IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF ETHAMBUTOL HYDROCHLORIDE IN PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

For the estimation of ethambutol HCL in tablet dosage form, a simple, sensitive, isocratic, and accurate reverse phase HPLC approach was presented. Tests of linearity, precision, accuracy, specificity, robustness, and ruggedness were used to validate the established approach. The technique works well enough to isolate the active component (API). The technique was effectively used for tablet dosage form discussion research. Accuracy, precision, specificity, repeatability, and sensitivity were all demonstrated by the approach. The investigation came to the conclusion that the suggested approach was suitable for both tablet dosage form assay and analysis of its dissolving samples.

KEYWORDS: RP-HPLC, Anti-tuberculosis drug, Ethambutol hydrochloride, Analytical estimation, Tablet dosage form.

INTRODUCTION

Quality assurance is a wide ranging concept covering all matters that individually or collectively influence that quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal product.[1]

Quality of the drug product is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare program worldwide. Quality is the total sum of all factors which contribute directly or indirectly to the safety; efficacy and acceptability of the product.[2]

Pharmaceutical analysis, a branch of pharmacy, plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulation and on finished products. Analytical chemistry has since long, occupied an important place in the development of science and technology. It is primarily concerned about determining the qualitative and quantitative composition of

material under study. The qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. The quantitative analysis deals about the content present in the sample. The development in analytical sciences has been more significant and prominent in recent years than the past. This has really broadened our vistas and helped to develop new methods of analysis. In pharmacy analytical chemistry is responsible for developing sensitive, reliable and more accurate methods for the estimation of drug in pharmaceutical dosage form. [3]

CHROMATOGRAPHIC TECHNIQUES:

Chromatography is separation of a mixture into individual components using a stationary phase and a mobile phase. The stationary phase may be solid or a liquid supported on a solid or gel or may be packed in column. The mobile phase may be gaseous or liquid. Chromatographic separation depends on relative movement of two phases. In chromatography one phase is fixed (stationary phase) and other is mobile (mobile phase) the mobile phase passes over the stationary phase. The separation of component is a result of the differential affinity of the components for the mobile phase and a stationary phase.

Classification:

1) Based on the nature of stationary and mobile phase

- Gas –solid chromatograph
- Gas- liquid chromatography
- Solid- liquid chromatography
- liquid-liquid chromatography

2) Based on the principle of separation

- Adsorption chromatography
- Partition chromatography

3) Based on the modes of chromatography

- Normal phase chromatography
- Reversed phase chromatography

4) Other types of chromatography

- Ion – exchange chromatography
- Exclusion chromatography

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is an analytical process utilizing special instruments designed to separate, quantify and analyses components of chemical mixture. Samples of interest are introduced to a solvent flow path; carried through a column packed with specialized materials for component separation; and component data is obtained through the combination of a detection mechanism coupled with a data recording system. A typical HPLC separation is based on the selective distribution of analytes between a liquid mobile phase and an immiscible stationary phase. The sample is first introduced by means of an injection port into the mobile phase stream that is delivered by a high-pressure pump. Next, the components of this sample mixture are separated on the column, a process monitored with a flow-through detector as the isolated components emerge from the column.

The analysis in HPLC is either in qualitative or quantitative determination of different components present in the sample.

TYPES OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

Normal phase HPLC:

Normal phase HPLC (NP-HPLC) was the first kind of HPLC chemistry used, and separates analytes based on polarity. This method uses a polar stationary phase and a non-polar mobile phase, and is used when the analyte of interest is fairly polar in nature. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes while more hydrophobic solvents tend to increase retention times.

Reversed phase HPLC:

RP-HPLC is the choice for the majority of samples. It consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with alkyl dimethyl silylchloride (RMe2SiCl), where R is a straight chain alkyl group such as octadecyl ($C_{18}H_{37}$) or octyl (C_8H_{17}).

Advantages of HPLC:

a) Capable of separating complex mixtures at low operating temperature.

b) Identification of an unknown solution.

c) Quantification of a compound in a known solution.

d) Capable of separating materials according to size and /or chemical properties.

e) Can be used to separate delicate or heat labile compound.

