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RP-HPLC-PDA Method for the Analysis of Terbutaline sulphate in Bulk, Dosage forms and in Dissolution samples

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ABSTRACT

The aim of the present work was to develop and validate a simple, efficient, economical and LC-MS compatible method for the analysis of Terbutaline sulphate in bulk, dosage forms and in dissolution samples of Terbutaline sulphate tablets by reverse phase high pressure liquid chromatography. A C_{18} reverse phase column (Phenomenex) of 250 x 4.6mm dimensions and 5µm particle size with mobile phase containing 15mM ammonium acetate: methanol (70:30% v/v) was used at isocratic mode binary pump and eluents were monitored at 220nm. The retention time of Terbutaline sulphate was 4.1min and showed a good linearity in the concentration range of 2-10µg/mL with a correlation coefficient >0.999. The validation characteristics included specificity, linearity, and limit of detection, limit of quantification, precision, robustness and stability. Validation acceptance criteria were met in all cases. The percent recoveries ranged between 98-102%, RSD < 2%. The method could be successfully used for the analysis of Terbutaline sulphate in bulk, dosage forms and in dissolution samples of marketed Terbutaline sulphate tablets.

INTRODUCTION

Terbutaline sulphate (TS) is an orally active β_2 sympathomimetic. Chemically it is 1, 3-benzenediol, 5-[2-[(1, 1-dimethyl ethyl) amino]-1-hydroxy-ethyl] sulphate (Maryadele & Neil, 2001) and used in the treatment of bronchial asthma (Sweetman, 2005), it acts through β_2 receptor stimulation leading to increased cAMP formation in bronchial cell which ultimately relaxes bronchi (Tripathi, 2010). Various analytical methods have been reported in the literature for quantitative determination of TS in combination with other drugs like Ambroxol HCl, Bromhexine, Guaifenesin by HPLC (Amit K et al., 2011; Giri R et al., 2012; Satyanarayana PVV et al., 2012; Senthil RM et al., 2011), Stability-indicating HPLC (Hanimi R et al., 2011; Porel A et al., 2011) Spectrophotometry (Patil KM et al., 1998), Fluorimetry (Rao HL et al., 1990) and by LC-MS (Kim H et al., 2003). Literature survey reveals that there were no validated RP-HPLC/PDA methods reported for the estimation of TS individually in bulk, tablet dosage forms and in dissolution samples. However, the so far reported HPLC methods for the estimation of TS in combination with other anti-histaminic and anti-asthmatics used non-volatile buffers in mobile phase which are not LC-MS compatible and also used high percent of organic solvents. Hence, the present investigation was aimed at developing a validated RP-HPLC-PDA method for the analysis of TS in bulk, dosage forms and *in vitro* dissolution samples of TS tablets which is LC-MS compatible and economical.

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MATERIAL AND METHODS

Chemicals

TS was gift sample from Hetero Drugs Ltd, India. Ammonium acetate, water and methanol were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade. Bricanyl® 5 (manufactured by Astra Zeneca Pharma India Limited, Bangalore) is a tablet containing Terbutaline Sulphate 5mg was commercially purchased.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex C_{18}- RP aqueous column (250 × 4.6mm, 5µ).

Chromatographic Conditions

Mobile phase consisting of 15mM ammonium acetate: methanol (70:30% v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min. before use. The flow rate was 1 mL/min and the injection volume was 20µL. PDA detection was performed at 220nm and the separation was achieved at ambient temperature.

Preparation of stock and standard solutions

The stock solution of TS strength 1mg/mL was prepared by dissolving 10 mg of drug in methanol and volume was adjusted to the mark with the same. An appropriate volume of the stock solution was then further diluted with water to get the required concentrations of standard solutions at a concentration range of 2-10µg/mL.

Validation of the HPLC method

The proposed method was validated as per ICH guidelines.

Linearity

A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of TS were prepared over a concentration range of 2-10µg/mL (2, 4, 6, 8, 10µg/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R^2) correlation coefficients (R) were calculated and the data was given in Table-1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision. Less than 2% RSD for peak areas indicates the precision of the developed method and the data was presented in Table-1.

