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A Simple Zero-Order Derivative UV Absorption Spectrophotometric Approach for Ethambutol Analysis

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Abstract: For the quantitative examination of ethambutol in pharmaceutical formulations, a brand-new zero-order absorption spectrophotometric technique has been created and verified. This technique shows maximum absorption at 638 nm for 2,6-dichlorophenolindophenol (DCPIP), which is used as a chromogenic reagent. According to Beers' law, the method's linearity was demonstrated over a concentration range of 30-90 µg/mL. It was determined that the quantitation and detection limits were 1.5 and 0.5 µg/mL, respectively. High accuracy was indicated by recovery studies, which showed an average recovery of 99. 5%. Reliability within and between days was found to be less than 2% according to precision studies. The method's dependability was confirmed by statistical analysis, and the outcomes met the recommendations of the International Conference on Harmonization (ICH). Ethambutol quantitation is made easy, sensitive, and precise with the help of the suggested zero-order absorption spectrophotometric method. A few sample preparation steps, quick analysis, and affordability are some of its benefits. Ethambutol concentrations in pharmaceutical products can be reliably monitored with this method, which is appropriate for use in pharmaceutical research and quality control applications. The method's effectiveness was validated by the validation parameters, indicating that it can serve as a competitive substitute for current analytical techniques. Overall, this work shows how routine ethambutol analysis in pharmaceutical formulations can benefit from the use of zero-order absorption spectrophotometry.

Keywords: Ethambutol dihydrochloride, DCPIP regents, validation, zero-order Derivative Abs. And, the spectrophotometric method.

I. INTRODUCTION

In the pharmaceutical industry, quality assurance is a critical step in preventing errors or defects in product outcomes. Quality assurance relies heavily on the development and validation of analytical methods to guarantee the precision, accuracy, and dependability of the methods. Developing analytical methods entails creating a methodical approach to ascertain a drug's identity, potency, purity, physicochemical properties, bioavailability, and stability. Choosing the most precise assay techniques to assess a medication's composition is part of this process[1]. The necessity to maintain high-quality products, the need to compete in a global market, and the advancement of analytical methods are the driving forces behind the need for method development. It attempts to separate and characterize contaminants and degraded materials in addition to purifying and quantifying the necessary drugs, to ensure that the performance parameters satisfy the specifications for a particular analytical problem. Method validation is essential. Evaluation of Boundaries of measurement and identification Range resilience involves precision, linearity, & sensitivity.

In pharmaceutical analysis, spectrophotometric techniques like UV-visible spectrophotometry are frequently employed. The laws of Beer-Lambert, which connect a solution's absorbance to its concentration, control these procedures. The process of derivative spectrometry is used to improve the sensitivity and selectivity of spectrophotometric techniques by eliminating matrix

interferences. Pharmaceutical analysis uses spectrophotometric techniques for a number of purposes, such as drug content determination, impurity identification, and protein and amino acid analysis. They are also employed in environmental analysis, clinical chemistry, and forensic toxicology[4,5].

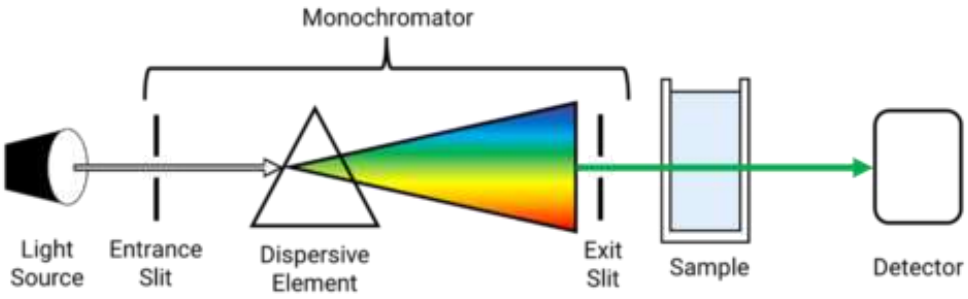
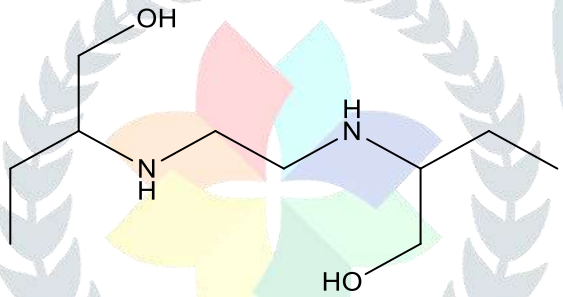


