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SPECTROPHOTOMETRIC DETERMINATION OF ETHAMBUTOL IN PURE AND PHARMACEUTICAL FORMS USING TRIPHENYL METHANE DYES

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Spectrophotometry, Ethambutol, Bromocresol green, Bromocresol purple, Bromophenol blue, Ion-pair complex, Validation

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ABSTRACT: Simple and sensitive extractive spectrophotometric methods for the determination of Ethambutol in pure form and in pharmaceutical formulations using triphenyl methane dyes have been developed. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with triphenyl methane dyes *viz.*, bromocresol green (BCG), bromocresol purple (BCP) and bromophenol blue (BPB) in acidic medium. The extracted complexes formed with BCG, BCP and BPB showed absorbance maxima at 420, 419 and 415 nm respectively. The stoichiometry of the ion- pair complex is found to be 1:1 in each case. Beer's law is obeyed in the concentration ranges 2.0-25, 3.0-30 and 4.0-40 µg/ml with BCG, BCP and BPB respectively. The effect of concentration of dye, pH, and interference of excipients have been studied for optimization. The limits of detection and quantification have been determined for all the three methods. These methods have been validated as per the guidelines of ICH. The results of analysis were validated statistically through recovery studies.

INTRODUCTION: Ethambutol is a white crystalline powder, soluble in chloroform, sparingly soluble in water, it has pKa 6.35 and 9.35, chemically called 2, 2'-(1, 2-Ethylenediimino) bis- 1-butanol [Fig.1]. It is an oral chemotherapeutic agent which is specifically effective against mycobacterium, which was the recent discovered (1961) first line treatment as anti-TB drug¹. It has excellent activity *in vitro* and *in vivo* against Mycobacterium tuberculosis.

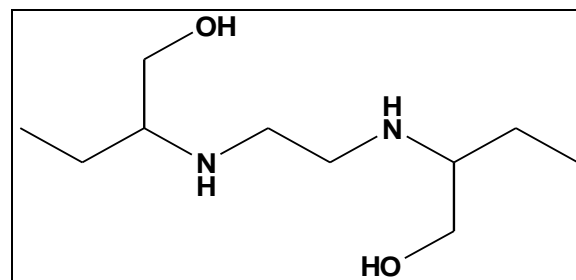


FIG. 1: CHEMICAL STRUCTURE OF ETHAMBUTOL

Ethambutol is bacteriostatic to both intracellular and extracellular organisms². Many reports have documented the efficacy of Ethambutol for the treatment of bronchopulmonary, urogenital and osteoarticular tuberculosis and *Mycobacterium avium* complex³. Microbiological and gas liquid chromatographic methods are available for assaying Ethambutol concentrations in serum and

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cerebrospinal fluid. In microbiological assay, when Ethambutol (EMB) is present along with other drugs in plasma, it cannot be assayed without the interference of other drugs⁴. Gas liquid chromatography methods using capillary columns are available but they involve a series of steps for preparation of the sample before analysis and needs internal standard⁵. Ethambutol has been routinely assayed using a non-aqueous titration method^{6,7}. It was observed that with certain pharmaceutical preparations, the values were higher than the stated contents. Another method for estimation of Ethambutol content in pharmaceutical preparations involves reaction with cupric chloride and measurement of the absorption in the visible range, which is again a time consuming procedure^{8,9,10}. In drug formulations containing EMB in addition to other anti-TB drugs, EMB has to be exclusively estimated without the interference of other drugs.

According to the literature available, methods found for the determination of Ethambutol in plasma were in High Performance Liquid Chromatography (HPLC)^{11,12,13} where, extraction process involves more time consuming and tedious steps like derivatization. Though methods are available for Liquid Chromatography Mass Spectrometer tandem Mass Spectrometry (LC-MS/MS)¹⁴⁻¹⁶, it is found that the chromatographic conditions and extraction processes were highly expensive as they use cartridges, and high solvent usages¹⁷. The assay method of Ethambutol hydrochloride in the tablets given in the Chinese Pharmacopoeia (CHP) involves repeated chloroform extraction from a basic aqueous solution and subsequent nonaqueous titration¹⁸.

A gas chromatography (GC)-mass spectrometric (MS) method has been used for the determination of Ethambutol in tablets¹⁹. The determination of Ethambutol by reverse-phase (RP) high-performance liquid chromatography (HPLC) is difficult because of its high pose, small molecule, and its lack of strong absorption by UV. Ethambutol derivatives have a strong UV absorbance and can be measured by RP-HPLC. A number of methods for the derivatization of Ethambutol have been reported. Ming Yan et al²⁰ reported the determination of Ethambutol hydrochloride in the combination tablets by

precolumn derivatization. The determination of Ethambutol in presence of fixed dose combination molecules from human plasma by direct injection to Liquid Chromatography Tandem Mass Spectrometry has also been reported²¹. A precise colorimetric procedure was proposed for the estimation of Ethambutol hydrochloride, based on reaction of the drug with 2,4-dinitro-1-fluorobenzene under stipulated conditions²². A method for estimation of Ethambutol in pharmaceutical preparations and biological fluid was standardized²³.