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the tablet solution. The overlay of diluent, placebo, standard and sample were presented in Figure-2. The retention times of drug substance and the drug product were observed. Absence of interference of excipients in the tablet indicates the specificity of the proposed method.

Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the synthetic solution of drug product (4µg/mL) and these solutions were analyzed by developed method in triplicate. The % recovery and the %RSD were calculated at each level of addition and the data was given in Table-1.

Table. 1: Linearity, Precision and Accuracy data of TS.

Validation data of TS	
Linearity (n=3)	Range 2-10 µg/mL y =23846x+1265.6 R=0.999 R^2=0.997
Precision (n=6)	**Average peak area of the standard sample (%RSD)** 148755 (0.069%)
Accuracy (n=3) **Level of addition**	**Mean Percent Recovery (%RSD)**
80%	101.13(1.404%) 99.36(0.058%) 99.76(1.331%)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., capacity factor, tailing factor, no. of theoretical plates and % assay were recorded. The flow rate of the mobile phase was 1mL/min. To study the effect of flow rate, the flow rate was changed by 20% and the effect of wavelength was studied by changing wavelength by ±1nm and the data was given in Table-2.

Table. 2: Robustness data of TS.

Chromatographic parameter	Retention time (min)	Theoretical plates #	Capacity factor (K')	Tailing factor (T_f)	% Assay
Flow rate (mL/min)					
0.8	5.06	35911	0.384	1.524	99.12
1.0	4.10	27027	0.395	1.499	99.54
1.2	3.47	36271	0.392	1.468	100.12
Wave length (nm)					
219	4.06	27000	0.361	1.482	100.76
220	4.06	27027	0.381	1.483	99.12
221	4.06	26999	0.360	1.483	100.21

System suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50µL. The system suitability test parameters were noted and % RSD was calculated.

Assay

Twenty tablets were weighed and finely powdered, the powder equivalent to 5mg of TS was accurately weighed and

transferred into a 5mL volumetric flask and dissolved in methanol and vortexed for 5min and volume was adjusted up to the mark with methanol. The above solution was centrifuged and then filtered using Nylon disposable syringe filter (13mm, 0.45µm). An aliquot of filtrate was diluted with water and analyzed in triplicate. The amount present in the each tablet was quantified by comparing the area of standard TS with that of the sample.

Dissolution Analysis

A calibrated dissolution apparatus (USP-1) was used with basket turning at 100 rpm and bath temperature maintained at 37±0.5°C. 900mL freshly prepared and degassed water was used as the dissolution medium. Dissolution samples were collected manually at 3, 5, 10, 15, 20, 30, 45 min.

At each time point, 5mL sample was removed and filtered through a nylon filter (0.45µm); an aliquot of filtrate was suitably diluted and analyzed by HPLC.

The % release of TS in the test samples was calculated by comparing test area with the peak area of the standard.

Filter compatibility study

In this study nylon filter (0.45µm) compatibility was evaluated. Sample solution was prepared and the solution was filtered using 0.45µm nylon filter. Filtered samples were injected and chromatograms were observed. The data was given in Table-3.

RESULTS AND DISCUSSION

Various HPLC, UV, MS, methods were published for the estimation of TS in combination with other drugs like ambroxol HCl, bromhexine, guaifenesin but so far no methods were reported on the quantification of TS individually in dosage forms and *in vitro* dissolution sample analysis of TS tablets. Hence, the present investigation was aimed to develop a simple, economical RP-HPLC-PDA method for the determination of TS in bulk, dosage forms and in dissolution samples.

