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Research Article

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ISONIAZID AND RIFAMPICIN IN COMBINED DOSAGE FORM

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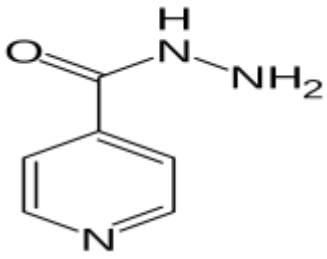
Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Isoniazid and Rifampicin in combined-dosage form. A Inertsil ODS (250*4.6*5µ) column with mobile phase containing water pH 4.5 adjusted with Sodium di hydrogen phosphate: Acetonitrile in the ratio of (400: 600, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 274 nm. The retention times of Isoniazid and Rifampicin were 2.953min and 3.382min, respectively. The correlation co-efficient for Isoniazid and Rifampicin was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Isoniazid and Rifampicin in formulations was found to be 100% and 100% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Isoniazid and Rifampicin in combined dosage form.
Key words: RP-HPLC, Isoniazid, Rifampicin.

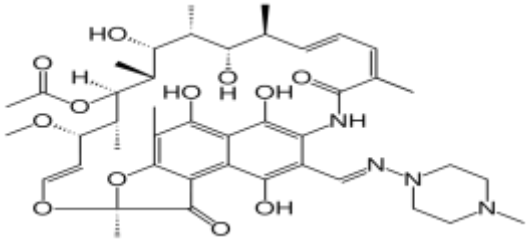
INTRODUCTION:

Isoniazid (isonicotinic acid hydrazide; INH), a whitish crystalline powder, It is the hydrazide of isonicotinic acid is a synthetic analog of pyridoxine. Isoniazid (INH), a first line antitubercular, is chemically 4-pyridinecarboxylic acid hydrazide or isonicotinic acid hydrazide having molecular formula $C_6H_7N_3O$ and molecular weight 137.14. It acts by inhibiting the synthesis of mycolic acids which get attached to arabinogalactan to form part of mycobacterial cell wall. It is an essential component of all antitubercular regimens, unless the patient is not able to tolerate it or bacilli are resistant. It is the first line antitubercular medication never used on its own to treat active tuberculosis because resistance quickly develops. It is widely used together with rifampicin, ethambutol and pyrazinamide among others, for the chemotherapy of tuberculosis.



Structure of Isoniazid

Rifampicin is a complex semisynthetic macrocyclic antibiotic derived from Streptomyces mediterranei, is a member of the rifamycin class of antibiotics used for the treatment of tuberculosis and other infectious diseases. Rifampicin having molecular formula $C_{43}H_{58}N_4O_{12}$ and molecular weight 822.94. It is categorized as one of the first line antituberculous agents. Tuberculosis remains a major health public problem and is the single most deadly infectious disease. It kills approximately two million people each year. Rifampicin is chemically (12*Z*, 14*E*, 24*E*)- (2*S*, 16*S*, 17*S*, 18*R*, 19*R*, 20*R*, 21*S*, 22*R*, 23*S*) - 1,2 -dihydro- 5, 6, 9, 17, 19 - pentahydroxy, 23 -methoxy- 2, 4, 12, 16, 18, 20,22 heptamethyl-8- (4-methylpiperazin -1 yliminomethyl) -1, 11 - dioxo 2, 7 (epoxypentadeca -1, 11, 13 trienimino) naphtha [2,1-*b*] furan -21-yl acetate[3]. Which is red-dish in colour,



Structure of Rifampicin

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and Inertsil ODS (250mmx4.6mm, particle size 5µm) column.

Chemicals and Reagents: Rifampicin, Isoniazid was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Sodium dihydrogen phosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of Sodium dihydrogen phosphate(pH 4.5 adjusted with Sodium dihydrogen phosphate) and Acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 400:600v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 274nm and the run time was 8min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

PREPARATION OF STANDARD SOLUTION:

Isoniazid: Accurately weighed quantity, 300 mg of Isoniazid was transferred into 50ml of volumetric flask and adds 30ml of water and sonicate for 15 min. make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

Rifampicin: Accurately weighed quantity, 600mg of Rifampicin was transferred into 50ml of volumetric flask and adds 30ml of water and sonicate for 15min. make up the volume with water. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with water.

PREPARATION OF SAMPLE SOLUTION:

Take accurately weighed sample powder equivalent to one tablet and transfer it into 50ml of volumetric flask and add 25ml of water and sonicated for 30mins and make up the volume with water and filtered through the 0.45µm filter paper Transfer above solution 5ml into 50 ml volumetric flask and make up the volume with water.

