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Research

Simultaneous Estimation of Rifampicin And Isoniazid by RP-HPLC

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Abstract

A New Simple, Accurate, Precise, Robust And Specific Rp-Hplc Method Has Been Developed And Validated For The Simultaneous Estimation Of Rifampicin And Isoniazid In Pure Form And Marketed Pharmaceutical Formulation. Chromatographic Analysis Of Rifampicin And Isoniazid In The Present Assay Was Carried Out Isocratically With A Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle Size Column And Mobile Phase Consisting Of Methanol: Acetonitrile V/V In The Ratio Of 45:55v/V. The Flow Rate Was 1.0ml/Min. The Effluent Was Monitored At A Wavelength Of 265nm At 36ºc Temperature With A Retention Time Of Rifampicin And Isoniazid Was Found To Be 2.343min And 3.288min Respectively. The Proposed Rp-Hplc Method Was Validated According To ICH Guidelines With Respect To Specificity, Precision, Linearity And Accuracy. The Percentage Rsd For Precision And Accuracy Of The Proposed Method For Rifampicin And Isoniazid Was Found To Be Less Than 2%. The Inter-Day And Intra-Day Precisions Were Found To Be Within Limits. The Method Was Linear Over The Concentration Range Of 30- 70µg/Ml (R2 -0.999), With Limits Of Detection And Quantification Of 2.65µg/Ml & 7.95µg/Ml For Rifampicin (R2 -0.999) And The Method Was Linear Over The Concentration Range Of (10-50µg/Ml) (R2 -0.999) Limits Of Detection And Quantification Of 3.4µg/Ml & 10.2µg/Ml For Isoniazid (R 2 -0.999). The Proposed Rp-Hplc Method Was Successfully Applied For Routine Analysis Of Rifampicin And Isoniazid In Pure Form And Marketed Pharmaceutical Formulation

Keywords: Rifampicin And Isoniazid, Rp-Hplc, Validation, Accuracy.

INTRODUCTION

Analytical chemistry[1] is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography.

Introduction to HPLC

HPLC[3] is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed, efficient ,accurate and highly resolved method of separation.

For the recent study Clonazepam and Propranolol was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low

Normal phase chromatography

In normal phase mode the stationary base (eg; silica gel) is polar in nature and the mobile phase is non polar. In this technique, non polar compound travel faster and are eluted first. This is because less affinity between solute and stationary phase and take more time to elute.

Reverse phase chromatography

The popularity of reversed phase liquid chromatography is easily explained by its unmatched simplicity, versatility and scope. Neutral and ionic analytes can be separated simultaneously. Retention in RPLC is believed to occur through nonspecific hydrophobic interaction of the solute with the stationary phase. The near universal application of RPLC stems from the fact that almost all organic compounds have hydrophobic regions in their structure and are capable of interacting with the stationary phase.

A decrease in the polarity of the mobile phase leads to a decrease in retention. It is also generally observed in RPLC that branched chain compounds are retained to a lesser extent than their straight chain analogues and that unsaturated compounds are eluted before their fully saturated analogs. A wide variety of RP-HPLC columns are available. Most columns are silica based. Silica offers good mechanical stability. A typical stationary phase is formed by chemically bonding a long-chain hydrocarbon group to porous silica. Typical ligands are n-octadecyl (C18), n-octayl (C8), n-butyl (C4), diphenyl (C2), and cyano propyl.