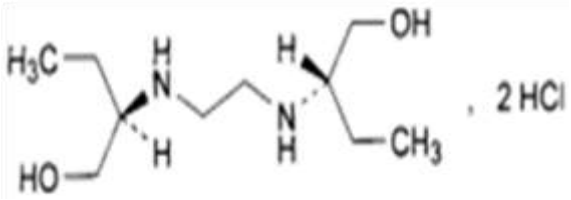
ANALYTICAL METHOD VALIDATION:

Analytical method validation according to USP is performed to ensure that an analytical methodology is accurate, specific, reproducible, precise and rugged over the specified range that an analyte will be analysed. Validation of method is the process by which a method is tested by a developer or user for reliability, accuracy and preciseness of it intended purpose.

DRUG PROFILE

- **ETHAMBUTOL HYDROCHLORIDE**

Parameter	Details
Drug Name	Ethambutol Hydrochloride USP
Brand Name	CONCOX 800 mg

Structural Formula	
Categories	Anti-tuberculosis
Molecular Formula	$C_{10}H_{26}Cl_2N_2O$
Molecular Weight	277.23 g/mol
IUPAC Name	(2S)-2-[2-[[(2S)-1-hydroxybutan-2-yl]amino]ethylamino] butan-1-ol; dihydrochloride
Appearance	White crystalline powder
Solubility	Freely soluble in water and soluble in alcohol and methanol.
Melting Point	198–202°C
Indication	Combination with other anti-tuberculosis drugs in the treatment of pulmonary tuberculosis
Dose	Available in 800 mg in Tablet dosage form
pka	It typically achieves peak plasma concentrations within 2-4 hours

EXPERIMENTALWORK:

IDENTIFICATION OF DRUG: Identification of drug sample was carried out by solubility study, melting point determination and FTIR.

SOLUBILITYSTUDY:

Table 1 Comparison of solubility of Ethambutol.

SOLVENT	**SOLUBILITY**	
	REPORTED	OBSERVED
Water	Freely soluble	Freely soluble
Ethyl Ether	Slightly soluble	Slightly soluble
Methanol	Slightly soluble	Slightly soluble
Ethanol	Slightly soluble	Slightly soluble

FTIR:

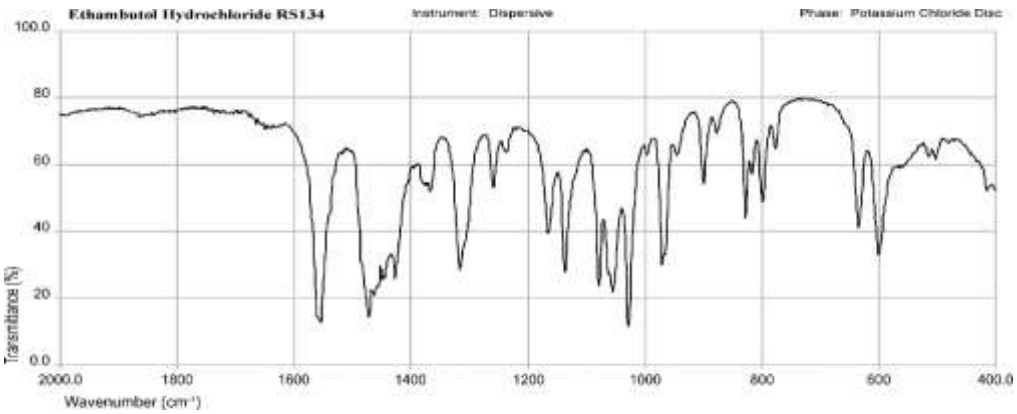


Fig 1: FTIR-spectra of Ethambutol

Table 2 Comparison of FTIR of Ethambutol

FUNCTIONAL GROUP	STANDARDIR RANGES (CM^{-1})	OBSERVEDRANGES (CM^{-1})
O-HSTRECH	2000-1800	1450
N-HSTRECH	1600-1400	1505
C-NSTRECH	1200-1000	1089
C-FSTRECH	800-600	750

Extract a quantity if the powdered tablets containing 50 mg of Ethambutol hydrochloride with 5 ml of methanol, filter and evaporate the filtrate to dryness. Use the residue for the test. The IR spectrum of the sample should concordant with the IR spectrum of the Ethambutol hydrochloride working standard.

