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Research Article

SPECTROPHOTOMETRIC ESTIMATION OF TERBUTALINE SULPHATE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple reproducible and efficient method for the determination of terbutaline sulphate in pharmaceutical dosage form has been developed using spectrophotometric method. The linear dynamic range was 4 to 20 mcg/ml with a coefficient of correlation 0.9993. The validity of the described procedure was assessed analytical parameters were calculate and a full statistical evaluation was performed. The proposed method was successfully applied to the determination of terbutaline sulphate in pharmaceutical formulations without any interference from common excipients. The reproducibility of the method is 99.87-100.66%.

KEYWORDS: Terbutaline sulphate, Spectrophotometry, Validation, Formulation.

INTRODUCTION
Terbutaline sulphate is (RS)-2-(tert – butyl amino) -1- (3, 5 – dihydroxy phenyl) ethanol sulphate [1]. It is used as β adrenoreceptor agonist and is usually given by orally up to 15 mg daily in divided dose1. It is *officially* in I.P., B.P, USP, E.P. several methods such as nonaqueous titration [1-4], HPLC [6,7] validation parameters[8,9], fluorimetric[10], colorimetric[11], LC[12,13] have been reported in the literature for its assay. The aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of terbutaline sulphate in pharmaceutical formulations USP also mention spectrophotometric method, but the time limit is very short (75 sec). By the proposed method the time is extended to 180 sec, hence this method is more convenient. So this method is daily practicable for analytical work.

MATERIALS AND METHODS
Instrument
The instrument used was UV visible recording spectrometer (Schimadzu – chemito) with 1cm matched Quartz cuvettes were used for all absorbance measurements. The detector was set a wavelength of 550nm.
Reagents and Solutions
All the solvents and reagents used are of analytical reagent grade.
Standard drug (100µg/ml): Accurately weighed 50mg of terbutaline sulphate was transferred into a 50ml calibrated flask, dissolved and completing to volume with distilled water. The solution was further diluted suitably with distilled water to get a solution of 100µg/ml concentration.
TRIS buffer solution (0.3m): Requisite amount of TRIS (hydroxyl methyl) amino methane buffer (TRIS buffer) pH 9.5 was dissolved in distilled water.
Antipyrine solution: 2% w/ L-amino antipyrine in solution was prepared in distilled water.
Potassium ferric cyanide solution: 8% w/v potassium ferric cyanide was dissolved in distilled water.
Hydrochloric acid solution (0.1M): Requisite volume of concentrated hydrochloric acid was diluted with distilled water.

Samples solution: Accurately weighed syrup equivalent to 2.5 mg of terbutaline sulphate was taken and suspended in 10ml of water which is in separating funnel. Extract the aqueous layer with four 30 ml portion of chloroform and discarded the organic layers .Then collected the aqueous layer into a 25ml calibrated flask. To the aqueous layer add 5 ml of 0.1M hydrochloric acid and shake for 15 minutes than make up the volume up to 25 ml with distilled water.

Procedure

To 5ml of each of blank sample and standard, add about 35ml of TRIS buffer, 1ml of Antipyrine reagent. Followed by 1 ml of ferric cyanide solution. Mix and makeup the volume up to 50 ml with TRIS buffer, measure the absorbance at 550nm exactly 3 minutes from the addition of ferric cyanide solution against reagent blank. A calibration curve is plotted between concentration and absorbance. Aliquots of sample solutions are treated similarity and the amount of drug present in the sample is determined from the calibration cure. The results are presented in Table 2 and figure1.

Recovery Studies

Recovery studies were carried out by adding various amount of pure drug to the pre-analyzed samples and the resulting mixture is reanalyzed by the proposed method and the results of recovery of studied are presented in Table 3.

RESULTS AND DISCUSSION

The effects of different parameters were studied and optimum conditions are included in the given procedure. It is evident from the absorbance values that the reaction is quite sensitive. The results of the recovery experiments indicate that the method is accurate and reproducible. Commonly used excipients did not show any interference. The results on presented in the table 1, table 2, table 3, table 4 & table 5 indicate that the method can be employed in the routine analysis of terbutaline sulphate in formulations.

Validation

Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as stability, linearity, sensitivity, Precision, accuracy, recovery, specificity, robustness and ruggedness were evaluated in developed methods.

Stability

The standard stock solutions of terbutaline sulphate were stored $4^{o}C$ for 2 months. During this period, the solutions were analyzed with UV spectrophotometric method.

Sensitivity

The limit of quantification (LOQ) is the lowest concentration of terbutaline sulphate on the calibration curve that can be quantified with acceptable precision and accuracy. The LOQ was found as 0.246 µg mL^{-1} (RSD = 1.51%) (n=5) for the proposed method.

