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UV SPECTROPHOTOMETRIC ESTIMATION AND VALIDATION OF THEOPHYLLINE AND TERBUTALINE SULPHATE IN BULK AND SYNTHETIC MIXTURE FORMULATION

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ABSTRACT

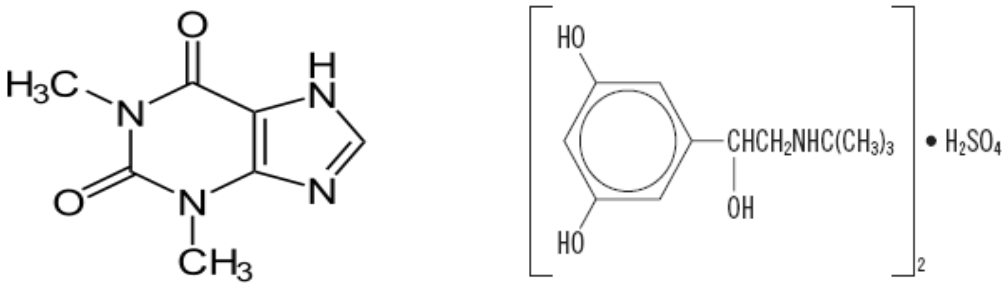
Two sensitive, precise, accurate, simple, and economical methods were developed and validated for simultaneous estimation of Theophylline and Terbutaline sulphate in Synthetic mixture form. Method A is simultaneous equation method, wherein the wavelengths selected for Theophylline is 274.8 nm and for Terbutaline sulphate is 295.6 nm. Whereas Method B is absorption ratio method wherein the wavelength selected for isosbestic point is 287.3 nm and 274.8 nm maximum absorption of Theophylline. In both methods, Theophylline and Terbutaline sulphate followed the linearity concentration range 4-24 mcg/ml for Theophylline and 2-12 mcg/ml for Terbutaline sulphate by these methods. Standard calibration curve for both Theophylline and Terbutaline sulphate with correlation coefficient (r^2) value is 0.998 for Theophylline and 0.997 for Terbutaline sulphate for method A whereas 0.998 for Theophylline and 0.997 for TES for method B. The proposed methods were validated according to ICH guidelines in terms of linearity, accuracy, precision, LOD and LOQ. Percentage assay was found to be in the range 100.20 – 100.75 for method A and 100.13-100.73 for method B. In precision % RSD was found to be 0.705 & 0.364 for Theophylline and 0.899 & 1.238 for Terbutaline sulphate for method A and 1.295 & 0.440 for Theophylline for method B and 0.548 & 2.433 for Terbutaline sulphate for method B. The mean percent recoveries was found to be in range 99.82-101.17% for method A and 100.14 – 100.96 for method B. LOD and LOQ values found to be 0.0496 & 0.150 for Theophylline and 0.442 & 1.342 for Terbutaline sulphate for method A and 0.0524 and 0.158 for Theophylline & 0.150 & 1.342 for Terbutaline sulphate for method B. Degradation conditions were carried under conditions of photolytic, sunlight and thermal degradation.

Keywords *– Theophylline, Terbutaline sulphate, Simultaneous equation method, Absorption ratio method, UV Spectroscopy, Validation.*

1. INTRODUCTION

The pharmaceutical formulations with combinations of drugs have shown an increasing trend to counteract the symptoms specific to one drug and formulation, and hence analytical chemist will have to accept the challenge of developing reliable and easy simultaneous methods because it does not require manual individual calculations and marginally give better results[1].

