ASSAY OF ETHAMBUTOL IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

Ethambutol tablets of 200 and 400 mg denominations were assayed by the standard non-aqueous titration method and a simpler calorimetric method. With the titrimetric method, assay values, appreciably higher than the stated content (117% or more), were obtained with the products of 4 companies, while all the values were within 6% of the stated content by the calorimetric method. Rifampicin and pyrazinamide interfered with the estimation of ethambutol by both methods: isoniazid, however, caused an overestimation with the titrimetric method only.

Introduction

The need for checking the stated content of a drug in a pharmaceutical preparation prior to administering to patients cannot be over-emphasized. Previous experience with cycloserine (Rao *et al,* 19681) and with a syrup formulation of isoniazid (Rao *et al.* 1971[2]) has shown that drug preparations could lose their potency when stored; for this reason, it is now customary at the Tuberculosis Research Centre to routinely assay the content of anti-tuberculosis drugs in various pharmaceutical preparations.

Ethambutol, the dextro-rotatory isomer of 2,2'–(ethylenediimino)-di-I-butanol dihydrochloride, has been routinely assayed using a non-aqueous titration method (Vaidyanathan, 1970[3], British Pharmacopoeia, 19804). It was observed that with certain pharmaceutical preparations, values appreciably higher than the stated content were obtained, and occasionally, the end-point of titration was not sharp. Another procedure for the determination of ethambutol content in pharmaceutical preparations described by the Lederle laboratories (Lederle Laboratories, 19685). involving reaction with cupric chloride and measurement of the absorption in the visible range, is time-consuming as it needs the use of celite columns. This procedure has been simplified primarily by avoiding the use of celite columns, and the results obtained with this method (calorimetric method) have been compared with those obtained by the non-aqueous titration method (titrimetric method).

Material and Methods

Ethambutol was procured from 8 different companies, namely, Biddle Sawyer Private Ltd., Cadilla Laboratories Private Ltd., Cyanamid India Ltd. (Lederle Division), Lupin Laboratories Private Ltd., Pharmaceutical Corporation of India Ltd., Pharmed Private Ltd., Sarabhai M. Chemicals and Themis Chemicals Ltd. Products of 5 of these companies for the 200 mg tablets and those of 7 companies for the 400 mg tablets were assayed by the titrimetric and the colorimetric methods, using 5 tablets of each. Each tablet was crushed to a fine powder after its weight was recorded. Two portions of the powder (each approximately, a third of the total tablet weight) were accurately weighed and used for assay by the two methods. The assays were undertaken after randomising and coding the samples independently for each method.

Pure ethamubutol dihydrochloride powder was obtained from Lederle laboratories; all reagents were of analytical grade.

Titrimetric method : To the portion of the powdered tablet in a 250 ml Erlenmeyer flask were added 50 ml of glacial acetic acid and the contents were stirred for 30 minutes on a rotary shaker. Ten ml of a 5% solution of mercuric acetate in glacial acetic acid were then added followed by 10 drops of a 0.1% solution of α -naphtholbenzein indicator in glacial acetic acid. The solution was titrated against 0.1 N perchloric acid in glacial acetic acid, the appearance of a green colour indicating the end-

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point of titration. One ml of 0.1 N perchloric acid is equivalent to 0.01386g of ethambutol dihydrochloride; the content of ethambutol in the tablet was calculated on the basis of the titre value and the amount of the powder taken for assay.

Calorimetric method: To the portion of the powdered tablet in a 250 ml Erlenmeyer flask were added 6 ml of distilled water. The contents were mixed, 0.5 ml of 10 N sodium hydroxide solution was added, the contents mixed again and left at room temperature for 5 minutes. To this mixture were then added 25 ml of chloroform and the contents were shaken on a rotary shaker for 30 minutes. The chloroform layer was separated and filtered through a Whatman No. 1 filter paper. To 5 ml of the chloroform extract were added 5 ml of a 1% solution of cupric chloride in methanol and the contents mixed. The absorption was measured at 687 nm in a Pye-Unicam SP 30 spectrophotometer using cells of 1 cm path-length with the spectrophotometer set to zero optical density with a distilled water blank processed similarly. Ethambutol dihydrochloride standards of 100 and 200 mg were set up simultaneously and processed similarly.

Results

The assay results of ethambutol tablets (both 200 and 400 mg denominations) by the titrimetric and the calorimetric methods are presented in the table.

