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Development and Validation of a Simultaneous Dissolution procedure for a dual active Terbutaline and Theophylline Tablet

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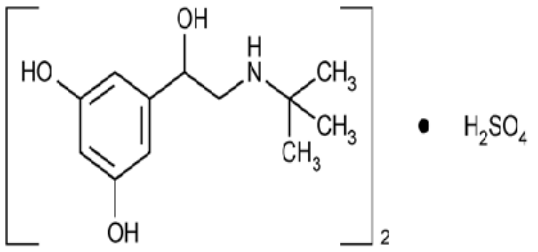
ABSTRACT: The aim of this work was to develop and validate a dissolution test for Terbutaline Sulfate 2.5 mg and Theophylline 100mg tablet using High Performance Liquid Chromatography method. The dissolution established conditions were: 900 mL of water, using a USP Type II Paddle apparatus at a stirring rate of 100 rpm. The drug release was evaluated by High Performance Liquid Chromatography method at 220 nm. The method was validated to meet requirements for a global regulatory filing. The validation included specificity, linearity, precision and accuracy. In addition, drug stability in medium was demonstrated. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard Pharmacopeial methods for the simultaneous determination of Terbutaline Sulfate 2.5 mg and Theophylline 100mg for the test dissolution using the HPLC systems.

Keywords: Dissolution, Validation, High Performance Liquid Chromatography.

INTRODUCTION

Terbutaline [1] and Theophylline [2] is used in the present research work. This formulation is used as a bronchodilator. 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. Terbutaline is a relatively selective beta2-adrenergic bronchodilator that has little or no effect on alpha-adrenergic receptors Theophylline, a xanthine derivative chemically similar to caffeine and theobromine, is used to treat asthma and bronchospasm [3].



Molecular weight: 548.7

Physical appearance: A white or almost white, crystalline powder

Melting point: 246-248 °C

Solubility: Freely Soluble in water, slightly soluble in ethanol (96%).

Dissociation Constant (pKa):8.8, 10.1, 11.2 @ 25°C

Fig. 1. Terbutaline Sulphate.

Chemical name: 5-[2-[1, 1-Dimethyl) amino]-1-hydroxyethyl]-1, 3- benzenediol sulphate.

Chemical formula: $C_7H_8N_4O_2$
Molecular weight: 180.2
Physical appearance: A white crystalline powder.
Melting point: 270°C to 274°C
Solubility: Slightly soluble in water, sparingly soluble in ethanol (96%). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.
Dissociation Constant (pKa): 8.77 @ 25°C.

Fig. 2. Theophylline.

Chemical name: 1, 3-dimethyl-2, 3, 6, 7-tetrahydro-1H-purine-2, 6-dione.

Dissolution testing is used to guide the development of new drug products and to assess the lot-to-lot variability of drug products. Analytical methods are validated to ensure that they are suitable for their intended use and provide accurate and reliable data. Validation of a dissolution method typically involves validation of the end analysis method [4].

The present research work describes a high performance liquid chromatographic method for the simultaneous determination of Terbutaline Sulphate and Theophylline in the tablets dosage form Theophylline 100mg and Terbutaline Sulphate 2.5mg using a reversed phase mode of separation. The separation was carried as per Optimized HPLC Chromatographic conditions.

MATERIAL AND METHODS

Material: Reference Standard of Theophylline and Terbutaline Sulphate were used. Tablets with (100mg Theophylline and 2.5 mg Terbutaline Sulphate) were procured from local market.

Reagents: 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. orthophosphate, citric acid, hydrochloric acid, sodium lauryl sulfate (SLS) (Sd fine chem pvt. Ltd, Mumbai) were used.

Instrumentation

Dissolution Tester: LABINDIA 2000 Auto Sampler

HPLC: Jasco, PU 980 HPLC isocratic pump with Jasco, AS - 2057 sampler and Jasco UV- Visible detector (UV-970). Column used Zorbax SB CN (10cm X 4.6 mm i.d. and 1.8 µm) with column oven temperature maintained at 40˚C. Mobile phase 0.01 % Ammonium acetate , Acetonitrile and Methanol in the ratio of (65:10:25) Flow rate 1.0 mL/min, Injection volume 20 µL and detection at 220.0 nm and recorded on Borwin chromatography software 1.21.