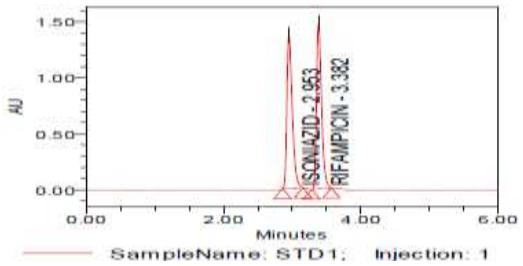


Fig. 1: Standard chromatogram for Isoniazid and Rifampicin

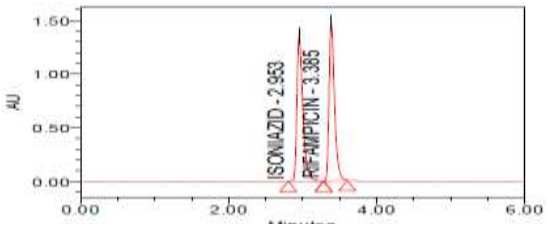


Fig. 2: Formulation chromatogram for Isoniazid and Rifampicin

METHOD VALIDATION:

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method**.**

Table1: System Suitability Parameters

Parameters	Isoniazid	Rifampicin
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	2.359	2.896
LOQ	7.864	9.6541
Theoretical plates	6642	10333
Tailing	1.478	1.428

Specificity: The specificity was established by preparing a Isoniazid and Rifampicin standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

ACCURACY AND PRECISION:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4).

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Table 2: Precision Studies

S. No.	Sample weight	Area (INH)	Area (RIF)	%Assay (INH)	%Assay (RIF)
1	1099.90	7463977	7567127	99	100
2	1099.90	7469647	7569942	99	100
3	1099.90	7460075	7562651	99	100
4	1099.90	7466034	7566522	99	100
5	1099.90	7465793	7561474	99	100
6	1099.90	7468156	7563286	99	100

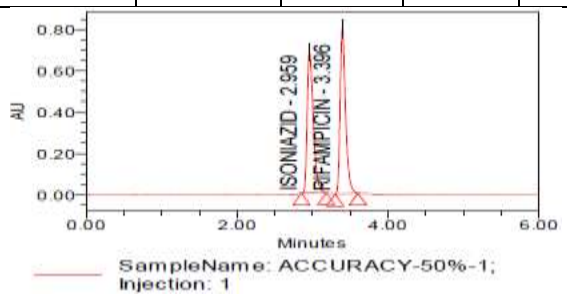


Fig. 3: Accuracy Chromatograms-50% of Isoniazid and Rifampicin

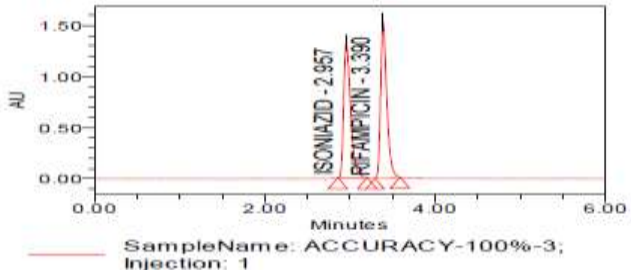


Fig. 4: Accuracy Chromatograms-100% of Isoniazid and Rifampicin

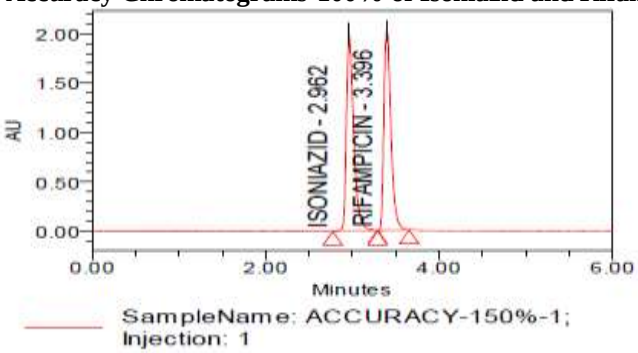


Fig. 5: Accuracy Chromatograms-150% of Isoniazid and Rifampicin

Table 3: Accuracy for Isoniazid

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	550.00	3734928	594.654	595.37	100	
50%	550.00	3730390	594.654	594.64	100	
50%	550.00	3732692	594.654	595.01	100	100
0%	550.00	3732341	594.654	594.95	100	
50%	550.00	3737404	594.654	595.34	100	
50%	550.00	3731148	594.654	594.76	100	
100%	1099.90	7466840	1189	1190.25	100	
100%	1099.90	7465149	1189	1189.98	100	100
100%	1099.90	7464778	1189	1189.92	100	
150%	1650.00	11136775	1784	1775.26	100	
150%	1650.00	11156975	1784	1778.48	100	
150%	1650.00	11175828	1784	1781.48	100	
150%	1650.00	11185718	1784	1783.06	100	100
150%	1650.00	11194409	1784	1784.45	100	
150%	1650.00	11174078	1784	1781.20	100	