Linearity range

Under the experimental conditions, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 4-20 µg/ml for the proposed method. The calibration graphs Figure 1 were constructed after analysis of 5 different concentrations with each concentration was measured six times. Each point of the calibration graph corresponded to the mean value obtained from 5 independent measurements. The regression equations (with standard error of intercept and slope) and correlation coefficients of the mean of 5 consecutive calibration curves are given in Table 1. The regression equation was $y = 0.040x+0.041$ where y is the absorbance and x is the concentration in µg mL^{-1} (r=0.9993).

Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of terbutaline sulphate in the linear range (3, 6 and 9 µg mL^{-1}) were analyzed in 6 independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision) from three measurements of every sample in each series. The precision of the analysis was determined by calculating the relative standard deviation (RSD %). The RSD values of intra-day and inter-day studies varied from 0.96 to 1.90% showed that the intermediate precision of the method was satisfactory (Table 4).

Accuracy and recovery

The accuracy of the method was determined by calculating the percentage relative error (Bias %) between the measured mean concentrations and added concentrations at the same concentration of terbutaline sulphate. Table 4 shows the results obtained for intra and inter-day accuracy. The results obtained for intra and inter-day accuracy were between 0.96-1.80 %. Observed concentration values are in good agreement with the expected ones. Recovery studies for the accuracy of the method were performed by spiking synthetic mixture with known amount of terbutaline sulphate.

Specificity

The spectra obtained from tablet and synthetic tablet solution was identical with that obtained spectrum from standard solution containing an equivalent concentration of terbutaline sulphate tablet solutions showed that the wavelength of maximum absorbance of terbutaline sulphate did not change. It was concluded that the excipients did not interfere with quantification of terbutaline sulphate this method and the proposed method could be considered specific. These data showed there was no spectral interaction in the analysis of terbutaline sulphate in pharmaceutical formulations is proposed method. Therefore, the calibration curve method, which is easier and quick than the standard addition method, was used in quantitative analysis of terbutaline sulphate. This value showed that no significant excipients interference, thus the procedures was able to determination of terbutaline sulphate in the presence of excipients. In the proposed method, there was no need for pre-separation and only centrifugation was applied to make the solution clear.

Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variables such wavelength (302 nm and 306 nm). Each deliberate small change was analyzed 7 independent series containing 15 µg/ml only one parameter was changed in the experiments at a time. The statistically comparison was done with Friedman analysis and no difference was found between result ($p = 0.062 > p = 0.05$) (Table 6). The results obtained from the various conditions were not different compared to the optimum conditions and none of these variables significantly affected the assay of terbutaline sulphate and the proposed method could be considered robust.

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 15 µg mL^{-1} of terbutaline sulphate using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts ($p = 0.075 > p = 0.05$) (Table 7).Thus, the proposed methods could be considered rugged.

Analysis of pharmaceutical formulations

The optimized spectrophotometric method was applied to the direct determination of terbutaline sulphate in tablet using calibration curve method without any sample extraction or filtration. The

Average amount present was determined by taking average of six replicate analysis and the amount present were found to be 79.918±0.570 mg/tab. The percentage RSD was found to be 0.937. The result shows that the proposed method was successfully applied for the assay of terbutaline sulphate in its pharmaceutical formulations (Table 8).

CONCLUSION

The developed spectrophotometric method was simple, sensitive, and specific for the determination terbutaline sulphate in pharmaceutical formulations. The linearity of the drug, which obeys beer's law, was 4-20 µg/ml and it shows that the regression was more than 0.999. The precision of the method was found to be 101.6%. It could be precisely detected and quantified at 0.0811µg/ml and 0.2460 µg/ml respectively. The proposed method will be suitable for the analysis of terbutaline sulphate in pharmaceutical formulations

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Table 1: Experimental parameters for the determination of Terbutaline Sulphate

S.NO.	Parameter	Range
1	λ max (nm)	550
2	Beer's law (µg/ml)	4-20
3	Reproducibility	99.87-100.66
4	Standard deviation	0.012-0.020
5	Coefficient of Variation	0.9993
6	% RSD	0.937
7	Molar absoriptivity ,L mol^{-1} cm^{-1}	1.1905×10^4
8	Regression equation(y)a	Y=0.040x +0.014
9	Sandell's sensitivity, µg/cm^2/0.001 A.U	0.0368844
10	Intercept	0.041
11	Limit of detection ,LOD µg/ml	0.0811
12	Limit of Quantification, LOQ, µg/ml	0.2460
13	Number of data points	5

Table 2: Linearity Study

Concentration (µg/ml)	Absorbance (A^o)
04	0.186
08	0.327
12	0.495
16	0.653
20	0.829

Table 3: Determination of terbutaline sulphate in pharmaceutical dosage forms using the proposed method and reference method

Sl. No	Dosage form	Labeled amount (mg)	Amount found *(mg)		(%) Recovery
			Proposed method	Reference method	
1	A	2.5	2.52	2.52	101.6
2	B	2.5	2.58	2.6	103.2
3	C	1.5	1.50	1.51	100.0

*Each value is an average of 6 determinations

Table 4: Precision and accuracy data of the developed spectrophotometric method for the analysis of terbutaline sulphate (n=5).