Methodology

Selection of appropriate dissolution Medium for the Quantitative determination of Terbutaline and Theophylline from drug product tablets.

Dissolution test conditions

Normally for basket and paddle apparatus the volume of the dissolution medium is 500 mL to 1000mL, with 900mL as the most common volume. The volume can be raised to between 2 and 4L, using larger vessels and depending on the concentration and the sink condition of the drug.

Different dissolution medium used during dissolution method development were [5-6].

1 Water
2 0.1 M HCL
3 Phosphate buffer (pH - 6.8)
4 Acetate Buffer (p H - 4.5)
5 0.5 % sodium dodecylsulfate in 0.01 M Sodium Phosphate pH -7.0.

Preparation of Dissolution Media

1. 0.1M HCl: 8.2 mL of Conc. HCL (37.5%) were pipette out & diluted to 1000 mL with water.
2. Phosphate buffer (pH - 6.8): Dissolved 6.8 g of KH_2PO_4 & 1g of NaOH were dissolved in 1000 mL water.
3. Acetate Buffer (pH 4.5): 2.99 g of Sodium Acetate Trihydrate & 1.66mL of Glacial Acetic Acid were transferred and diluted to 1000 mL with Water.
4. 0.5 % sodium dodecyl sulfate in 0.01 M Sodium Phosphate (pH 7.0): Dissolved 1.38 g of sodium dihydrogen phosphate (NaH_2PO_4, $2H_2O$) was dissolved in 1.0 Ltr of water & pH was adjusted to pH 7.0 with NaOH. Sodium dodecyl sulfate (SDS) was added so that the conc. is 0.5% w/v.

In present research work USP TYPE II (PADDLE TYPE) was used for the dissolution with 100 rpm speed and the volume of the dissolution medium was 900 mL. Dissolution profiling was done up to 60 min. Samples were drawn at 15 min, 20 min, 30 min, 45 min and 60 min.

Dissolution on Tablets was performed in different disso medium and its percentage release of Terbutaline Sulfate and Theophylline was calculated by using standard prepared using respective dissolution medium [7-8].

Preparation of standard solution

Standard Solution of Theophylline (100.0µg/mL) and Terbutaline Sulfate (3.0µg/mL):

Terbutaline Sulfate Stock solution (Solution A): 30 mg of Terbutaline Sulfate was weighed and transferred in to 100 mL volumetric flask. To it 70 mL of dissolution medium was added and the solution was sonicated for 5 mins. Finally, it was diluted up to the mark with dissolution medium.

Standard Preparation (Solution B): 20.0 mg of Theophylline was weighed and transferred in to a 200 mL volumetric flask containing 2 mL of solution A. To it 150.0 mL of dissolution medium was added and the solution was sonicated for 5 mins. Finally, it was diluted up to the mark with dissolution medium.

Method Validation

The method validation is a process to demonstrate that the analytical method developed is specific, accurate, precise and suitable for its intended use. The parameters that were considered for the validation of the dissolution method for the Terbutaline Sulfate and Theophylline in Tablets (Terbutaline Sulfate 2.5 mg and Theophylline 100mg) were,

A. Specificity and system suitability
B. Linearity and Range
C. Precision
   a. Repeatability
   b. Intermediate Precision
D. Accuracy
E. Robustness
F. Solution stability

A. Specificity and System Suitability

Blank (Dissolution medium) & Placebo (Excipients) were injected into the chromatograph to check the interference at the retention time corresponding to peak of Theophylline and Terbutaline. Individual identification solution of Theophylline and Terbutaline sulfate were injected to identify the peaks. The Standard solution was prepared and injected. The parameters such as similarity factor, tailing factor, theoretical plates and % RSD for peak area response and retention time of Theophylline and Terbutaline was determined.

