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New RP HPLC method for the simultaneous estimation of terbutaline and theophylline in pharmaceutical dosage form

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ABSTRACT

A simple and selective LC method is described for the determination of Terbutaline and Theophylline dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 20Mm Phosphate buffer (KH2PO4) pH: 3.5 Acetonitrile (80:20v/v/v), with detection of 250 nm. Linearity was observed in the range 1.25-3.75 µg /ml for Terbutaline (r^2 =0.9975) and 50-150/ml for Theophylline (r^2 =0.9994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key words: Phosphate buffer (KH2PO4) pH: 3.5 Acetonitrile (80:20v/v/v), Terbutaline and Theophylline

INTRODUCTION

High Performance Liquid Chromatography is the most widely used of all the analytical separation techniques. The reasons for its popularity are its sensitivity, ready adaptability to quantitative determination, suitable for non-volatile and thermally fragile species, wide applicability to variety of substances such as amino acids, carbohydrates, nucleic acids, proteins, hydrocarbons, terpenoids, pesticides, steroids, metal-organic species and inorganic species. As high pressures (around 3000 psi) are used for the separation of the analytes down the column, it is often termed as High Pressure Liquid Chromatography. [4, 5, 6]

Types of HPLC

HPLC is classified into various types

Based on polarity of stationary and mobile phase

- Normal Phase Chromatography
- Reverse Phase Chromatography

Based on the principle of separation

- Adsorption Chromatography
- Partition Chromatography
- Ion Pair Chromatography
- Size Exclusion Chromatography
- Chiral Phase Chromatography

Based on elution technique

- Isocratic Elution
- Gradient Elution

Based on scale of operation

- Analytical HPLC
- Preparative HPLC

Based on the polarity of the stationary phase and the mobile phase, it is of two types:

Normal Phase (NP) HPLC

In this type, the stationary phase is polar and the mobile phase is non-polar, polar compounds are retained for a longer periods because of more affinity towards the stationary phase, hence non-polar compounds travel faster and are eluted first.

Reverse Phase (RP) HPLC

In this type, the stationary phase is non-polar and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3, 4,5,6]

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of TERBUTALINE & THEOPHYLLINE in pharmaceutical dosage form.

Plan of work

- Solubility determination of Terbutaline & Theophylline in various solvents and buffers.
- Determine the absorption maxima of the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of 80 volumes of Phosphate buffer pH 3.5:20volumes of Acetonitrile. The mobile phase was sonicated for 10min to remove gases.

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of TERBUTALINE

50 mg of Terbutaline was weighed and transferred in to 500ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution ofTHEOPHYLLINE

50mg of Theophylline was weighed in to 500ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSION

Solubility Studies

These studies are carried out at 25 ^{0} C

Terbutaline

Soluble in methanol, sparingly soluble in DMSO, insoluble in Water,.

Theophylline

Freely Soluble in Methanol. Slightly Soluble in Water and DMF.

Wavelength determination

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 1, 2 and 3 and the absorption curve shows characteristic absorption maxima at 241 nm for Terbutaline and Theophylline 278 and 250 nm for the combination.

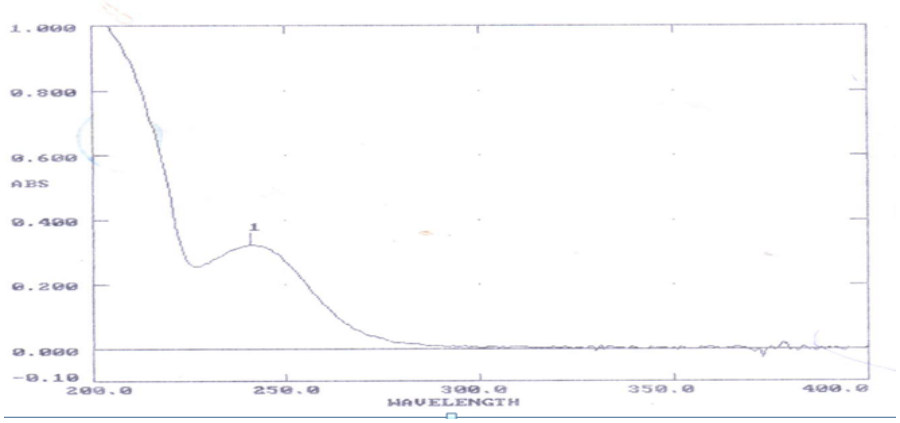


Fig. 1: UV-VIS spectrum of terbutaline

Obeservation

λ_{max} was found to be 241 nm for Terbutaline shown in the figure 1

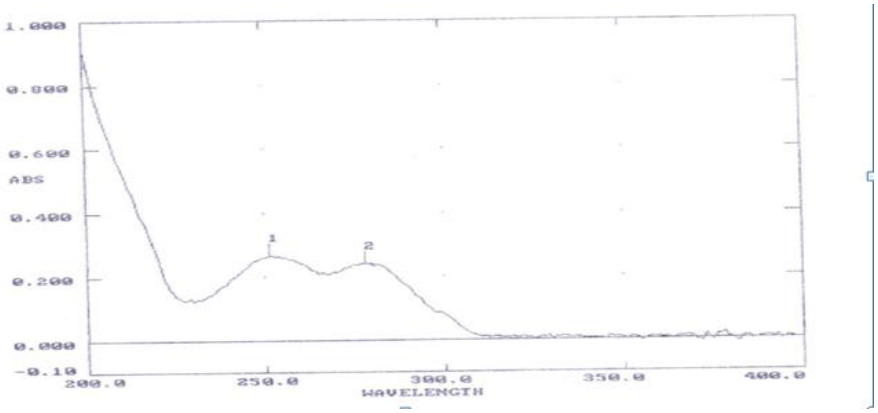


Fig. 2: UV-VIS spectrum of Theophylline

Observation

λ_{max} was found to be 278 nm for Theophylline shown in the figure 2

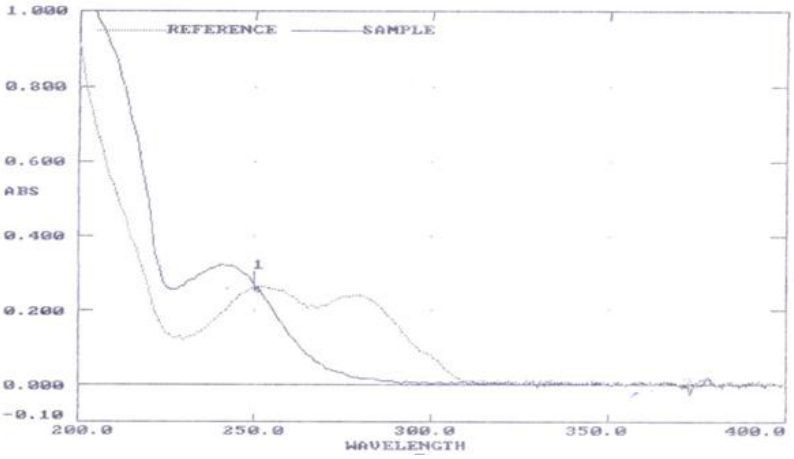


Fig. 3: UV-VIS spectrum of Terbutaline and Theophylline and the isosbestic point was 250 nm