Figure 1 UV – Visible Spectroscopy

A key antitubercular medication used in combination treatments to treat Mycobacterium tuberculosis infections is ethambutol hydrochloride. It is an efficient part of treatment regimens for tuberculosis because of its mechanism of action, which involves inhibiting the synthesis of bacterial cell walls. Its poor UV absorption, however, makes direct absorbance measurement difficult and error-prone due to interference from pharmaceutical excipients.

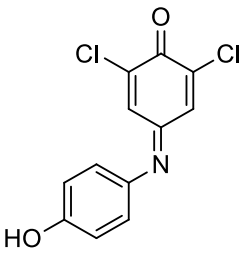


2,2'-(ethane-1,2-diyldiazanediyl)di(butan-1-ol)

Figure 2: Ethambutol Hydrochloride

2,6-Dichlorophenolindophenol (DCPIP) has been developed as a chromogenic reagent in a spectrophotometric method to overcome this limitation. The formation of a colored complex by the reaction of DCPIP and ethambutol HCl allows for accurate quantitation at a particular wavelength. This technique is the best means of detecting ethambutol HCl in pharmaceutical preparations because it is more sensitive and accurate than traditional methods.

A dependable and effective method of evaluating ethambutol HCl is to use DCPIP as a chromogenic reagent, which guarantees the effectiveness and caliber of antitubercular drugs. The linearity, accuracy, and resilience of this approach have been verified, qualifying it for regular use in pharmaceutical quality control labs. Manufacturers can guarantee the precise determination of ethambutol HCl by utilizing this spectrophotometric method, which will ultimately aid in the development of potent treatments for tuberculosis[6-16].



2,6-dichloro-4-[(4-hydroxyphenyl)imino]cyclohexa-2,5-dien-1-one

Figure 3: Dichlorophenol indophenol (DCPIP)

II. Materials and methods:

Drug: Ethambutol Hydrochloride

Materials:

The solvents used were acetonitrile, chloroform, and distilled water.

Equipment used:

The instrument used for the estimation was a Shimadzu model 1900i UV-Visible Spectrophotometer UV-1900 with 1cm matched quartz cells.

Get the Reagent Ready: -

2, 6-Dichlorophenol indophenol (DCPIP) solution 0 percent Weight/Volume Percentage in chloroform was made by Weight measurement, 100 mg of DCPIP sodium, which is equal to 100 mg of DCPIP. Then the mixture was transferred to a separatory funnel with roughly 30 ml of water and allowed to settle. Following five consecutive 20 ml portions of chloroform, the solution was acidified with five milliliters of 2N hydrochloric acid to extract the reagent. After passing through anhydrous sodium sulphate and a 20 ml water wash, the extracts were diluted to a 100 ml volume using chloroform as a final step.

Setting up the Standard Solution: -

A precise measurement of the drug salt equal to da. A 100 ml volumetric flask containing 100 mg of base was filled with acid that had been diluted to volume with water. After transferring a 10 ml volume to a 100 ml separatory funnel, the sample was alkalinized using a sodium hydroxide solution and extracted using five consecutive 20 ml portions of chloroform. After being dried on the chloroformic solutions were collected in a 100 mL calibrated flask containing anhydrous salt sulphate.

Volumetric container and made up to volume using Chloroform (0-point 1 mg ml -1). Chloroformic extracts were gathered in a 100 ml calibrated container and made up to volume using chloroform (0-point 1 mg ml -1).