Method Development

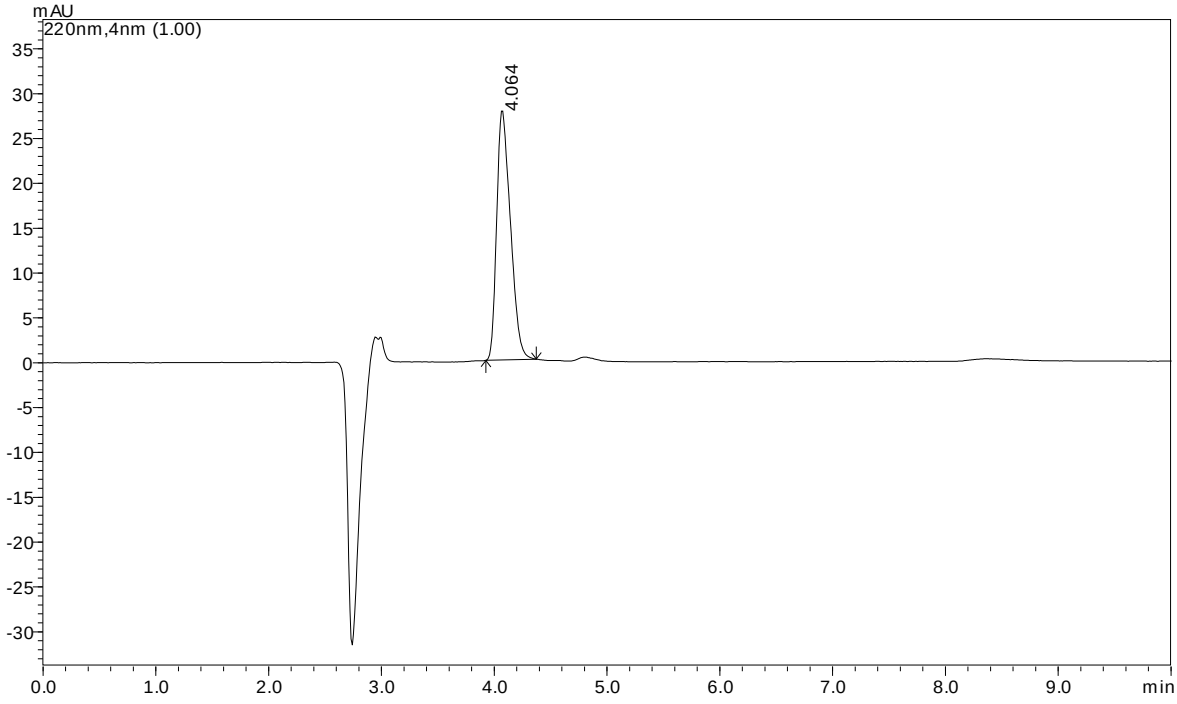
Mobile phase optimization initially carried with Phenomenex C_{18} column (250 x 4.6 mm) using formic acid (0.02% v/v) and methanol as mobile phase in different ratios, TS was eluted before solvent peak at 1 mL/min flow rate. In other trial 15mM ammonium acetate and methanol (50:50 v/v) was used and the peak observed was not with symmetry. Finally the mobile phase of 15mM ammonium acetate and methanol was selected at a ratio of 70:30% v/v at flow rate of 1mL/min using water as diluent, in this condition a sharp peak was obtained and tailing factor was within the limits and the peak eluted within 10 min run time. The retention time was 4.1min for TS. For quantitative analytical purpose wavelength was set at 220 nm, which provided better reproducibility with minimum or no interference. The method was validated as per ICH guidelines. The peak purity index was found to be greater than 0.9999 and this indicating peak purity of the drug sample used in the analysis and shown in Figure-1 along with UV spectra.

Method validation

The method has been validated as per ICH-Guidelines for following parameters.

Linearity

The range of reliable quantification was set at the concentrations of 2-10µg/mL of TS. This range was selected based on 80-120% of the standard concentration used for accuracy and were analyzed in triplicate. Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the range used. The data from the calibration curve was given in Table-1.