MATERIALS AND METHODS

Instruments used

Table 1: Instruments used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

Chemicals used

Table 2: chemicals used

S.No	Chemical	Brand names
1	Rifampicin (Pure)	Sura labs
2	Isoniazid (Pure)	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Rifampicin and Isoniazid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5ml of the above Rifampicin and 0.3ml of the Isoniazid stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile in proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra. Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Column : Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size
Column temperature : 36˚C
Mobile phase : Methanol: Acetonitrile (45:55v/v)
Flow rate : 1ml/min
Wavelength : 265nm
Injection volume : 20µl
Run time : 5 minutes

Method validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 450ml (45%) of HPLC Methanol and 550ml of Acetonitrile (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Validation parameters

System suitability

Accurately weigh and transfer 10 mg of Rifampicin and Isoniazid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rifampicin and 0.3ml of the Isoniazid stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Repeatability

Accurately weigh and transfer 10 mg of Rifampicin and Isoniazid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rifampicin and 0.3ml of the Isoniazid stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy

Accurately weigh and transfer 10 mg of Rifampicin and Isoniazid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.25ml of the above Rifampicin and 0.15ml of the Isoniazid stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

RESULTS AND DISCUSSION

Trails

Trail 1

Mobile phase : Methanol: TEA Buffer (60:40%v/v)
Column : Apollo (4.6mm ×150mm, 5µm particle size) Make; waters
Flow rate : 1.0ml/min
Wavelength : 265 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 10 minutes

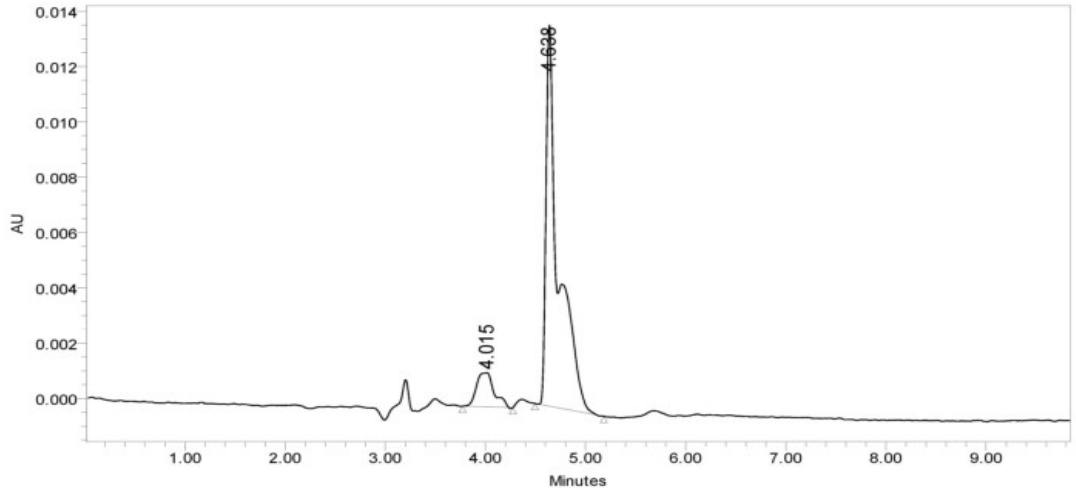

Fig 1: Chromatogram for Trail 1

Table 3: Peak Results for Trail 1

S.No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Rifampicin	4.015	26534	895		0.96	1205
2	Isoniazid	4.638	535242	65847	2.16	2.08	1154

Observation: This trial shows improper separation of sample peaks and less plate count, improper baseline in the chromatogram. So more trials were required for obtaining good peaks.

Trail 2:

Mobile phase : Acetonitrile: TEA Buffer (75:25 v/v)
Column : X-Bridge C18 (4.6mm×250mm) 5µm Particle size
Flow rate : 1.2 ml/min
Wavelength : 265nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 6 minutes