Observation

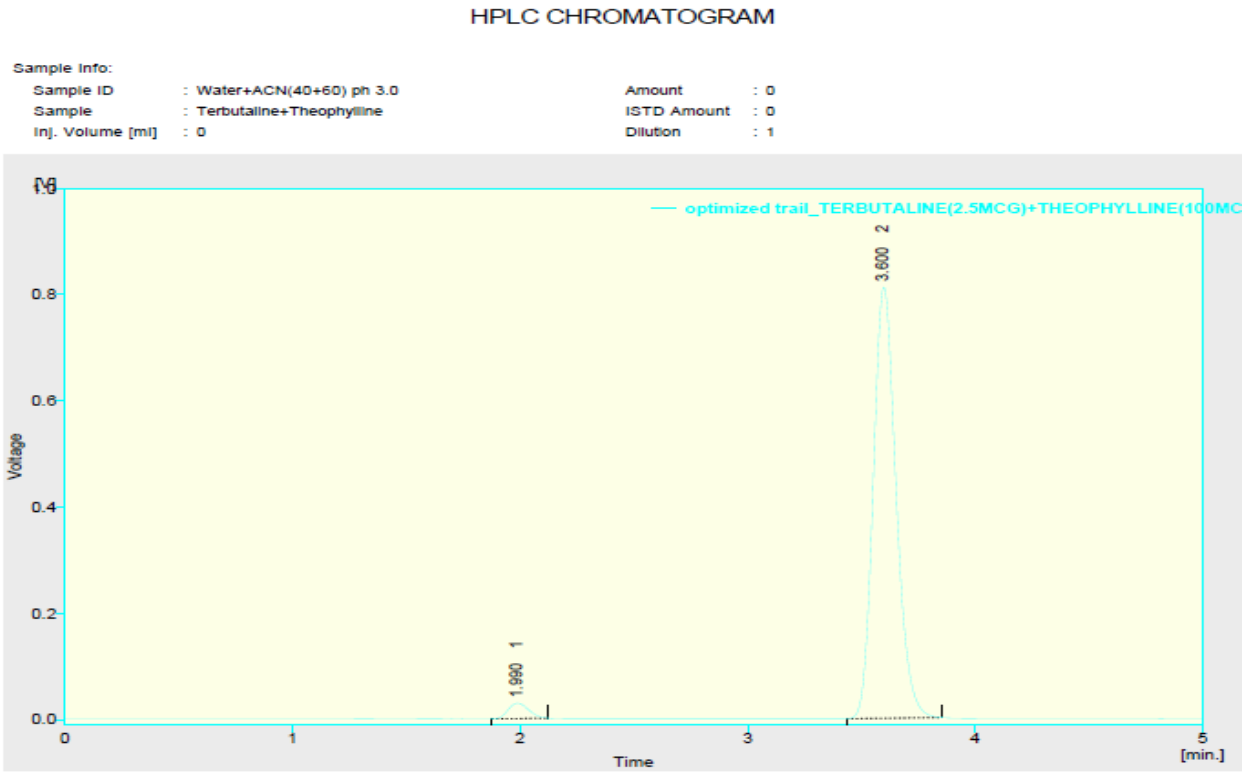
The Isosbestic point was found to be 250nm for Terbutaline and Theophylline in combination and was shown in figure 3

METHOD DEVELOPMENT OF TERBUTALINE & THEOPHYLLINE

Trial- 4

Preparation of mixed standard solution

weigh accurately 2.5mg of Terbutaline and 100 mg of Theophylline in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 2.5 µg/ml of Terbutaline and 100 µg/ml of Theophylline is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



Result Table (Uncal - optimized trail_TERBUTALINE(2.5MCG)+THEOPHYLLINE(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.990	179.164	28.814	3.1	3.4	0.10
2	3.600	5641.595	811.392	96.9	96.6	0.11
	Total	5820.759	840.206	100.0	100.0	

Column Performance Table (From 50% - optimized trail_TERBUTALINE(2.5MCG)+THEOPHYLLINE(100MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	1.990	0.103	1.250	0.00	2055	20546	-
2	3.600	0.110	1.296	0.00	5934	59338	8.882

Fig. 4: Chromatogram of terbutaline and theophylline by using mobile phase.

Observation

The peaks showed more efficiency and more resolution. Hence this method was optimised.

Table 1: Optimized chromatographic conditions

Mobile phase	**Phosphate buffer (KH2PO4) pH: 3.5 Acetonitrile (80:20v/v/v),**
pH	3
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	250nm
Injection volume	20 µl
Run time	10 min
Retention time	About 2.337 min for Terbutaline and 4.028min for Theophylline

ASSAY

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 2.5mg of Terbutaline and 100 mg of Theophylline in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 2.5 µg/ml of Terbutaline and 100 µg/ml of Theophylline is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5tablets (each tablet contains 2.5mg of Terbutaline and 100mg of Theophylline) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Terbutaline (25µg/ml) and Theophylline (1000µg/ml) were prepared by dissolving weight equivalent to 2.5mg of Terbutaline and 100 mg of Theophylline and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 2.5µg/ml of Terbutaline and 100 µg/ml of Theophylline was made by adding 1ml of stock solution to 10 ml of mobile phase.

