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Original Article

Stability Indicating Method Development and Validation for the Simultaneous Estimation of Ethambutol and Isoniazid in Bulk and Pharmaceutical Dosage form by using RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Isoniazide and Ethambutol in Tablet dosage form. Chromatogram was run through kromasil. Mobile phase containing Buffer and Acetonitrile taken in the ratioμ250mm x 4.6 mm, 5 58:42 was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0. 1% OPA solution. Temperature was maintained at 30°C. Optimized wavelength for Isoniazide and Ethambutol was 220nm. Retention time of Isoniazide and Ethambutol were found to be 2.430min and 2.989min. %RSD of the Isoniazide and Ethambutol were and found to be 0.9 and 0.8 respectively. %assay was obtained as 99.72% and 100.21% for Isoniazide and Ethambutol respectively. LOD, LOQ values are obtained from regression equations of Isoniazide and Ethambutol were 1.56ppm, 0.07ppm and 4.72pm, 0.21ppm respectively. Regression equation of Isoniazide is y = 2462x + 20382, and y = 5037x + 89279.Of Ethambutol

Key Words: Isoniazide, Ethambutol, RP-HPLC.

1. INTRODUCTION

Ethambutol (commonly abbreviated EMB or simply E) is a medication primarily used to treat tuberculosis. It is usually given in combination with other tuberculosis drugs, such as isoniazid, rifampicin and pyrazinamide.

Isoniazid, also known as isonicotinylhydrazide (INH), is an antibiotic used as a first-line agent for the prevention and treatment of both latent and active tuberculosis. It is effective against mycobacteria, particularly *Mycobacterium tuberculosis*.

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In the proposed work, attempt shall be made to develop a new HPLC method for simultaneous estimation of Isoniazide and Ethambutol to develop a validated method according to ICH guidelines. To apply validated method for the estimation of Isoniazide and Ethambutol in pharmaceutical formulation

2. MATERIALS AND METHODS

Isoniazide and Ethambutol, Combination Isoniazide and Ethambutol capsules, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instruments:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Isoniazide and Ethambutol solutions.

Preparation of buffer: Buffer:

1 ml of Ortho phosphoric acid was taken in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water. pH was adjusted by triethylamine to 3.8

Standard Preparation:

(300µg/ml Isoniazid& 600µg/ml Ethambutol) Accurately Weighed and transferred 30mg&60mg of Isoniazid and Ethambutol working Standards into a 10ml and 10ml clean dry volumetric flask respectively, add 5ml and 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 70ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions Isoniazide and Ethambutol are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 75ppm, 150ppm, 225ppm, 300ppm, 375ppm, 450ppm of Isoniazide and 150ppm, 300ppm, 450ppm, 600ppm, 750ppm, 900ppm of Ethambutol

Accuracy:

Standard Preparation:

(300µg/ml Isoniazid& 600µg/ml Ethambutol) Accurately Weighed and transferred 30mg&60mg of Isoniazid and Ethambutol working Standards into a 10ml and 10ml clean dry volumetric flask respectively, add 5ml and 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of 50% Spiked Solution:

weight equivalent to 600mg of tablet powder was transferred into a 100 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution:

weight equivalent to 1200mg of tablet powder was transferred into a 100 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents. Preparation of 150% Spiked Solution: weight equivalent to 1800 mg of tablet powder was transferred into a 100 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents. into the system and the chromatograms were recorded to assess the stability of the sample.

METHOD DEVELOPMENT

Trials were done by changing columns and Mobile phases and were reported below.

TRIAL: 1

Column Used : ODS 250 x 4.6 mm, 5µ.

Mobile phase : Water: Acetonitrile (50:50A)

Flow rate : 1ml/min

Wavelength : 220nm

Temperature : 30 C

Injection Volume : 10µl

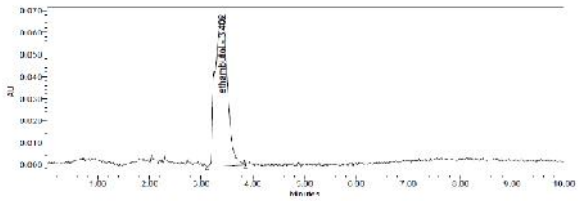

Fig 1: Trial 1 chromatogram

Observation: Etambutol peak was eluted but isoniazide peak was not eluted. So further trail was carried out.

TRIAL: 2

Column Used : ODS 250 x 4.6 mm, 5µ.

