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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TERBUTALINE AND ETOPHYLLINE

Yash S. Nandwani, Santosh V. Gandhi*, Shreeyash R. Tapale

AISSMS College of Pharmacy, Kennedy Road, Pune-411 001, India.

*Corresponding author e-mail: santoshvgandhi@rediffmail.com

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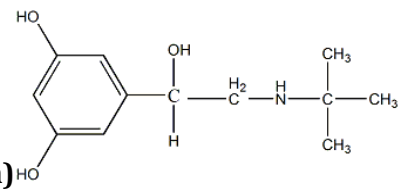
ABSTRACT:
The present work describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric (zero crossing) method for the simultaneous determination of Terbutaline and Etophylline in fixed dose combination.The first order derivative absorption at 280 nm (zero cross point for Etophylline) and 272.5 nm (zero cross point for Terbutaline) was used for Terbutaline and Etophylline determination, respectively. The linearity of Terbutalineand Etophyllinewas observed in the range of 5-30 µg/ml with correlation coefficient (r^2) of 0.9981 and 0.9979, respectively.The mean % recoveries were found to be in the range of 100.09 % and 96.86 % for Terbutaline and Etophylline, respectively. The method was validated as per ICH Q2(R1) guidelines.

Key words: Derivative spectroscopy, Terbutylline, Etophylline, Validation.

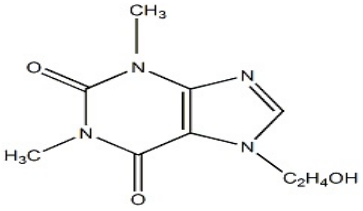
1. INTRODUCTION:

Terbutaline (TER) is 5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1,3-diol (Fig 1 a). TER is selective beta 2 adrenergic bronchodilator[1]. Literature survey reveals UV [2], HPLC [3-4] methods for estimation of TER in alone and in combination with other drugs. Etophylline (ETO) is chemically 1H-Purine-2,6-dione, 3,7- dihydro-7-(2-hydroxyetyl)-1,3- dimethyl (Fig 1 b). ETO is the hydroxyl ethyl ester of theophylline. It belongs to xanthine group of drugs. It acts on respiratory tract by inhibiting phosphodiesterase, which degrades cyclic nucleotides, hence increased amount of intra cellular CAMP molecule causing smooth muscle relaxation [5]. Literature survey reveals HPLC [6-7] and visible spectrophotometry [8] methods for determination of ETO with other drugs in combination.ETO is not official in any pharmacopeia while TER is official in IP and USP [9-10].

The purpose of present work describes the development of a simple, precise, accurate and economic spectro-photometricmethod for the simultaneous estimation of TER and ETO in fixed dose combination. As there is no reported method on the above combination,this work was undertaken. The developed method was validated in accordance with ICH guidelines and successfully employed for the assay of TER and ETO in fixed dose combination.



a)



b)

Fig. 1: Structure of a) Terbutaline and b) Etophylline

2. MATERIALS AND METHODS

2.1 Instrumentation

A double beam UV/Visible spectro-photometer (Jasco V550, Japan) with matching pair of 1 cm quartz cells was used to record all spectra. Band width was kept 2 nm and scanning was carried at speed of 400 nm/min. Spectra manager software (version 1.53.01) was used to do all data collection. MS-Excel was used to do all statistical calculations.

2.2 Reagents and Materials

TER and ETO bulk powder were kindly gifted by Himalaya Meditek Pvt. Ltd., Dehradun, India. Bulk drug were used without further purification. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

2.3 Preparation of standard stock solution

Stock solution of TER and ETO were prepared by dissolving accurately weighed 10 mg of standard drugs in 10 ml of methanol, separately. The concentration of TER and ETO were 1000 µg/ml from which further 1 ml was pipetted and diluted to 10 ml to achieve concentration of 100 µg/ml of TER and ETO, respectively.

2.4 Methodology

The working standard solutions of TER and ETO were prepared separately in methanol having concentration of 10 µg/ml by dilution of stock solution. They were scanned in the wavelength range of 200-400 nm against solvent methanol as blank. The absorption spectra thus obtained were derivatised to first order. From the overlain spectra of both the drugs (Fig. 2) wavelengths selected for quantitation were 280 nm for ETO (zero cross point of TER) and 272.5 nm for TER (zero cross point of ETO).

