Biological Forum – An International Journal **5**(1): 33-41 (2013)

ISSN No. (Print) : 0975-1130
ISSN No. (Online) : 2249-3239

Simultaneous Quantitative Determination of Terbutaline and Theophylline from Drug Product by RP-HPLC Method

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(Received 15 October, 2012, Accepted 25 November, 2012)

ABSTRACT: An isocratic reversed-phase HPLC method with VWD detector has been developed for the simultaneous assay evaluation of Terbutaline and Theophylline in bulk drug. The analysis was performed using Zorbax SB CN (10cm X 4.6 mm i.d. and 1.8 µm) as a stationary phase with column oven temperature 40°c and UV detection at 220nm. The separation was achieved using isocratic program of buffer (Buffer used was of 0.01% Ammonium acetate in water), Acetonitrile and Methanol in the ration (65:10:25). The method was optimized based on the peak shapes and resolution of Terbutaline and Theophylline. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of linearity, precision, accuracy, specificity, robustness and solution stability. The sample concentration were injected was 25 µg/ml and 200 µg/ml for Terbutaline and Theophylline respectively. The method is linear within the range of 100 to 300 µg/ml for Theophylline and 12.50 to 37.50 µg/ml for Terbutaline.

Keywords: Terbutaline; Theophylline, RP-HPLC.

INTRODUCTION

Terbutaline is a relatively selective beta2-adrenergic bronchodilator that has little or no effect on alpha-adrenergic receptors. The drug has exerts a preferential effect on beta2-adrenergic receptors but stimulates beta-adrenergic receptors less selectively. Terbutaline appears to have a greater stimulating effect on beta-receptors of the bronchial, vascular, and uterine smooth muscles (beta2 receptors) than on the beta-receptors of the heart (beta1 receptors). This drug relaxes smooth muscle and inhibits uterine contractions, but may also cause some cardiostimulatory effects and CNS stimulation. Terbutaline [1] (trade names Brethine, Bricanyl, or Brethaire) is a β2-adrenergic receptor agonist, used as a fast-acting bronchodilator (often used as a short-term asthma treatment) and as a tocolytic to delay premature labour. It is A white or almost white, crystalline powder freely Soluble in water, slightly soluble in ethanol (96%). The pharmacologic effects of terbutaline are at least in part attributable to stimulation through beta-adrenergic receptors of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic- 3',5'- adenosine monophosphate (c-AMP). Increased c-AMP levels are associated with relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells. Theophylline, a xanthine derivative chemically similar to caffeine and theobromine, is used to treat asthma and bronchospasm. Theophylline [2] has two distinct actions in the airways of patients with reversible (asthmatic) obstruction; smooth muscle relaxation (*i.e.,* bronchodilation) and suppression of the response of the airways to stimuli (*i.e.,* non-bronchodilator prophylactic effects). Theophylline is structurally classified as a methylxanthine. It occurs as a white, odorless, crystalline powder with a bitter taste. Slightly soluble in water, sparingly soluble in ethanol (96%). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids. Theophylline relaxes the smooth muscle of the bronchial airways [3] and pulmonary blood vessels [4] and reduces airway responsiveness to histamine, methacholine, adenosine, and allergen. Theophylline competitively inhibits type III and type IV phosphodiesterase (PDE) [5], the enzyme responsible for breaking down cyclic AMP in smooth muscle cells [6], possibly resulting in bronchodilation. Theophylline also binds to the adenosine A2B receptor and blocks adenosine mediated bronchoconstriction.

Tablet is also used as a bronchodilator [7]. It helps open up the airways in your lungs to make it easier to breathe. This medicine is used to treat the symptoms of asthma, bronchitis, and emphysema. It is available for oral administration as tablets containing 2.5 mg of Terbutaline sulphate and 100 mg of Theophylline anhydrous.

Each tablet contains the following inactive ingredients: Starch, Magnesium Stearate, Micro crystalline Cellulose [8], Lactose, Silicon dioxide colloidal [9], Talc and Sodium starch glycolate.