Theophylline (1,3 dimethyl, 2-3-6-7-tetrahydro-1-H Purine-2,6- dione. Theophylline is Xanthine dr. which is used for the treatment of asthma and bronchial obstructive diseases. It acts competitively inhibiting the Type 3 and Type 4 phosphodiesterase, the enzyme responsible for breaking down the cyclic AMP in smooth muscle cells, possibly resulting in bronchodilation.



a) Theophylline **b) Terbutaline sulphate**

Fig.1: Chemical structure

Terbutaline sulphate (2-[terbutylamino]1-(3-5 dihydroxyphenyl) ethanol sulphate. Terbutaline sulphate is a beta-adrenergic receptor agonist which is used in the treatment of bronchospasm, bronchitis, asthma, and emphysema. It acts by stimulation through the beta adrenergic receptors, the enzyme that catalyzes the conversion of triphosphate (ATP) to 3, 5-adenosine monophosphate (cAMP), The increased cAMP levels are associated with the relaxation of smooth muscles of the bronchi [2-4].

According to literature survey revealed that most of the methods such as bioanalytical, spectrometric, HPLC, RP-HPLC are reported for the THE as well as TES either single or in combination with other drugs. But not any UV spectrophotometric method is reported for this combination [5-17].

2. MATERIALS AND METHODS

2.1 Reagents and chemicals

A Shimadzu UV Visible double beam spectrophotometer (UV Model – 1800) and 1 cm UV matched quartz cells were used. A Shimadzu electronic analytical balance (Shimadzu model no. AA2200) and Ultrasonicator (HMG India) were used. Pharmaceutically pure samples of THE and TES were obtained from Blue cross pharma, Nashik Pvt. Ltd. and Bidwai pharmaceuticals Pvt. Ltd. Nanded, respectively. Sodium hydroxide (NaoH) AR grade was used as a solvent.

2.2 Preparation of Standard stock solution

Accurately 10 mg each of THE and TES were weighed separately and transferred to different 100 ml volumetric flask, volume was made up to the mark with 0.1 N NaoH. The standard stock solutions (100 µg/ml) further dilutes separately to obtain working standard of concentrations 10 µg/ml for THE and 10 µg/ml for TES each.

2.3 Study of spectra and selection of wavelength

Each working standard concentration were scanned in the range 200 – 400nm in 1 cm cell against 0.1 N NaoH as a blank. Maximum absorbing wavelength of THE and TES were selected from spectral data and isobestic wavelength selected from overlain spectra of zero order. The absorbance peak is showed at 274.8nm for THE and 295.6nm for TES and Overlain spectra shows isobestic point at 287.3 nm, respectively.

2.4 Method 1st: Simultaneous estimation method

For each drug dilutions were done to obtained 20 µg/ml for THE and 10 µg/ml for TES from standard stock solution using 0.1 N NaoH and were scanned separately in the UV range from 200-400 nm. Represent the overlain spectrum of both the drug. The wavelength of THE and TES for simultaneous equations were 274.8 nm and 295.6 nm, respectively.

Working standard solutions (100 µg/ml) of both the drug were diluted to prepare solutions having concentrations 4,8,12,16,20,24 µg/ml and 2, 4, 6, 8, 10, 12 µg/ml of both THE and TES respectively. All the solutions were measured at both the wavelengths and calibration curves were constructed. The absorbances were measured at selected wavelengths and absorptivity's (A 1%, 1cm) for both drugs at both wavelengths were determined as a mean of 5 independent determinations. Concentration in the sample were obtained by the following equations:

$C_X = (A_1ax_2 – A_2ax_1) / (ax_2ay_1 – ax_1ay_2)$ ……. (1^{st})

$C_Y = (A_2ay_1 – A_1ay_2) / (ax_2ay_1 – ax_1ay_2)$ ……. (2^{nd})

Where, C_X and C_Y are the concentration in g/100 ml of THE and TES respectively.

ax_1 is the absorptivity of TES at 295.6 nm, ax_2 is the absorptivity of THE at 274.8 nm

ay_1 is the absorptivity of TES at 295.6 nm, ax_2 is the absorptivity of THE at 274.8 nm

A_1 and A_2 are the absorbances of mixture at 274.8 nm and 295.6 nm, respectively.

2.5 Method 2nd: Q Analysis (or) Absorption ratio method

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being λ max of one of the two components. From the overlay spectra of two drugs, THE and TES shows an isoabsorptive point at 287.3 nm and the second wavelength which is used is 274.8 nm, which is λ max of THE.