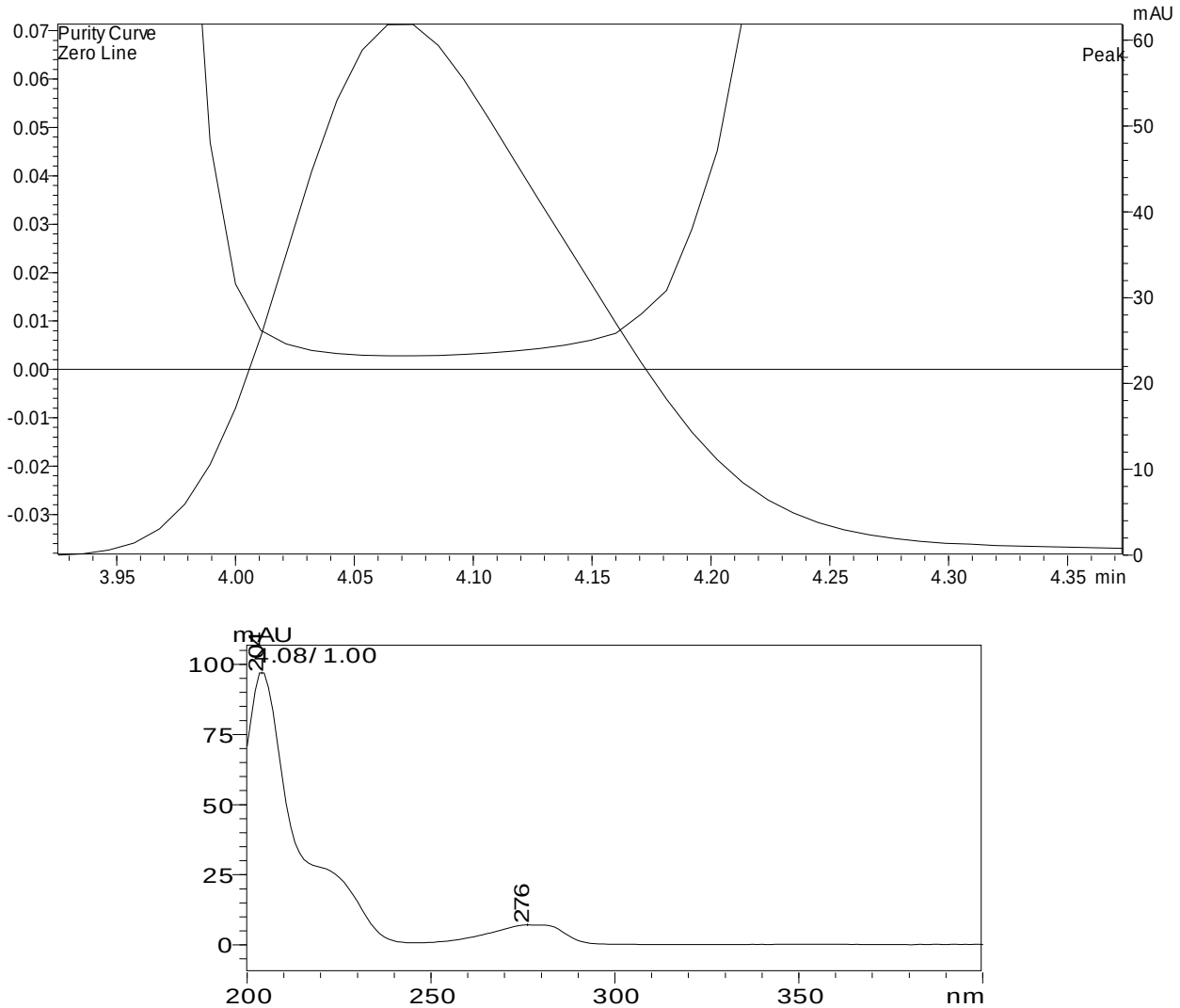


Fig. 1: A - Chromatogram of TS (10 µg/mL) with B - Peak purity index and C - UV spectrum.