Dissolution:

Weight of standard: 30.1 mg

Preparation of standard: 30.1m Volume make up to 100 ml with HPLC grade water

Table 3: Area of Standard

Sr. No.	Area of Standard
1	391862
2	393072
3	390074
4	38157
5	390054
6	391089
Average	390885
Standard Deviation	1422.208
RSD %	0.364

Weight of sample: 37.4mg

Preparation of sample: 37.4mg volume made up to 100 ml with HPLC grade water

Table 4: Area of Sample

Sr. No.	Area of sample
1	383862
2	385260
Average	384561

Weight of sample one tablet → 900ml media from this dilute 10ml to 25 ml with media.

The dissolution method for Ethambutol hydrochloride tablets involves a paddle method in water, with 900mL of medium at 50 revolutions per minute.

The assay typically uses a spectrophotometric ion-pair extraction method, employing bromocresol green as a dye. Specifics include withdrawing aliquots, filtering, and using a standard solution for comparison.

The observed Dissolution chromatograms:

BLANK

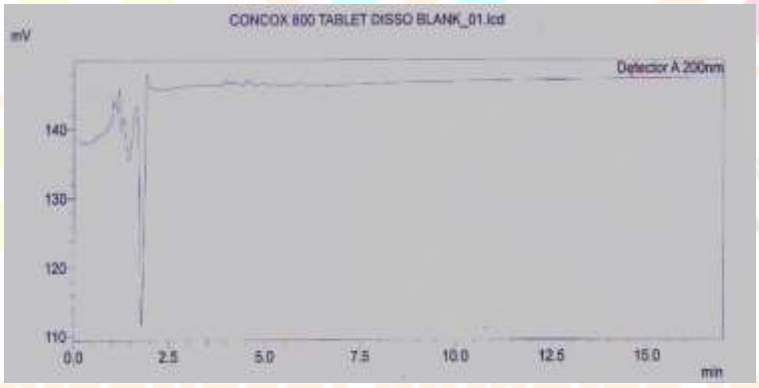


Fig 2: Dissolution Blank peak

STANDARD 1

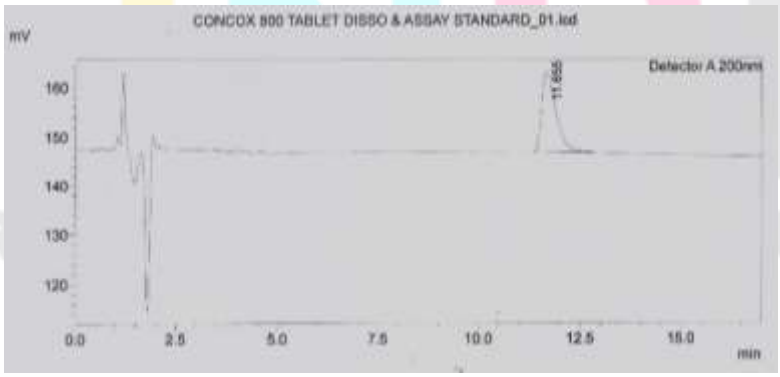


Fig 3: Dissolution and Assay Standard Peak 1

STANDARD 2

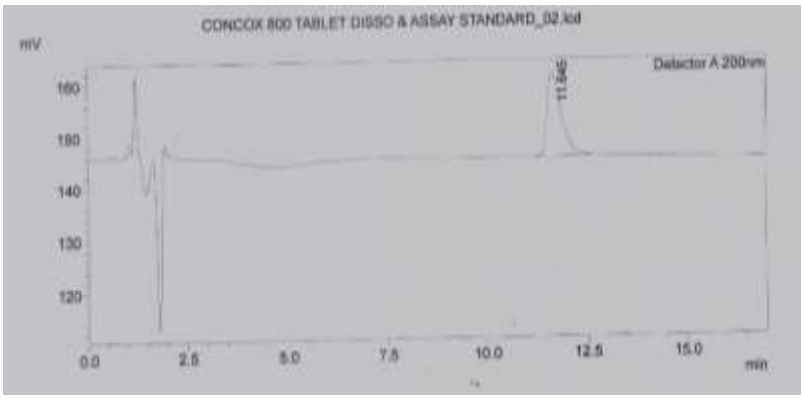


Fig 4: Dissolution and Assay Standard Peak 2

STANDARD 3

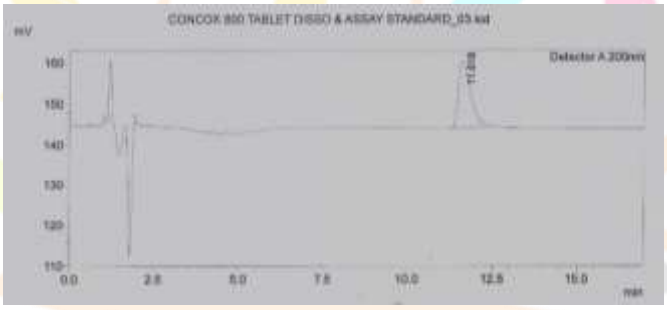


Fig 5: Dissolution and Assay Standard Peak 3

STANDARD 4

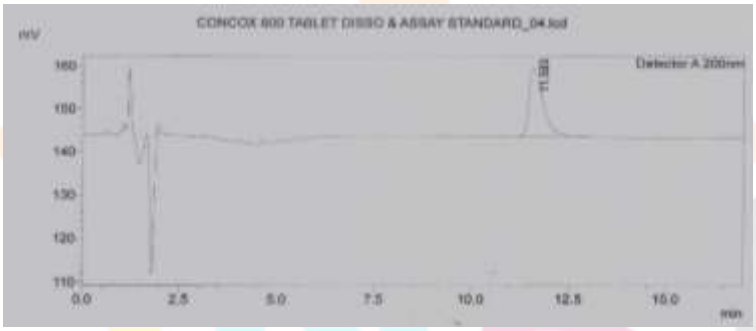


Fig 6: Dissolution and Assay Standard Peak 4

STANDARD 5

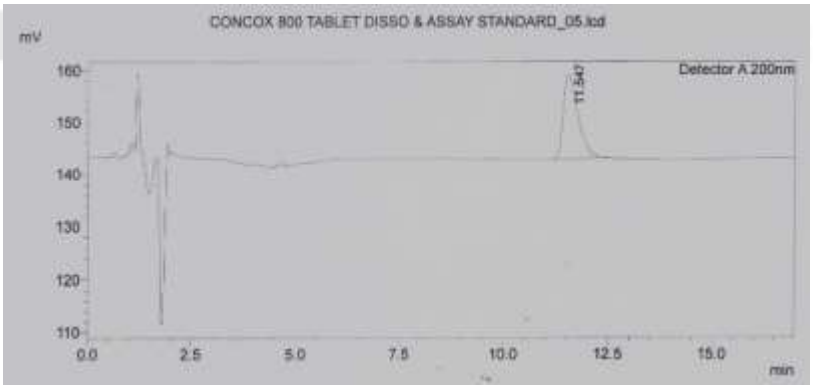


Fig 7: Dissolution and Assay Standard Peak 5

STANDARD 6

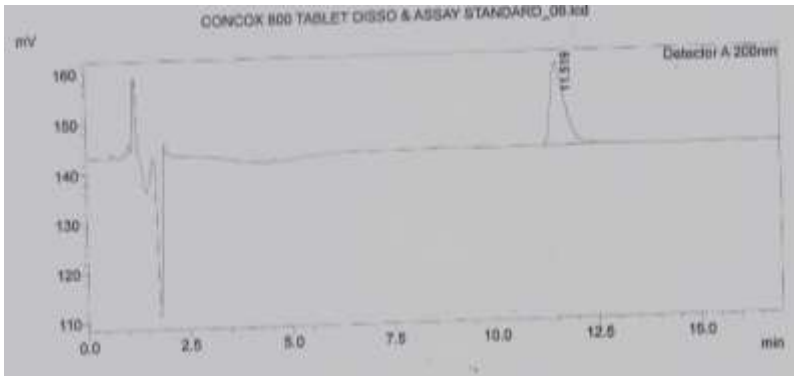


Fig 8: Dissolution and Assay Standard Peak 6

DISSOLUTION AND ASSAY BRACKETING SAMPLE:

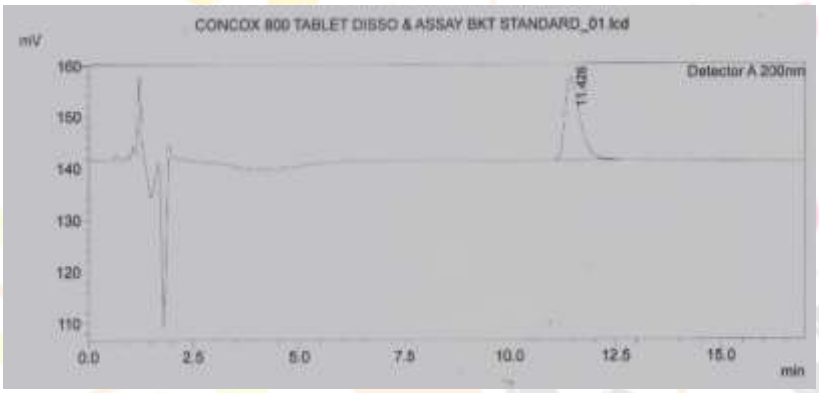


Fig 9: Dissolution and assay bracketing sample 1

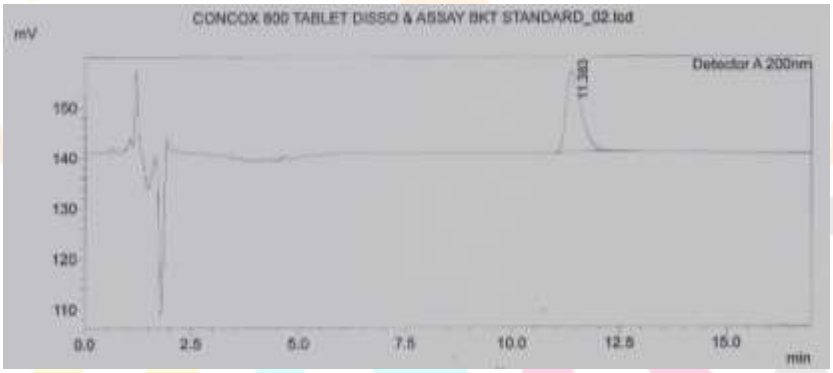


Fig 10: Dissolution and assay bracketing sample 2

VALIDATION PARAMETERS

ASSAY METHOD PRECISION:

Standard preparation:

Refer to the method for assay (attached)

Sample preparation:

Refer to the method for assay (attached)

Procedure:

Perform the assay as per the procedure specified in the attached method for assay for establishing assay method precision (Method precision)

Acceptance criteria:

1) Not less than 95.0 % and not more than 105.0 % of the stated amount of Ethambutol hydrochloride.

2) The relative standard deviation of 6 determinations should not be more than 2 0%.

SPECIFICITY:

Specificity is the ability to assess clearly the analyte in the presence of components which may be expected to be present. Typically these might include degradant, matrix, etc. In the case of assay, demonstration of specificity requires that it can be shown that the procedure was unaffected by the presence of excipients. Specificity of an analytical method was its ability to measure accurately and specifically the analyte of interest without interference from blank and placebo.

LINEARITY AND RANGE:

Linearity:

Linearity is the ability of the method to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

The linearity was performed by analyzing standard at 5 different concentration levels ranging from 80% to 120% with respect to analyte concentration as per the method specified.

Three replicate titrations were performed & the mean burette reading for each level was calculated. Linearity graph was drawn between concentration in mg/ml & burette reading is shown below.

Acceptance criteria:

1) The correlation coefficient value should be between 0. 99 & 1.00.

2) The values of slope, intercept & Residual Square of sum is to be reported.

Range:

The range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written The range is normally expressed in the same units as the test results (eg . percent, parts per million) obtained by the analytical method

Linearity experiment:

Standard preparation of Ethambutol:

Weigh accurately about 30.0 mg of Ethambutol hydrochloride working standard into a 100 ml volumetric flask, dissolve and dilute to volume with mobile phase and mix.