An alternative method for Ethambutol determination by UV spectrophotometry in pharmaceutical formulations using complexation with Cu(II) was optimized²⁴. Another method of determination depends on the interaction of Ethambutol with Co(II) ions in the presence of ammonia solution²⁵. Spectrofluorimetric quantification of Ethambutol hydrochloride in bulk and oral dosage forms was reported²⁶. Micro determination of Ethambutol with AHC (Ammonium Hexanitrate Cerate) was also carried out and reported²⁷.

The chemical features of the Ethambutol drug molecule offers a lot of scope for the development of new methods for its determination with better sensitivity, specificity, precision and accuracy. The reported chromatographic techniques require expensive experimental set-up and are not affordable in every laboratories for routine analysis. Although several analytical methods for the estimation of Ethambutol are available in literature, a little attention was paid to the development of spectrophotometric methods for its determination using dyes. Spectrophotometry is considered as the most convenient analytical technique because of its inherent simplicity, low cost, and wide availability in most quality control laboratories.

So the present study reports on newly developed and validated spectrophotometric methods for the estimation of Ethambutol in bulk and pharmaceutical formulations using triphenyl methane dyes *viz.*, bromocresol green (BCG), bromocresol purple (BCP) and bromophenol blue (BPB) in acidic medium. The developed methods

involve formation of coloured chloroform extractable ion-pair complexes of the drug with dyes in acidic medium. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quantitative analysis of Ethambutol.

MATERIALS AND METHODS:

Ethambutol was procured from Srinu Pharmaceuticals Limited, Hyderabad as a gift sample. The dyestuffs viz., BCG, BCP and BPB (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers of pH 2.5, 3.0 and 3.5 were prepared by mixing 50ml of 1.0M sodium acetate solution with calculated volume of 1.0 M HCl solution and diluted to 250 ml with doubly distilled water. The pH of each solution was adjusted to an appropriate value with the aid of a pH meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai was used throughout the work.

The spectra (Fig. 2-4) of ion-pair complexes have been recorded on Elico double beam SL 210 spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.