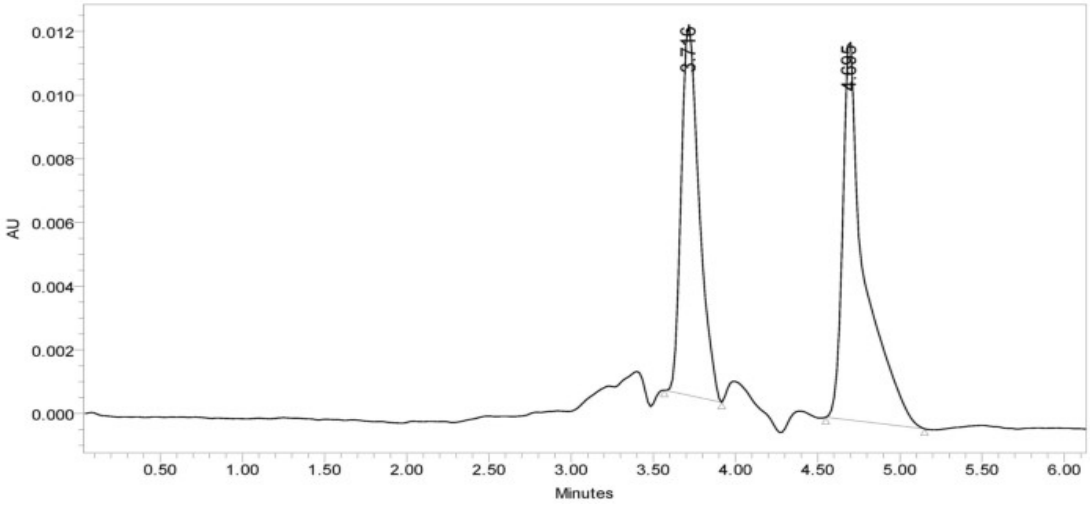

Fig 2: Chromatogram for Trail 2

Table 4: Peak Results for Trail 2

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	3.716	82754	35264		4.25	2548
2	Isoniazid	4.695	98547	32548	4.53	3.24	3682

From the above chromatogram it was observed that the void peaks are obtained and sample peaks are not separated and show less plate count in the chromatogram. So it's required more trials to obtain well peaks.

Trail 3:

Mobile phase : Acetonitrile: Water (55:45 % v/v)
Column : Zorbax C18 (4.6mm×250mm, 5µm)
Flow rate : 1.0 ml/min
Wavelength : 265 nm
Column temp : 45ºC
Injection Volume : 10 µl
Run time : 8 minutes

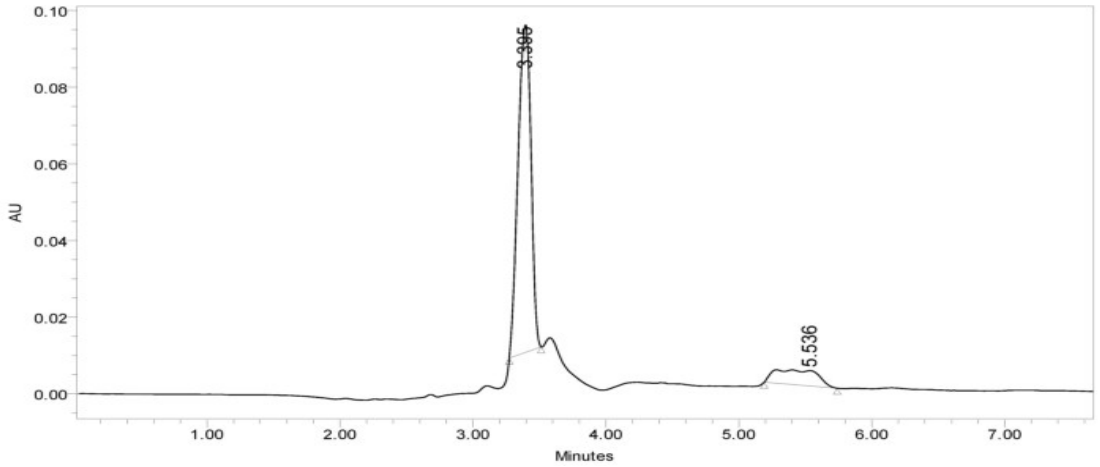

Figure 3: Chromatogram for Trail 3

Table 5: Peak Results for Trail 3

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	3.395	6584572	684575		2.16	2365
2	Isoniazid	5.536	6258	3568	5.46	3.24	3458

Observation: This trial show very less plate count and sample peaks are not well separated, so more trials were required for obtaining good peaks.

