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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Isoniazid and Ethambutol in Combined Tablet Dosage Form

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the Isoniazid and Ethambutol in Tablet dosage form. Chromatogram was run through Inertsil ODS C185µm (4.6 x 250mm). Mobile phase containing Phosphate buffer and Acetonitril in the ratio of 30:70 was pumped through column at a flow rate of 1ml/min. Buffer used at pH 4.6. Temperature was maintained at Ambient. Optimized wavelength for Isoniazid and Ethambutol was 255n. Retention time of Isoniazid and Ethambutol were found to be 2.399min and 3.907min. The % purity of Isoniazid and Ethambutol was found to be 100.7% and 101.4% respectively. The system suitability parameters for Isoniazid and Ethambutol such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0.The linearity study for Isoniazid and Ethambutol was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively.

Keywords: *Lopinavir, Ritonavir, RP-HPLC*

A R T I C L E I N F O

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1. Introduction

Isoniazid is a chemically named as pyridine-4-carbohydrazide [1]. Isoniazid is a prodrug and must be activated by bacterial catalase. Specficially, activation is associated with reduction of the mycobacterial ferric KatG catalase-peroxidase by hydrazine and reaction with oxygen to form an oxyferrous enzyme complex. Once activated, isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. At therapeutic levels isoniazid is bacteriocidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms [2]. Specifically isoniazid inhibits InhA, the enoyl reductase from Mycobacterium tuberculosis, by forming a covalent adduct with the NAD cofactor [3].

Ethambutol is a chemically named as (2S)-2 - [(2 - {[(2S)-1-hydroxybutanyl] amino}amino]butan-1-ol[4]. Ethambutol inhibits arabinosyl transferase which is involved in cell wall biosynthesis. By inhibiting this enzyme, the bacterial cell wall complex production is inhibited. This leads to an increase in cell wall permeability [5].

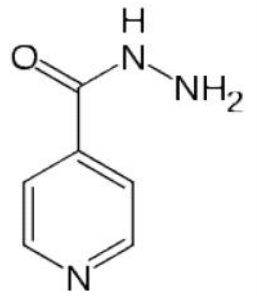

Figure 1: Structure of Isoniazid

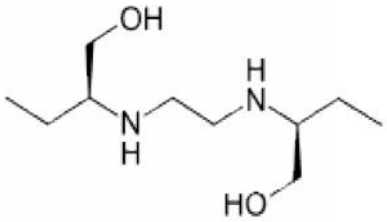

Figure 2: Structure of Ethambutol

2. Materials and Methods

Method development for simultaneous estimation of Isoniazid and Ethambutol in Pharmaceutical dosage forms includes the following steps:

1. Selection of detection wavelength (λmax)
2. Selection of column
3. Selection of mobile phase
4. Selection of flow rate
5. Preparations and procedures

1. Selection of Detection wavelength:

10 mg of Isoniazid and Ethambutol was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overla y spectrum was used for selection of wavelength for Isoniazid and Ethambutol. The isobestic point was taken as detection wavelength [6].

2. Selection of column:

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5µm, Make: Waters)].

3. Selection of mobile phase:

Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will achieved [7].

4. Selection of flow rate:

Flow rate selected was 1ml/min

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities
5. Preparations and procedures:

Preparation of Phosphate buffer :(PH: 4.6):

Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration [8].

Diluant Preparation: Mobile phase is used as Diluant.

Preparation of the individual Isoniazid standard preparation:

10mg of Isoniazid working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Ethambutol standard preparation:

10mg of Ethambutol working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Ethambutol and Isoniazid (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant [9].

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for Ethambutol and Isoniazid peaks are measured and the %Assay are calculated by using the formulae.

System Suitability:

Tailing factor for the peaks due to Ethambutol and Isoniazid in Standard solution should not be more than 2.0.Theoretical plates for the Ethambutol and Isoniazid peaks in Standard solution should not be less than **2000.**

Assay calculation:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim of Lopinavir e mg/ml.