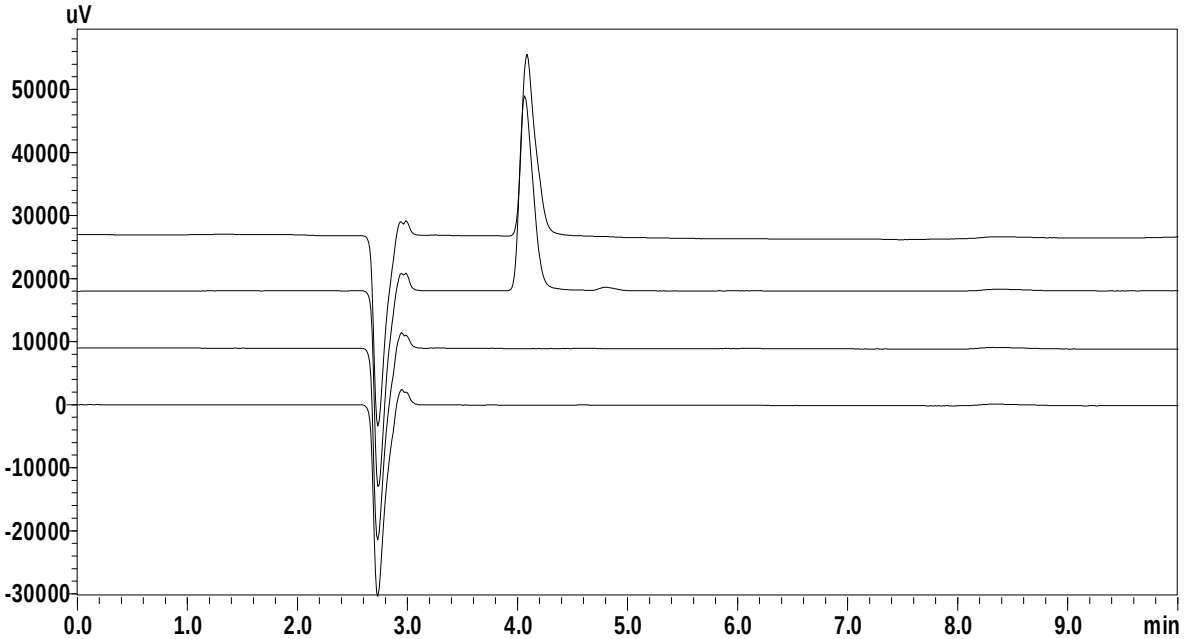


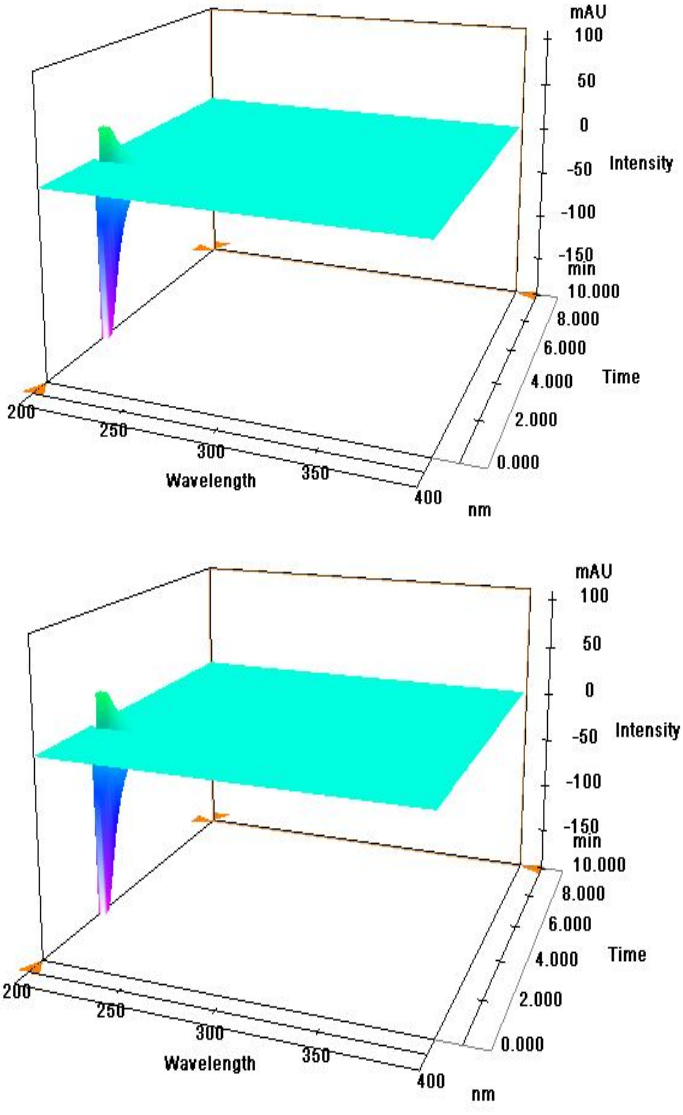
Fig. 2: Overlay of the Diluent (A), Placebo (B), Standard (C) and Sample (D) chromatograms.