B. Linearity and Range for Terbutaline Sulfate and Theophylline

The working range for Terbutaline Sulfate and Theophylline was determined by using working standard solution in the concentration range of 10.0 µg/mL to 150.0 µg/mL for Theophylline and for 0.3 µg/mL to 4.5 µg/mL for Terbutaline Sulfate separately. 20 µL of each of these solutions were injected into the chromatographic system under the optimized chromatographic conditions and the peak areas were recorded for each concentration of Terbutaline Sulfate and Theophylline. The analysis was performed in triplicate for all the levels.

C. Precision

a) Repeatability: Precision of an analytical procedure express the closeness or the agreement between a series of measurements obtained from multiple analysis of the same homogenous sample under the prescribed condition. Precision study was continued after specificity and system suitability study. Dissolution was performed on six Tablets for Terbutaline Sulfate and Theophylline Six independent aliquots withdrawn from six different vessels. The % release of Theophylline and Terbutaline Sulfate (%) for each tablet was determined. The peak area values and retention times of Terbutaline Sulfate and Theophylline standard were recorded for five replicate injections. The mean peak area, mean retention time, standard deviation and relative standard deviation (% R.S.D) were calculated

b) Precision: Intermediate precision: Intermediate precision of samples were carried out on different system and on different day using same samples of repeatability study.

The Difference of the average value between the dissolution result in two conditions (repeatability and intermediate precision) was calculated.

D. Accuracy

Accuracy was carried out by spiking analyte in the placebo (Excipients) preparations. Spiking was performed at four concentration levels each in triplicate. The % recovery of Theophylline and Terbutaline Sulfate in placebo (Excipients) was determined at concentrations of 10 %, 50%, 100% and 150% of the working level. The % RSD of % recovery for each level of Theophylline and Terbutaline Sulfate was determined.

E. Robustness

The robustness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Robustness of the in-vitro dissolution method was performed by intentionally changing the disso conditions such as bowl temperature, RPM, Volume of disso medium.

Blank (Dissolution medium) & Placebo (Excipients) was injected into the chromatograph to check the interference at the retention time corresponding to peak of Theophylline and Terbutaline.

The standard solution of Terbutaline Sulfate & Theophylline was prepared twice and injected. The parameters such as similarity factor, tailing factor, theoretical plates and % RSD for peak area responses and retention time of Terbutaline & Theophylline were determined.

Dissolution was performed on six Tablets for Terbutaline Sulfate and Theophylline by using optimized dissolution parameters

Six independent aliquots withdrawn from six different vessels. The % release of Theophylline and Terbutaline Sulfate (%) for each tablet was determined. The % RSD for % release of Theophylline and Terbutaline Sulfate of all six samples was observed within the acceptance criteria.

1. Robustness Experiments were carried out by changing bowl temperature (36.5°C)
2. Robustness Exp II was carried out by changing bowl temperature (37.5°C)
3. Robustness Exp III was carried out by changing flow rate of mobile phase from 1.0 ml / min to 0.9 ml / min
4. Robustness Exp V was carried out by changing RPM (98)
5. Robustness Exp VI was carried out by changing RPM (102).

F. Stability of Sample / Standard Solution

The % released of Terbutaline Sulfate & Theophylline was determined initially and then at the predetermined time interval of 4, 8, 12, 16, 20 and 24 hrs.

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.

RESULTS AND DISCUSSION

Method Development: Selection of appropriate dissolution Medium for the Quantitative determination of Terbutaline and Theophylline from tablets.

Table 1: % Release of Terbutaline Sulphate and Theophylline in Water.