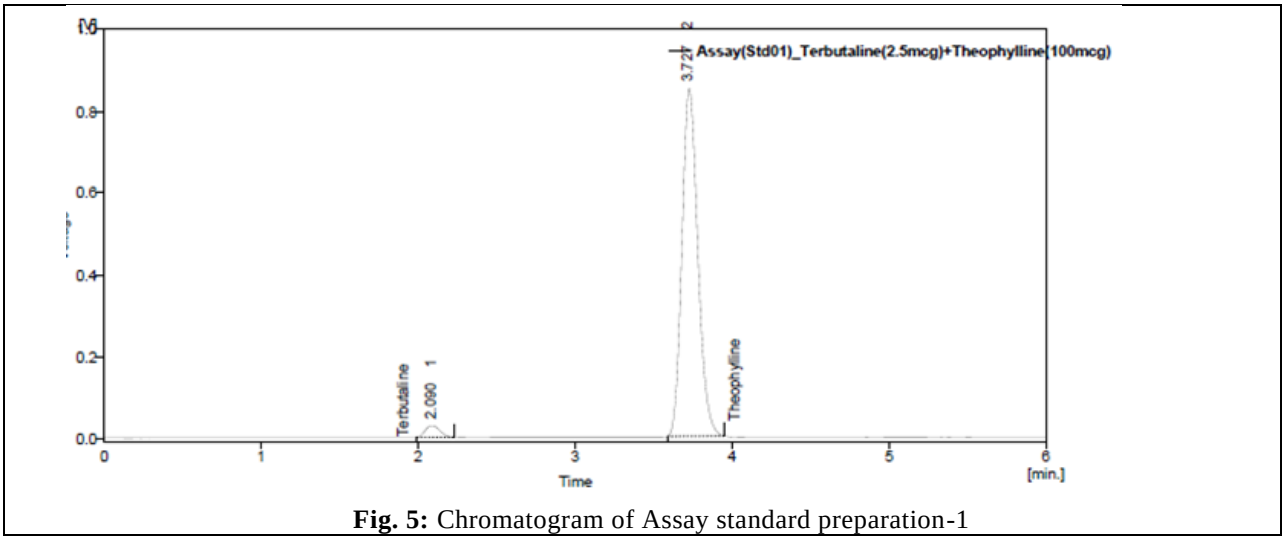


Fig. 5: Chromatogram of Assay standard preparation-1

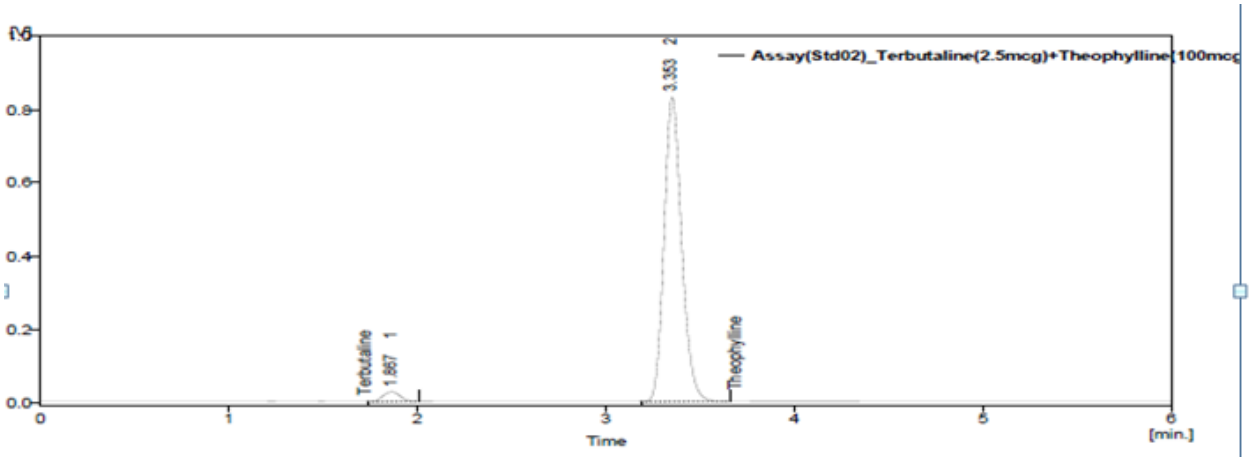


Fig. 6: Chromatogram of Assay standard preparation-2

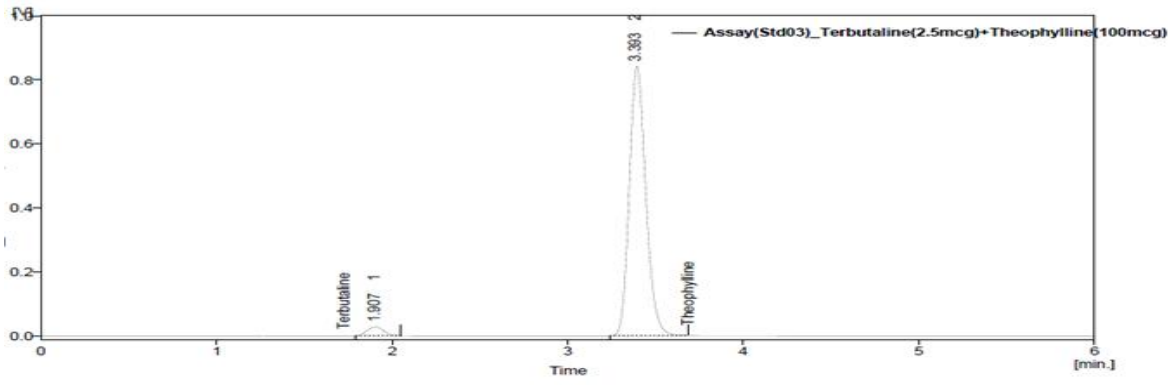


Fig. 7: Chromatogram of Assay standard preparation-3

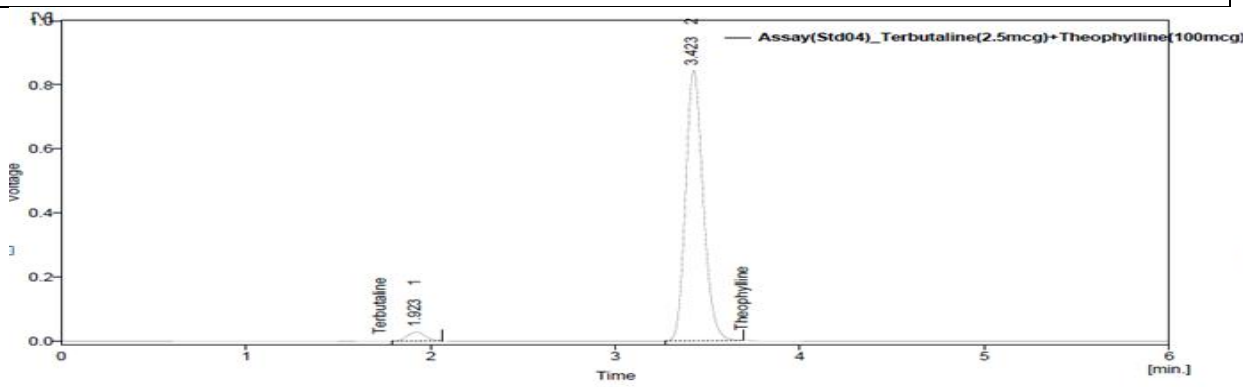


Fig. 8: Chromatogram of Assay standard preparation-4

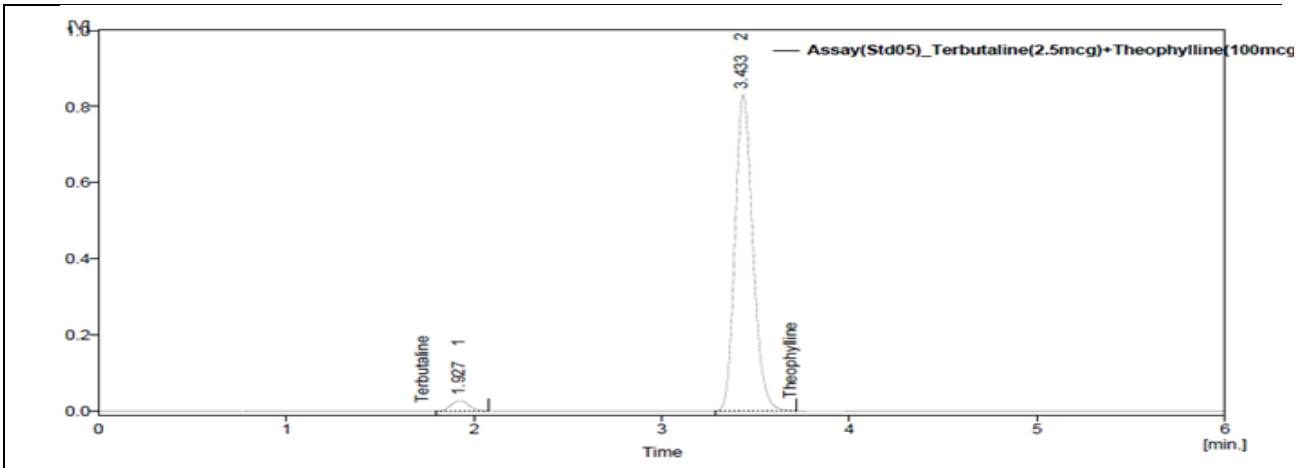


Fig. 9: Chromatogram of Assay standard preparation-5

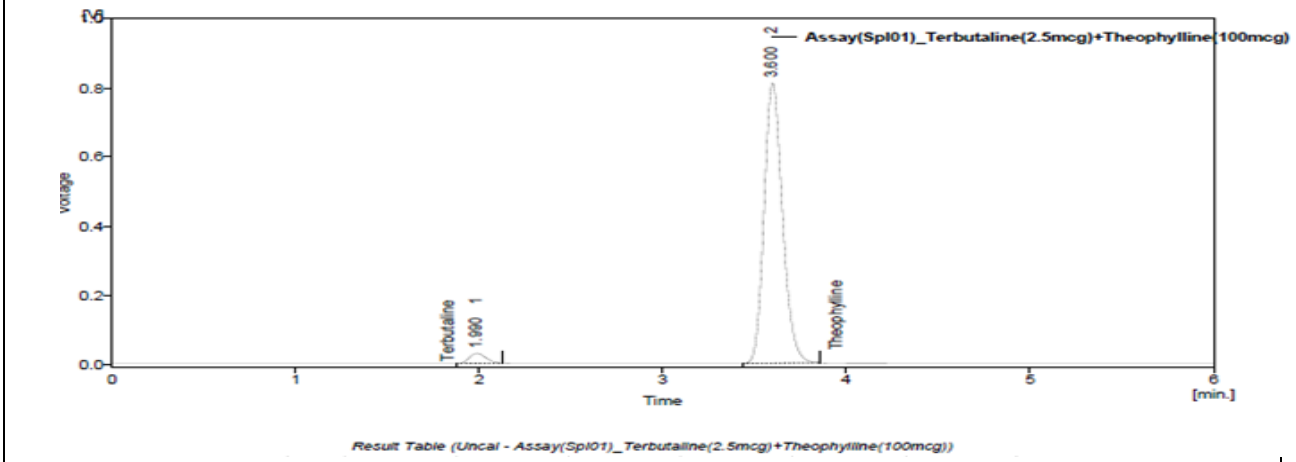


Fig. 10: Chromatogram of Assay sample preparation-1

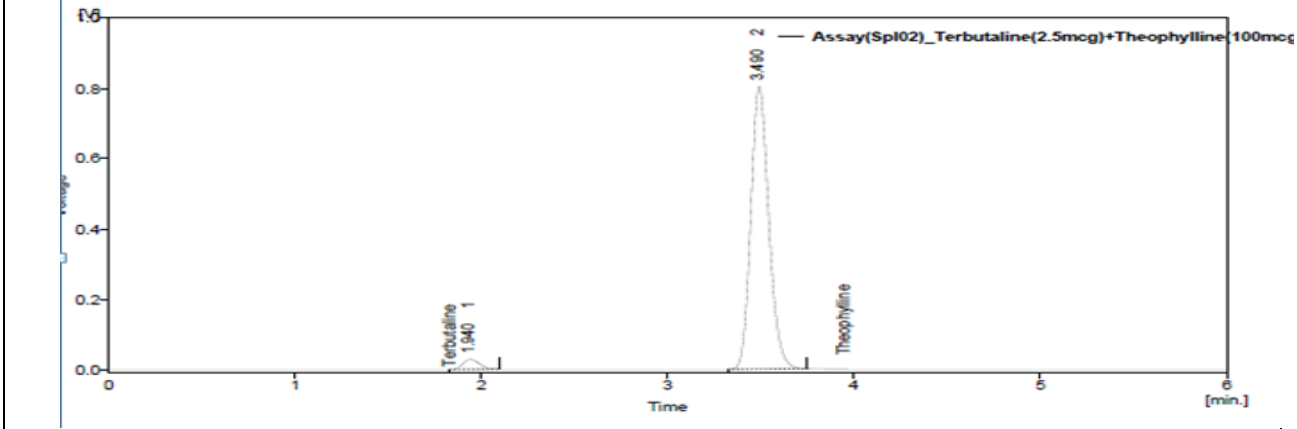


Fig. 11: Chromatogram of Assay sample preparation-2

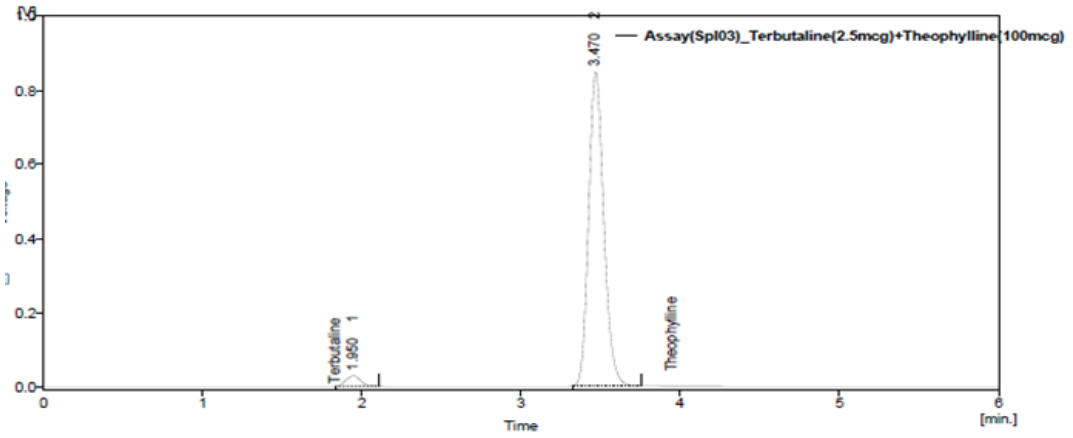


Fig. 12: Chromatogram of Assay sample preparation-3

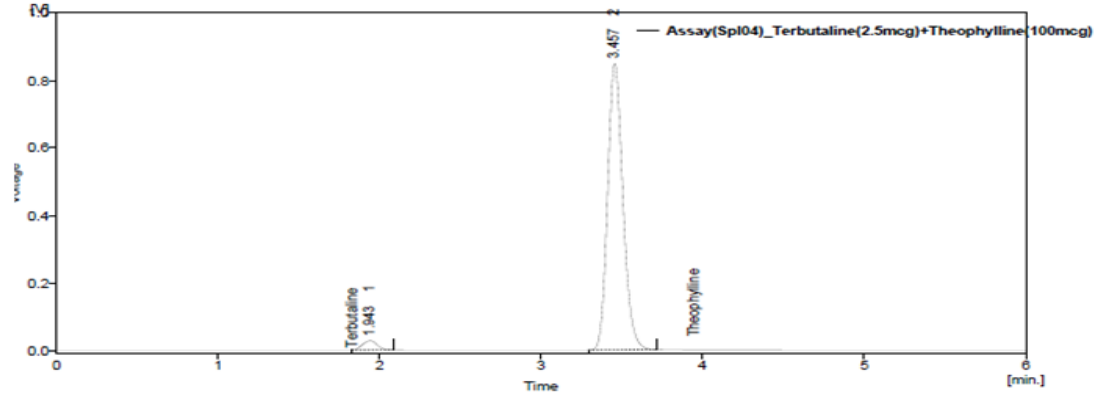