Calculation: (For Ritonavir)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{100} \times \frac{P}{WT} \times \frac{Avg.\ Wt.}{Label\ Claim} \times 100$$

Where:

AT = average area counts of sample preparation.

As = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim of drug in mg/ml.

2.3 Analytical Method Validation

Accuracy:

Preparation of standard solution (Isoniazid and Ethambutol): Accurately weighed 10 mg of Ethambutol and 10mg of Isoniazid working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks. About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents.

Preparation of Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately 5mg of Ethambutol and 5mg of Isoniazid working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 3ml and 0.3ml of the above Ethambutol and Isoniazid stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant [10].

For preparation of 100% solution (With respect to target Assay concentration): Accurately 10mg of Ethambutol and 10mg of Isoniazid working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 3ml and 0.3ml of the above Ethambutol and Isoniazid stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately 15mg of Ethambutol and 15mg of Isoniazid working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 3ml and 0.3ml of the above Ethambutol and Isoniazid stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant [11].

Procedure:

The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Ethambutol & Isoniazid and the individual recovery and mean recovery values were calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

Precision

Repeatability:

Preparation of standard stock solution:

Accurately 10 mg of Ethambutol and 10mg of Isoniazid working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further it was pipette (3ml and 0.3ml) into a 10ml volumetric flask and diluted up to the mark with diluents [12].

Procedure:

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

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2.

B) Intermediate Precision (Ruggedness):

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of standard stock solution:

Accurately 10 mg of Ethambutol and 10mg of Isoniazid working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further this Stock was pipette (3ml and 0.3ml) into a 10ml volumetric flask and dilute up to the mark with diluents [13].

Procedure

The standard solution was injected for five times and the area for all five injections measured in HPLC. The %RSD for the

area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five sample injections results should not be more than 2%.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

LOD:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

Where

σ - Standard deviation (SD) S – Slope

LOQ:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines [14].

Formula:

LOQ = 10 σ /Slope

Where

σ - Standard deviation

S – Slope

Linearity

Preparation of stock solution:

Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Ethambutol and Isoniazid (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Ethambutol&10pm of Isoniazid):

1ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

Preparation of Level – II (40ppm of Ethambutol&20ppm of Isoniazid):

2ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant [15].

Preparation of Level – III (60ppm of Ethambutol&30ppm of Isoniazid):

3ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

Preparation of Level – IV (80ppm of Ethambutol&40ppm of Isoniazid):

4ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

Preparation of Level – V (100ppm of Ethambutol&50ppm of Isoniazid)

5ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

Procedure:

Each level was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Isoniazid and Ethambutol respectively [16].

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 3ppm of Isoniazid and 300ppm of Ethambutol was prepared and analyzed using the varied flow rates along with method flow rate.

b) The organic composition in the mobile phase was varied from 65% to75 % standard solution 3 µg/ml of Isoniazid and 300 µg/ml of Ethambutol were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability:

5 mg of Isoniazid and 500 mg of Ethambutol working standard was accuratel y weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluant and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Isoniazid and Ethambutolwas pipetted out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluant.

3. Results and Discussion

Validation Parameters:

3.1 Precision: The accuracy study was performed for 50%, 100% and 150 % for Isoniazid and Ethambutol. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