MATERIAL AND METHODS

A. Drug and reagents

Pure Standard Used Theophylline and Terbutaline Sulphate were used. Tablets with (100mg Theophylline and 2.5 mg Terbutaline Sulphate) were procured from local market. HPLC grade Acetonitrile and Methanol of Qualigens (99.0 %), AR grade Ammonium acetate of Merck were used throughout the quantitative determination. Distilled water was obtained from MILLI Q water purifying system (Millipore, U.S.A). All solvents were filtered through 0.5µ (Millipore) membrane and degassed in ultrasonic bath.

B. Apparatus and equipment

LC was carried out on Jasco, PU 980 HPLC isocratic pump , AS - 2057 sampler HPLC system with UV- Visible detector (UV-970). The output signal was monitored and processed using Borwin chromatography software 1.21. In all the studies, separations were achieved on a Zorbax SB CN(10cm X 4.6 mm i.d. and 1.8 µm) procured from LCGC (Banglore, INDIA). Other small equipment were PCI sonicator (22L500/CC/DTC made in), precision analytical balance (Mettler Toledo, Schwerzenbach, Switzerland).

C. Chromatographic conditions

The separation was achieved using Zorbax SB CN (10cm X 4.6 mm i.d. and 1.8 µm) and isocratic program of buffer (Buffer used was of 0.01% Ammonium acetate in water), Acetonitrile and Methanol in the ration (65:10:25). The flow rate was set at 1.0 ml/min and column was maintained at 40^{o}C. The injection volume was set 5µl and detector was set at a wavelength of 220 nm.

D. Preparation of Mobile Phase

- **Buffer (0.01 % Ammonium acetate):** Weigh 100 mg ammonium acetate in 1000 ml volumetric flask. Add 500 ml of distilled water, sonicate to dissolve and make the volume with distilled water up to the mark.
- **Mobile Phase:** Prepare a filtered and degassed mixture of Buffer, Acetonitrile and methanol in the ratio of (65:10:25) v/v
- **Preparation of Diluent :** The diluent was prepared by mixing distilled water and methanol in the ratio of (50:50) v/v

E. Preparation of standard solution for method Validation

Standard Solution of Theophylline (200.0µg/ml) and Terbutaline Sulphate (25.0 µg/ml):

- **Terbutaline Sulphate Stock solution (Solution A):**
  25 mg of Terbutaline Sulphate was weighed and transferred in 50 mL volumetric flask to it 20 ml of the diluent was added and the solution was sonicated for 5 mins. Finally it was diluted up to the mark with the diluent.
- **Standard Preparation (Solution B):**
  20.0 mg of Theophylline was weighed and transferred in 100 mL volumetric flask containing 5 ml of **solution A**. To it 60.0 ml of diluent was added and the solution was sonicated for 5 mins. Finally it was diluted up to the mark with diluent.

F. Preparation of sample solution for method Validation:

Average weight of a tablet: 0.336 gm (Determined on 20 tablets)

Sample Stock Solution:

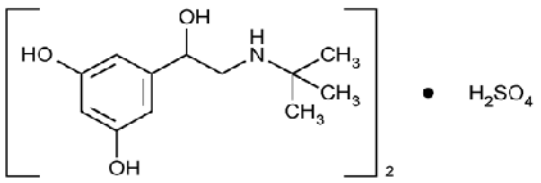
Weigh and crush 20 tablets. Weigh accurately about 0.336 gm of tablet powder equivalent to average weight of a single tablet (equivalent to 2.5 mg of Terbutaline Sulphate and 100 mg of Theophylline) and transfer into 100 mL volumetric flask. Add about 50 ml of diluent, sonicate for 20 mins at an ambient temperature with intermittent swirling, cool and dilute upto the mark with diluent, mix well.

For Terbutaline content (25.0µg/mL): Filter sample stock solution through 0.45µ syringe filter and inject the same.

For Theophylline content (200.0µg/mL): Pipette out 5.0 ml from sample stock solution transfer to 25 ml volumetric flask. Dilute upto the mark with diluent, mix well. Filter through 0.45 µ syringe filter and inject.