	% Release of Terbutaline Sulfate					**% Release of Theophylline**				
Sample	15min	20min	30min	45min	60min	15min	20min	30min	45min	60min
1	28.7	43.6	81.4	94.3	98.7	36.3	52.7	86.6	96.6	101.3
2	29.7	45.7	82.3	93.3	99.3	35.3	50.3	87.5	95.3	102.4
3	29.6	44.6	83.7	94.3	100.3	34.3	53.0	87.0	97.6	99.3
4	28.5	44.3	84.3	93.6	101.0	34.9	52.0	85.7	96.9	100.3
5	29.3	45.0	84.3	94.9	99.7	36.0	52.4	86.6	96.3	100.9
6	29.0	44.3	83.4	93.9	99.7	34.3	51.9	87.3	95.9	99.9
Mean	29.1	44.6	83.2	94.0	99.8	35.2	52.0	86.8	96.4	100.6
SD	0.5	0.7	1.2	0.6	0.8	0.9	1.0	0.6	0.8	1.1
% R.S.D	1.7	1.6	1.4	0.6	0.8	2.4	1.8	0.7	0.8	1.1

Table 2: % Release of Terbutaline Sulphate and Theophylline in 0.1N HCL.

Sample	% Release of Terbutaline					% Release of Theophylline				
	15min	20min	30min	45min	60min	15min	20min	30min	45min	60min
1	12.3	23.7	34.6	51.3	61.3	20.2	29.4	52.3	67.2	74.3
2	12.6	24.6	33.7	50.0	62.0	17.4	29.6	52.1	67.5	75.1
3	14.3	27.0	34.3	51.0	62.1	19.9	29.9	51.5	67.9	75.4
4	13.6	24.3	33.4	50.3	60.6	18.7	27.4	52.4	69.3	75.0
5	14.3	23.7	34.3	51.1	61.3	19.9	30.3	50.3	68.5	73.6
6	12.4	22.1	33.2	50.1	62.0	20.4	30.1	52.4	66.4	72.3
Mean	13.2	24.2	33.9	50.6	61.5	19.4	29.4	51.8	67.8	74.3
SD	0.9	1.6	0.6	0.6	0.6	1.2	1.1	0.8	1.0	1.2
% R.S.D	6.9	6.6	1.7	1.1	1.0	6.0	3.6	1.6	1.5	1.6

Table 3: % Release of Terbutaline Sulfate and Theophylline in Acetate Buffer pH 4.5.

Sample	% Release of Terbutaline Sulphate					% Release of Theophylline				
	15min	20min	30min	45min	60min	15min	20min	30min	45min	60min
1	16.4	39.0	61.2	81.3	91.3	15.9	24.7	41.8	56.9	60.4
2	14.9	37.9	60.6	81.0	90.3	13.7	24.6	42.1	58.6	60.9
3	16.5	38.2	59.6	79.6	92.6	14.9	22.3	42.2	58.3	58.3
4	16.0	38.3	61.3	80.2	91.5	14.7	24.9	43.1	57.6	61.9
5	16.3	37.6	59.6	82.5	92.9	13.8	24.7	42.6	56.4	61.6
6	16.2	36.9	61.5	80.6	90.3	15.0	24.3	40.4	58.3	62.3
Mean	16.0	38.0	60.6	80.9	91.5	14.6	24.2	42.0	57.7	60.9
SD	0.6	0.7	0.8	1.0	1.1	0.8	1.0	0.9	0.9	1.4
% R.S.D	3.7	1.9	1.4	1.3	1.2	5.5	3.9	2.2	1.5	2.4

Table 4: % Release of Terbutaline Sulfate and Theophylline in Phosphate Buffer pH 6.8.

Sample	% Release of Terbutaline Sulfate					% Release of Theophylline				
	15min	20min	30min	45min	60min	15min	20min	30min	45min	60min
1	18.4	40.3	70.3	86.5	97.3	15.1	37.1	60.2	78.3	90.2
2	19.9	42.6	70.7	84.3	93.3	14.9	38.6	59.3	76.5	89.7
3	19.9	42.3	68.3	85.8	97.3	13.3	38.2	58.2	78.7	91.3
4	19.6	39.5	69.9	83.3	96.5	15.2	36.3	57.4	79.6	87.3
5	20.1	38.3	64.2	86.9	94.3	14.4	37.6	61.2	81.2	86.4
6	17.4	42.6	70.2	85.2	96.9	15.7	34.1	60.9	81.2	91.3
Mean	19.2	40.9	68.9	85.3	95.9	14.8	37.0	59.5	79.2	89.3
SD	1.1	1.8	2.4	1.4	1.7	0.9	1.6	1.5	1.8	2.1
% R.S.D	5.7	4.4	3.5	1.6	1.8	5.8	4.4	2.5	2.3	2.3

Conclusion: From Dissolution experiments data it was concluded that Water is discriminative dissolution medium for in-vitro study of Terbutaline Sulfate and Theophylline.