Fig. 13: Chromatogram of Assay sample preparation-4

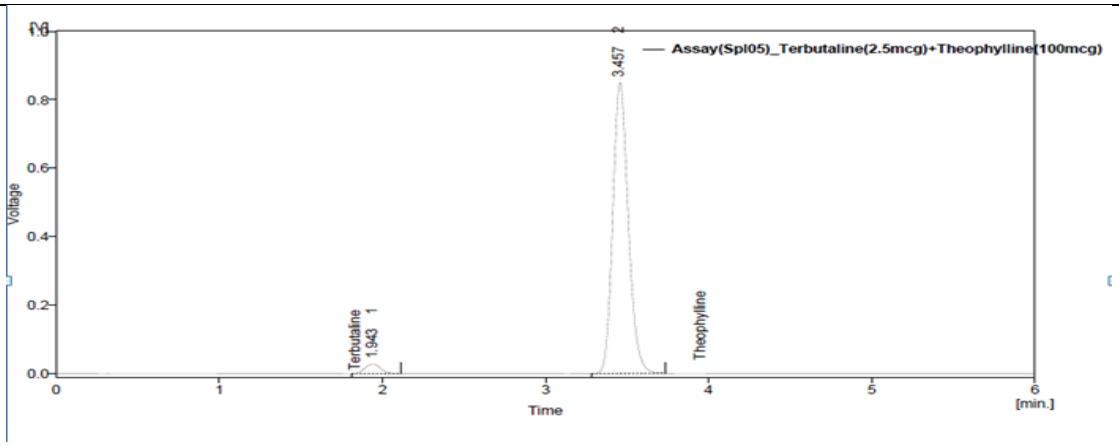


Fig. 14: Chromatogram of Assay sample preparation-5

Table No.2: Assay Results

TERBUTALINE			**THEOPHYLLINE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	187.838	179.164	5678.797	5541.595

Injection-2	175.570	175.851	5436.955	5508.623
Injection-3	176.601	184.101	5494.221	5523.567
Injection-4	179.819	180.499	5575.961	5406.592
Injection-5	179.681	185.581	5483.936	5614.223
Average Area	179.902	181.039	5533.974	5518.92
Tablet average weight	150.75mg		150.75mg	
Standard weight	2.5mg		100mg	
Sample weight	150.75mg		150.75mg	
Label amount	2.5mg		100mg	
std. purity	99.2		99.3	
Amount found in mg	2.495		99.03	
Assay(%purity)	**99.60**		**99.03**	

Observation

The amount of Terbutaline and Theophylline present in the taken dosage form was found to be 99.60 and 99.03% respectively.

VALIDATIONS

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of mixed standard solution

2.5 µg/ml of Terbutaline and 100 µg/ml of Theophylline solution is prepared with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5tablets (each tablet contains 2.5mg of Terbutaline and 100mg of Theophylline) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Terbutaline (25µg/ml) and Theophylline (1000µg/ml) were prepared by dissolving weight equivalent to 2.5mg of Terbutaline and 100 mg of Theophylline and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 2.5µg/ml of Terbutaline and 100 µg/ml of Theophylline was made by adding 1ml of stock solution to 10 ml of mobile phase.