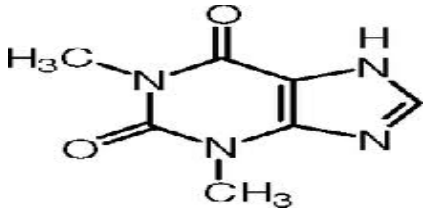
METHOD DEVELOPMENT AND COLUMN SELECTION

Chemical structure of Terbutaline Sulphate and Theophylline are shown in (Fig.1 and 2). Reverse phase chromatography columns are efficient, stable and reproducible. Due to their high reproducibility and stability coupled with wide versatility, reverse phase chromatographic separations have become preferred mode of separation by HPLC. Aqueous eluents having high optical transparency as well as low flammability and toxicity can be used to accomplish most of the separation goals. Selectivity is most easily achieved in reverse phase chromatography [10] by the variation of the bonded groups [dimethyl silane (C2), butyl silane (C4), octyl silane (C8), octadecyl silane (C18), phenyl, cyanopropyl, nitro, amino etc]. Selectivity may be further enhanced by a variety of modifications possible in the aqueous mobile phases such as the variation in pH, the addition of buffers.and.the.organic.modifiers. A reverse phase mode of separation was employed taking into account the polar nature of Terbutaline and Theophylline, its solubility in water and methanol. Hence a reverse phase mode of separation was chosen for HPLC.determination.



Terbutaline Sulphate : 5-[2-[1, 1-Dimethyl) amino]-1-hydroxyethyl]-1, 3- benzenediol sulfate

Fig. 1. Chemical structure of Terbutaline Sulphate.



Theophylline : 1, 3-dimethyl-2, 3, 6, 7-tetrahydro-1H-purine-2, 6-dione

Fig. 2. Chemical structure of Theophylline.

The column is selected depending on the nature of the solute and the information about the sample. The number of theoretical plates (N) is an important characteristic of a column. N- Defines the ability of the column to produce sharp, narrow peaks for achieving good resolution. N is dependent on specific experimental factors. In the method development, peak shape is equally important. Columns that provide symmetrical peaks are always preferred. Agilent Zorbax SB CN column with dimensions of 100mm X 4.6 mm i.d. and 1.8 µm particle size was employed as the stationary phase as it gave desired performance.

Acetonitrile and Methanol are the most popular solvents in HPLC. Methanol and Acetonitrile are water miscible, have low viscosity, low surface tension and are readily available in pure form, hence they are used as components of the mobile phase. With water and methanol in the ratio (50:50) v/v the Rt of Theophylline was around 7.0 min retention time and the Rt of Terbutaline peak was around 8.0 min. Both the peaks merged with each other and hence peak could not be resolved. The best resolution was obtained with mobile phase with the composition 0.01 % Ammonium acetate, Acetonitrile and Methanol in the ratio of (65:10:25).

In the present research work, simultaneous determination of Terbutaline and Theophylline was performed using a UV Visible detector as both Terbutaline and Theophylline have absorbance in UV region as shown in Fig 3. it was concluded that the simultaneous quantification of Terbutaline and Theophylline could be performed at 220 nm.

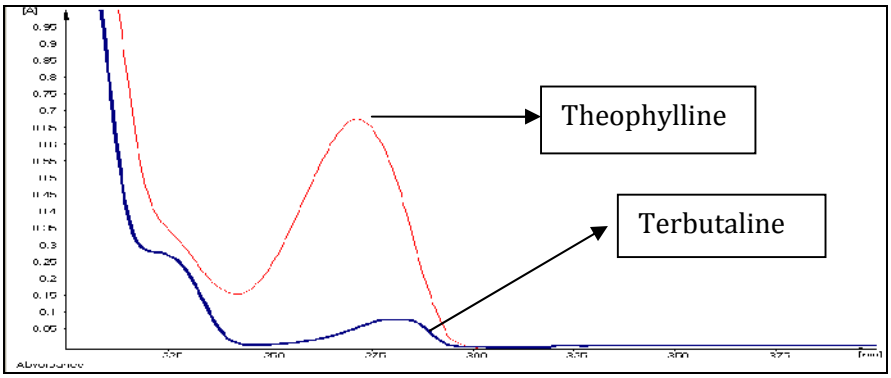


Fig. 3. UV scan of Theophylline and Terbutaline.