Method Validation

1. Specificity And System Suitability

Table 5: System suitability parameter for Terbutaline & Theophylline.

Peak Name	Tailing factor	Theoretical plate (As per USP)	Resolution between Theophylline & Terbutaline
Terbutaline	1.2	2755	--
Theophylline	1.4	4586	3.8

The value of percent relative standard deviation for peak area and retention time are less than 2.0%, and system suitability criteria for the system is suitable for the chromatographic analysis.

2. Linearity & Range for Terbutaline Sulfate and Theophylline

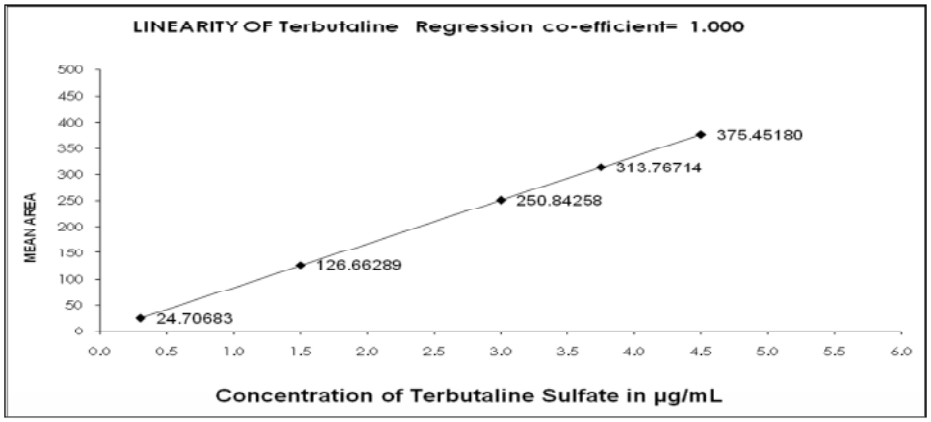


Fig. 3: Graph of Peak Area Vs Conc. of Terbutaline Sulfate.

Table 6: Regression analysis data of Terbutaline Sulfate.

Slope	83.45916
Y-Intercept	0.5
% Y-Intercept	0.20
Regression Coefficient (R^2)	1.000

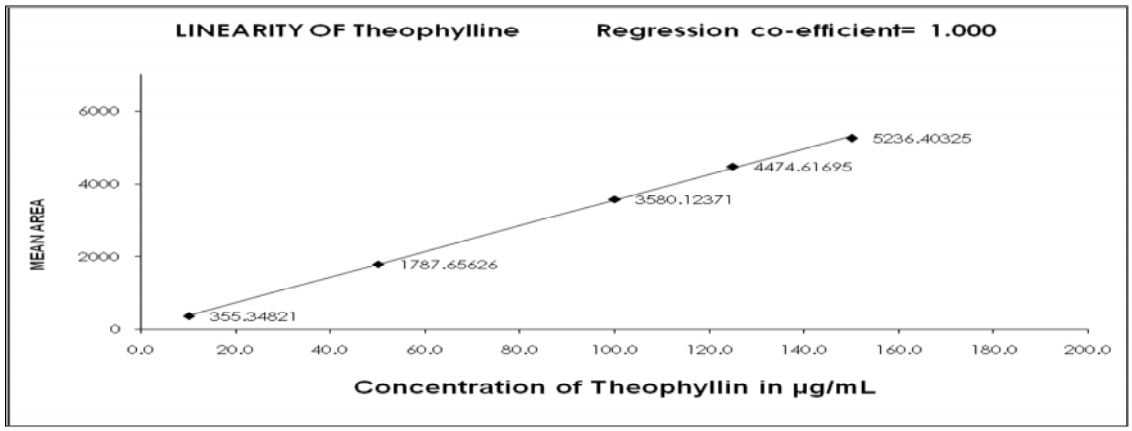


Fig. 4: Graph of Peak Area Vs Conc. of Theophylline.