2.5 Validation of proposed method

The proposed method was validated according to the ICH Q2 (R1) guidelines.

2.5.1 Linearity

The calibration curves were plotted over a concentration range of 5-30 µg/ml for TER and ETO. Accurately measured standard stock solutions (Concentration 100 µg/ml) of both drugs (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-order derivative absorbance (D1) was measured at 272.5 nm for TER and 280 nm for ETO. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated. The overlain first derivative spectra of TER and ETO (5-30 µg/ml each) in their linear range is given in Fig. 3. The absorbance data for calibration graph is given in Table 1 and calibration graph in Fig. 4 and Fig. 5.

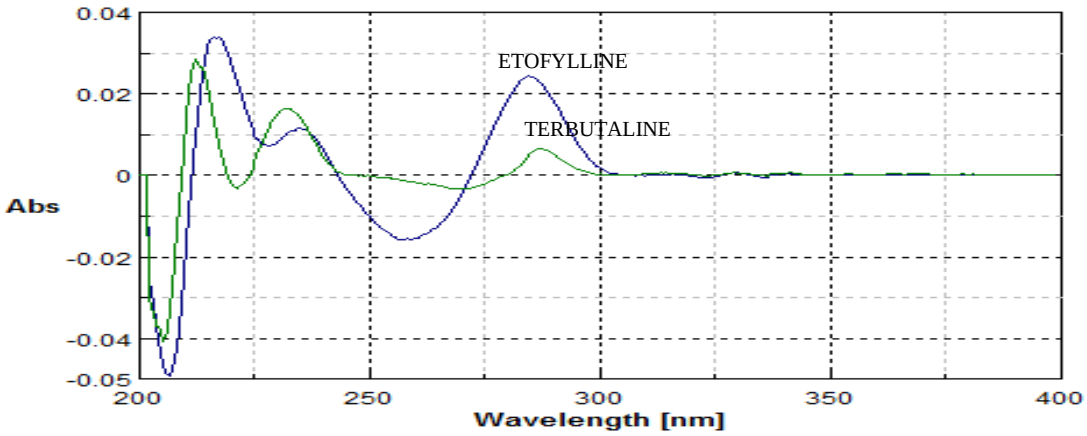


Fig. 2: Overlaid first derivative spectra of TER (10 µg/ml) and ETO (10 µg/ml)

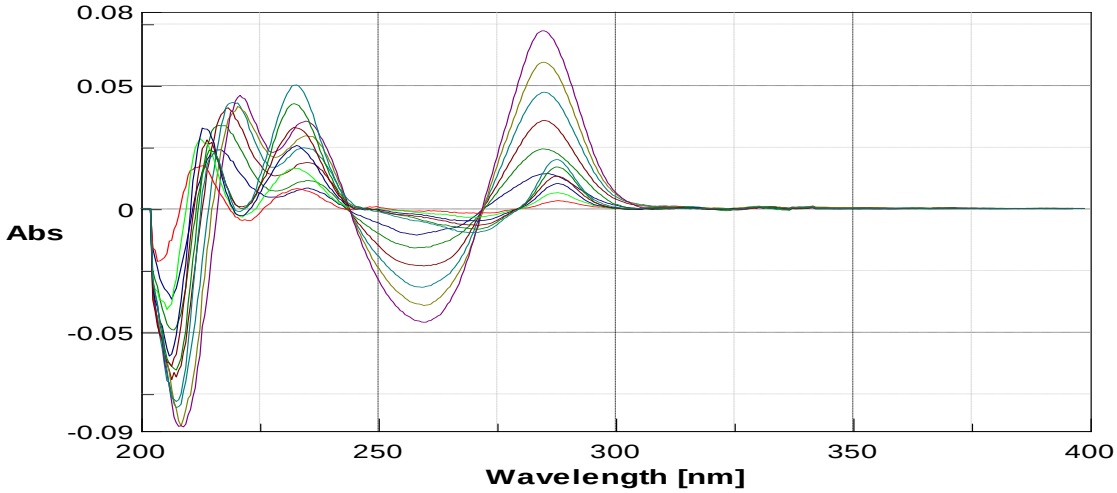


Fig. 3: Overlain first derivative spectra of TER and ETO (5-30 µg/ml each) in their linear range