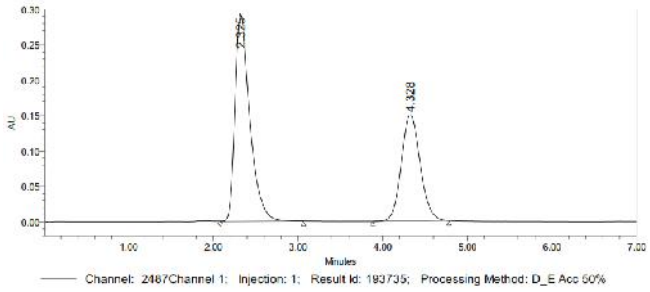

Fig 3: Chromatogram showing accuracy 50% injection-1

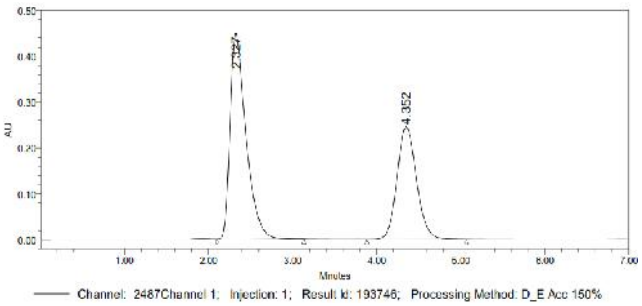

Fig 4: Chromatogram showing accuracy 150%injection-1

3.2. Precision

i) Repeatability

ii) Intermediate precision (Ruggedness)

Repeatability

The precision study was performed for five injections of Isoniazid and Ethambutol. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD.