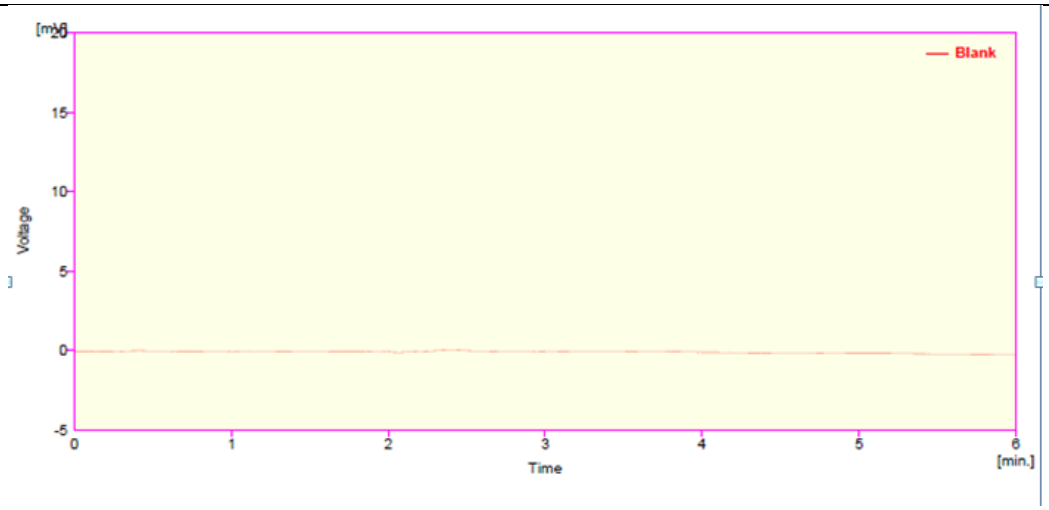


Fig. 15: Blank chromatogram for specificity by using mobile phase

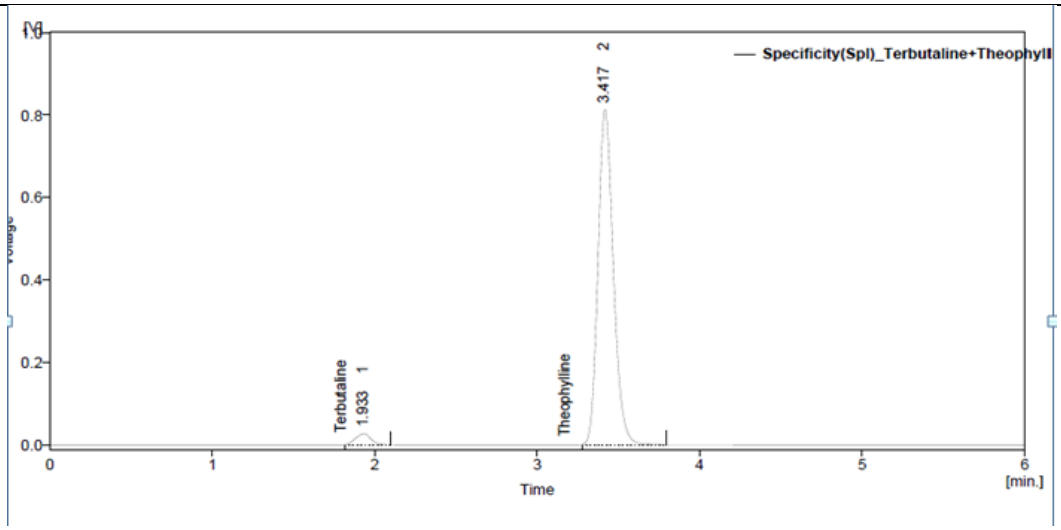


Fig. 16: Chromatogram for specificity of Terbutaline & Theophylline sample

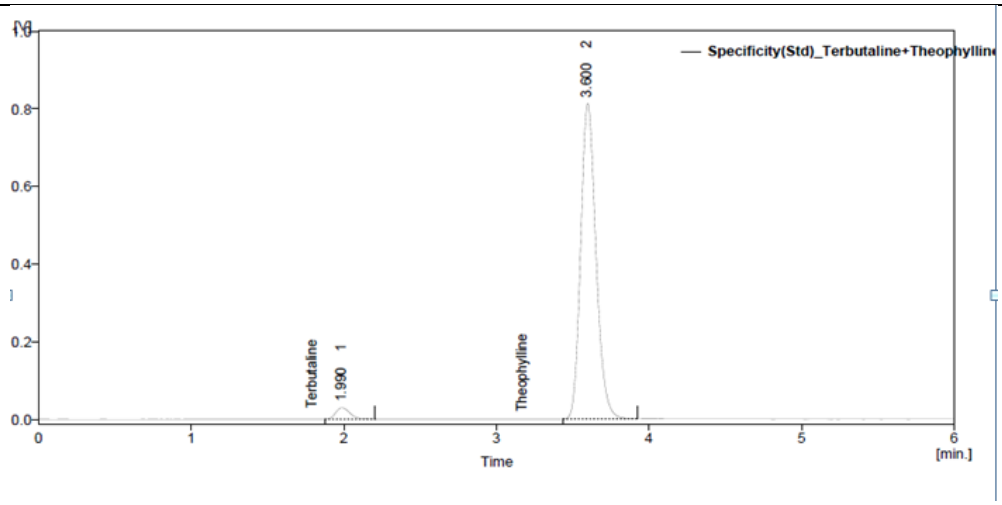


Fig. 17: Chromatogram for Specificity of Terbutaline & Theophylline standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the Terbutaline & Theophylline peaks.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 2.5 mg of Terbutaline and 100 mg of Theophylline in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. Further take 1ml into 10ml volumetric flask and make upto 10ml with mobile phase.

Table 3: Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
			Terbutaline	Theophylline

Preparation 1	0.0125	0.5	1.25	50
Preparation 2	0.0185	0.75	1.85	75
Preparation 3	0.025	1	2.5	100
Preparation 4	0.0315	1.25	3.15	125
Preparation 5	0.0375	1.5	3.75	150

