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DIFFERENTIAL SCANNING CALORIMETRY AND INFRARED SPECTROMETRY METHODS FOR THE QUANTIFICATION OF ETHAMBUTOL HYDROCHLORIDE IN ORAL DOSAGE FORMS

N. Delhiraj [*1] and A. M. Sekar [2]

Department of Pharmaceutical Analysis [1], School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai - 600119, Tamil Nadu, India.
Department of Pharmaceutical Chemistry [2], GRT Institute of Pharmaceutical Education and Research, Tiruttani, Tiruvallur - 631209, Tamil Nadu, India.

Keywords:

Beer's law concentration, Ethambutol hydrochloride, Differential scanning Calorimetry (DSC), Infra red spectroscopy (IR).

Correspondence to Author:
Dr. N. Delhiraj

Professor,
Department of Pharmaceutical Analysis, School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai - 600119, Tamil Nadu, India.

E-mail: pharmaraj1981@gmail.com

ABSTRACT: A new, simple, and sensitive differential scanning calorimetry and infrared spectrophotometric technique was developed to estimate ethambutol hydrochloride in tablet dosage form. In the first method the concentration range of ethambutol hydrochloride was discovered to be 0.5 mg to 2.0 mg using differential scanning calorimetry (DSC), with the peak region of an exothermic thermogram at 75.8 °C and a continuous heating rate of 5 °K min-1. Second method which involves the measurement of ethambutol hydrochloride by Infra Red (IR) spectroscopy, the concentration range was determined to be 1.0 mg to 2.5 mg. The correlation coefficients for these techniques were determined to be 0.999 and 0.9919 for both DSC and IR methods respectively. The analytical results were statistically validated by the ANOVA technique and recovery trials. The recovery trials show no influence from other chemicals in the formulation. As a result, these two procedures are easy, precise, accurate and specific, and they might be employed for regular analysis.

INTRODUCTION: Ethambutol hydrochloride [1] is a chemotherapeutic agent that has been found to be effective against Mycobacterium tuberculosis **Fig. 1**. It is used as the first line of defence against pulmonary tuberculosis, along with other anti-tubercular drugs such as rifampicin, isoniazid, and pyrazinamide. Ethambutol was developed in response to a need for antibiotics that were effective against isoniazid-resistant Mycobacterium tuberculosis strains.

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Ethambutol enters cells and inhibits arabinosyltransferases (embA, embB, and embC), preventing the formation of cell wall components such as arabinogalactan and lipoarabinomannan, resulting in cell division inhibition, mycolic acid accumulation, trehalose monomycolate and trehalose dimycolate accumulation, and interference with mycobacterial interaction with host cells.

According to the published literature [2-16] to determine the drug in raw materials, dosage forms, and biological fluids, a few sophisticated analytical techniques such as High-Performance Liquid Chromatography, UV spectrophotometry, and electrophoretic methods were used. Most of these methods include more time-consuming and labor-

intensive phases, such as extraction and derivatization. As a result, the current study describes a novel, simple, and precise Fourier Transform- Infrared spectrophotometric method [17, 18]. Differential Scanning Calorimetry method [19, 20] for detecting the content in bulk and pharmaceutical dosage formulations.

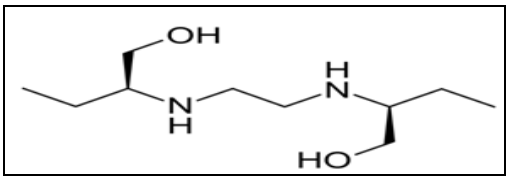

FIG. 1: STRUCTURE OF ETHAMBUTOL

MATERIALS AND METHODS:

Chemical and Reagents: Ethambutol reference standard obtained from by Orchid Pharma in Chennai, India. IR grade potassium bromide (KBr) and Analytical grade internal standard potassium thiocyanate (KSCN) were given by Merck (Darmstadt, Germany).