RESULTS AND DISCUSSION

A. Method Validation [11]

Specificity. Blank (Diluent) & Placebo (Excipients) injected into the chromatograph to check the interference at the retention time corresponding to the peak of Theophylline and Terbutaline. Individual identification solution of Theophylline and Terbutaline injected to identify the peaks. There were no interfering peaks in the chromatogram of the Blank (Diluent) & Placebo (Excipients) at the retention time corresponding to the peak of Theophylline and Terbutaline.

Linearity. Five Solutions containing concentrations of Theophylline and Terbutaline Sulphate in the range of 50% to 150% of the working level (i.e. 100 µg/mL to 300 µg/mL of Theophylline and 12.5 µg/mL to 37.5 µg/mL of Terbutaline Sulphate) were injected into the chromatograph. The peak area responses were found to be linear with respect to the concentration. The regression coefficient (r^2), the %Y-intercept, % RSD of the peak area response, % RSD of retention time of each level, and % RSD of responses factor were within the acceptance range. The mean responses recorded for each analyte were plotted against concentration. The correlation coefficient for Theophylline and Terbutaline Sulphate was found to be 1.00 and 1.00 respectively, which indicates good linearity. (Fig. 6 for Theophylline and Fig. 7 for Terbutaline Sulphate).

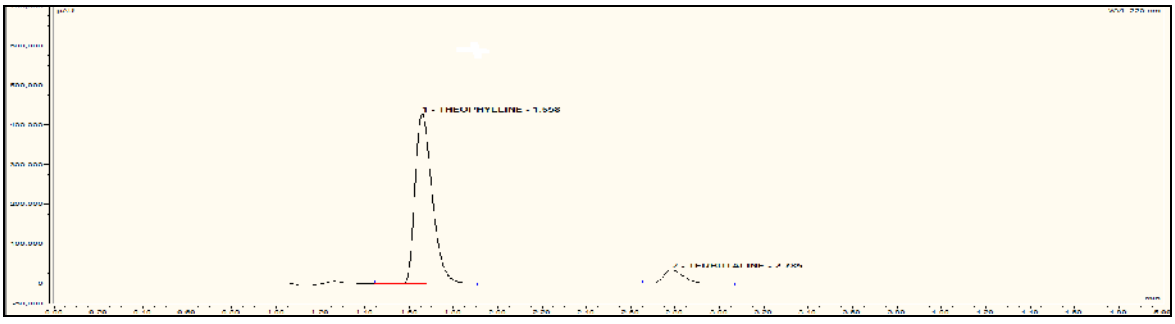


Fig.4. A typical chromatogram for Theophylline and Terbutaline standard solution.

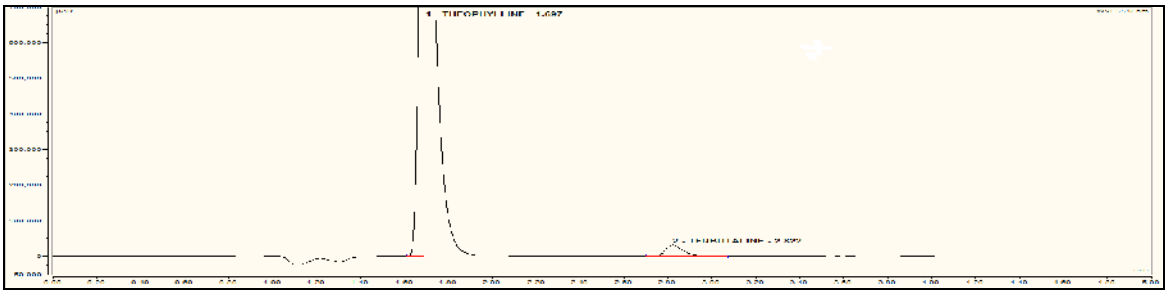


Fig. 5. A typical chromatogram for Theophylline and Terbutaline sample solution**.**