Trail 4:

Mobile phase : Methanol: Acetonitrile (35:65)
Column : Develosil C18 5µm (4.6mm×250mm) Make; waters
Flow rate : 0.8 ml/min
Wavelength : 265 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 10 minutes

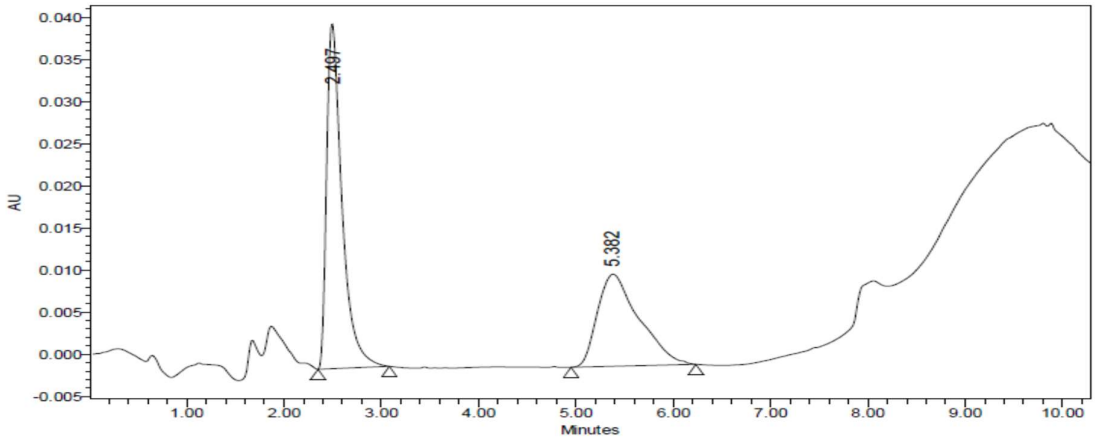

Figure 4: Chromatogram for Trail 4

Table 6: Peak Results for Trail 4

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	3.395	6584572	684575		2.16	2365

2	Isoniazid	5.536	6258	3568	5.46	3.24	3458

This trial show very less plate count and sample peaks are not well separated, so more trials were required for obtaining good peaks.

Trail 5:

Mobile phase : Methanol: Acetonitrile (20:80 v/v)
Column : Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size
Flow rate : 1.0 ml/min
Wavelength : 265 nm
Column temp : 40ºC
Injection Volume : 20 µl
Run time : 9 minutes

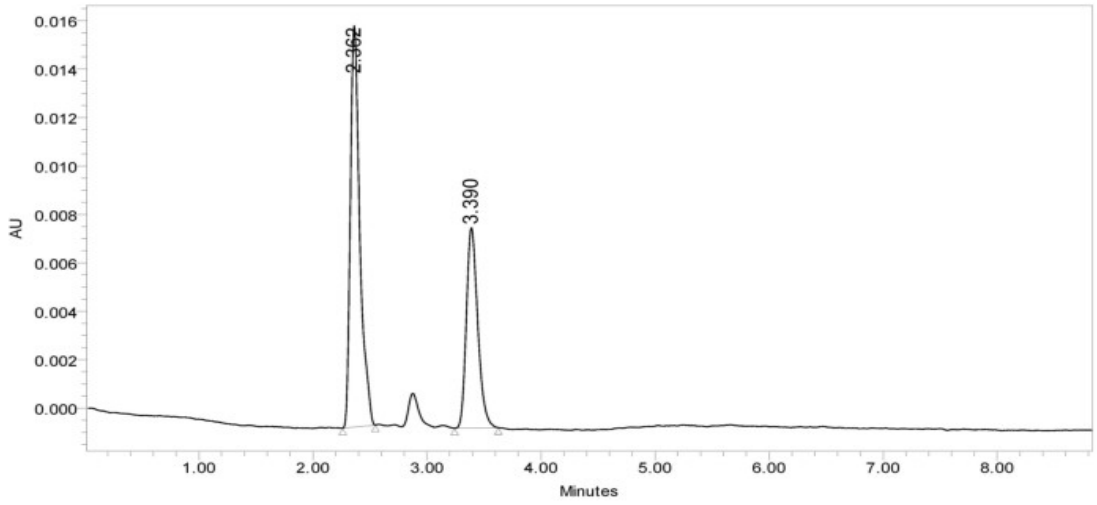

Fig 5: Chromatogram for Trail 5

Table 7: Peak Results for Trail 5

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	2.362	1036528	896854		1.09	1365
2	Isoniazid	3.390	635894	6524	5.34	2.2	1854