Method A: Differential Scanning Calorimetry Method:

Preparation of Standard and Sample Solutions: To determine the content, the calibration curve was plotted. Four distinct dosages of standard drug (0.5mg-2.0mg) were properly weighed for the DSC experiment. The experiment was conducted in a stream of nitrogen (about 50 cm^3 min^{-1}) at a heating rate of 5 K min-1 in a temperature range of -4°C to 174°C using the Netzsch DSC-204 and Proteus software, with an empty aluminum pan serving as a reference. The calibration curve was generated by plotting the peak area at 75.80°C (exothermic thermogram) versus concentration.

Preparation of Standard and Sample Solutions: 20 tablets were weighed and crushed to a fine powder, Tablet powder was accurately weighed equivalent to 1.0 mg of ethambutol and used for DSC thermogram recording. Interpolating the sample peak area on the calibration curve at 75.80°C (exothermic thermogram) yielded drug concentration in the formulation.

Method B: IR-KBr Disc Method:

Preparation of Standard Solutions: Potassium thiocyanate (KCNS) was preground, dried, then reground with dry KBr to obtain a thiocyanate concentration of roughly 0.2% by weight as an internal standard. The final mixture was kept in the presence of phosphorous pentoxide.

For the calibration curve, known weights of the standard substance were mixed with a known weight of the KBr-KCNS mixture, as shown in **Table 1**, pipetting out the required quantity in a china dish from a 20% w/v alcoholic solution of the standard drug, and evaporating the solvent from the residue. A known amount of the KBr-KCNS mixture was added, dried using an infrared light, and homogenised with an agate mortar and pestle.

The discs were manufactured using a KBr press and a hydraulic press (Model no. CAP-15T), and the infrared spectrum was captured using an Agilent in absorbance mode. Technology instrument (FTIR model Cary 630). The calibration curve was generated by plotting the absorbance of the IR absorption at 2971 cm^{-1} (the dominant band) against the chemical concentration.

TABLE 1: CONCENTRATION OF KBR/KCNS MIXTURE AND STANDARD

KBr/KCNS (mg)	50	50	50	50	50
Reference Standard (mg)	0.0	1.0	1.5	2.0	2.5

Preparation of Sample Solutions: 20 tablets were weighed, the average weight was established, and the pills were ground to a fine powder. A tablet powder containing 1.5mg of drug was precisely weighed and mixed with the KBr-KCNS mixture before being homogenised with a mortar and pestle. The resultant powder was transferred to a KBr press with a Hydraulic press (Model no.CAP-15T) to form a disc, and the infrared spectrum was recorded in absorbance mode with an Agilent Technology Instrument (Model no. Cary 630 FTIR). The concentration was estimated by interpolating the sample absorbance using the EMB's linearity curve.

Recovery Studies: The recovery studies were carried out using spiked samples, which were created by adding a predetermined amount of standard medicines to the respective sample. After 50 and 100% of standard drugs were supplied, the absorbance and peak area of the sample were measured. The recovery percentage was calculated.

The recovery study was undertaken at two levels to check the precision and correctness of the above-mentioned procedures.

RESULTS AND DISCUSSION: In DSC analysis, **Fig. 2** depicts the DSC thermogram of various concentrations of reference standard and oral dosage form at a heating rate of 5 k min^{-1} and chilling under a nitrogen stream. **Fig. 3** shows the calculated peak area value for the reference standard, as well as the calibration curve between the peak area at 75.80 degrees Celsius (Exothermic thermogram) and concentration. The correlation coefficient for EMB was calculated to be 0.9993 using equation line y= 47.668x+1.81. The linearity values of peak area at 75.8°C (Exothermic thermogram) versus concentration are shown in **Table 2.**