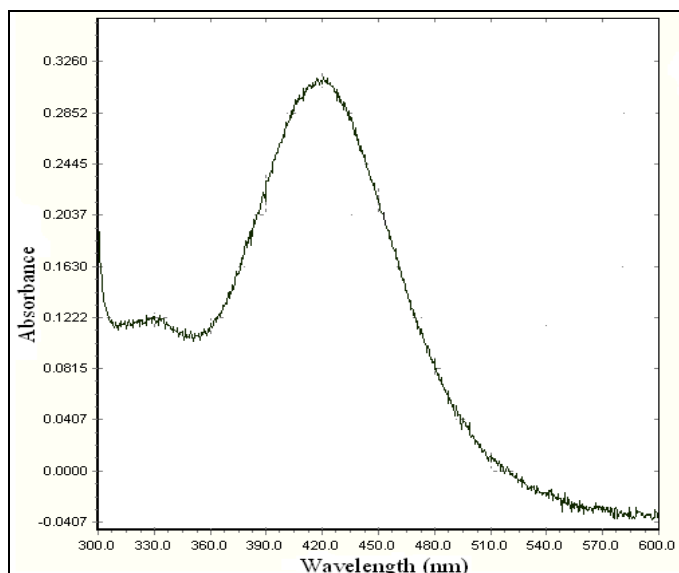


FIG. 2: ABSORPTION SPECTRUM OF ETHAMBUTOL-BROMOCRESOL GREEN (BCG) COMPLEX EXTRACTED INTO 10 ML CHLOROFORM

[drug] = 25 $\mu\text{g ml}^{-1}$ + 5 ml of 0.025% BCG + 5 ml of pH 2.5 buffer

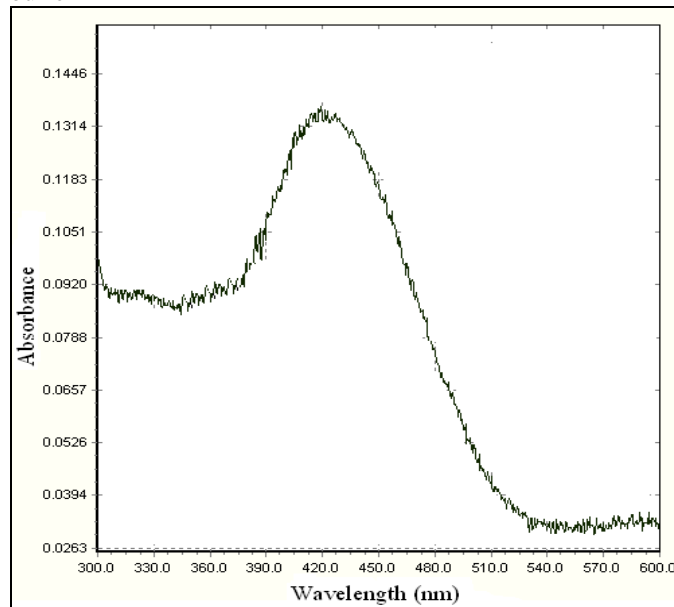


FIG. 3: ABSORPTION SPECTRUM OF ETHAMBUTOL-BROMOCRESOL PURPLE (BCP) COMPLEX EXTRACTED INTO 10 ML CHLOROFORM

[drug] = 25 $\mu\text{g ml}^{-1}$ + 5 ml of 0.025% BTB + 5 ml of pH 3.0 buffer

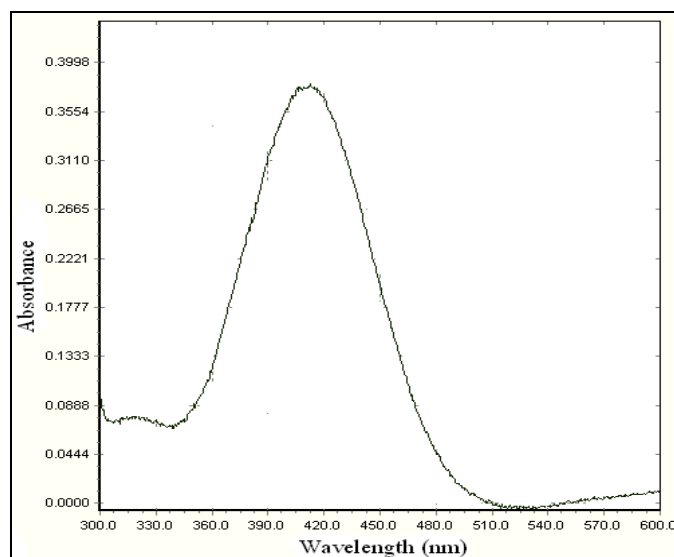


FIG. 4: ABSORPTION SPECTRUM OF ETHAMBUTOL-BROMOPHENOL BLUE (BPB) COMPLEX EXTRACTED INTO 10 ML CHLOROFORM

[drug] = 25 $\mu\text{g ml}^{-1}$ + 5 ml of 0.025% BCP + 5 ml of pH 3.5 buffer

Calibration curve:

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (pH 2.5, 3.0 and 3.5), 5 ml of dye were added and total volume was made up to 20 ml with water. 10 ml of chloroform was added and the contents were shaken for 5 min. The two layers

were allowed to separate for 5 min. The organic layer was separated and absorbance of yellow colored solution which is stable atleast for 3 hrs is measured at 420nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage

form. The calibration graphs (Fig.5) are linear over the concentration ranges and are within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in Table 1.