Precision

Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 6µg/mL level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas, this shows the closeness of the data values to each other, indicating the precision of the method.

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard, sample (Formulation) individually to examine any interference, from the overlay of chromatograms as shown in (Figure-2) and the 3D plots of placebo and formulation in (Figure-4) it can be inferred that there were no co-eluting peaks at the retention time of TS, this shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analysis and the peak purity indices for sample and standard was found to be greater than 0.999 and this confirms specificity of the method.



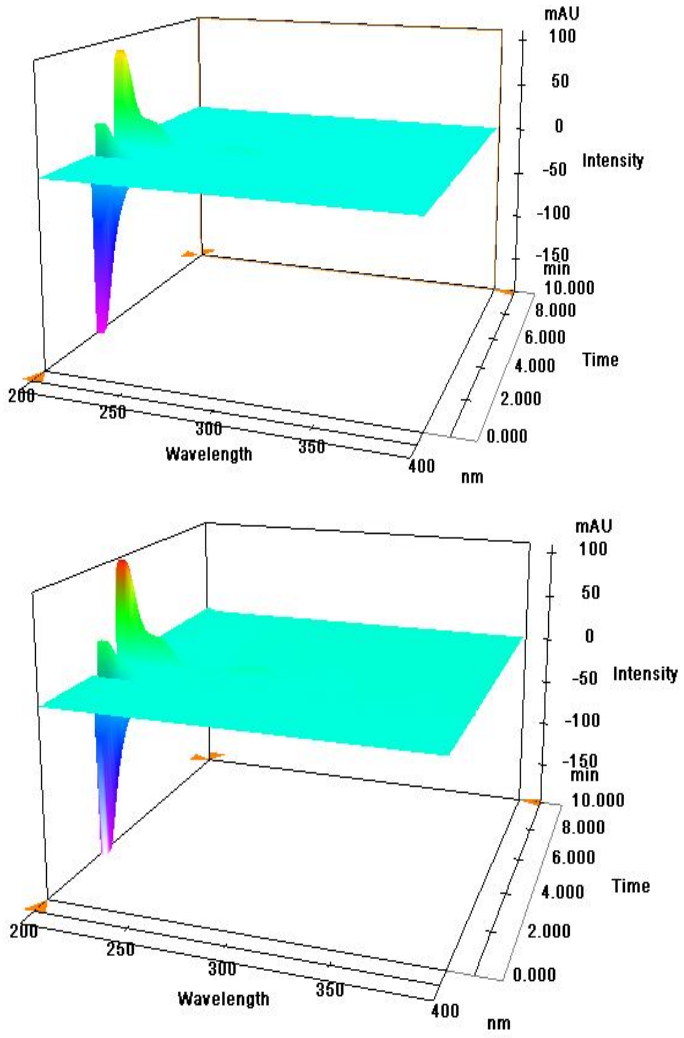


Fig. 4: 3D plots of Placebo (1), Diluent (2), Sample (3) and Standard (4).

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80%, 100%, 120% to the drug product solution of 4µg/mL and these solutions were analyzed in triplicate in each level of addition. The %RSD and the %Recovery were within the acceptable limit in all cases. It is evident from the results of accuracy study given in Table-1, that the proposed method enables very accurate quantitative estimation of TS.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).The limit of detection for TS was found to be 0.063µg/mL, the drug peak could be detected without any base line disturbances at this concentration. The limit of quantification for TS was found to be 0.192 µg/mL.