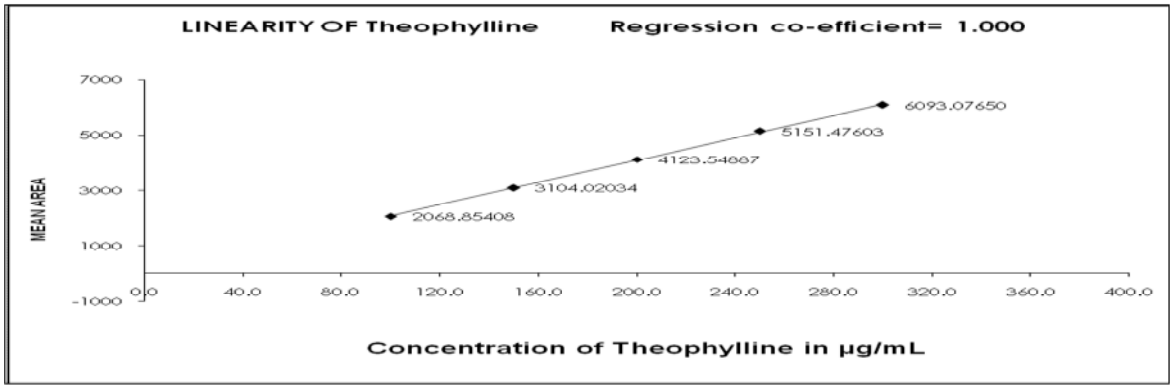


Fig. 6. Linearity curve Graph of Peak Area Vs Conc. of Theophylline.

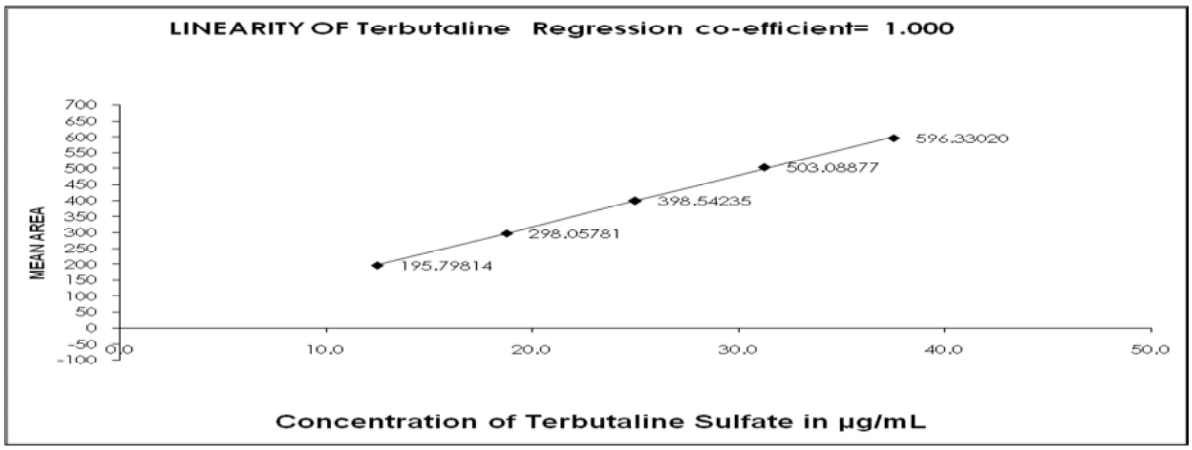


Fig. 7. Linearity curve Graph of Peak Area Vs Conc. of Terbutaline Sulphate.