The chromatogram shows void peaks, and improper baseline. The trial shows tailing effect so go for further trials to obtain good separation of the peak.

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Acetonitrile (45:55 v/v)
Column : Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 36ºC
Injection Volume : 20 µl
Run time : 5 minutes

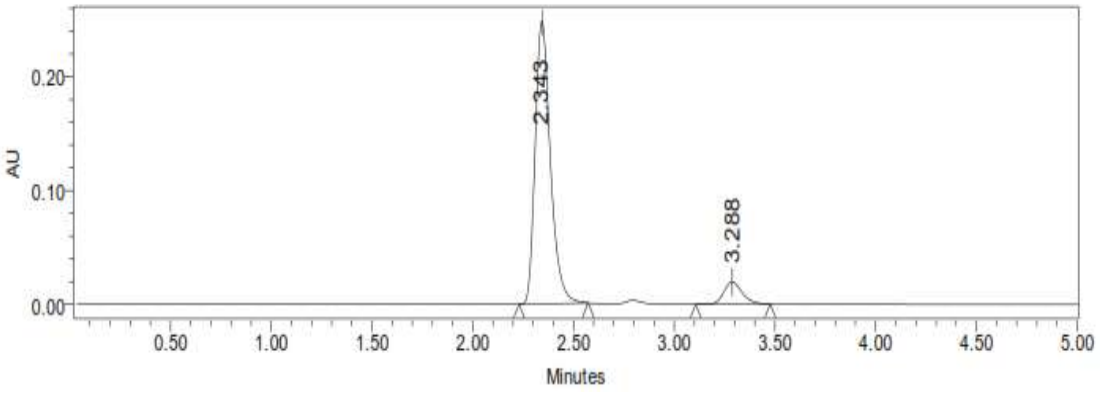

Fig 6 : Optimized Chromatographic Condition

Table 8: Peak Results for Optimized Chromatographic Condition

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	2.343	7584689	352654		1.46	6584
2	Isoniazid	3.288	42568	3658	7.52	1.38	8789

From the above chromatogram it was observed that the Rifampicin and Isoniazid peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

Mobile phase : Methanol: Acetonitrile (45:55 v/v)
Column : Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 36ºC
Injection Volume : 20 µl
Run time : 5 minutes

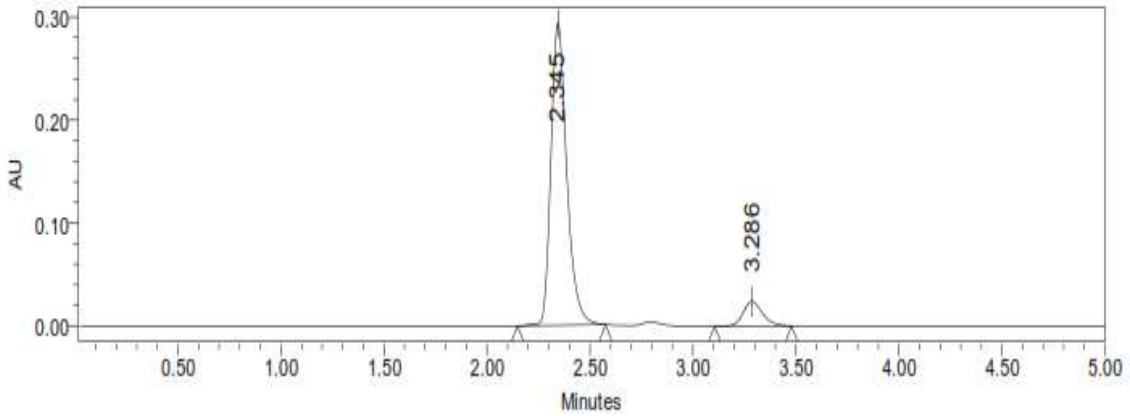

Fig 7: Optimized Chromatogram (Sample)