Preparation of linearity solutions:

Follow the scheme of Weight of Ethambutol taken for assay as mentioned in the following table

Sr. No.	Volume taken (mL)	Diluted to (mL)	Concentration in ppm Ethambutol
1	1	100	150
2	2	100	200
3	3	100	250
4	4	100	300
5	5	100	350

Table 5: Linearity solutions

Procedure:

Run on HPLC each of the above solutions. Plot area as ordinate (Y-axis) and analyte concentration (as □g/mL) as the abscissa (X-axis) for Ethambutol HCL. Determine correlation coefficient.

Acceptance criteria:

The correlation coefficient should be between 0.99 to 1.00

ACCURACY STUDY:

Accuracy is the closeness of the test results obtained by the method to the true value. The accuracy as performed by spiking sample with standard at three different concentration levels ranging from 5 % to 20 % with respect to analyte concentration as per the method specified then detecting the content-Accuracy was calculated as per the percentage of recovery by the assay of known amount of Ethambutol which was added in the sample.

Acceptance criteria: The % recovery should be between 98.0% and 102.0%.

Recovery experiment for Concox-800 (Ethambutol Tablets BP)

The recovery experiment will be carried out at three levels

Standard preparation of Concox-800 (Ethambutol Tablets BP) :

Weigh accurately about 30.0 mg of Ethambutol hydrochloride working standard into a 100 ml volumetric flask, dissolve and dilute to volume with mobile phase and mix.0

Sample Preparation:

Weigh accurately about sample equivalent 30 mg of Ethambutol hydrochloride and transfer into a 100ml volumetric flask. Add about 30ml of mobile phase and dissolve the contents completely by through shaking and filter.

First level:

Weigh accurately about sample equivalent 30 mg of Ethambutol hydrochloride and transfer into a 100ml volumetric flask. Add about 30ml of mobile phase and dissolve the contents completely by through shaking and filter. Add 4 ml of standard stock solution & dilute with diluent to volume & mix.

Second level:

Weigh accurately about sample equivalent 30 mg of Ethambutol hydrochloride and transfer into a 100ml volumetric flask. Add about 30ml of mobile phase and dissolve the contents completely by through shaking and filter. Add 5 ml of standard stock solution & dilute with diluent to volume & mix.

Third level:

Weigh accurately about sample equivalent 30 mg of Ethambutol hydrochloride and transfer into a 100ml volumetric flask. Add about 30ml of mobile phase and dissolve the contents completely by through shaking and filter. Add 6 ml of standard stock solution & dilute with diluent to volume & mix.

Procedure: As per the method enclosed. Calculate the mean standard deviation and relative standard deviation at different levels of Ethambutol.

Acceptance criteria:

The % recovery at each level should be between 95.0% & 105.0% Overall recovery should be between 95.0%

& 105.0% the relative standard deviation should not be more than 3.0%

RUGGEDNESS:

Intralaboratory ruggedness (Intermediate precision):

Intermediate precision expresses with in laboratory variation with different analysts or equipment within same laboratory using the same lot of drug product as specified under assay precision.

Inter laboratory ruggedness (Reproducibility): Reproducibility expresses precision between laboratories as in collaborative studies. It is normally expressed as a lack of influence on the test results of inherent operational and environmental variables of the analytical method.

Acceptance criteria:

The average of Intra laboratory & Inter laboratory ruggedness should be within ± 3% of the previous analysis. & the RSD should not be more than 3%.

ROBUSTNESS:

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- Stability of analytical solutions;
- Extraction time.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers);
- Temperature;
- Flow rate.

Acceptance criteria:

Difference in the results obtained by changed method should not be observed by more than 2% from unchanged method.

RESULT AND DISCUSSION:

The following parameters were considered for the analytical method validation of title ingredients. The study had been carried out to gather validation data for the assay method for the determination of Ethambutol in Concox-800, by titrimetric method.

- Assay method precision (Method precision)
- Specificity

- Linearity and range of Ethambutol
- Accuracy (Recovery) and Precision of Ethambutol
- Intermediate Precision
- Robustness

ASSAY METHOD PRECISION (REPEATABILITY) FOR ETHAMBUTOL

Sample	**% of Ethambutol**
1	101.04
2	101.02
3	101.01
4	101.03
5	101.02
6	101.03
Mean	**101.025**
Standard Deviation(±)	**0.0104**
Relative Standard Deviation (%)	**0.0103**

Table 6: Assay method precision for Ethambutol HCL

Conclusion: The method precision data given above demonstrate that the % RSD is 0.0103, which is well within the acceptance criteria & hence method is precise.