Working standard solutions was prepared in 0.1 N NaoH and absorbance at isoabsorptive wavelength is at 287.3 nm and 274.8 nm (λmax of THE). Solution having the concentration 4,8,12,16,20,24 µg/ml and 2,4,6,8,10,12 µg/ml for both THE and TES respectively. All the solutions were measured at selected wavelengths and calibration curve was constructed.

The concentration of drugs in the mixture can be calculated by using following equation:

$C_X = Q_m – Q_y / Q_x – Q_y \times A / ax_1$…… (1)

$Cy = Q_m – Q_x / Q_y – Q_x \times A / ay_1$…… (2)

Where, $Q_m = A_2 / A_1$

$Q_x = ax_2 / ax_1$

$Q_y = ay_2 / ay_1$

A_1 = Absorbances of the mixture at 274.8nm

A_2 = Absorbance of the mixture at 295.6nm

ax_1 & ax_2 are the absorptivities of TES at 295.6 nm

ay_1 & ay_2 are the absorptivities of THE at 274.8 nm

2.6 Method Validation

Method was validated according to ICH guidelines

2.6.1 Linearity

The linearity was at different concentrations of THE and TES for both the method. Concentration range was found to be 4-24 µg/ml for THE and 2-12 µg/ml for TES. Result of linearity are shown in table 2.

2.6.2 Precision

The intraday and interday precision of proposed methods were determined by analyzing the corresponding responses 3 times on the same day and on 3 different days, 3 different concentrations of standard solutions of THE and TES for both methods. Result of precision are shown in table 3.

2.6.3 Accuracy

The accuracy of the method was determined by calculating recovery of THE and TES by the standard addition method. Known amounts of standard solutions of THE and TES were added at 80, 100 and 120 % level to prequantified sample solutions of THE and TES. (20 µg/ml for THE and 10 µg/ml for TES), the amounts of THE and TES were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times at each level result for both methods. Result of accuracy are shown in table 4.

2.6.4 Limit of Detection and Limit of Quantitation

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the drug were derived by calculating the signal to noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines. Result of LOD & LOQ are shown in table 5.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = standard deviation of the intercept of calibration curve

S = slope of the calibration curve.

2.7 Analysis of Synthetic Mixture

The excipients as per tablet formulation were calculated and added to the THE + TES mixture (2:1 w/w) and mixture was sonicated for 20 minutes after the addition of 0.1 N NaoH. The solution was filtered through Whatman filter paper. The working solution was prepared by dilution with 0.1 N NaoH to obtain the concentration of 20 µg/ml of THE and 10 µg/ml of TES. The working sample solution of synthetic mixture was analyzed by both UV Spectrophotometric methods. The absorbance of sample solution was measured at 274.8 nm and 295.6 nm for simultaneous estimation and the sample solution was measured at 274.8 nm and 287.3 nm for absorption ratio method. The analysis procedure was repeated 6 times with the synthetic mixture. Results of synthetic mixture formulation are shown in table-1.

2.8 Forced degradation studies

2.8.1 Photolytic degradation

Pure drugs were exposed to UV radiations for 4 hours. The sample after exposure to light were accurately weighed 20 mg of THE and 10 mg of TES, transferred to 100 ml volumetric flask diluted with 0.1 N NaoH to get THE 20 µg/ml & TES 10 µg/ml and absorbance was measured at 274.8 nm, 295.6 nm for THE and TES respectively. Finally, absorbance of sample was compared with standard absorbance and percent degradation was calculated.

2.8.2 Thermal degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 80^0c for 4 hours. The sample after exposure to heat were accurately weighed 20 mg THE and 10 mg of TES, transferred to 100 ml volumetric flask, diluted with 0.1 N NaoH to get THE 20 µg/ml & TES 10 µg/ml and absorbance was measured at 274.8 nm and 295.6 nm for THE & TES respectively. Finally, absorbance of sample was compared with standard absorbance and percent degradation was calculated.