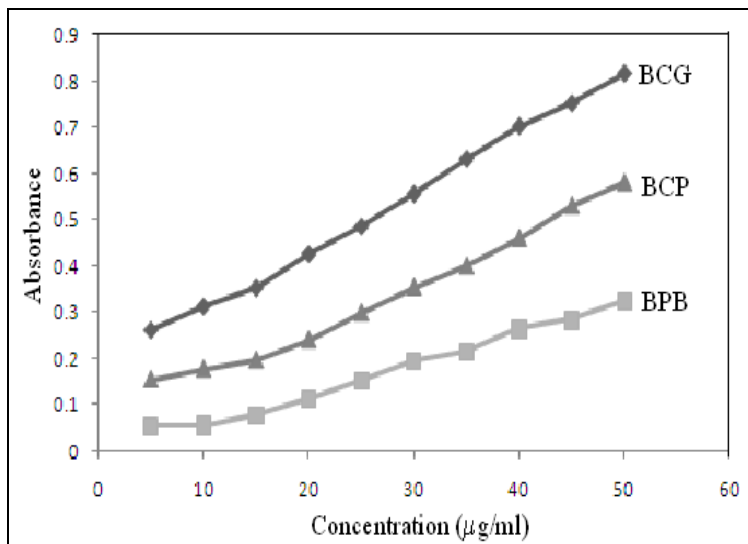


FIG. 5: CALIBRATION GRAPHS FOR DRUG-BCG, BCP & BPB ION-PAIR COMPLEXES

TABLE 1: OPTICAL CHARACTERISTICS AND STATISTICAL ANALYSIS FOR THE REGRESSION EQUATION OF THE PROPOSED METHODS

Parameters	Extraction methods with ^b		
	BCG	BCP	BPB
λ_{max} (nm)	420	419	415
Beer's law limit ($\mu\text{g ml}^{-1}$)	2.0-25	3.0-30	4.0-40
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	18532	20237	21590
Formation constant, K, M^{-1}	1.28×10^6	1.53×10^6	1.65×10^6
Sandell sensitivity ($\mu\text{g cm}^{-2}$)	0.0249	0.0152	0.0137
Slope (specific absorptivity), b	0.0401	0.0655	0.0728
Intercept (a)	0.0212	0.0221	0.0325
Correlation coefficient (r)	0.999	0.998	0.995
Standard deviation of intercepts (% n=6)	0.0062	0.0071	0.0102
Limit of detection, μgml^{-1}	0.5102	0.3577	0.4623
Limit of quantification, μgml^{-1}	1.5461	1.0839	1.4010
Regression equation ^a	$Y=0.0401C \pm 0.0212$	$Y=0.0655C \pm 0.0221$	$Y=0.0728C \pm 0.0325$

^aWith respect to $Y=bc+a$, where C is the concentration ($\mu\text{g ml}^{-1}$) and Y is absorbance

^bSix replicate samples

Procedure for the assay of pure drug:

Five different solutions of pure drug in the range of calibration curve were selected and the recovery

experiments were performed. The recoveries and their relative standard deviations are tabulated in Table 2.

TABLE 2: APPLICATION OF PROPOSED METHODS FOR THE ANALYSIS OF ETHAMBUTOL IN PURE FORM

Taken ($\mu\text{g ml}^{-1}$)	Proposed methods			Reference method			
	Found ($\mu\text{g ml}^{-1}$)			Recovery %		Recovery %	
	BCG	BCP	BPB	BCG	BCP	BPB	
4	4.09	3.98	4.11	101.25	99.5	102.75	101.25
8	7.96	7.91	8.18	99.5	98.87	102.25	99.82
12	12.06	11.95	12.10	100.5	99.58	100.83	101.0
16	16.05	16.01	15.96	100.3	100.06	99.75	101.02

20	20.03	20.10	19.98	100.15	100.5	99.9	101.15
							100.06
							101.12
							99.98
RSD (%)				0.6296	0.6160	1.343	0.6019
Mean±SD				100.34	99.70	101.09	100.67
				±0.6318	±0.6149	±1.3583	±0.6060
t-test				0.0785	0.0405	1.1632	
F-test				0.9200	0.9711	0.1990	

Procedure for the assay of dosage forms:

Ten tablets of Combutor 10 mg each are powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 ml standard volumetric flask and diluted with

doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are tabulated in **Table 3**.

TABLE 3: APPLICATION OF PROPOSED METHODS FOR THE ANALYSIS OF ETHAMBUTOL IN PHARMACEUTICAL FORM

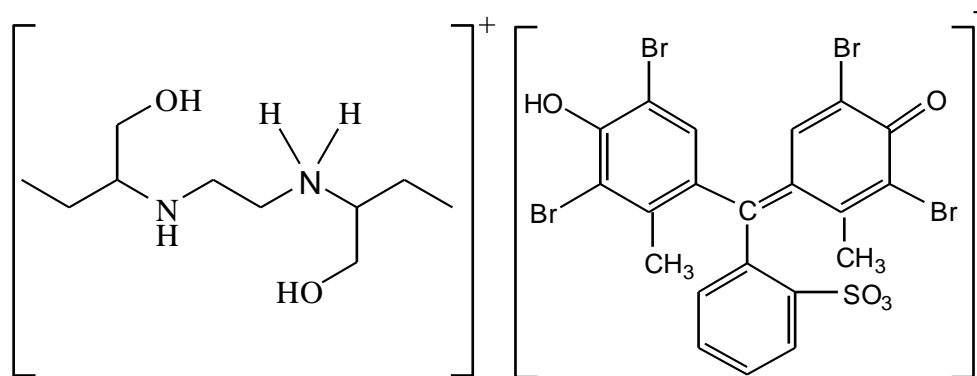
Taken ($\mu\text{g ml}^{-1}$) Combutor 10 mg	Proposed methods			Reference Method			
	Found ($\mu\text{g ml}^{-1}$)			Recovery (%)			
	BPB	BTB	BCP	BPB	BTB	BCP	Recovery(%)
5	4.98	5.08	4.97	99.6	101.6	99.4	98.98
10	10.05	9.98	10.12	100.5	99.8	101.2	101.25
15	14.98	15.05	15.15	99.86	100.33	100	101.24
20	20.02	20.15	19.98	100.1	100.75	99.9	99.88
25	25.02	24.98	24.95	100.08	99.84	99.8	100.25
							100.25
							99.96
							101.34
							99.82
RSD (%)				0.3223	0.7415	0.6762	0.7976
Mean±SD				100.03	100.46	100.06	100.38
				±0.3224	±0.7450	±0.6767	±0.8007
t-test				1.5775	0.1351	0.1584	
F-test				1.2376	1.1550	1.4000	