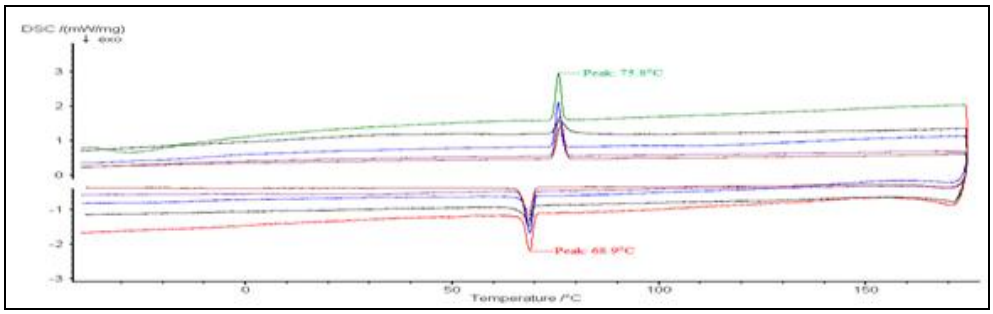

FIG. 2: DSC THERMOGRAM OF DIFFERENT CONCENTRATION OF ETHAMBUTOL HYDROCHLORIDE REFERENCE STANDARD AND ORAL DOSAGE FORM

TABLE 2: THE AREA OF PEAK AT 75.8°C EXOTHERMIC THERMOGRAM

S. no.	Concentration of Drug	Peak Area*
1	0.5 mg	25.46
2	1.0 mg	50.12
3	1.5 mg	72.58
4	2.0 mg	97.42

*Each value is the mean of three determinations.

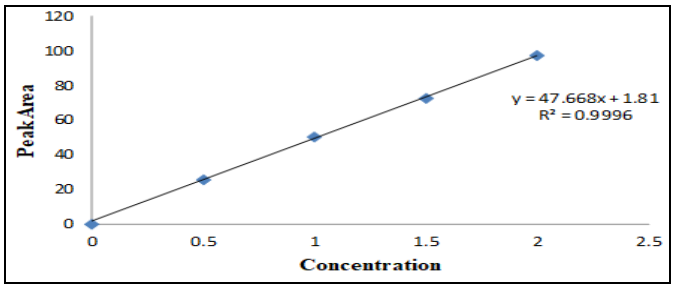

FIG. 3: CALIBRATION CURVE FOR STANDARD ETHAMBUTOL

In IR analysis, **Fig. 4** illustrates the IR spectra of drug in conventional and oral dosage forms. **Fig. 5** represents the calibration curve drawn between concentration and absorbance value, while **Table 3** gives the absorbance value of standard at wavelength 2971 cm^{-1}. With the equation line y= 538x+0.036, the coefficient of correlation was calculated to be 0.9919.

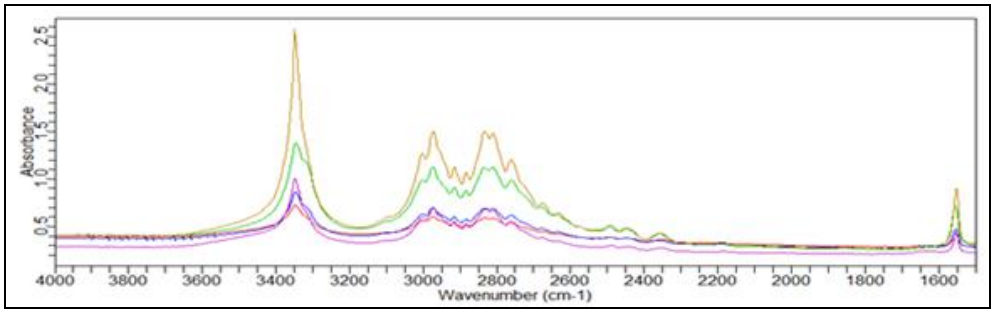

FIG. 4: IR SPECTRA OF STANDARD & ORAL DOSAGE FORM ETHAMBUTOL HYDROCHLORIDE

TABLE 3: CONCENTRATION OF A PEAK AREA AT 2971 CM^{-1} WAVE NUMBER

S. no.	Concentration of Drug	Absorbance
1	1.0 mg	0.62
2	1.5 mg	0.77
3	2.0 mg	1.12
4	2.5 mg	1.40

*Each value is the mean of three determinations.