2.8.3 Sunlight degradation

Sunlight degradation was carried out by exposing pure drugs to sunlight for 4 hours. The sample after exposure to heat were accurately weighed 20 mg of THE and 10 mg of TES, transferred to 100 ml volumetric flask, diluted with 0.1 N NaoH to get THE 20 µg/ml & TES 10 µg/ml and absorbance of sample was compared with standard absorbance and percent degradation was calculated.

Results of degradation studies are shown in table no.6

3. RESULTS AND DISCUSSION

The validated spectrophotometric method for simultaneous estimation of THE and TES in synthetic mixture form has been developed using 0.1 N NaoH as a solvent. The proposed method for simultaneous estimation of THE and TES in bulk & synthetic mixture form was found to be simple, accurate, economical, rapid. From the overlay spectra λ max of THE 274.8 nm and λ max of TES 295.6 nm respectively and isoabsorptive point observed at 287.3 nm. In both method linearity for detector response was observed in the concentration range 4-24 µg/ml for THE and 2-12 µg/ml for TES respectively. Standard calibration curve for THE and TES with correlation coefficient (r_2) value in the range of 0.998 for THE and 0.997 for TES in method A, whereas 0.998 for THE and 0.999 for TES in method B. In precision % RSD was found to be 0.705 & 0.364 for THE and 0.899 & 1.238 for TES in method A and 1.295 & 0.440 for THE and 0.548 & 2.433 for TES in method B. The mean percent recoveries was found to be in range 99.82-101.17% for method A and 100.14 – 100.96 for method B. LOD and LOQ values found to be 0.0496 & 0.150 for THE and 0.442 & 1.342 for TES for method A and 0.0524 & 0.158 for THE & 0.150 & 1.342 for TES for method B. Degradation conditions were carried under conditions of photolytic, sunlight and thermal degradation.

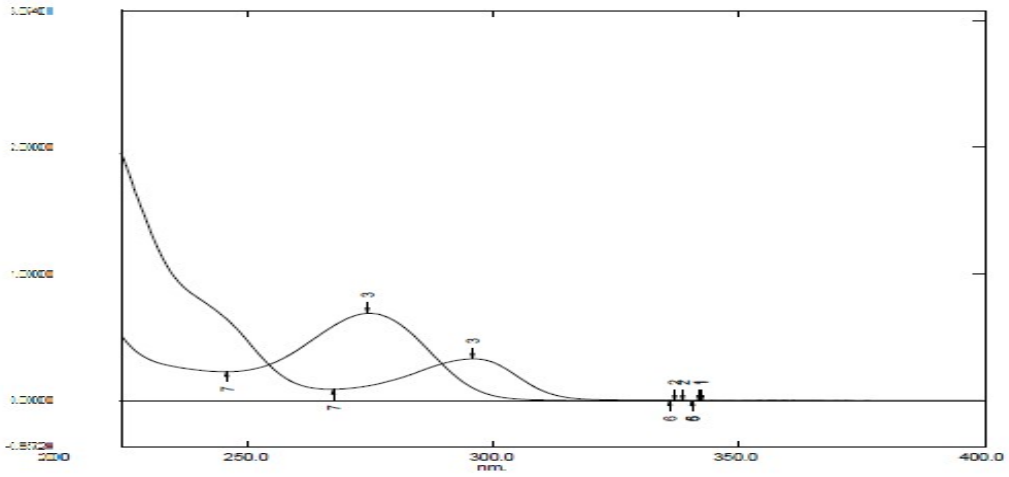
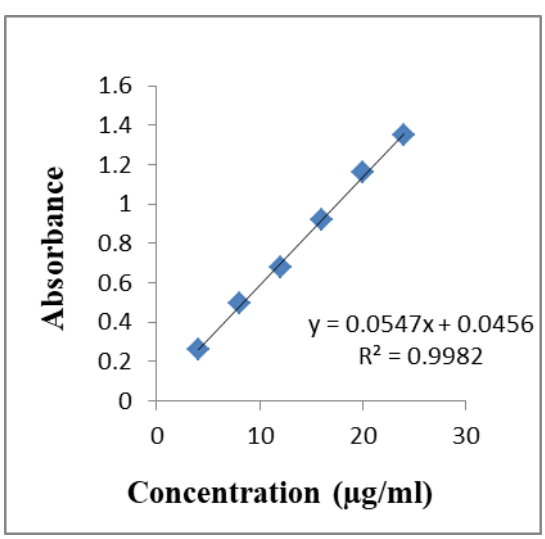
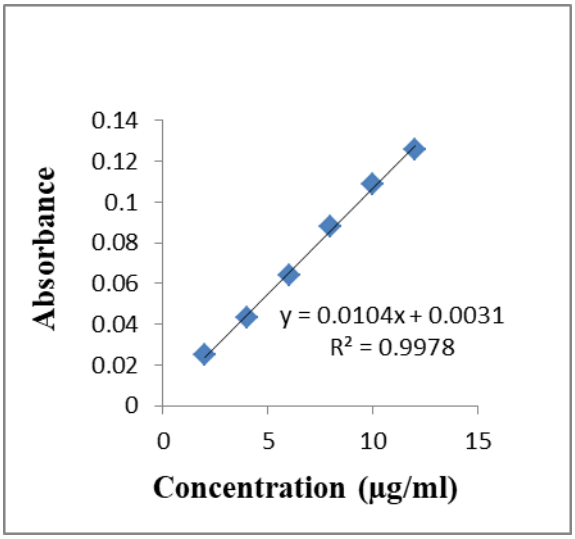