RESULTS AND DISCUSSION:

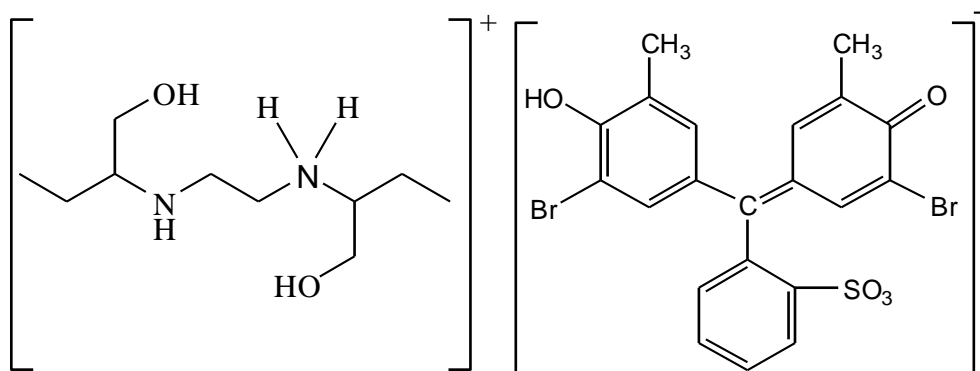
Formation of Ion-pair complexes:

Ethambutol forms ion-pair complexes in acidic buffer with dyestuffs such as bromocresol green (BCG), bromocresol purple (BCP) and bromophenol blue (BPB) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BCG, BCP and BPB absorbed maximally at 420, 419 and 415 nm respectively (**Fig. 2-4**). The reagent blank under similar conditions showed no absorption. Ethambutol contains secondary amine groups which are protonated in acid medium, while

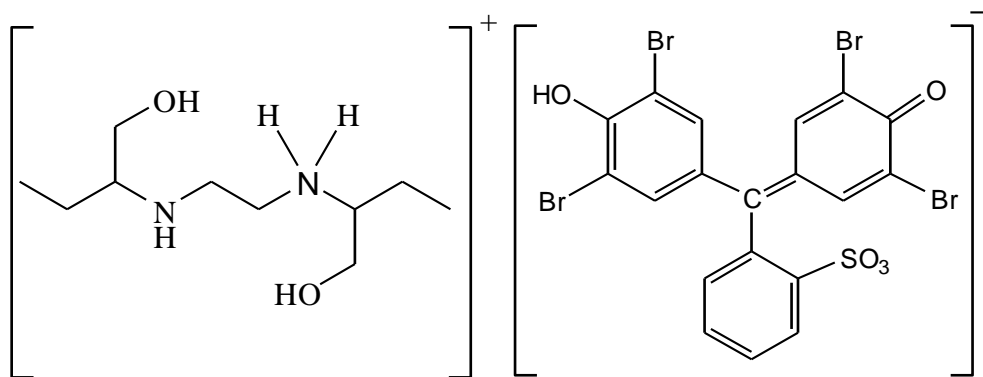
sulphonic acid group present in BPB, BTB and BCP undergoes dissociation in the pH range 1-5. The colour of the ion-pair complex is attributed to opening of lactoid ring and subsequent formation of quinoid group in dye moiety. It is appropriate to mention that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated Ethambutol forms ion-pairs with dyestuffs which are quantitatively extracted into chloroform. **Fig. 6** shows the possible structures of ion-pair complexes.



Ethambutol-Bromocresol green ion-pair complex



Ethambutol-Bromocresol purple ion-pair complex



Ethambutol-Bromophenol blue ion-pair complex

FIG. 6: ETHAMBUTOL-DYE ION-PAIR COMPLEXES

Stoichiometry:

In order to establish molar ratio between Ethambutol and dyestuffs used, the Job's method²⁸ of continuous variation has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations ($8 \times 10^{-5}M$) were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and

plotted against the mole fraction of the drug, $[drug]/ [drug] + [dyestuff]$ (Fig. 7). This measurement showed that 1:1 complex was formed with each dyestuff. The formation constants^{29,30} were also estimated and found to be 1.39×10^6 , 1.61×10^6 and $1.71 \times 10^6 K M^{-1}$ for complexes with BCG, BCP and BPB respectively.

