of expected

sample concentration covering the specified range was determined & expressed as recovery values. The results were shown in table 4.

**Table 4:** Accuracy data for ISO and RFP

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total amount (µg/ml)	Amounts of sample found (µg/ml)	% Recovery
ISO	0%	10	0	10	9.9001	99.001
	80%	10	8	18	17.870	99.281
	100%	10	10	20	20.118	100.5
	120%	10	12	22	22.131	100.6
RFP	0	10	0	10	9.93	99.39
	80	10	8	18	17.95	99.76
	100%	10	10	20	20.02	100.1
	120%	10	12	22	22.17	100.8

**LOD and LOQ**

The LOD and LOQ for ISO and RFP was found and calculated and result given in table no.7

**Robustness**

The robustness of the method was established by making deliberate minor variation in the following method parameters. The retention time was unaffected by changes in parameters and RSD was found to be less than 2%

Mobile Phase Ratio : ± 2.0 ml

Flow rate: ± 0.02 units

**Assay**

A synthetic mixture containing 300mg of ISO equivalent was placed in a 10ml volumetric flask methanol was introduced and sonicated for 2-3 minutes, and the volume was brought up to the mark. Whatmann filter paper no. 42 was used to filter the mixture. As a result, the final solution included 1000 µg/ml ISO and 1000 µg/ml RFP. To make a solution containing ISO(100 µg/ml)and RFP(100 µg/ml) 1.0 ml of the above solution was pipette out and transferred to a 10 ml volumetric flask and volume was made up to the mark with mobile phase. This solution was used for Assay. Estimation of ISO and RFP in synthetic mixture chromatogram was recorded and the concentration of ISO and RFP was obtained by solving the regression equation. Result is shown in table 5.

**Table 5:** Analysis of synthetic mixture

Synthetic mixture	Concentration (µg/ml)		Amount obtain (µg/ml)		%Assay of ISO±S.D. (n=5)	%Assay of RFP ±S.D. (n=5)
	ISO	RFP	ISO	RFP		
	10	10	9.96	9.95	99.62±0.1591	99.50±0.2179

**Table 6:** Summary of RP-HPLC Method

Parameters	ISO	RFP
Linearity (µg/ml)	5-25 µg/ml	5-25µg/ml
Regression Equation (y = mx + c)	Y=19422x - 77801.7	Y= 13853x – 49841
Regression coefficient (R <sup>2</sup> )	0.9960	0.9951
Correlation co-efficient (r)	0.9979	0.9975
Repeatability (%R.S.D.) (n=6)	0.2566	0.2492
Intraday precision (%R.S.D.) (n=3)	0.33861 - 0.62398	0.2226 - 0.3091
Interday precision (%R.S.D.) (n=3)	0.56279 - 0.84134	0.44642 - 0.6365
LOD (µg/ml) (n=5)	0.20110	0.18813
LOQ (µg/ml) (n=5)	0.60941	0.57012
% Recovery (n=3)	99.001-100.6	99.36-100.8
Assay (%) ± S.D. (n=5)	99.62 ± 0.1591	99.50 ± 0.2179



## CONCLUSION

The proposed HPLC method provides a rapid, accurate, precise result for Isoniazid and Rifapentine in synthetic mixture. In conclusion, the developed method is good recommended for the assay of Isoniazid and Rifapentine in combine dosage form.

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