TABLE

ASSAY OF ETHAMBUTOL BY COLORIMETRIC AND TITRIMETRIC METHODS

Preparation*	Mean @ assay values (mg) of ethambutol by two methods							
	200 mg tablet+				400 mg tablet+			
	Colorimetric		Titrimetric		Colorimetric		Titrimetric	
	mg	%+	mg	%+	mg	%+	mg	%+
I	187	*94*	184	*92*	406	*102*	380	*95*
II	200	*100*	179	*90*	383	*96*	386	*96*
III	200	*100*	216	*108*	413	*103*	419	*105*
IV	204	*102*	303	*151*	382	*96*	499	*125*
V	208	*104*	256	*128*	--	–	–	–
VI	–	–	–	–	377	*94*	376	*94*
VII	–	–	–	–	397	*99*	467	*117*
VIII	–	–	–	–	426	*106*	467	*117*

* Listed according to increasing order of assay results (for 200 mg) by colorimetric method: for names of companies, see text.

@ Mean of 5 tablets. + Stated content.

The accepted limits for ethambutol are 95-105% of the stated content (British Pharmacopoeia, 198C[4]). Considering the 200 mg tablets, the mean assay values by the colorimetric method were within the accepted range for 4 preparations, and just beyond the limit (94%) for the other. With the titrimetnc method, the mean assay values were beyond the accepted limits for all preparations, and in 2 of them the values were appreciably higher than the stated content (128% and 151%). With the 400 mg tablets, the mean values by the colorimetric method were within the accepted limits for 5 preparations, and just beyond the range (94% and 106%) for the other 2 preparations. With the titrimetric method, the values were within the accepted limits for 3 preparations, just beyond the limit (94%) for another, and appreciably higher than the stared content (117%, 117% and 125%) for the remaining 3 preparations.

The mean assay values by the 2 methods were similar for the products of 4 companies; the values were significantly higher ($P \leq 0.01$) by the titrimetric method for the products of the remaining 4 companies The co-efficient of variation for replicate estimates (for both 200 and 400 mg denominations) ranged from 2.4 to 8.2% for assay by the colorimetric method. and from 2.1 to 12.4% by the titrimetric method.

Interference due to some anti-tuberculosis drugs : Interference in the assay procedures due to isoniazid, rifampicin and pyrazinamide was also examined. The concentrations set up corresponded to combinations of ethambutol 400 mg with isoniazid 100 mg, rifampicin 150 mg and pyrazinamide 500 mg. Results (not tabulated) indicated that with the titrimetric method, isoniazid caused a considerable overestimation (about 100%), and it was difficult to discern the end-point in the presence of rifampicin and pyrazinamide, the former due to deep red colour, and the latter due to formation of a precipitate. With the colorimetric method, there was no interference due to isoniazid, but rifampicin and pyrazinamide caused appreciable over-estimation (about 45% and 30%. respectively).

Discussion

With the non-aqueous titration method for the assay of ethambutol (Vaidyanathan, 1970[3], British Pharmacopoeia, 1980[4]) the end-point of titration was sometimes difficult to discern. Moreover, certain excipients, probably the basic ones, and isoniazid

appeared to cause a considerable over-estimation, and the titrimetric method could not be used in the presence of rifampicin and pyrazinamide. The principle of the colorimetric method of assay, viz.. that of complexing ethambutol with cupric chloride and measuring the absorption at 687 nm, is similar to that described earlier (Lederle Laboratories, 1968[5]), but the modified method described here is simpler and less time-consuming as it avoids the use of celite columns. While isoniazid and excipients (from products of 8 different companies) did not interfere in the assay of ethambutol by the modified colorimetric method, rifampicin and pyrazinamide appeared to have caused appreciable over-estimation. In view of these observations, it is, therefore, recommended that the content of ethambutol in pharmaceutical preparations (in the absence of rifampicin and pyrazinamide) be determined by the colorimetric method as described in this paper.

REFERENCES

1. Rao. K.V.N., Eidus, L., Evans. C., Kailasam, S., Radhakrishna, S., Somasundaram.,P.R., Stott, H., Subbammal, S. and Tripathy, S. P. (1968). Deterioration of cycloserine in the tropics, Bulletin of worid Health organisation *39:* 781-789.

2. Rao. K.V.N., Kailasam, S., Menon, N.K. and Radkhakrishna. S. (1971). Inactivation of isoniazid by condensation in a syrup preparation. Indian J Med Res *59:* 1343-1353.

3. Vaidyanathan, T. S. (1970). Methods for the assay of Ethambutol in Tablets. Tuberculosis Chemotherapy Centre, Madras, Report on Research activities (1970-1971). P-8

4. British Pharmacopoeia (1980). Ethambutol tablets. Her Majesty's Stationery Office, London, Vol. II, 767-768.

5. Lederle Laboratories (1968). Myambutol(R) Ethambutol hydrochloride–oral antituberculosis therapy, II edition, American Cyanamid Company, New York, p 64-65.

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