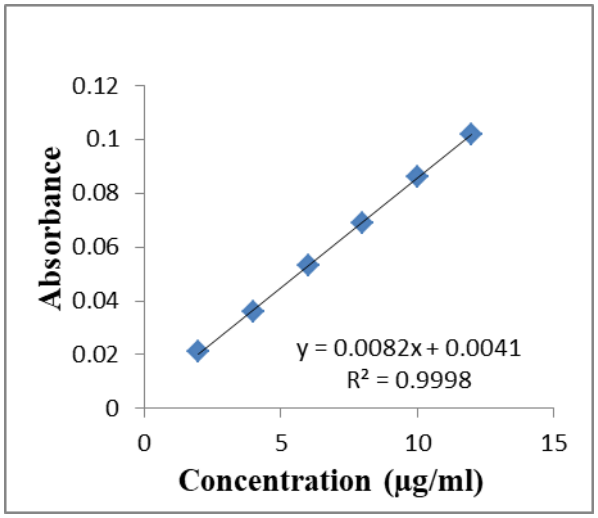
Fig. 2: Overlay spectrum of Theophylline and Terbutaline sulphate



a) Theophylline at 274.8 nm



b) Terbutaline sulphate at 295.6 nm



Terbutaline sulphate at 287.3 nm

Fig. 3: Calibration curve of THE & TES

Table 1: Recovery study data

Level of Recovery	Amount present (mg)		Added Conc.(mg)		Simultaneous equation method				Absorption ratio method			
					Amount recovered (mg)		% Recovery		Amount recovered (mg)		% Recovery	
	THE	TES	THE	TES	THE	TES	THE	TES	THE	TES	THE	TES
80%	20	10	16	8	36.36	17.90	101	99.4	36.29	18.08	100.80	100.44
	20	10	16	8	36.45	17.89	101.25	99.38	36.31	18.13	100.86	100.72
	20	10	16	8	36.28	18.01	100.55	100.77	36.28	18.03	100.77	100.16
100%	20	10	20	10	40.52	19.96	101.03	99.80	40.30	20.09	100.75	100.45
	20	10	20	10	40.49	19.84	101.22	99.2	40.27	19.97	100.67	99.85
	20	10	20	10	40.46	20.27	101.15	101.35	39.15	20.01	99.72	100.09
120%	20	10	24	12	44.60	21.77	101.3	98.72	42.28	22.01	100.66	100.04
	20	10	24	12	44.69	22.03	101.5	100.13	42.23	21.87	101.15	99.40
	20	10	24	12	44.71	21.94	101.6	99.72	42.28	22.03	101.4	100.13