Accuracy. Theophylline and Terbutaline Sulphate analytes were spiked in placebo solution at 50%, 100% and 150%. Each spiked solution was prepared in triplicate and injected. The recovery percentage and %RSD were calculated for each analyte. Recovery of Theophylline and Terbutaline Sulphate ranged from 98.65-100.77% and 99.09-101.47% respectively. The results are shown in Table 1 and 2 respectively.

Table 1 : Results of Theophylline accuracy.

Accuracy Level	Weight of Theophylline std. taken in accuracy level (in mg)	Total Volume	Amount added (in ppm)	Area of Theophylline in acc. Level	Amount found (in ppm)	% recovery
Acc 1/1	50.2	100	100.40	2075.48558	101.02	100.6
Acc 1/2	50.1	100	100.20	2074.12558	100.95	100.7
Acc 1/3	49.9	100	99.80	2070.99659	100.80	101.0
Acc 2/1	100.1	100	200.20	4150.25848	202.00	100.9
Acc 2/2	99.8	100	199.60	4144.52969	201.72	101.1
Acc 2/3	99.7	100	199.40	4152.33695	202.10	101.4
Acc 3/1	151.2	100	302.40	6210.25485	302.26	100.0
Acc 3/2	150.7	100	301.40	6201.25362	301.83	100.1
Acc 3/3	150.4	100	300.80	6208.25362	302.17	100.5

Table 2 : Results of Terbutaline accuracy.

Accuracy Level	Volume of (502 ppm) Stock taken in accuracy level (in mL) A	Total Volume	Amount added (in ppm)	Area of Terbutaline in acc. Level	Amount found (in ppm)	% recovery
Acc 1/1	2.5	100	12.55	198.54623	12.53	99.8
Acc 1/2	2.5	100	12.55	199.12548	12.56	100.1
Acc 1/3	2.5	100	12.55	202.01210	12.74	101.6
Acc 2/1	5.0	100	25.10	399.02584	25.17	100.3
Acc 2/2	5.0	100	25.10	400.12596	25.24	100.6
Acc 2/3	5.0	100	25.10	402.35690	25.38	101.1
Acc 3/1	7.5	100	37.65	599.85698	37.84	100.5
Acc 3/2	7.5	100	37.65	589.25963	37.18	98.7
Acc 3/3	7.5	100	37.65	600.25979	37.87	100.6

System and method precision. Precision study was continued after specificity and system suitability study. The sample solution was prepared six times. The content of Theophylline and Terbutaline Sulphate (%mg/tab) for each preparation was determined. The % RSD for the content of Theophylline and Terbutaline Sulphate of all six samples was observed to be within limit. The variation in the results for the two analytes were expressed in terms of % RSD. The values calculated were found to be below 2.0% RSD for analytes, indicating satisfactory method precision. A typical chromatogram for Theophylline and Terbutaline standard solution and sample solution is shown in Fig. 4 and Fig. 5. The results are shown in Table 3 and Table 4.

Table 3 : Results of repeatability for Theophylline content.

Obs. No.	Sample. Wt.(mg)	Peak Area of Theophylline	Conc. of Theophylline µg/mL	%Content	Theophylline mg /tab
1	336.5	4138.26465	198.55	99.28	99.28
2	334.5	4158.52693	200.72	100.36	100.36
3	339.1	4172.17432	198.64	99.32	99.32
4	336.5	4189.25632	201.00	100.50	100.50
5	340.1	4200.52986	199.40	99.70	99.70
6	336.2	4140.46631	198.83	99.42	99.42
Mean	337.2	4166.53640	199.52	99.76	99.76
S.D	2.06	25.48	1.08	0.54	0.54
%R.S.D	0.61	0.61	0.54	0.54	0.54

Table 4 : Results of repeatability for Terbutaline Sulphate content.