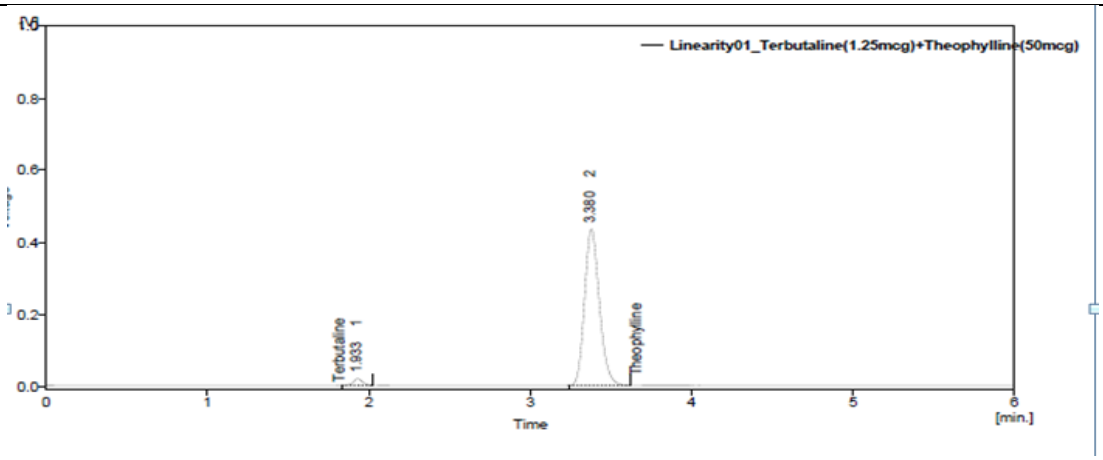


Fig. 18: Chromatogram of Terbutaline and Theophylline preparation-1

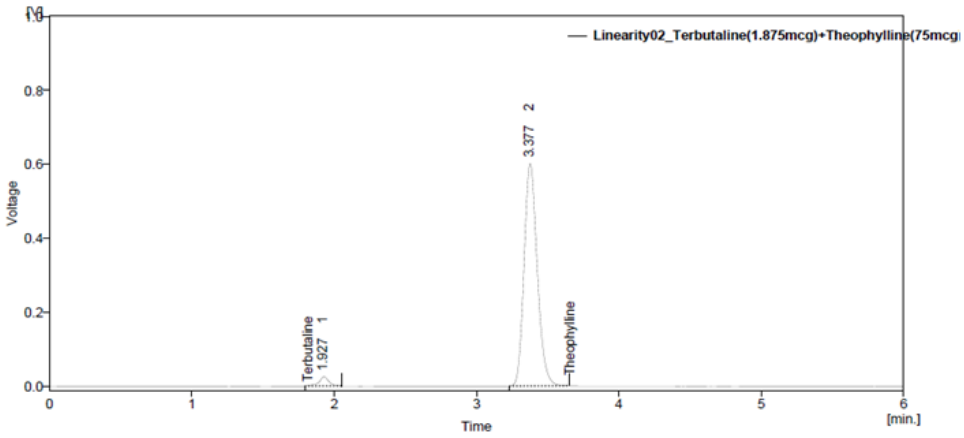


Fig. 19: Chromatogram of Terbutaline and Theophylline preparation-2

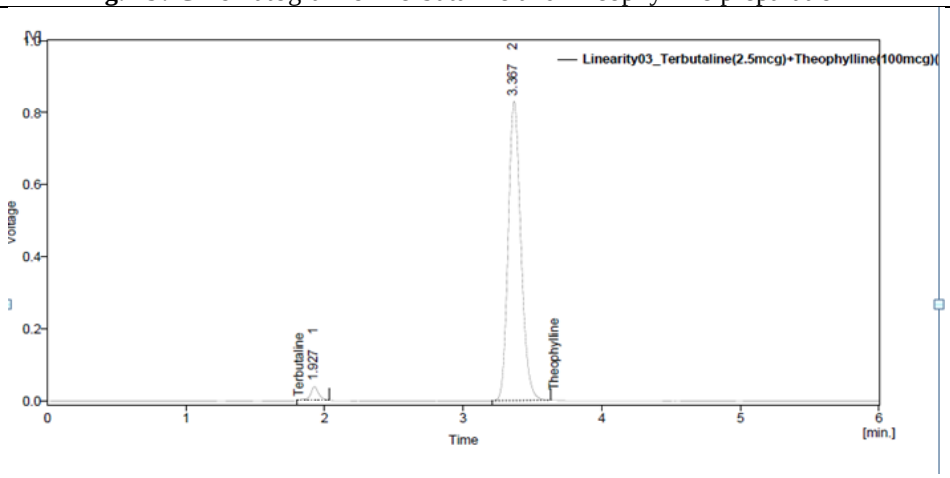


Fig. 20: Chromatogram of Terbutaline and Theophylline preparation-3

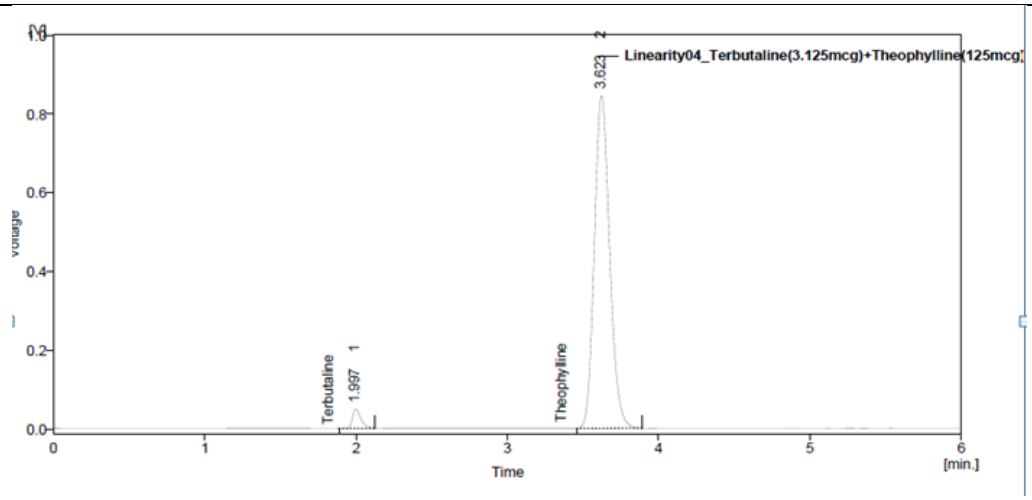


Fig.21: Chromatogram of Terbutaline and Theophylline preparation-4

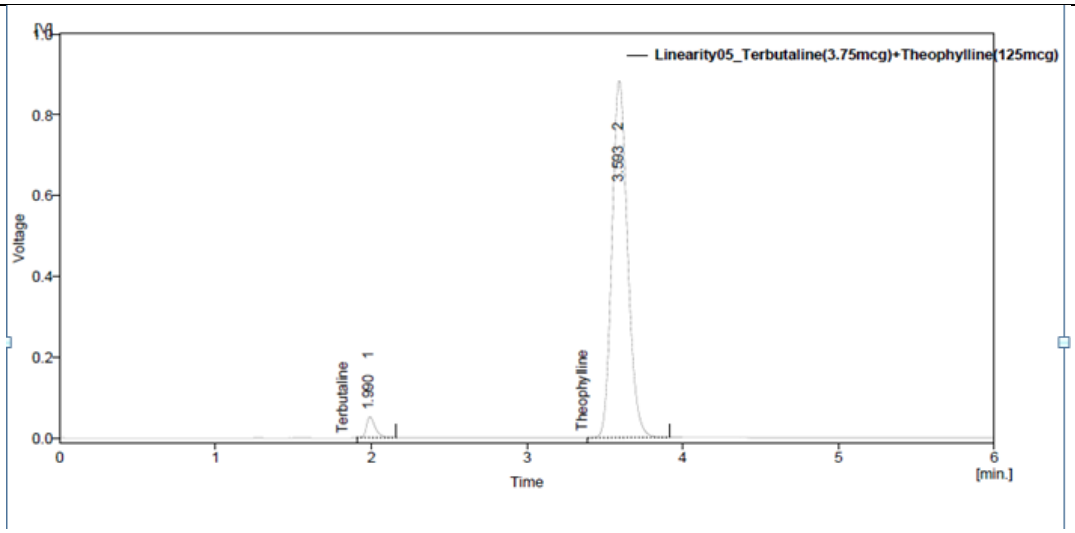


Fig. 22: Chromatogram of Terbutaline and Theophylline for preparation-5

Table 4: linearity of Terbutaline

S.No.	Conc.(µg/ml)	Area
1	1.25	78.029
2	1.85	122.306
3	2.5	154.766
4	3.15	190.241
5	3.75	218.291

Table 9.3.8: linearity of THIOCOLCHICOSIDE

S.No.	Conc.(µg/ml)	Area
1	50	2790.728
2	75	3866.934
3	100	5285.723
4	125	6121.454
5	150	6361.533

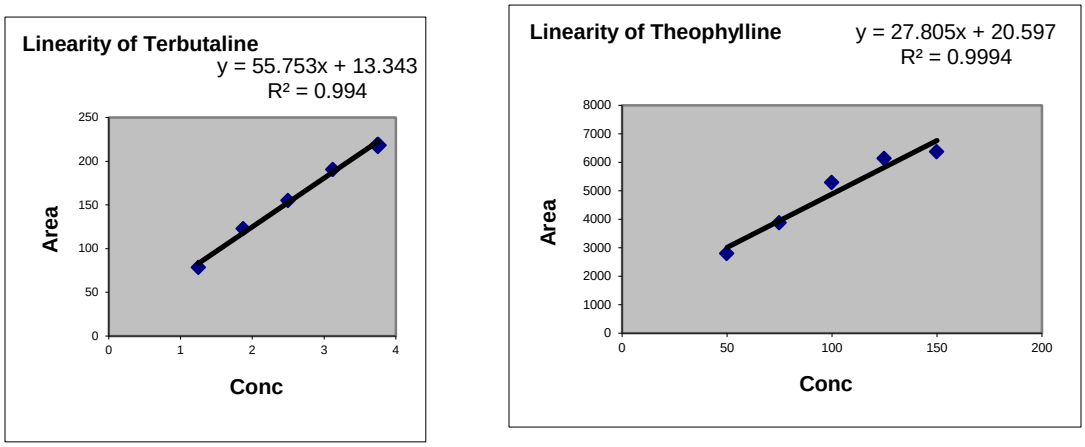


Fig. 23: Linearity graph of Terbutaline And Theophylline

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Terbutaline and Theophylline is 0.998 and 0.999. The relationship between the concentration of Terbutaline and Theophylline and area of Terbutaline and Theophylline is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 75%, 100%, 125%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 75%, 100% & 125%.