Linearity & Range:

LINEARITY OF ETHAMBUTOL

Sample No	**Concentration in ppm**	**Area**
1	50	218169
2	100	436325
3	150	624485
4	200	812464
5	250	944687
Correlation Coefficient		**0.9932**

Table 7: Linearity of Ethambutol HCL

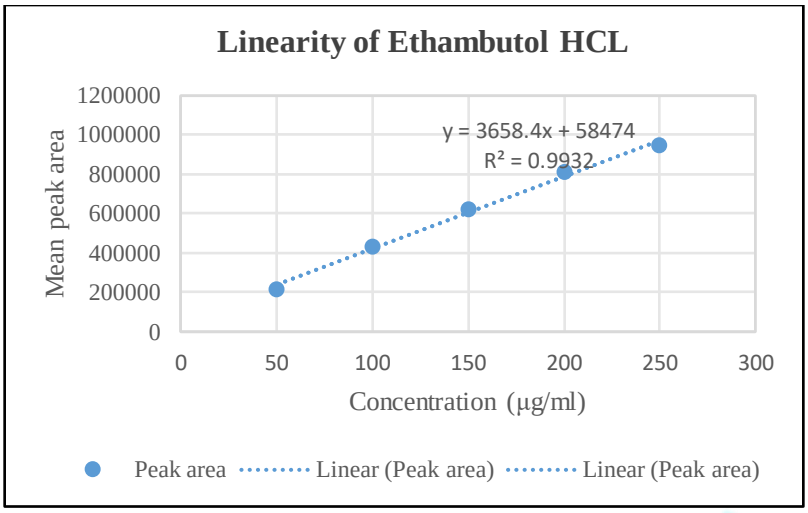


Fig 11: Linearity of Ethambutol

Conclusion:

The linearity study shows that correlation coefficient is 1.000 within the acceptance criteria therefore the method is linear & selected sample concentration is in working range.

RECOVERY EXPERIMENT FOR ETHAMBUTOL

Level of Addition	Amount of Ethambutol (%)	Amount of Ethambutol added %	Amount of Ethambutol found	% Recovery	Mean & Standard deviation (±)	Relative Standard Deviation (%)
Initial	101.04 101.02 101.01	--	--	--	101.02 0.015	0.015
First level	101.04 101.02 101.01	50 50 50	151.54 151.56 151.57	101.00 101.08 101.12	101.06 0.061	0.060
Second level	101.04 101.02 101.01	100 100 100	201.07 200.97 200.87	100.03 99.95 99.86	99.94 0.085	0.085
Third level	101.04 101.02 101.01	150 150 150	250.63 250.64 250.62	99.72 99.74 99.74	99.73 0.011	0.011
Overall recovery (%)				100.43		
± SD				0.701		
RSD (%)				0.698		

Table 8: Recovery experiment for Ethambutol HCL

RUGGEDNESS OF ASSAY METHOD OF

ANALYSIS FOR THE DETERMINATION Of ETHAMBUTOL IN CONCOX-800

Sr.No.	% of Ethambutol		
	Analysts-1	Analysts-2	Analyst- 3
1	101.11	101.01	101.03
2	101.14	101.01	101.04
3	101.12	101.03	101.04
4	101.14	101.05	101.05
5	101.13	101.06	101.15
Mean	**101.128**	**101.032**	**101.062**
SD	**0.013**	**0.022**	**0.049**
% RSD	**0.012**	**0.022**	**0.049**

Table 9: Ruggedness of assay method of Analysis for the determination of Ethambutol

RUGGEDNESS FOR ETHAMBUTOL **% ASSAY OF ETHAMBUTOL IN CONCOX-800**

Analyst	**% of Ethambutol**
Analyst-1	101.128
Analyst-2	101.032
Analyst-3	101.062
Mean	**101.074**
SD	**0.0491**
%RSD	**0.0485**

Table 10: Ruggedness for Ethambutol % assay of Ethambutol

ROBUSTNESS:

The parameter selected for the determination of robustness of the method is

1) Change in Flow rate

% ASSAY OF ETHAMBUTOL

Tablet number	**Initial**	**Parameters changed & observations**	
		Set I	**Set II**
		Change in Flow rate (1.8 ml)	**Change in Flow rate (2.2ml)**
1	101.04	101.10	101.11
2	101.02	101.11	101.11

3	101.01	101.12	101.12
4	101.03	101.14	101.14
5	101.02	101.15	101.15
Mean (%)	101.024	101.124	101.126
SD (±)	0.0114	0.0207	0.0181
RSD (%)	0.0112	0.0205	0.0179

Table 11 :% assay of Ethambutol

Result: Deviation for Set I = 0.0205 %, Set II = 0.0179 %

SUMMARY OF RESULTS

Sr. No.	Experiment	Acceptance criteria	Results	Remark
01	Assay (Method) precision	The relative standard deviation of 06 determinations should not be more than 2.0%	RSD = 0.0103 %	Method is précise
02	Linearity & range	The correlation coefficient should be between 0.99 to 1.00	correlation coefficient = 0.9932	Linearity is found over the specified range
03	Accuracy (Recovery)	The % recovery at each level should be between 90.0% & 110.0%.	The % recovery at each level is between 90.0% & 110.0%.	Method is accurate
		Overall recovery should be between 90.0% & 110.0% The relative standard deviation should not be more than 3.0%	Overall recovery = 100.43% RSD = 0.698%	
04	Robustness	Difference in the results obtained by changed method should not be observed by more than 2% from unchanged method.	Set I = 0.0205 % Set II =0.0179 %	Method is robust
05	Ruggedness	RSD should not be more than 3%.	RSD = 0.0485 %	Method is rugged
06	Limit of Detection	The lowest amount of analyte that can be detected.	0.5 ppm	-

07	Limit of Quantitation	The lowest amount of analyte that can be quantitatively determined.	1 ppm	-

Table 12: Summary of results

Conclusion:

The validation exercise demonstrates that the assay method of Ethambutol in Concox-800 stands validated.

SUMMARY

Ethambutol hydrochloride (ETB) is a first-line antitubercular drug commonly used in the treatment of tuberculosis (TB) in combination with other medications. It acts by inhibiting the synthesis of the bacterial cell wall, thereby halting the growth of Mycobacterium tuberculosis. Accurate measurement of ethambutol hydrochloride in pharmaceutical dosage forms is crucial to ensure proper dosing and therapeutic efficacy while avoiding toxicity. The formulation of ethambutol hydrochloride in tablets requires stringent quality control to ensure that the drug content is consistent with the labelled amount. High-Performance Liquid Chromatography (HPLC) is a widely accepted analytical technique for the quantification of pharmaceutical compounds due to its precision, accuracy, and ability to handle complex matrices. Anti-tuberculosis: Describes a drug or effect that works against tuberculosis. (A contagious bacterial infection that usually affects the lungs) [1] Analytical chemistry deals with art of determining the composition of material in term of element or compound contained. Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the productionand evaluation of by analytical chemist to save time, to avoid chemical separation or to obtain increased accuracy. In this era it is the need of the time that more reliable method are to be developed on the widely available handy instrument, which are not time consuming and validated for various factor. The method is developed for the individual drug like the instrumental techniques which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances. [2]

The most characteristic feature of the development in the methodology of pharmaceutical and biomedical analysis during the past 25 years is that HPLC became undoubtedly the most important analytical method for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations during the process of their discovery, development and manufacturing. It has been vast expanding areas of knowledge as the instrument automation is in ever increasingin power and scope. Further all the manual techniques in the line of analytical studies had steadily been transferred to the instrumental techniques. Analytical methods are generally classified as Physical and Chemical analysis. Physical analysis includes measurement of particlesize, dimension, thickness of a solid dosage forms etc.

CONCLUSION:

A simple, sensitive, isocratic and accurate reverse phase HPLC method was described for determination of Ethambutol HCL in tablet dosage form. The developed method was validated by testing Linearity, Precision, Accuracy, Specificity, Robustness, and Ruggedness. The method is good enough to separate out API (active

ingredient) the method was successfully applied for discussion studies of tablet dosage form. The study concluded that the developed method was valid for analysis of it method from its dissolution samples and for assay of tablet dosage form.

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