Mobile phase : Buffer: Acetonitrile (50:50A)

Buffer : 0.01N KH2PO4 solution

Flow rate : 1ml/min

Wavelength : 220nm
Temperature : 30 C
Injection Volume : 10µl

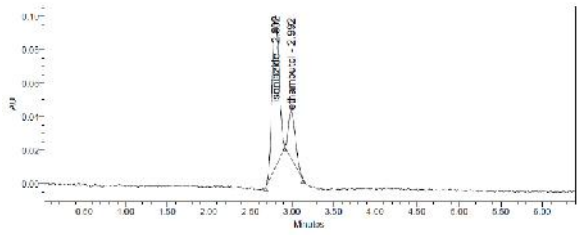

Fig 2: Trial 2 chromatogram

Observation: Resolution was not passed. So further trials are carried out.

TRIAL: 3

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Mobile phase : buffer: Acetonitrial (70:30A)
Buffer : 0.1%OPA
Flow rate : 1ml/min
Wavelength : 220nm
Temperature : 30 C
Injection Volume : 10µl

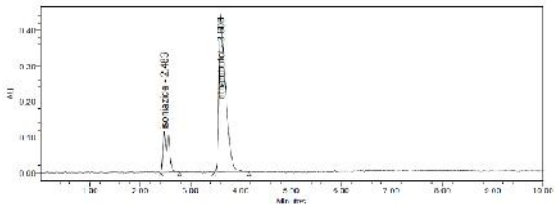

Fig 3: Trial 3 chromatogram

Observation: Isoniazide peak shape was not good so trail was carried out.

TRIAL: 4

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Mobile phase : buffer: Acetonitrile (60:40A)
Buffer : 0.1%OPA
Flow rate : 1ml/min
Wavelength : 220nm
Temperature : 30 C
Injection Volume : 10µl

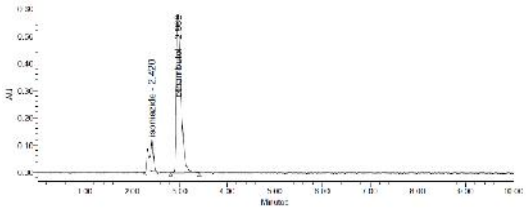

Fig 4: Trial 4 chromatogram

Observation: peak shape was not good. so further trail was carried out.

TRIAL: 5

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Mobile phase : buffer: Acetonitrile (60:40A)
Buffer : 0.1%OPA
Flow rate : 1ml/min
Wavelength : 220nm
Temperature : 30 C
Injection Volume : 10µl

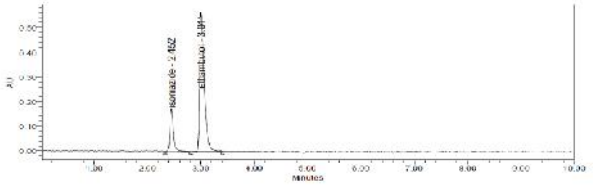

Fig 5: Trial 5 chromatogram

Observation: peak shape was good. so further trail was carried out

OPTIMIZED METHOD

Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Buffer : 0.1%OPA
Mobile phase : buffer: Acetonitrile (58:42A)
Flow rate : 1.0ml/min
Diluent : water:acn: 50:50
Wavelength : 220nm
Temperature : 30 C
Injection Volume : 10µl

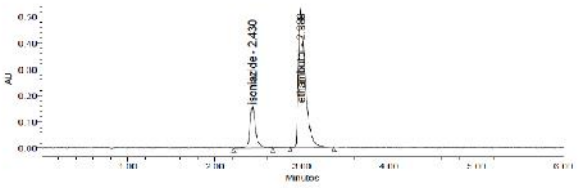

Fig 6: Optimized chromatogram of Isoniazide and Ethambutol

Observation: peak shape and retention time is good**.**

3. RESULTS AND DISCUSSION

1. System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines

System suitability studies of Isoniazide and Ethambutol method

Property	Isoniazide	Ethambutol
Retention time (tR)	2.430min	2.989min
Theoretical plates (N)	7455 + 63.48	6290 ± 63.48
Tailing factor (T)	1.33 ± 0.117	1.784 0.117