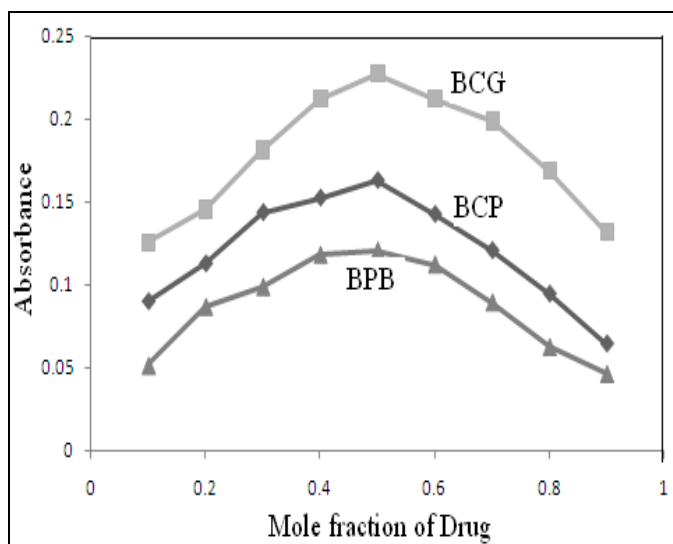


FIG. 7: CONTINUOUS-VARIATIONS STUDY OF DRUG-DYE SYSTEMS
[drug] = [dye] = 8×10^{-5} M

Optimization of the factors affecting the absorbance:

The influence of pH on the ion-pair formation of Ethambutol with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in Fig.8. It is evident that absorbance of complexes with BCG, BCP and BPB was found to be constant within the pH ranges 2.0-3.0, 2.5-3.5 and 3.0-4.0 respectively. Thus, all the absorbance measurements were made at pH 2.5, 3.0 and 3.5 with BCG, BCP and BPB respectively.

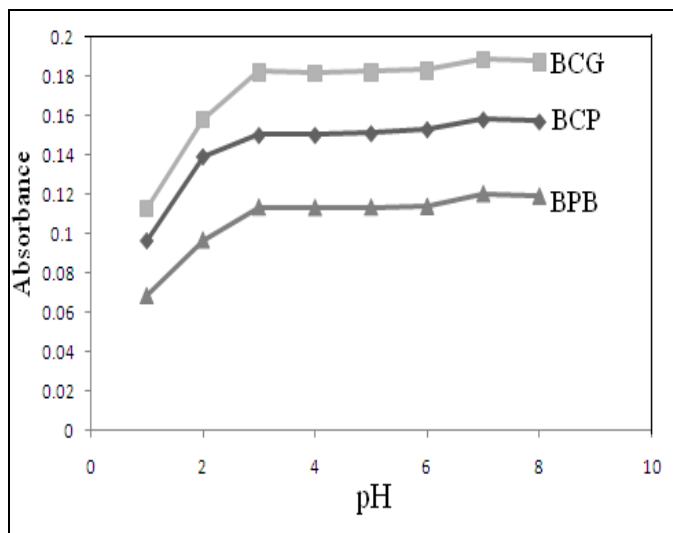


FIG. 8: EFFECT OF pH
[Drug] = $8 \mu\text{g ml}^{-1}$, [Dye] = 5ml of 0.025%

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Ethambutol ($8 \mu\text{g ml}^{-1}$). It is

apparent from Fig. 9 that the maximum absorbance, in each case, was found with 3.5 ml of dyestuff, beyond which absorbance was constant. Thus, 5 ml of each dyestuff was used for ion-pair formation throughout the experiment.