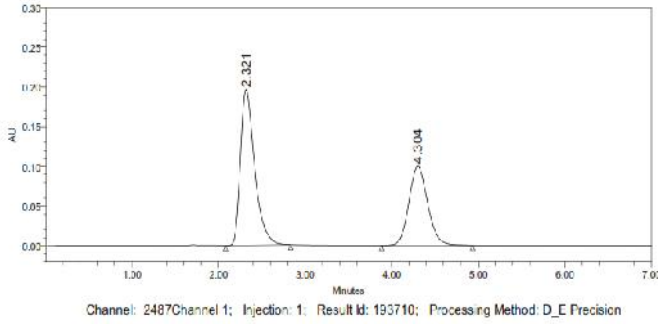

Figure 5: Chromatogram of Standard Inj-1

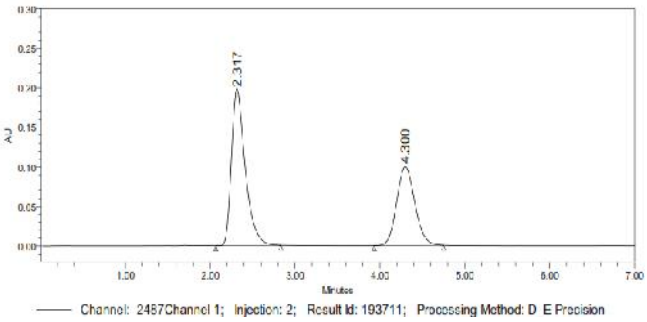

Figure 6: Chromatogram of Standard Inj-2

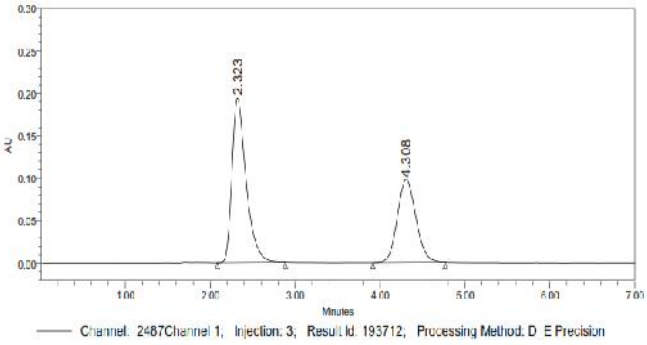

Figure 7: Chromatogram of Standard Inj-3

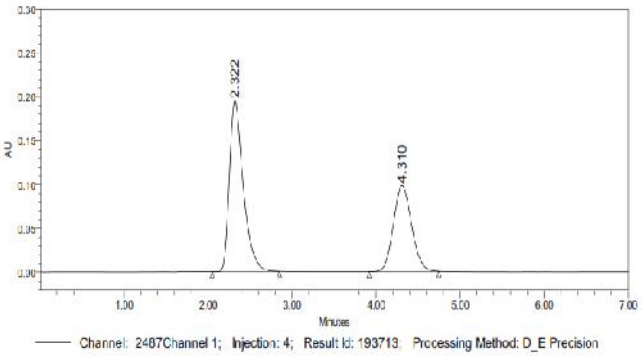

Figure 8: Chromatogram of Standard Inj-4

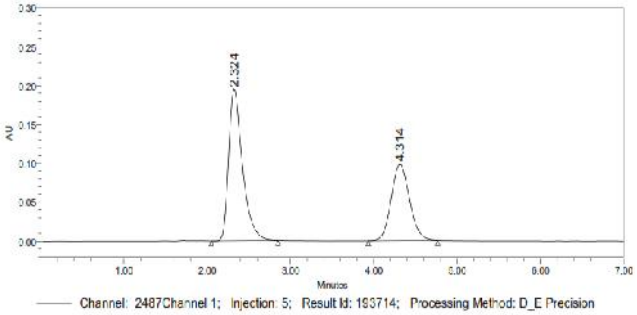

Figure 9: Chromatogram of Standard Inj-5

Table 5: Repeatability results of Isoniazid

Name: Isoniazid

	Name	RT	Area	Height (µV)
1	Isoniazid	2.321	2235319	196999
2	Isoniazid	2.317	2240678	198254
3	Isoniazid	2.323	2249490	195128
4	Isoniazid	2.322	2245822	196164
5	Isoniazid	2.324	2251694	195887
Mean			2244601	
Std. Dev.			6656.8	

	Name	RT	Area	Height (µV)
% RSD			0.30	

Table 6: Repeatability results of Ethambutol

Name: Ethambutol

	Name	RT	Area	Height (µV)
1	Ethambutol	4.304	1501417	100275
2	Ethambutol	4.300	1486940	100079
3	Ethambutol	4.308	1490656	98257
4	Ethambutol	4.310	1487329	98165
5	Ethambutol	4.314	1490384	98153
Mean			1491345	
Std. Dev.			5881.4	
% RSD			0.39	

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%

The Method precision study was performed for the %RSD of Isoniazid and Diazepam was found to be 0.3 and 0.3 (NMT 2).

Intermediate precision / Ruggedness: The intermediate precision study was performed for five injections of Isoniazid and Ethambutol. Each standard injection was

injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

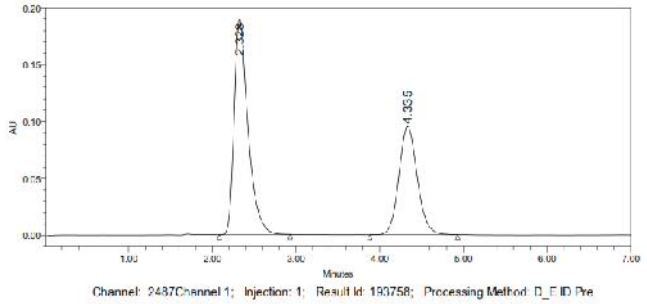

Figure 10: Chromatogram of Standard Inj-1(ID Precision)

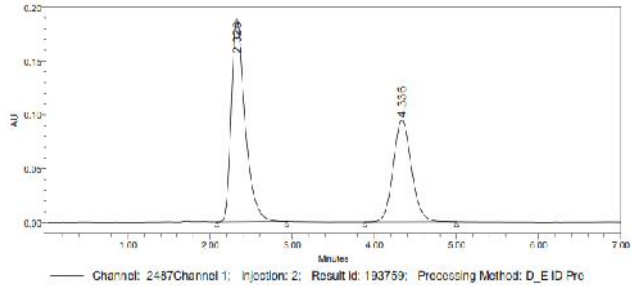

Figure 11: Chromatogram of Standard Inj-2(ID Precision)