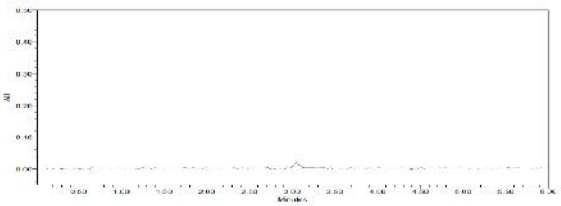

Fig 7: Chromatogram of blank

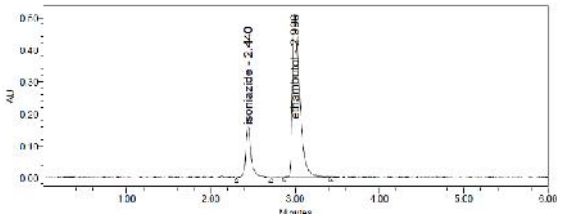

Fig 8: Typical chromatogram of Isoniazide and Ethambutol

2. Linearity: Six Linear concentrations of Isoniazide (75-450ppm) and Ethambutol (150-900ppm) are prepared and Injected. Regression equation of the Isoniazide and Ethambutol are found to be,

y =2462x +20382, and y = 5037x +89279 and regression co-efficient was 0.999.

Calibration data of Isoniazide and Ethambutol method

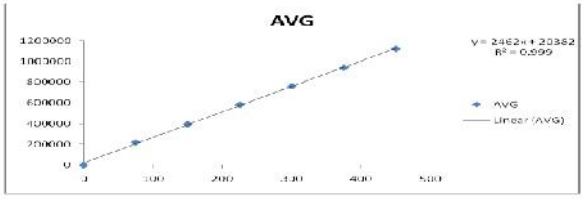

Fig 9: Calibration curve of Isoniazide

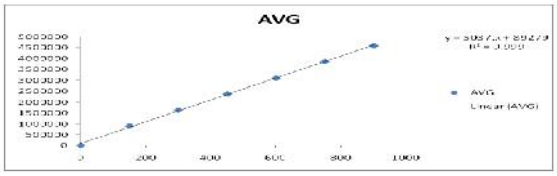

Fig 10: Calibration curve of Ethambutol

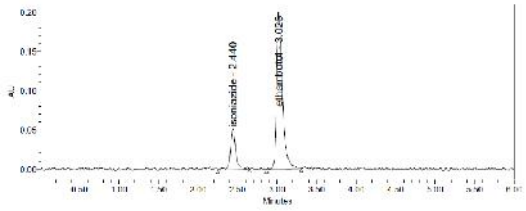

Fig 11: Linearity 25% Chromatogram of Isoniazide and Ethambutol

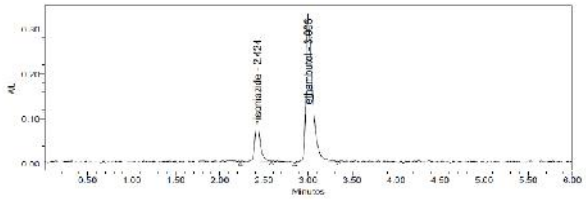

Fig 12: Linearity 50% Chromatogram of Isoniazide and Ethambutol

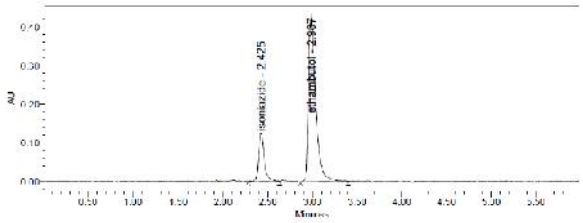

Fig 13: Linearity 75% Chromatogram of Isoniazide and Ethambutol

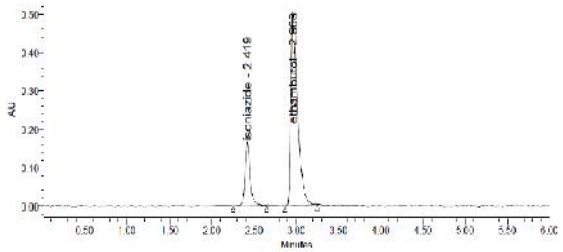

Fig 14: Linearity 100% Chromatogram of Isoniazide and Ethambutol

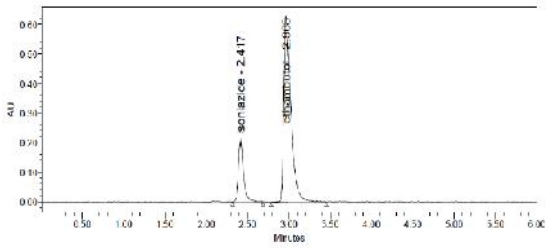

Fig 15: Linearity 125% Chromatogram of Isoniazide and Ethambutol

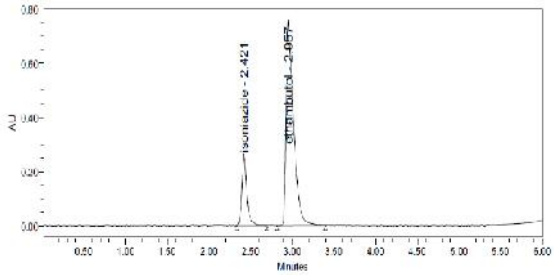

Fig 16: Linearity 150% Chromatogram of Isoniazide and Ethambutol

3. Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Isoniazide and Ethambutol were found to be 0.9% and 0.8% respectively.

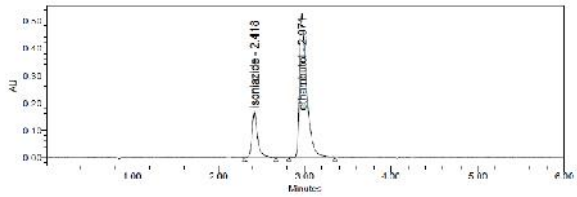

Fig 17: Repeatability Chromatogram of Isoniazide and Ethambutol

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Isoniazide and Ethambutol were 1.4% and 0.9%.

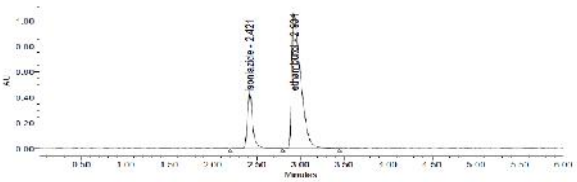