Table 2: Linearity data for THE and TES (4-24 µg/ml and 2-12 µg/ml)

Name of the drug	Simultaneous equation method				Absorption ratio method			
	Linearity range (µg/ml)	r^2	Slope	Intercept	Linearity range (µg/ml)	r^2	Slope	Intercept
THE	4-24	0.998	0.054	0.045	4-24	0.998	0.054	0.045
TES	2-12	0.997	0.010	0.003	2-12	0.999	0.008	0.004

Table 3: Precision data for THE and TES

Sr. No.	Interval of Time	Simultaneous equation method				Absorption ratio method			
		Concentration (µg/ml)		% Recovery		Concentration (µg/ml)		% Recovery	
		THE	TES	THE	TES	THE	TES	THE	TES
I	Intra-day	20	10	99.4	98.8	20	10	101.2	101.09
II		20	10	100.65	98.71	20	10	101.8	100.02
III		20	10	100.60	100.3	20	10	99.3	100.32
I	Inter-day	20	10	100.64	99.5	20	10	101.6	97.29
II		20	10	100.45	101.7	20	10	101.9	101.83
III		20	10	101.16	99.6	20	10	101.02	101.09

Table 4: Recovery study data of THE and TES

Level of Recovery	Amount present (mg)		Added Conc.(mg)		Simultaneous equation method				Absorption ratio method			
					Amount recovered (mg)		% Recovery		Amount recovered (mg)		% Recovery	
	THE	TES	THE	TES	THE	TES	THE	TES	THE	TES	THE	TES
80%	20	10	16	8	36.36	17.90	101	99.4	36.29	18.08	100.80	100.44
	20	10	16	8	36.45	17.89	101.25	99.38	36.31	18.13	100.86	100.72
	20	10	16	8	36.28	18.01	100.55	100.77	36.28	18.03	100.77	100.16
100%	20	10	20	10	40.52	19.96	101.03	99.80	40.30	20.09	100.75	100.45
	20	10	20	10	40.49	19.84	101.22	99.2	40.27	19.97	100.67	99.85
	20	10	20	10	40.46	20.27	101.15	101.35	39.15	20.01	99.72	100.09
120%	20	10	24	12	44.60	21.77	101.3	98.72	42.28	22.01	100.66	100.04
	20	10	24	12	44.69	22.03	101.5	100.13	42.23	21.87	101.15	99.40
	20	10	24	12	44.71	21.94	101.6	99.72	42.28	22.03	101.4	100.13

Table 5: LOD and LOQ data of THE and TES

	Simultaneous equation method		Absorption ratio method	
Name of the drug	LOD(µg/ml)	LOQ(µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
THE	0.0496	0.150	0.0524	0.158
TES	0.442	1.342	0.150	1.342

Table 6: Degradation study data

Sr. No.	Condition	% Degradation		% Assay	
		THE	TES	THE	TES
1.	Photolytic degradation (UV radiation, room temp. 6hrs)	85.82%	58.27%	14.18%	41.73%
2.	Thermal degradation (60^0c, room temp., 6hrs)	86.93%	70.24%	13.07%	29.76%
3.	Sunlight degradation (keep under sunlight, 4 hrs)	55.93%	37.14%	44.07%	62.86%

4. CONCLUSION

The validated spectrophotometric method was found to be simple, accurate, precise, rapid, selective for the concurrent estimation of drug THE and TES simultaneously in combined synthetic mixture form. The method was validated for various parameters including linearity, accuracy, precision. The developed method can be successfully used for simultaneous estimation of THE and TES in pharmaceutical application.

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