Table 9: Optimized Chromatogram (Sample)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rifampicin	2.345	7689854	365874		1.47	6689
2	Isoniazid	3.286	43652	3756	7.53	1.39	8857

- Resolution between two drugs must be not less than 2.

- Theoretical plates must be not less than 2000.
- Tailing factor must is not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 10: The Accuracy Results for Rifampicin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	446515.67	25	25.234	100.936%	100.52%
100%	842246.67	50	50.175	100.350%	
150%	1239288	75	75.198	100.264%	

Table 11: The Accuracy Results for Isoniazid

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	21758.33	15	15.063	100.420%	100.19%
100%	43069.33	30	30.060	100.200%	
150%	64262	45	44.974	99.942%	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Method validation

System Suitability

Table 12: Results of system suitability for Rifampicin

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rifampicin	2.343	7586598	356985	6582	1.46
2	Rifampicin	2.343	7594825	358746	6598	1.47
3	Rifampicin	2.342	7589842	359864	6574	1.46
4	Rifampicin	2.344	7579854	358745	6592	1.47
5	Rifampicin	2.342	7593659	35629	6539	1.46
Mean			7588956			
Std. Dev			6036.45			
% RSD			0.079543			

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 13: Results of system suitability for Rifampicin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Isoniazid	3.281	42653	3785	8758	1.39	7.52
2	Isoniazid	3.285	43265	3698	8859	1.38	7.53
3	Isoniazid	3.282	42856	3685	8754	1.39	7.52
4	Isoniazid	3.282	42875	3785	8839	1.38	7.52
5	Isoniazid	3.282	43689	3854	8755	1.39	7.53
Mean			43067.6				
Std. Dev			411.967				
% RSD			0.956559				

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Rifampicin and Isoniazid in drug product.

Assay (Standard)

Table 14: Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Rifampicin	2.344	7585684	358694		1.46	6598	1
2	Isoniazid	3.286	42658	3652	7.54	1.38	8759	1
3	Rifampicin	2.344	7568953	356985		1.47	6574	2
4	Isoniazid	3.283	42987	3687	7.55	1.39	8763	2
5	Rifampicin	2.344	7586945	356874		1.46	6553	3
6	Isoniazid	3.283	42653	3692	7.54	1.38	8774	3

Assay (Sample)

Table 15: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Rifampicin	2.344	7698542	369855		1.48	6698	1
2	Isoniazid	3.282	43658	3789	7.56	1.39	8859	1
3	Rifampicin	2.342	7685424	365472		1.49	6725	2
4	Isoniazid	3.282	43852	3792	7.57	1.42	8878	2
5	Rifampicin	2.342	7698365	369856		1.50	6692	3
6	Isoniazid	3.282	43987	3785	7.58	1.43	6752	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Rifampicin and Isoniazid in pharmaceutical dosage form was found to be 99.89 %.

Chromatographic data for linearity study

Rifampicin

Concentration µg/ml	**Average Peak Area**
30	4856985
40	6452874
50	7989858
60	9485746
70	11158754