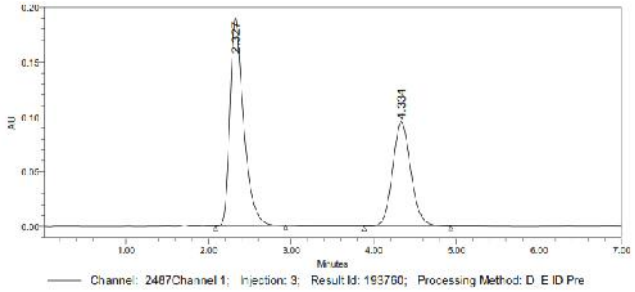

Figure 12: Chromatogram of Standard Inj-3(ID Precision)

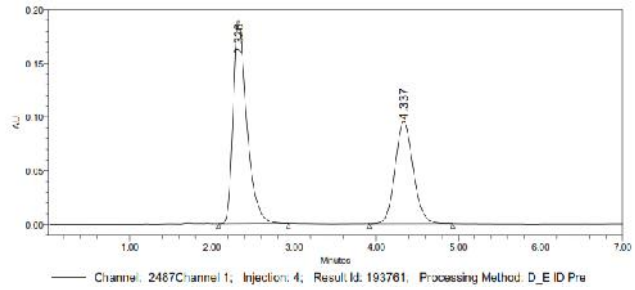

Figure 13: Chromatogram of Standard Inj-4(ID Precision)

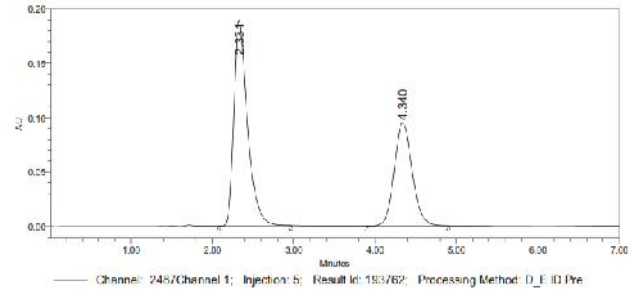

Figure 14: Chromatogram of Standard Inj-5(ID Precision)

Table 7 Ruggedness results of Ethambutol

Name: Ethambutol

	Name	RT	Area	Height (µV)
1	Ethambutol	4.335	1456296	95623
2	Ethambutol	4.336	1457422	95150
3	Ethambutol	4.334	1456513	95165
4	Ethambutol	4.337	1454579	95298
5	Ethambutol	4.340	1451483	95251
Mean			1455259	
Std. Dev.			2347.6	
% RSD			0.16	

Table 8: Ruggedness results of Isoniazid

Name: Isoniazid

	Name	RT	Area	Height (µV)
1	Isoniazid	2.328	2194758	189693
2	Isoniazid	2.326	2195700	190025
3	Isoniazid	2.327	2196191	189862
4	Isoniazid	2.326	2195326	190700
5	Isoniazid	2.331	2200951	189426
Mean			2196585	
Std. Dev.			2496.0	

	Name	RT	Area	Height (µV)
% RSD			0.11	

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%. The intermediate precision was performed for %RSD of Isoniazid and Ethambutol was found to be 0.1 and 0.1 respectively (NMT 2).

3.3. Linearity:

Plotting of calibration graphs: The resultant areas of linearity peaks are plotted against Concentration.