Robustness

As part of the robustness, a deliberate change in the flow rate and wavelength was made to evaluate the impact on the method. Retention times were significantly changed with flow rate and no change in the retention time was observed in wavelength change. Percent assay values were also estimated under these changed conditions and the results were given in Table-2. The parameters like capacity factor, theoretical plate number and assay were not changed and were within the limits. These results indicated that the method is robust in terms of changed flow rate and wavelength.

System suitability

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting five times a 6µg/mL standard concentration of TS at different injection volumes ranging from 10µL to 50µL. The %RSD values for system suitability test parameters like retention time [R_t = 4.07 (0.46%)], tailing factor [T_f = 1.50 (0.10%)] and theoretical plate number [# = 4242 (1.82%)] were less than 2% indicating the present conditions were suitable for the analysis of TS in tablets.

Assay

Assay of TS tablets was performed by the proposed method and the % assay of the formulation was calculated as an average of 3 determinations, which was about 99.72 ± 0.057. These results indicate that the present HPLC method can be successfully used for the assay of TS in bulk and dosage forms.

Stability of the stock solution

The stability of the stock solution was determined by analyzing the samples under refrigeration (8±1°C) at different time intervals up to 48hrs. The % variation in assay values at different time intervals were found to be less than 2% of the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8±1°C.

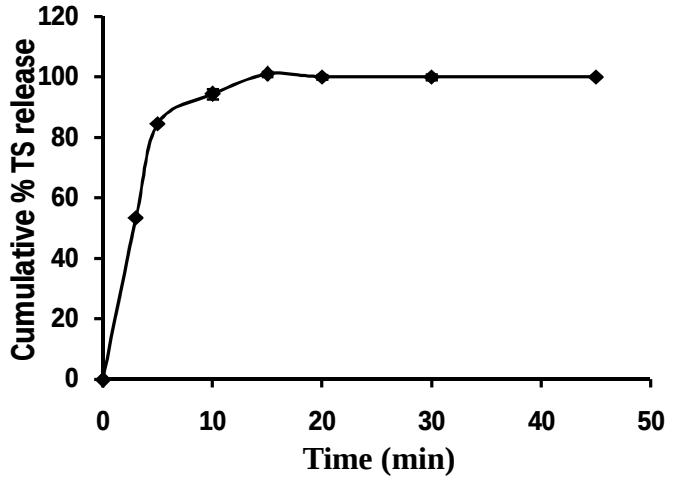


Fig. 3: Dissolution profile of marketed Terbutaline sulphate tablets.

Dissolution analysis of marketed product

The validated method was used for the *in vitro* dissolution analysis of TS tablets. The %drug release was found to meet USP specification of NLT 75% (Q) of the labeled amount of TS dissolved in 45min, proving that the developed method can be successfully applied for the routine *in-vitro* dissolution sample analysis of TS. The dissolution profile was presented in Figure-3.

Filter compatibility study

Compatibility of 0.45µm nylon filter was studied. Standard sample solution and dissolution samples were filtered and analysed and the variation in the assay value was calculated and tabulated in Table-3. After the analysis it was found that nylon filters are suitable for filtration.

Table. 3: Filter compatibility study.

Sample name	Peak area of TS	% Difference
Standard sample	235679	-
Samples filtered through 0.45µm nylon filter	235618	0.025

CONCLUSION

In this work, a simple and efficient RP-HPLC-PDA method was developed for the analysis of TS in bulk, dosage forms and in dissolution samples of TS tablets. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of TS in tablets and in dissolution samples using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of TS without interference from diluents and placebo. By this method, using USP dissolution conditions it is possible, simply and exactly to determine TS in dissolution medium without any additional pre-treatment. Therefore, this method can be employed in quality control to estimate the amount of TS in bulk, dosage forms and in analysis of dissolution samples of marketed TS tablets.

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