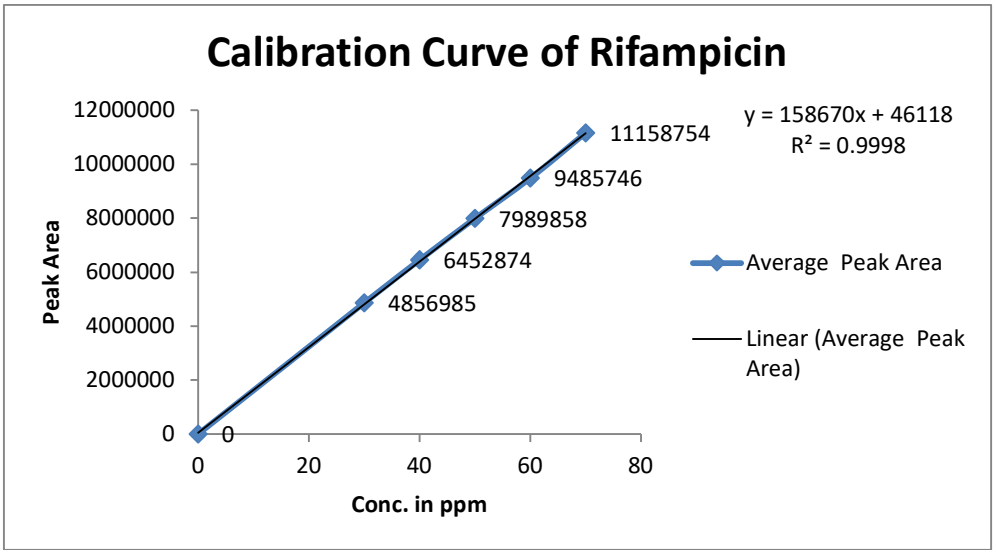

Fig 8: calibration graph for Rifampicin

Isoniazid

Concentration µg/ml	Average Peak Area
10	14659
20	28857
30	43459
40	57454
50	70898

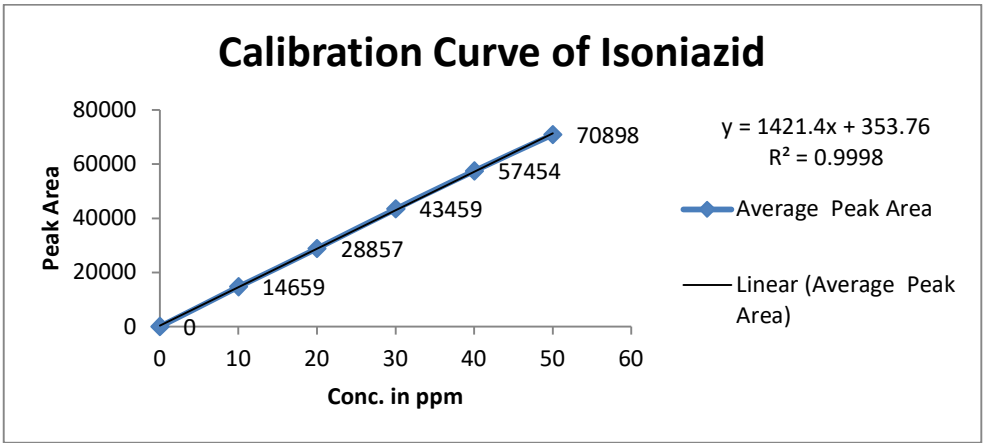

Fig 9: calibration graph for Isoniazid

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 16: Results of Intermediate Precision for Rifampicin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing

1	Rifampicin	2.344	7698524	369852	6698	1.48
2	Rifampicin	2.343	7625892	378564	6755	1.49
3	Rifampicin	2.345	7625487	365874	6692	1.48
4	Rifampicin	2.344	7699854	385472	6687	1.49
5	Rifampicin	2.342	7854752	363659	6658	1.49
6	Rifampicin	2.343	7748574	368785	6785	1.48
Mean			7708847			
Std. Dev			85888.72			
% RSD			1.114158			

- %RSD of five different sample solutions should not more than 2.