Inter-day				Intra-day		
Added µg/ml	**Found [a], µg/ml**	**Precision RSD%**	**Accuracy [b] Bias**	**Found [a] µg/ml**	**Precision RSD %**	**Accuracy Bias**
3.0	3.05±0.02	1.80	1.66	3.02±0.02	1.54	0.86
6.0	6.02±.0.02	1.69	0.33	5.95±0.06	1.21	-0.83
9.0	8.98±0.04	1.14	-0.22	8.92±0.04	0.96	-0.88

Found [a] x= mean± standard error, RSD % = Relative standard deviation, Accuracy [b]= [(found – Added)/Added] ×100

Table 5: The results of percentage recovery value in synthetic mixture of terbutaline sulphate for proposed method (added terbutaline sulphate for tablet 15mg) (n=7)

Found, 15 mg	Recovery, %
14.72	98.60
15.18	100.40
14.96	99.80
14.84	99.20
15.34	101.70
14.82	99.10
15.06	100.30

X: 14.99±0.08, 99.87%, SD: 0.22, RSD%:1.10, X: Mean ± standard error, SD: standard Deviation, RSD %: Relative standard deviation.

Table 6: Robustness data of developed method (n=7).

Solution	Found, µg/ml	RSD%
Standard,10.00µg mL	10.25±0.05	1.41
Sodium hydroxide , 0.1M	10.25±0.07	1.71
Wave length, 302nm	10.32±0.06	1.42
Wave length, 306nm	9.96 ±0.05	1.42
Friedman analysis: P=0.062> P=0.05		

X: Mean ±standard error, X RSD %: Relative standard deviation.

Table7: The ruggedness of proposed method (Added of terbutaline sulphate amount of 10.00 µg mL^{-1}) (n=7).

1. Analyst Found , µg mL^{-1}	2. Analyst found, µg mL^{-1}
X:9.96 ±0.02	X:10.06±0.05
SD:0.04	SD:0.12
RSD %:0.40	RSD %: 1.19

Wilcoxon paired test: p=0.075> p= 0.05, X: mean ±Standard error, RSD %: Relative standard deviation.

Table 8: Assay of pharmaceutical formulations containing terbutaline sulphate analyzed by proposed method (n=6).

Solution	Amount found (mg)	% found	%RSD
80 mg	79.3862	99.23	0.937
	80.4805	100.06	
	78.8013	98.50	
	80.5330	100.66	
	79.6703	99.58	
	80.6402	100.80	
	79.918	99.89	

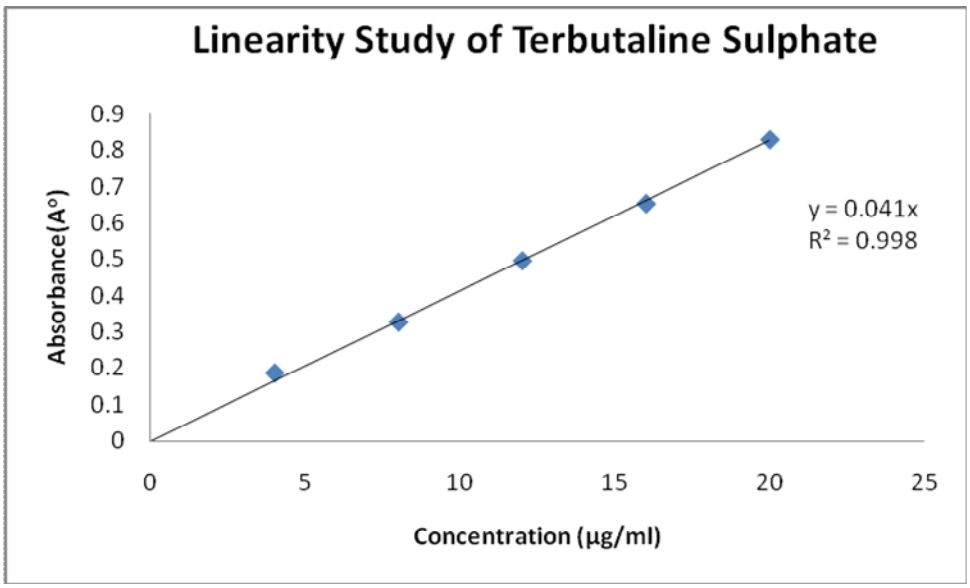

Figure 1: Linearity study of terbutaline sulphate

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