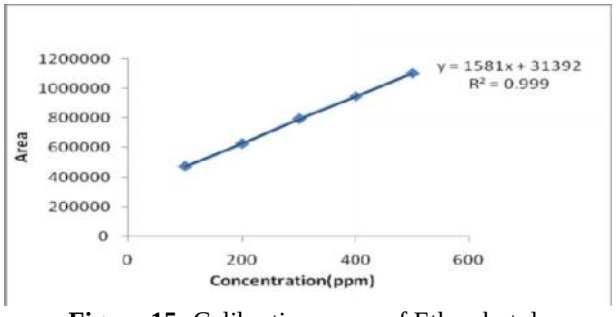

Figure 15: Calibration curve of Ethambutol

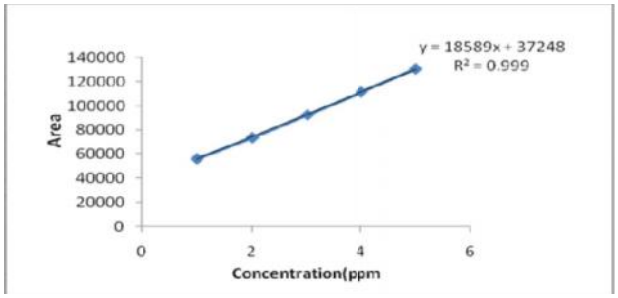

Figure 16: Calibration curve of Isoniazid

Table 1: List of Instruments

S.No	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	PDA Detector	Waters 996		Lab India
3	UV double beam spectrophotometer	UV 3000	UV Win 5	Satorius
4	Digital weighing balance	BSA224SCW		Lab India
5	pH meter	AD102U	-	-
6	Ultra sonicator	SE60US	-	-

Table 2: List of Chemicals

S.No	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R
5	Isoniazid and Ethambuto lAPI	-	-
6	Eurepa mf tablets	Local Pharmacy	-

4. Conclusion

A new method was established for simultaneous estimation of Isoniazid and Ethambutol by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Isoniazid and Ethambutol by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orth ophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399mins and 3.907mins. The % purity of Isoniazid and Ethambutol was found to be 100.7% and 101.4% respectively. The system suitability parameters for Isoniazid and Ethambutol such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Isoniazid & Ethambutol was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Isoniazid and Ethambutol in API and Pharmaceutical dosage form.

5. References

[1] G.R. Chatwal, S.K. Anand, Text book of Instrumental Methods of Chemicaln Analysis, Himalaya Publishing House,5th Ed, **2002**, p.2.566-2.570.

[2] G.W. Ewing, Text book of Instrumental Methods of Chemical Analysis, Mc Graw-Hill Book Company, 5th Ed, p.375-385.

[3] B.K. Sharma, Textbook of Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 23rd Ed, p.288-289.

[4] G. Vidyasagar, Textbook of Instrumental Methods of Drug Analysis, Pharmamed Press, **2009**, pp.106-120.

[5] H. H Willard, L. L Merritt, J. A Dean and F. A Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed,**1986**, pp.592-596.

[6] H.H. Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium, **2008**, pp.45-58.

[7] D.A. Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis, Brooks/Cole, Cengage Learning India Private Limited, **2007**, pp.900-906.

[8] R. E. Schirmer, Textbook of Modern Methods of Pharmaceuticals, CRC press, 2nd Ed, P.242-244.

[9] LR. Snyder, JJ Kirkland, LG. Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Ed, **1997**, p. 1-56.

[10] Ranjith singh, HPLC Method Development and Validation- an Overview, J Pharm. Educ. Res. **2013**, 4: 26-33.

[11] ICH: Q2B, Analytical Validation – Methodology (**1996**)

[12] S.K. Dhal and R. Sharma et al, Development and Validation of RPHPLC Method for Simultaneous Determination of Pyridoxine Hydrochloride, Isoniazid, Pyrazinamide and Rifampicin in pharmaceutical Formulation. Chem. Anal. **2009**, 54, 1487.

[13] Ganga Prasad chenna et al, Development and Validation of RP-HPLC Method for Quantitative estimation of Pyrazinamide in Bulk and Pharmaceutical dosage forms. International Journal of Pharm Tech Research

[14] Bhandari and Kauret A Sensitive HPLC Method for Determination of Isoniazid in Rat Plasma, Brain, Liver and Kidney. J Chromat Separation Techniq. **2012**, 3(4): 332-337

[15] T. T. Mariappan A Validated Reversed-Phase (C18) HPLC Method for Simultaneous Determination of Rifampicin, Isoniazid and Pyrazinamide in USP Dissolution Medium and Simulated Gastric Fluid. Research gate. **2013**, 4(3): 232-237.