Table 7: Regression analysis data of Theophylline.

Slope	35.16812
Y-Intercept	27.2
% Y-Intercept	0.76
Regression Coefficient (R^2)	1.000

From the above graph, it is observed that Terbutaline Sulfate shows linear response in the conc. range of 0.3 µg/mL to 4.5 µg/mL. And it is also observed that Theophylline shows linear response in the conc. range of 10 µg/mL to 150 µg/mL.

3. Precision

a) Repeatability: The % RSD for % release of Theophylline and Terbutaline Sulfate of all six samples was observed within the acceptance criteria.

Table 8: Results of repeatability study.

Samples	**% Release of Terbutaline Sulfate**	**% Release of Theophylline**
1	100.23	102.23
2	100.25	100.21
3	99.65	101.08
4	100.39	100.31
5	99.55	102.34
6	99.68	102.19
Mean	99.96	101.39
SD	0.37	0.99
% R.S.D	0.37	0.98

b) Precision - Intermediate precision

Table 9: Difference between Repeatability and Intermediate precision results.

SET	**% Release of Terbutaline**	**% Release of Theophylline**
Repeatability	99.96	101.39
Intermediate precision	99.99	101.02
Difference	0.04	0.38

The % RSD for the Theophylline and Terbutaline Sulfate content of all six samples was within the acceptance criteria. The Difference of the average value between the dissolution result in two conditions (repeatability and intermediate precision) was observed within the acceptance criteria.

4. Accuracy

The % recovery for Terbutaline Sulfate & Theophylline and % RSD of % recovery for each level of Theophylline and Terbutaline Sulfate obtained was within acceptance criteria.

Table 10: Terbutaline Sulfate % recovery of individual levels.

% Recovery for	Accuracy level 1	Accuracy level 2	Accuracy level 3	Accuracy level 4
1	101.5	102.0	99.3	100.7
2	97.1	99.4	98.7	99.2
3	99.3	99.2	98.8	101.5
Mean	99.3	100.2	98.9	100.5
SD	2.18	1.56	0.32	1.16
% RSD	2.2	1.6	0.3	1.2
Minimum	97.1	99.2	98.7	99.2
Maximum	101.5	102.0	99.3	101.5

Table 11: Theophylline % recovery of individual levels.

% Recovery for	Accuracy level 1	Accuracy level 2	Accuracy level 3	Accuracy level 4
1	100.3	102.3	101.7	98.3
2	98.3	100.6	100.3	99.2
3	100.2	100.8	99.8	99.8
Mean	99.6	101.2	100.6	99.1
SD	1.14	0.93	1.02	0.72
% RSD	1.1	0.9	1.0	0.7
Minimum	98.3	100.6	99.8	98.3
Maximum	100.3	102.3	101.7	99.8

As the % recovery for both Terbutaline Sulfate and Theophylline is close to 100.0% hence the method is accurate.

5. Robustness

Table 12: Difference between Repeatability and Robustness Exp-I.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness I	99.70	100.78
Difference of Average value	0.26	0.61

Table 13: Difference between Repeatability and Robustness Exp-II.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness II	100.62	101.23
Difference of Average value	0.66	0.17

Table 14: Difference between Repeatability and Robustness Exp-III.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness I	98.77	100.06
Difference of Average value	1.19	1.34

Table 15: Difference between Repeatability and Robustness Exp-IV.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness II	101.26	101.26
Difference of Average value	1.30	0.13

Table 16: Difference between Repeatability and Robustness Exp-V.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness I	100.69	100.85
Difference of Average value	0.73	0.55

Table 17: Difference between Repeatability and Robustness Exp-VI.

SET	% Release of Terbutaline	% Release of Theophylline
Repeatability	99.96	101.39
Robustness II	100.69	100.90
Difference of Average value	0.73	0.49

The % RSD for % release of Theophylline and Terbutaline Sulfate of all six samples was observed within the acceptance criteria.