Table 1: Absorbance data for calibration curve of TER and ETO

Concentration	Absorbance of TER	Absorbance of ETO
5	-0.00208	0.00952
10	-0.00376	0.01728
15	-0.00566	0.02546
20	-0.00716	0.03392
25	-0.00882	0.04432
30	-0.01094	0.05134

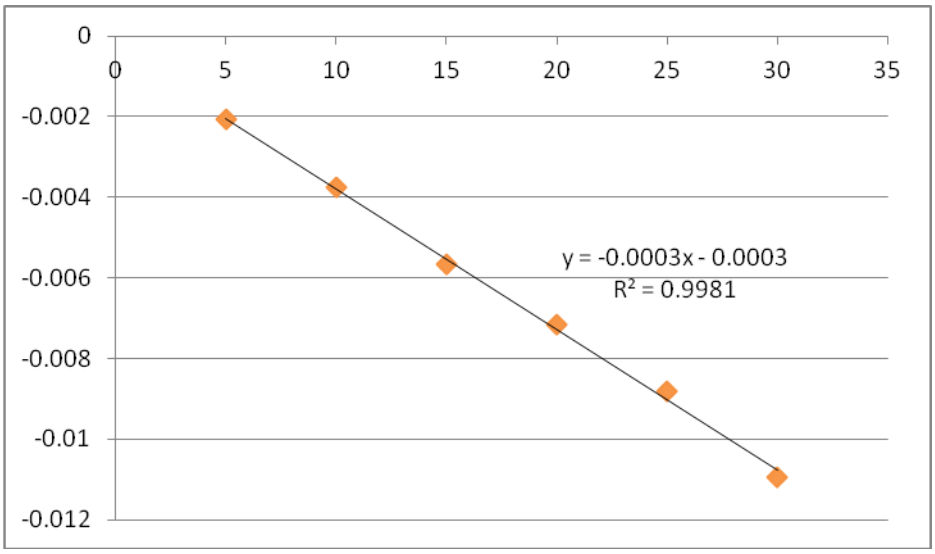


Fig. 4: Calibration graph for TER

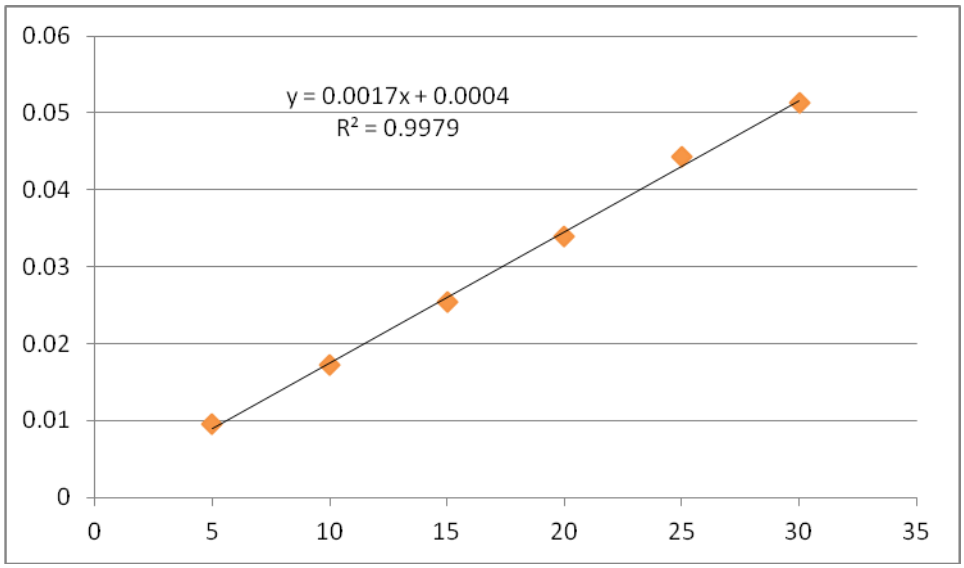


Fig. 5: Calibration graph for ETO

2.5.2 Analysis of drugs in blend sample (Assay)

Accurately weighed finely powdered Lactose (150 mg), Magnesium stearate (5 mg) and Starch (25 mg) were blended with TER (20 mg) and ETO (800 mg) to form synthetic blend of drugs. For proper and uniform homogenization blend were prepared in large quantities. This blend further used to perform assay and to determine accuracy (recovery study).