Table 4: Accuracy for Rifampicin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	550.00	3787241	1196.509	4.99	100	100
50%	550.00	3787256	1196.509	4.99	100	
50%	550.00	3784484	1196.509	4.99	100	
50%	550.00	3782636	1196.509	4.99	100	
50%	550.00	3786030	1196.509	4.99	100	
50%	550.00	3781367	1196.509	4.98	100	
100%	1099.90	7563618	2392.800	9.95	99	100
100%	1099.90	7564196	2392.800	9.98	100	
100%	1099.90	7567456	2392.800	9.98	100	
150%	1650.00	11320350	3589.526	14.93	100	100
150%	1650.00	11355456	3589.526	14.92	99	
150%	1650.00	11395916	3589.526	14.88	99	
150%	1650.00	11309982	3589.526	14.96	100	
150%	1650.00	11337854	3589.526	14.93	100	
150%	1650.00	11330288	3589.526	14.94	100	

LINEARITY AND RANGE:

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for Isoniazid and y = 19288x (R^2=0.99) for Rifampicin. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Isoniazid and Rifampicin shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

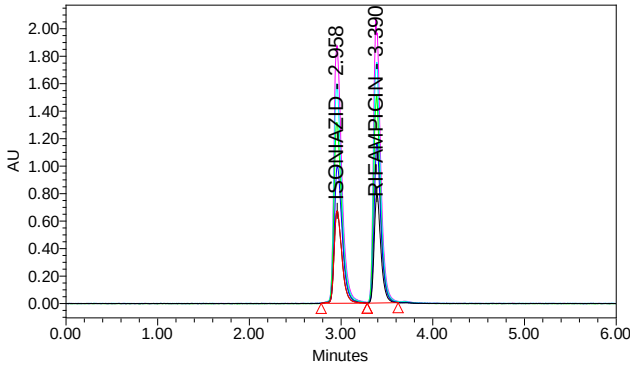


Fig 6: Overlay chromatograms of Linearity for Isoniazid and Rifampicin

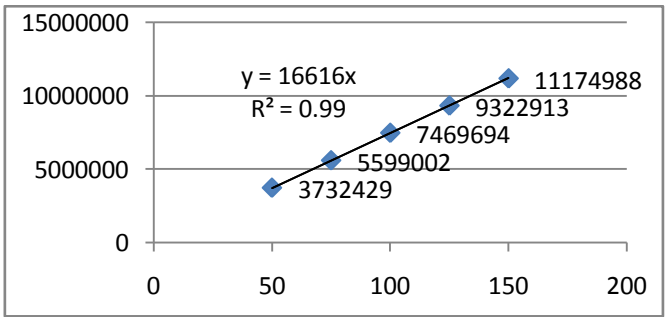


Fig. 7: Linearity Curve for Isoniazid

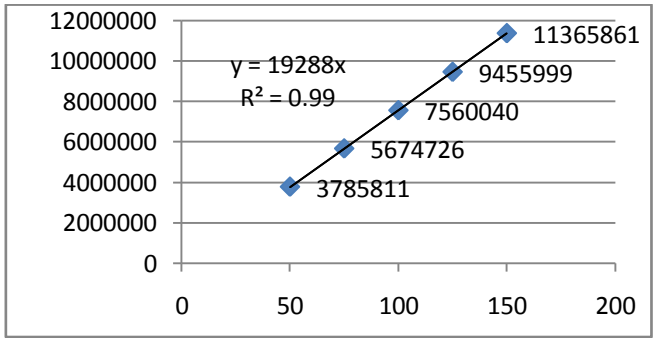


Fig. 8: Linearity Curve for Rifampicin

Limit of detection & Limit of quantifications (LOD & LOQ):

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Isoniazid and Rifampicin. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels and the results are tabulated in Table 02.

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ

Where σ = residual standard deviation of response

S = slope of the calibration curve

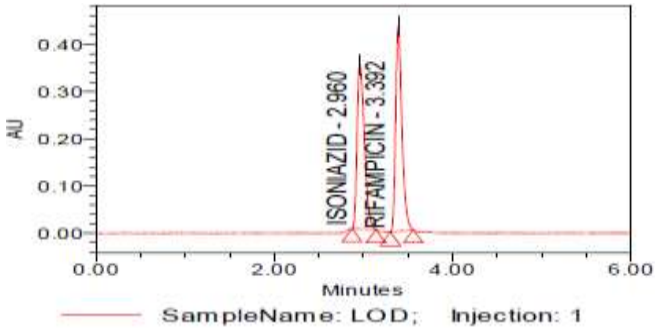


Fig.9: LOD Chromatograms for Isoniazid and Rifampicin

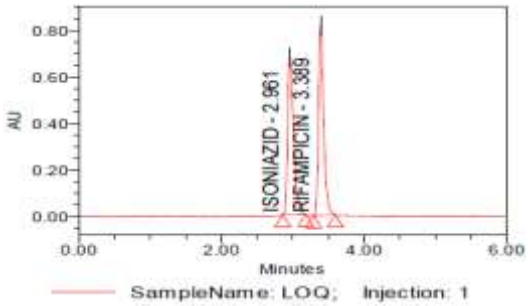


Fig.10: LOQ Chromatograms for Isoniazid and Rifampicin

ROBUSTNESS:

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust (Table-5&6).