Graphs used for calibration: -

Separate sets of the 10 mL volumetric flasks were filled with aliquot volumes containing DCPIP 0.03-0. 15 mg base. Two milliliters of DCPIP solution were added to the first set, and acetonitrile was then diluted to volume. After 10 minutes at room temperature (250 c), the flasks were left to develop a bluish violet color. The optical density (OD) of this color was determined at 553 nm and 578 nm compared to a reagent blank that was treated in the same way.

The measurement of wavelength: -

Following the process of serial dilution that the concentration. Underwent after preparing the standard stock solution. is 30µg /ml,40µg/ml,50 µg/ml,60µg/ml, 70µg/ml, 80µg/ml, and 90µg/ml. Once the 30µg/ml solution was concentrated on the drug's wavelength was checked using a chloroform solvent. A sample of the 10µg/ml solution was taken, and when it was worked on in spectrum mode, it showed greater absorbance in the range of 553nm–578nm for zero order.

Drug stability in a chosen solvent: - By keeping an eye on the medication solution's absorbance (30 µg/ml), Time-course stability studies were conducted in the chosen solvent, chloroform were determined. Five minutes later, the absorption of determined. Spectral Absorbance did not change much as observed, indicating that the solution was stable. The stability data is given in the Table.

Sr no	Time (min)	Absorbance
1	0	0.900
2	5	1.200
3	10	1.500
4	15	1.900

Table 1: Zero-order derivative stability data for Ethambutol Hydrochloride

Linearity:

An appropriate volume of the standard Ethambutol Stock solution was diluted in a 10-milliliter volumetric flask, and a dilution was prepared using chloroform at a concentration of 100 µg/ml to create working standard solutions with concentrations of 30

µg/ml,40 µg/ml, 50µg/ml, 60 µg/ml, 70 µg/ml, 80µg/ml, and 90 µg/ml. The difference in absorbance of Ethambutol hydrochloride was measured in a zero-order derivative mode instrument at 553 nm, and the drug's calibration curve was plotted.

Sr. No	Conc. Of Ethambutol (µg/ml)	Absorbance
1.	30	0.100
2.	40	0.200
3.	50	0.220
4.	60	0.300
5.	70	0.400
6.	80	0.500
7.	90	0.600

Table 2: Standard calibration table for Ethambutol hydrochloride by zero-order spectrum method

III. Results and discussion:

A quantitative determination of certain basic drugs as n-donor was carried out using the halogenated quinone 26-dichlorophenol indophenol as an acceptor reagent. Ethambutol was determined spectrophotometrically using this reaction. Ethambutol is an n-donor, thus reacts with DCPIP, an acceptor, to produce a blue-violet Chromogen that has an Acetonitrile solution exhibiting strong UV absorption at 578 nm. This could be explained by the radical anion DCPIP, which is created when all of the electrons in a donor moiety are completely transferred to an acceptor moiety in a polar solvent.

In order to achieve maximum color development, the ideal parameter was investigated. The greatest color formation in a total volume of 10ml was found to occur in a volume of 2ml of a 0.1 percent w/v solution of DCPIP. It took ten minutes at room temperature (250 c) to reach the maximum color intensity. For an additional half hour, the color stability persisted, and the ideal parameter was investigated to achieve the greatest amount of color development.

Quantitative estimation of zero-order derivative absorption for ethambutol Hydrochloride in pharmaceutical

With a Shimadzu 1900i Spectroscopy UV-visible spectrophotometer, the standard solutions of ethambutol hydrochloride in DCPIP reagents and chloroform were scanned at a range of wavelengths from 800 nm to 400 nm. At N=5 zero zero-order derivative spectra were obtained, and a Standardization curve of ethambutol hydrochloride was discovered to be linear at conc. It was thus evident that ethambutol hydrochloride could detect the presence of chloroform in pharmaceutical products without the need for any extraneous substance to be added. The range of the DCPIP reagent was 30µg/ml to 90µg/ml at 578nm.