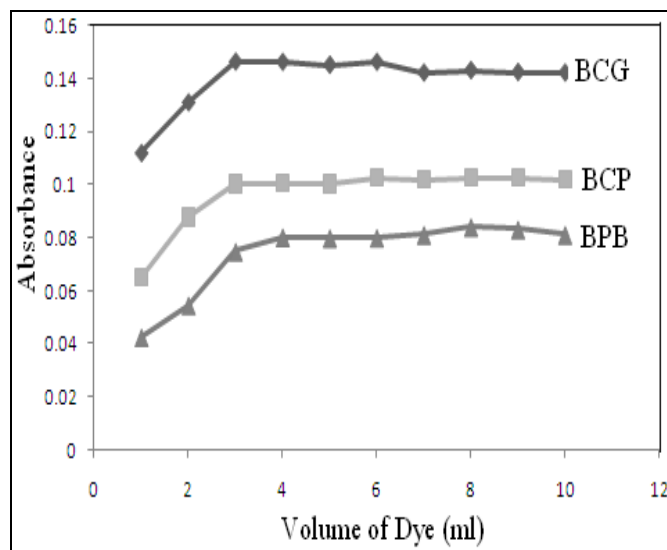


FIG. 9: INFLUENCE OF THE VOLUME OF 0.025% DYE
[Drug] = $8 \mu\text{g ml}^{-1}$

A systematic study of the effect of foreign species present along with Ethambutol on the determination of Ethambutol at $8 \mu\text{g ml}^{-1}$ levels was undertaken. This study was carried out by following the proposed procedures for a 10 ml sample system, by adding a known amount of foreign species to an Ethambutol solution of $8 \mu\text{g ml}^{-1}$. Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

TABLE 4: INTERFERENCE STUDY

Sl.no	Excipients	Tolerance limit ($\mu\text{g ml}^{-1}$)
1	Microcrystalline cellulose	88
2	Starch	155
3	Lactose	132
4	Silicon dioxide	68
5	Titanium dioxide	36
6	Magnesium stearate	32

Validation of the proposed method:

All the three proposed methods have been validated in terms of guideline proposed by

International Conference on Harmonization³¹ viz. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student *t*-test and variance *F*-test have been performed in comparison with a reference method. **Table 1** summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries. To test the reproducibility of the proposed methods, six replicate determinations of 10 μ g ml⁻¹ of Ethambutol were made. The coefficient of variation was found to be less than 1.2% for all the procedures.

The proposed methods have been successfully applied to the determination of Ethambutol in pharmaceutical preparations. The performance order of the proposed methods is BCG>BCP>BPB. The results obtained and shown in **Table 2** and **Table 3** were compared to those obtained by a reference method³¹ by means of *t*-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level.

CONCLUSION: Ethambutol forms ion-pair complexes with acidic triphenylmethane viz., bromocresol green, bromocresol purple and bromophenol blue in 1:1 proportion. These complexes are extractable into chloroform and their maximum absorbances offer a basis for assay of the drug. The developed methods are simple, sensitive, reproducible and can be used for routine analysis of Ethambutol in pure and pharmaceutical dosage forms.

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REFERENCES:

- Hans LR: Interventions for Tuberculosis Control and Elimination. International Union Against Tuberculosis and Lung Disease (IUATLD), March 2002.
- Bailey WC, Albert RK, Davidson PT, Farer LS, Glassroth J, Kendig EJ, Loudon RG, Inselman LS: Treatment of tuberculosis and other mycobacterial disease, An official statement of the American Thoracic Society. American Review of Respiratory Disease 1983; 127:790-796.
- American Thoracic Society, Diagnosis and treatment of disease caused by non-tuberculous mycobacteria. American Journal of Respiratory and Critical Care Medicine 1997; 156:S1-25.
- Gangadharam PRJ, Candler ER: Microbiological assay of Ethambutol. Antimicrobial Agents and Chemotherapy 1997; 3:57-63.
- Ching SL, Leslie Z: Micro and macro GLC determination of Ethambutol in biological fluids. Journal of Pharmaceutical Sciences 1978;67:470-473.
- Vaidyanathan TS: Methods for the assay of Ethambutol in Tablets. Tuberculosis Chemotherapy Centre, Madras. Report on research activities; 1970. p.38.
- Ethambutol Tablets. British Pharmacopoeia. Vol II. Her Majesty's Stationery Office, London; 1980. p.767.
- Lederle Laboratories. Myambutol- Ethambutol hydrochloride-oral antituberculosis therapy. 2nd ed. American Cyanamid Company, New York; 1968. p.64.
- Prema G, Narayanan ASL, Raghupathi Sharma G, Somasundaram PR: Assay of Ethambutol in pharmaceutical preparations. Lung India 1984; 1:143-145.
- Magare BK, Mehete AR, Magar BK, Bhosale VN: Development of Spectrophotometric method for the determination Cu (II) ion using Ethambutol hydrochloride drug as an analytical reagent. Journal of Chemical, Biological and Physical Sciences Section A 2014; 4(2):913-917.
- Chenevier P, Massias L, Gueylard D, Farinotti R: Determination of Ethambutol in plasma by high-performance liquid chromatography after pre-column derivatization. Journal of Chromatography B, Biomedical Sciences and Applications 1998; 708:310-315.
- Breda M, Marrari P, Pianezzole E, Strolin BM: Determination of Ethambutol in human plasma and urine by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography A 1996; 729:301-307.
- Ranganath MK, Chandramouli R, Sandeep K, Prasad K: Method development and validation of Anti-tubercular drugs in fixed dose formulation by HPLC technique. International Journal of Universal Pharmacy and Biosciences 2013; 2(4):432-439.
- Kumud S, James DT, Suresh K, Ramesh N, Sasijith SL: Method Development and Validation of Ethambutol in Human Plasma by LCMS/MS. International Journal of Research in Pharmaceutical and Biosciences 2011;1:1-6.
- Conte JE Jr, Lin E, Zhao Y, Zurlinden E: A high-pressure liquid chromatographic-tandem mass spectroscopic method for the determination of Ethambutol in human plasma, bronchoalveolar Lavage fluid, and Alveolar cells. Journal of Chromatographic Science 2002; 40:113-118.
- Chen X, Song B, Jiang H, Yu K, Zhong D: A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of isoniazid and Ethambutol in human plasma. Rapid Communications in Mass Spectrometry 2005; 19:2591-3596.