Fig 18: Accuracy 150% Chromatogram of Isoniazide and Ethambutol

5. LOD: Limit of detection was calculated by std deviation method Isoniazide and Ethambutol and LOD for Isoniazide and Ethambutol were found to be 1.56 and 0.07respectively.

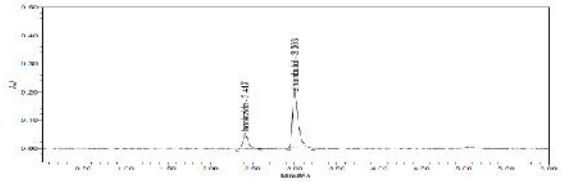

Fig 19: LOD Chromatogram of Isoniazide and Ethambutol

6. LOQ: Limit of Quantification was calculated by std deviation method Isoniazide and Ethambutol and LOQ for Isoniazide and Ethambutol were found to be 4.72 and 0.21 respectively.

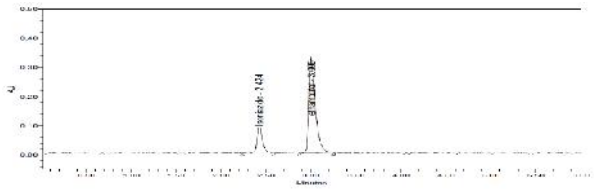

Fig 20: LOQ Chromatogram of of Isoniazide and Ethambutol

Summary

Parameters	Isoniazide	Ethambutol
Calibration range (mcg / ml)	75-150 ppm	350-800ppm
Optimized wavelength	220nm	220nm
Retention time	2.430min	2.989min
Regression equation (y)	y = 2462x + 20382	y = 5037x + 89279
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD)	0.9	0.8
% Assay	99.72%	100.21%
Limit of Detection (mcg / ml)	1.56ppm	0.07ppm
Limit of Quantization (mcg / ml)	4.72ppm	0.21ppm

4. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Isoniazide and Ethambutol in Tablet dosage form. Retention time of Isoniazide and Ethambutol were found to be 2.430min and 2.989min. %RSD of the Isoniazide and Ethambutol were and found to be 0.9 and 0.8 respectively. %assay was obtained as 99.72% and 100.21% for Isoniazide and Ethambutol respectively. LOD, LOQ values are obtained from regression equations of Isoniazide and Ethambutol were 1.56ppm, 0.07ppm and 4.72ppm, 0.8ppm respectively. Regression equation of Isoniazide is y = 2462x + 20382, and y = 5037x + 89279 Of Ethambutol .Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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