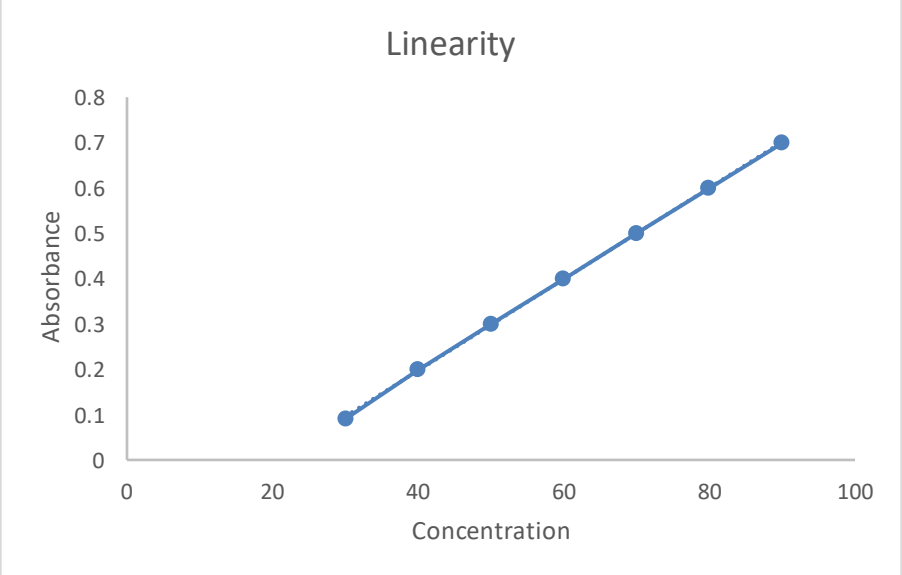


Figure 4: linearity graph of zero-order absorbance of DCPIP reagent

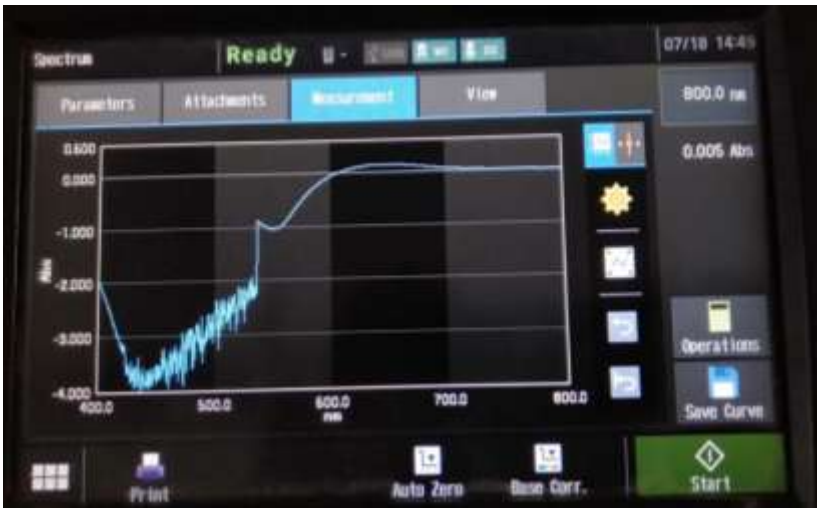


Figure 5: Zero derivative spectrum of Ethambutol hydrochloride conc. 30 µg/ml

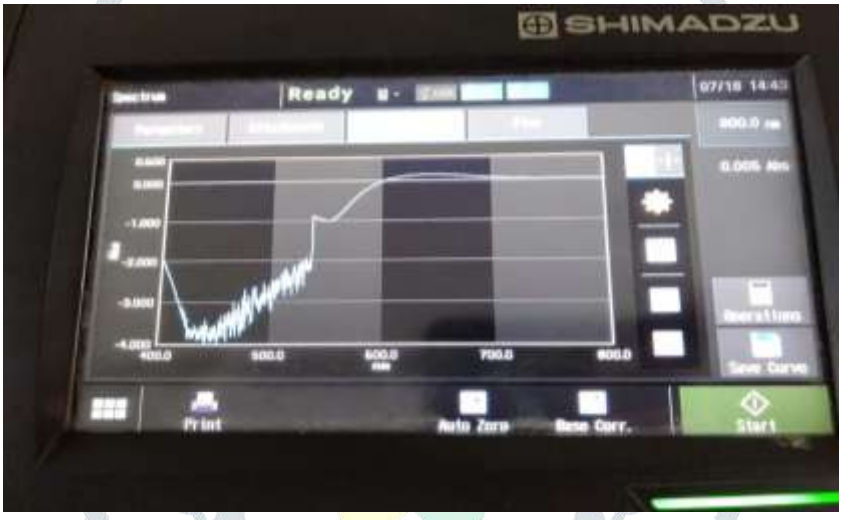


Figure 6: Zero derivative spectrum of Ethambutol hydrochloride, conc. .60 µg/ml

Figure 7: Zero derivative spectrum of Ethambutol hydrochloride conc. 90µg/ml

Parameters	Ethambutol hydrochloride
Linearity range (concentration)	30-90 µg/ml
Slope	0.0101
Intercept	0.2079
Regression coefficient(r^2)	0.9998

Table 3: The zero-order spectrum method yielded both the optical and regression parameters for the calibration curve

Accuracy: -

The accuracy of the analytical process is defined as the degree of agreement between the value recognized as a conventional true value or a recognized reference value and the value found. Sometimes this is referred to as truth. Over the designated range of the analytical procedure, accuracy should be established.

Level of % Recovery	Amount Present (mg/ml)	Amount of Stand. Added (mg/ml)	Total amount present + stand.	Total Amount Recovered (mg/ml)	%Recovery	% Mean Recovery	SD	CV
80	20	16	36	38	99.72	9.78	3138	0.0031
80	20	16	36	36.1	100.2			
80	20	16	36	35.8	99.44			
100	20	20	40	39.8	99.50	99.75	0.2041	0.002
100	20	20	40	40	100			
100	20	20	40	39.9	99.75			
120	20	24	44	43.8	99.54	9.91	.2763	0.0028
120	20	24	44	44	100			
120	20	24	44	44.1	100.2			

Table 4: Ethambutol hydrochloride accuracy parameter for the method of zero-order derivative.