Obs. No.	Sample. Wt.(mg)	Peak Area of Terbutaline	Conc. of Terbutaline Sulphate µg/mL	%Content	Terbutaline Sulphate mg /tab
1	336.5	399.06039	25.07	100.29	2.51
2	334.5	397.16687	25.10	100.41	2.51
3	339.1	395.26523	24.64	98.57	2.46
4	336.5	393.25621	24.71	98.83	2.47
5	340.1	399.25632	24.82	99.28	2.48
6	336.2	396.01398	24.90	99.61	2.49
Mean	337.2	396.66983	24.87	99.50	2.49
S.D	2.06	2.31	0.19	0.75	0.02
%R.S.D	0.61	0.58	0.75	0.75	0.75

Stability in analytical solution

The content of Terbutaline Sulphate & Theophylline was determined initially and then at the predetermined time interval of 4, 8, 12, 16, 20 and 24 hrs. The % relative difference for the content of Terbutaline Sulphate & Theophylline between initial value and predetermined time interval was calculated and found to be within acceptance criteria. Standard solution was also injected initially and then at the predetermined time interval of 4, 8, 12, 16, 20 and 24 hrs. The system suitability parameters such as tailing factor, theoretical plates, % RSD for retention time and peak area response of Terbutaline & Theophylline in Standard was determined and was found to be within acceptance criteria. No extraneous peaks were observed in the chromatogram of sample and standard till 24hrs interval. This shows that the standard and sample is stable upto 24 hrs. The results are shown in Table 5.

Table 5 : Results of Summary of Solution Stability Study.

Solution Stability Condition	Content of Theophylline mg/tablet	% Relative difference with initial Theophylline content	Content of Terbutaline Sulphate mg/tablet	% Relative difference with initial Terbutaline Sulphate content
Initial	100.08	--	2.51	--
4 Hrs	100.05	0.03	2.49	0.02
8 Hrs	100.02	0.06	2.48	0.03
12 Hrs	100.04	0.04	2.47	0.04
16 Hrs	100.02	0.06	2.47	0.04
20 Hrs	99.98	0.10	2.46	0.05
24 Hrs	99.75	0.33	2.46	0.05

Robustness. Robustness was carried out by changing,
Exp I - The column oven temp by 40°C to 38°C.
Exp II - The column oven temp by 40°C to 42°C.
Exp III - The flow rate of mobile phase from 1.0 ml to 0.9 ml.
Exp IV - The flow rate of mobile phase from 1.0 ml to 1.1 ml.

Specificity, System suitability and the Precision of Samples were performed. Blank (diluent) & Placebo (Excipients) was injected into the chromatograph to check the interference at the retention time corresponding to peak of Theophylline and Terbutaline. There was no interfering peak in the chromatogram of the Blank (diluent) & Placebo at the retention time corresponding to the peak of Theophylline and Terbutaline. The results are shown in Table 6 and Table 7.

Table 6 : Summary of Robustness Study for Theophylline.

Condition	% R.S.D Peak Area	% R.S.D for RT	% R.S.D Content	% Relative difference of average content from Repeatability & Robustness
Flow Rate (0.9 mL/min)	0.22	0.20	0.32	0.25
Flow Rate (1.1 mL/min)	0.50	0.31	0.31	0.34
Column Oven Temperature 38˚C	0.01	0.20	0.25	0.37
Column Oven Temperature 42˚C	0.06	0.09	0.20	0.32

Table 7 : Summary of Robustness Study for Terbutaline.

Condition	% R.S.D Peak Area	% R.S.D for RT	% R.S.D Content	% Relative difference of average content from Repeatability & Robustness
Flow Rate (0.9 mL/min)	0.16	0.13	0.39	1.28
Flow Rate (1.1 mL/min)	0.31	0.65	0.38	1.45
Column Oven Temperature 38˚C	0.05	0.67	0.30	1.37
Column Oven Temperature 42˚C	0.08	0.67	0.22	1.20

CONCLUSION

The proposed LC method is selective for the Simultaneous quantitative determination of Terbutaline and Theophylline from tablet. Hence this method is useful for the detection Terbutaline and Theophylline in routine analysis.

ACKNOWLEDGEMENTS

The author wish to thank Dr. Padmakar A. Sathe and Dr. Sudesh Bhure for reviewing and giving suggestions for the manuscript and also grateful for their encouragement and support to carry out this work.

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