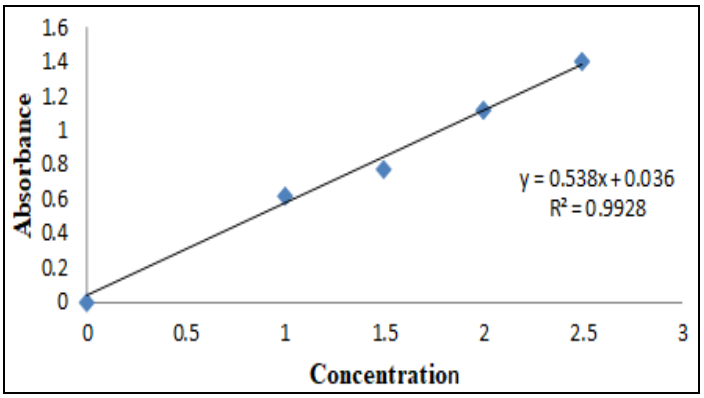

FIG. 5: CALIBRATION CURVE OF ETHAMBUTOL VERSUS PEAK AREA

Table 4 shows the optical properties, such as the concentration range of Beer's law. **Table 4** summarizes the findings of the regression features such as slope (b), intercept (c), and correlation coefficient. The recovery percentages of the two procedures range between 98 and 100% w/w. The correlation values for the two procedures were 0.999 and 0.9919, respectively, and the recovery trials show no influence from other chemicals in the formulation. As a result, these two procedures are easy, precise, accurate, time-consuming, and specific, and they might be employed for regular analysis. **Table 5** shows the test results obtained using the proposed method. Validation studies for the suggested methodologies were conducted, and the results are shown in **Tables 6** and **7**. DSC and IR measurements do not require any prior extraction and are unaffected by drug solubility.

TABLE 4: OPTICAL PARAMETERS OF ETHAMBUTOL HYDROCHLORIDE BY DSC AND IR METHOD

Parameters	Method A Heat-Flux DSC method	Method B KBr Disc method using Internal standard
Beer's law limit (mg)	0.5– 2.0	1.0 – 2.5
Regression equation (y = mx + c)	47.668x+1.81	0.538x+0.036
Slope (m)	47.668	0.538
Intercept (C)	1.81	0.036
Correlation coefficient	0.9993	0.9919
LOD (µg/mL)	0.250608	0.441636
LOQ (µg/mL)	0.759419	1.33829

TABLE 5: RESULT OF TABLET ASSAY

S. no.	Method	Label claim	Amount found (mg)*	Percentage Assay	SD	SE	%RSD
1	Method A	100 mg	99.02	99.02	0.541223	0.31249	0.546553
2	Method B	100 mg	99.56	99.56	1 .495337	0.863359	1.501895

*Each value is a mean of 3 determinations.

TABLE 6: RECOVERY STUDY

S. no.	Method	Label claim	Amount of drug added (mg)*	Amount of drug recovered (mg)*	Percentage Recovery
1	Method A	100mg	0.5	0.496	99.20
			1.0	0.994	99.46
2	Method B	100mg	0.75	0.742	98.93
			1.5	1.48	98.66

*Each value is a mean of 3 determinations.

TABLE 7: ANOVA CALCULATION FOR DSC METHOD

Source of Variation	SS	Df	MS	F-Ratio	P-value*
Between sample	7234.8421	1	7234.8421	15.27087	0.007912
Within sample	2842.6047	6	473.7675		

*The result is significant of $P < 0.05$.

TABLE 8: ANOVA CALCULATION FOR IR METHOD

Source of Variation	SS	Df	MS	F-Ratio	P-value*
Between sample	1.5051	1	1.5051	6.41896	0.044465
Within sample	1.4069	6	0.2345		

*The result is significant of $P < 0.05$.

CONCLUSION: The proposed methods for measuring the drug, ethambutol hydrochloride in bulk and pharmaceutical dose forms are simple, precise, exact, time-efficient, specific, and selective.

In contrast to the chromatographic technique, these methods are less expensive and faster, and they do not need complex equipment. As a result, it is suitable for regular analysis in bulk and medicinal dose forms.

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CONFLICT OF INTEREST: The authors of this research work have no conflict of interest

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