Precision: -

The accuracy of an analytical procedure is defined as the degree of scatter between a set of measurements made by repeatedly sampling some homogeneous sample under the given circumstances. By using four separate containers to carry four different samples of the drug ethambutol hydrochloride. Several analysts in the same lab evaluated the precision (inter-day). An overview of the four analysts' precision values is given in the table.

Sample No.	Assay of Ethambutol Hydrochloride % of labelled amount (Inter-day precision)			
	Time I	Time II	Time III	Time IV
1	99.07	99.96	98.99	99.97
2	99.70	100.03	99.57	98.00
3	99.02	98.96	100.4	99.70
4	99.04	99.87	99.26	99.96

Mean	99.57	99.67	99.58	99.92
S.D.	0..4617	0.4321	0.5767	0.8124
CV	0.0045	0.0043	0.0046	0.0008

Table 5: Ethambutol hydrochloride precision in the first derivative method.

IV. Conclusion:

The 2, 6-dichlorophenolindophenol (DCPIP) chromogenic reagent-based zero-order absorption spectrophotometric method provides a straightforward, sensitive, precise, and economical method for quantifying ethambutol in pharmaceutical formulations. The approach is a suitable and viable substitute for current analytical techniques for quality control and pharmaceutical research applications due to its high accuracy, precision, and reliability, which have been confirmed by statistical analysis and ICH recommendations.

V. Conflict of interest:

The authors have no conflicts of interest regarding this investigation.

VI. Acknowledgement:

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