Table 17: Results of Intermediate precision for Isoniazid

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Isoniazid	3.281	41528	3526	8652	1.36	7.50
2	Isoniazid	3.285	41856	3542	8647	1.35	7.51
3	Isoniazid	3.282	41685	3587	8624	1.36	7.51
4	Isoniazid	3.286	41698	3562	8697	1.37	7.50
5	Isoniazid	3.283	41782	3485	8642	1.35	7.52
6	Isoniazid	3.287	41986	3582	8625	1.36	7.51
Mean			41755.83				
Std. Dev			157.4578				
% RSD			0.377092				

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rifampicin and Isoniazid. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Rifampicin and Isoniazid were injected by changing the conditions of chromatography. There was no significant changes in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 18: Results for Robustness

Rifampicin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	7584689	2.343	6584	1.46
Less Flow rate of 0.9 mL/min	7985452	2.911	6856	1.45
More Flow rate of 1.1 mL/min	7425416	2.014	6421	1.43
Less organic phase	7254841	2.361	6325	1.48
More organic phase	7365872	2.038	6184	1.49

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Isoniazid

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	42568	3.288	8789	1.38
Less Flow rate of 0.9 mL/min	49865	4.075	8985	1.36
More Flow rate of 1.1 mL/min	41258	3.089	8457	1.35
Less organic phase	43569	4.422	8624	1.37
More organic phase	44587	3.015	8526	1.35

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Rifampicin and Isoniazid was done by RP-HPLC. The mobile phase was optimized with consists of Methanol: Acetonitrile mixed in the ratio of 45:55% v/ v. A Symmetry C18 (4.6mm×150mm, 5.0 µm) Particle size or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Rifampicin and Isoniazid were found to be from 30-70µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Rifampicin and Isoniazid. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

REFERENCES

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
A. Braith Wait and F.J. Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
2. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
3. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
4. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
5. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
6. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
7. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
8. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
9. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
10. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
11. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
12. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
13. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
14. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
15. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
16. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
17. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology (2003), 5, PP 110-114.
18. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
19. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).
20. Aliya Thahaseen*, Dr. Yeluri Rama Chandra Reddy, Development And Validation Of Liquid Chromatographic Method For The Simultaneous Estimation Of Isoniazid And Rifampicin In Combined Dosage Form, International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS), 2014; 4(1):40-46.
21. Sushruta S Hakkimane1, Bharath Raja Guru1,2*, Nano Formulation Analysis: Analytical Method Development of Isoniazid And Simultaneous Estimation Of Antitubercular Drugs Isoniazid And Rifampicin By Reverse Phase High Pressure Liquid Chromatography, Asian J Pharm Clin Res, Vol 10, Issue 5, 2017, 330-335.
22. Yateen Shah, S. Khanna, K.C. Jindal, V.S. Dighe, Determination of rifampicin and isoniazid in pharmaceutical formulations by HPLC, Drug Development and Industrial Pharmacy, Volume 18, 1992 - Issue 14, Pages 1589-1596.
23. Laura Carolina Luciani-Giacobbe1, María Laura Guzman1, Rubén Hilario Manzo1, María Eugenia Olivera1*, Validation of a simple isocratic HPLC-UV method for Rifampicin and Isoniazid quantification in human plasma, Journal of Applied Pharmaceutical Science Vol. 8(07), pp 093-099, July, 2018.

24. B. Prasanthia, b, J. Vijaya Ratnaa , and R. S. Ch. Phanib, Development and Validation of RPHPLC Method for Simultaneous Estimation of Rifampicin, Isoniazid and Pyrazinamide in Human Plasma1, 2015, Vol. 70, No. 8, pp. 1015–1022.
25. Chellini, Paula R.; Lages, Eduardo B.; Franco, Pedro H.C.; Nogueira, Fernando H.A.; César, Isabela C.; Pianetti, Gerson A., Journal of AOAC International, Volume 98, Number 5, September-October 2015, pp. 1234-1239(6).
26. S.K. Dhal and R. Sharma*, Development and Validation of RPHPLC Method for Simultaneous Determination of Pyridoxine Hydrochloride, Isoniazid, Pyrazinamide and Rifampicin in pharmaceutical Formulation, Chem. Anal. (Warsaw), 54, 1487-1500 (2009).