17. Gong Z, Basir Y, Chu D, McCort-Tipton M: A rapid and robust liquid chromatography/tandem mass spectrometry method for simultaneous analysis of anti-tuberculosis drugs-Ethambutol and pyrazinamide in human plasma. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life sciences* 2009; 877:1698-1704.
18. The Pharmacopoeia Commission of PRC. The Pharmacopoeia of the People's Republic of China: Chemical Industry Publishing House, Beijing, China; 2005. p.456.
19. TL Ng: A gas chromatographic mass spectrometric study of the trimethylsilylation of Ethambutol and a tablets assay method based on the trimethylsilyl derivative. *Journal of Chromatographic Science* 1982; 20: 479-482.
20. Ming Yan, Tao Guo, Hongtao Song, Qingchun Zhao, Yin Sui: Determination of Ethambutol hydrochloride in the combination tablets by precolumn derivatization, *Journal of Chromatographic Science* 2007;45:269-273.
21. Chaitanya Krishna A, Saravanan RS, Sathiya Raj M, Jeevanantham S, Baskaran R: Determination of Ethambutol in presence of fixed dose combination molecules from human plasma by direct injection to Liquid Chromatography Tandem Mass Spectrometry. *Clinical Pharmacology & Biopharmaceutics* 2012; 1(2):101-106.
22. Shingbal DM, Naik SD: Colorimetric determination of Ethambutol hydrochloride. *Journal – Association of Official Analytical Chemists* 1982; 65(4): 899-900.
23. Gurumurthy P, Gayathri T N, Bhagavathy S, Venkatesan P: Standardization of the method for estimation of Ethambutol in pharmaceutical preparations and biological fluid. *Indian Journal of Experimental Biology* 2004; 42(1):68-73.
24. Adriana FF, Luiza FM, Juliana PV: Ethambutol analysis by Copper complexation in pharmaceutical formulations: Spectrophotometry and Crystal structures. *Journal of Brazilian Chemical Society* 2011; 22(5):867-874.
25. Abdel MIM, Fardous AFM, Noha NA, Seham MB: Ethambutol – Cobalt(II) ions complexation spectral characteristics and applications for quantitative analysis. *Pakistan Journal of Pharmaceutical Sciences* 2015; 28(2):603-609.
26. Sekar AM, Anbazhagan S, Baskar A, Saranya N, Rajakumari D: Spectrofluorimetric quantification of Ethambutol hydrochloride in bulk and oral dosage forms. *Indo American Journal of Pharmaceutical Research* 2014; 4(7):3349-3354.
27. Pratibha Singh, Siddhartha Singh, Chauhan RPS, Afshan Suraiya: Micro determination of some antituberculous drugs with AHC reagent. *American International Journal of Research in Formal, Applied and Natural Sciences* 2014; 14:73-75.
28. Vosburgh WC, Cooper GR: The identification of complex ions in solution by spectrophotometric measurements. *Journal of American Chemical Society* 1941; 63:437-442.
29. Likussar W, Boltz DF: Theory of continuous variations plots and a new method for spectrophotometric determination of extraction and formation constants. *Analytical Chemistry* 1971; 43:1265-1272.
30. Momoki K, Sekino J, Sato H, Yamaguchi N. Theory of curved molar ratio, plots and new linear plotting method. *Analytical Chemistry* 1969; 41:1286-1299.
31. ICH (International Conference on Harmonization) of Technical Requirement for the Registration of Pharmaceuticals for Human use, Validation of analytical procedures: definitions and Terminology Genera. 1996.

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