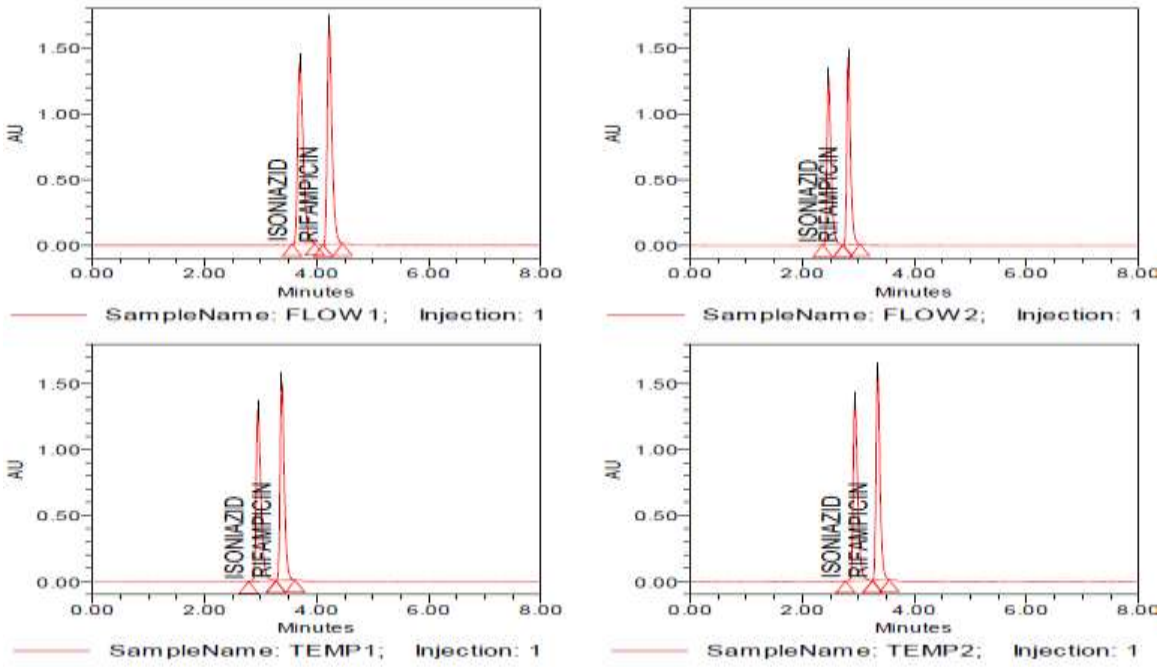


Fig.11:Robustness Chromatograms for Isoniazid and Rifampicin

Table 5: Robustness for Isoniazid

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Isoniazid	3.698	9510824	1.506	6559
2	Flow2	1.2ml/min	Isoniazid	2.469	6457737	1.560	5502
3	Temp1	25^{o}C	Isoniazid	2.953	7737925	1.533	5788
4	Temp2	35^{o}C	Isoniazid	2.940	7743794	1.534	6664

Table 6: Robustness for Rifampicin

S No	Sample name	change	Name	RT	Area	Tailing	Plate count
1	Flow1	0.8ml/min	Rifampicin	4.220	9831647	1.487	11558
2	Flow2	1.2ml/min	Rifampicin	2.824	6567128	1.451	9081
3	Temp1	25^{o}C	Rifampicin	3.377	7793869	1.425	10675
4	Temp2	35^{o}C	Rifampicin	3.344	7759290	1.445	11482

RESULTS AND DISCUSSION:

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Isoniazid and Rifampicin standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Isoniazid and Rifampicin studies. The method accuracy was evaluated by recovery studies. Isoniazid and Rifampicin recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient. Linear correlation was found to be Y= 16646x for Isoniazid and y = 19288x for Rifampicin. The intra day and inter day variations was calculated in terms of %RSD and results was found to be intra day and inter day respectively. Method robustness results were given by table 5&6.

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Isoniazid and Rifampicin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Isoniazid and Rifampicin in pure and its pharmaceutical dosage forms.

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