The % RSD for the Theophylline and Terbutaline Sulfate content of all six samples was within the acceptance criteria. The Difference of the average value between the dissolution result in two condition (repeatability and Robustness Exp I) was observed within the acceptance criteria.

6. Stability of standard and sample solution

The % relative difference for % released of Terbutaline Sulfate & 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 additional peaks were observed for samples after the specified predetermined time interval when compared with the initial run. Hence the sample solution was found to be stable at 24 hours at laboratory conditions.

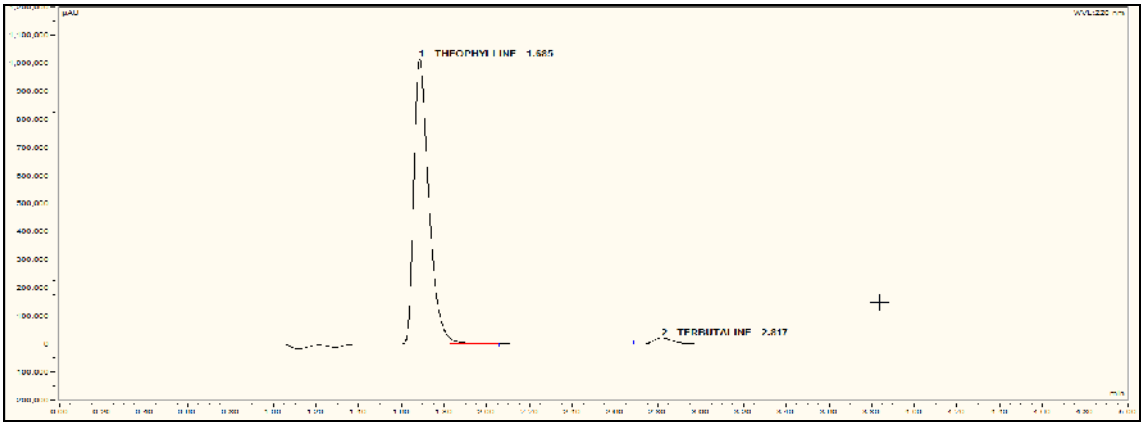


Fig. 5: A typical chromatogram for Theophylline and Terbutaline standard solution.

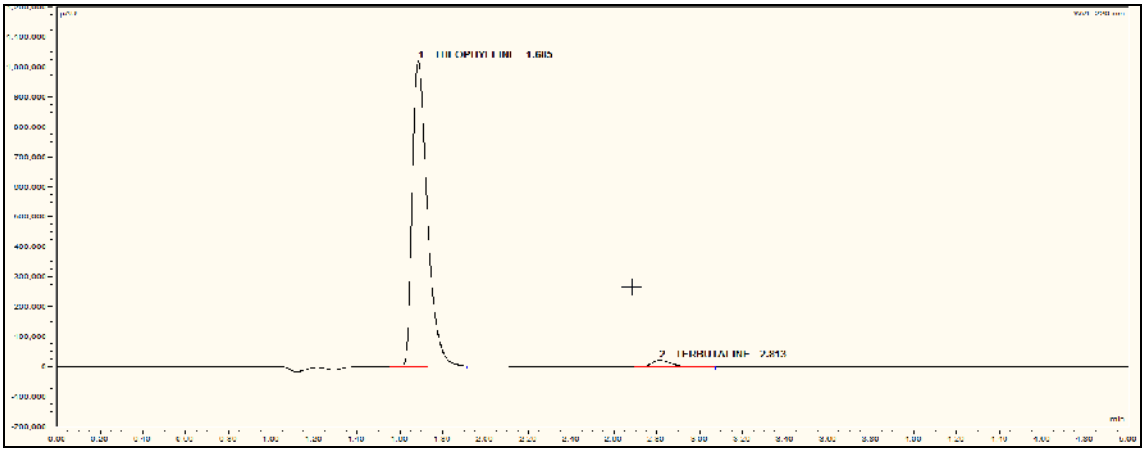


Fig. 6: A typical chromatogram for Theophylline and Terbutaline sample solution.

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