

## DETERMINATION OF ASSAY FOR ETHAMBUTOL IN ETHAMBUTOL HYDROCHLORIDE TABLETS BY RP-HPLC

### ABSTRACT

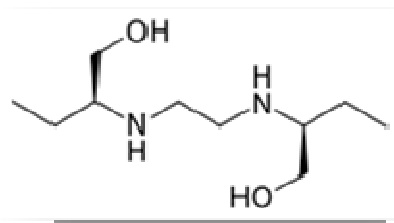
HPLC is an advanced analytical instrument to analysis pharmaceutical drugs very accurately. We are developed a HPLC method to analyze Ethambutol by RP-HPLC technique. Our target is to develop an economically cheep method with very accurate. The mobile phase of developed method is methanol–water–glacial acetic acid (70:30:0.2 v/v/v). U.V detector wave length is 210 nm, flow rate is 1ml/min.

**KEY WORDS:** Ethambutol, RP-HPLC, Assay, 210 nm,

### INTRODUCTION

Ethambutol is a bacteriostatic antimycobacterial drug prescribed to treat tuberculosis. It is usually given in combination with other tuberculosis drugs, such as isoniazid, rifampicin and pyrazinamide.

Ethambutol is bacteriostatic against actively growing TB bacilli. It works by obstructing the formation of cell wall. Mycolic acids attach to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall.



**Figure 1: Structure of Ethambutol**

Ethambutol is used with other medications to treat tuberculosis (TB). Ethambutol is an antibiotic and works by stopping the growth of bacteria. This antibiotic treats only bacterial infections. It will not work for viral infections (such as common cold, flu). Unnecessary

use or misuse of any antibiotic can lead to its decreased effectiveness. Take this medication by mouth with or without food, usually once daily or as directed by doctor. This medication may sometimes be taken twice weekly. The dosage is based on age, weight, medical condition, and response to treatment. Antibiotics work best when the amount of medicine in the body is kept at a constant level. The possible side effects like Optic neuritis (hence contraindicated in children below six years of age), Red-green color blindness, Peripheral neuropathy, Arthralgia, Hyperuricaemia, Vertical nystagmus, Milk skin reaction are occur during the usage of Ethambutol.

### Experimental Procedure:

Working standard of Ethambutol was obtained from well reputed research laboratories; formulation tablet was purchased from local market. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

### Apparatus

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used.

### Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Ethambutol an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

### Chromatographic conditions:

For the analysis of Ethambutol, the method described by Ming Yan et al method was followed i.e. the mobile phase consists of methanol–water–glacial acetic acid (70:30:0.2 v/v/v). The flow rate was 1.0 mL/min with UV detection of 210 nm at room temperature.

Parameter	Condition
Mobile Phase	methanol–water–glacial acetic acid (70:30:0.2 v/v/v)
Stationary Phase	Chromosil C18
Wavelength	210nm
P <sup>H</sup> of the mobile phase	4.7
Flow Rate	1ml/min
Pump Pressure	8±5
Concentration	20ppm
Runtime	5min
Retention time	2.42min

### Standard and sample solutions

A 10 mg amount of Ethambutol reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (Combuto1-1000) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Ethambutol was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 40ppm.

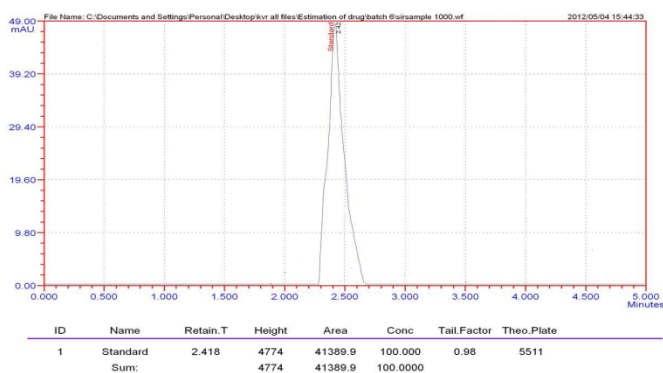


Figure.2: Standard chromatogram of Ethambuto

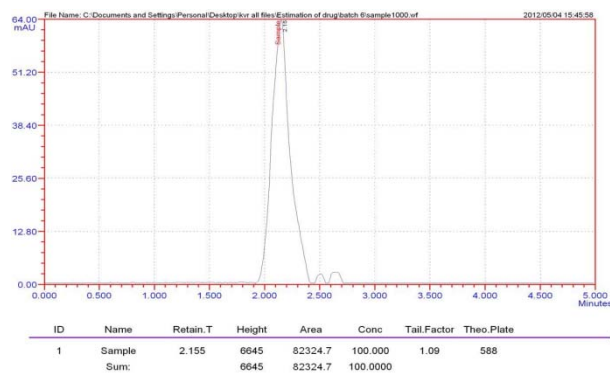


Figure.3: Formulation chromatogram of Ethambutol

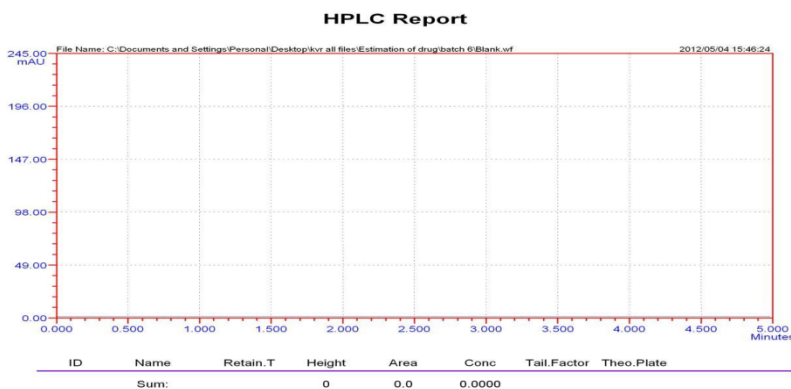


Figure.4: Blank chromatogram of Ethambutol

Brand Name	Label Claim	Concentration	Amount Found	% Assay
Combuto1	1000mg	40 ppm	39.78	99.45

Table 1: Formulation results of Ethambutol

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