The quantity of blend equivalent to 10 mg of TER was weighed, dissolved in 10 ml of methanol and filtered. The 1 ml of filtrate (concentration 1000 µg/ml of TER) was further diluted to 10 ml (concentration 1000 µg/ml of TER). This Solution was further diluted to get 10 µg/ml of TER. In similar manner dilutions were made to get another solution having concentration 10 µg/ml of ETO. First derivative absorbance spectra of both the solutions were recorded and

quantification was done through calibration curve. The analysis procedure was repeated six times.

2.5.3 Accuracy study

The accuracy of the method was determined by calculating recovery of TER and ETO by the standard addition method. Known amounts of standard solutions of TER and ETO were added at 50, 100 and 150 % level to prequantified sample solutions of both drugs (10µg/ml for TER and ETO each). The experiment was repeated for three times.

2.5.4 Precision

The precision of the method was demonstrated by intra-day (repeatability) and inter-day (intermediate precision) variation studies. For the intra-day studies, 6 replicates at assay concentrations (10µg/ml for TER and ETO each) were analyzed in a day and percentage relative standard deviation (%RSD) was calculated.

For the inter day variation studies, 3 different concentrations (5, 10 and 15 µg/ml for both drugs) were analyzed on 3 consecutive days and % RSD was calculated. The results obtained for intraday (RSD – 0.98 %) and inter day (RSD - 1.3 %) variations were found to be within limits (less than 2% RSD).

2.5.5 Limit of detection and limit of quantification

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

3. RESULTS AND DISCUSSION

The two drugs can be quantified without separation using first derivative spectroscopy. The wavelengths selected for quantitation were 280 nm for ETO (zero cross point of TER) and 272.5 nm for TER (zero cross point of ETO). The results found linear in the range of 5-30 µg/ml for both the drugs. The developed method was found to be accurate, precise and reproducible as results were in limit. Table 2 represents the results of assay performed. The summary of accuracy results is depicted in Table 3. Summary of results is presented in Table 4.

Table 2: Assay of formulation (synthetic mixture prepared in house)

S. No.	Concentration (µg/ml)		% Assay	
	TER	ETO	TER (272.5 nm)	ETO (280 nm)
1	10	10	100.42	98.64
2	10	10	99.11	99.94
3	10	10	98.86	100.41
4	10	10	101.2	98.89
5	10	10	100.14	98.96
6	10	10	101.81	99.63
		Mean	100.257	99.412
		SD	1.150	0.692
		RSD	1.147	0.696

Table 3: Accuracy study of TER and ETO by proposed method and their % RSD

Level %	Sample		Standard		%Recovery , ±%RSD	
	TER (µg/ml)	ETO (µg/ml)	TER (µg/ml)	ETO (µg/ml)	TER	ETO
50	10	10	5	5	100.873±0.535	100.890± 1.068
100	10	10	10	10	99.370± 1.028	100.843± 0.930
150	10	10	15	15	100.493± 1.196	99.940± 0.521

Table 4: Summary of results by proposed first derivative method

Parameters	Terbutaline (TER)	Etofylline (ETO)
λ Determination (Ist Derivative)	272.5 nm	280 nm
Linearity	5-30 µg/ml	5-30 µg/ml
Accuracy		
50 %	100.873 ± 0.535	100.890 ± 1.068
100 %	99.370 ± 1.028	100.843 ± 0.930
150 %	100.493 ± 1.196	99.940 ± 0.521
LOD	0.773 µg/ml	0.121 µg/ml
LOQ	2.345 µg/ml	0.369 µg/ml
Precision (% RSD)		
Intraday	0.136 %	0.114 %
Interday	0.270 %	0.248 %
% Assay	100.257 ± 1.147	99.412 ± 0.696

4. CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 5-30µg/ml for TER and ETO. The results of the analysis of fixed dose combination by the proposed method are reproducible and reliable. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of TER and ETO. The method can be used for the routine analysis of these drugs in pharmaceutical formulation.

5. ACKNOWLEDGEMENT

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