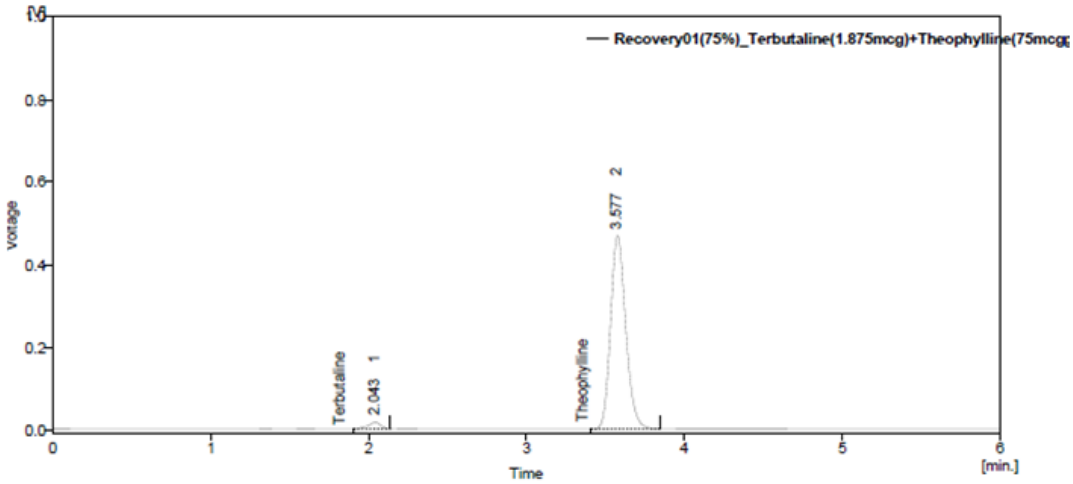


Fig. 24: Chromatogram of 75%recovery (injection 1)

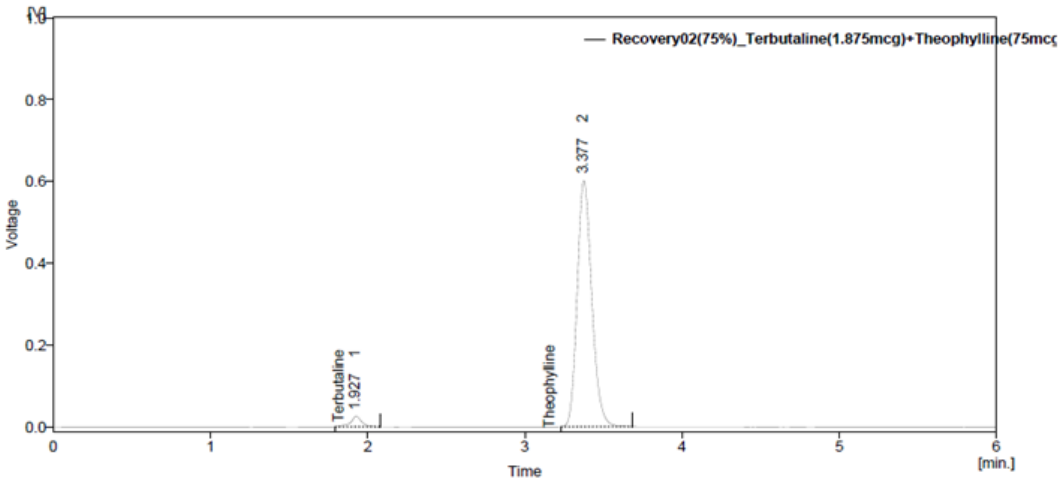


Fig. 25: Chromatogram of 75% recovery (injection 2)

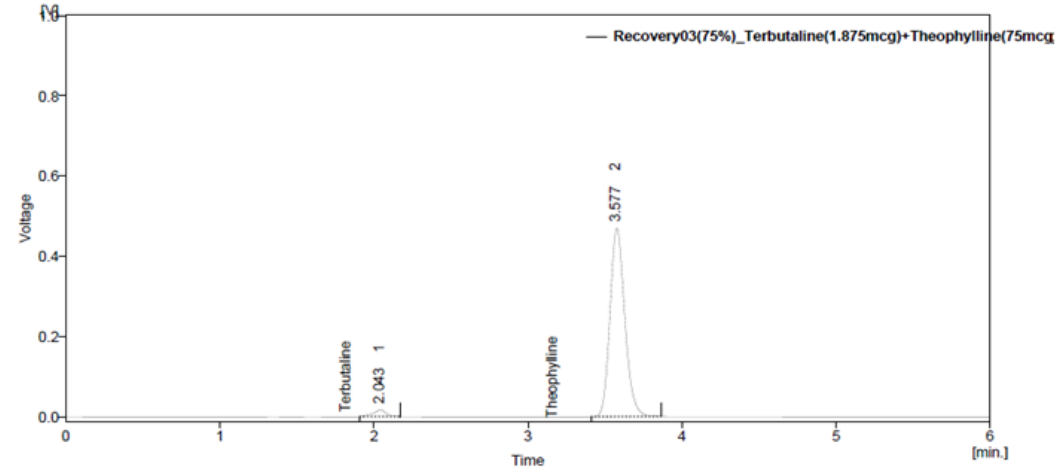


Fig. 26: Chromatogram of 75% recovery (injection 3)

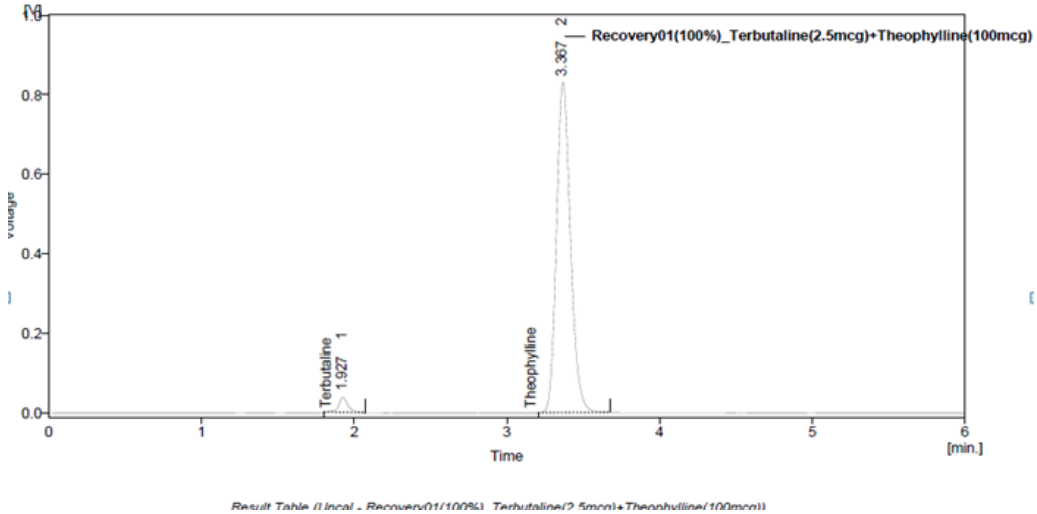


Fig. 27: Chromatogram of 100% recovery (injection 1)

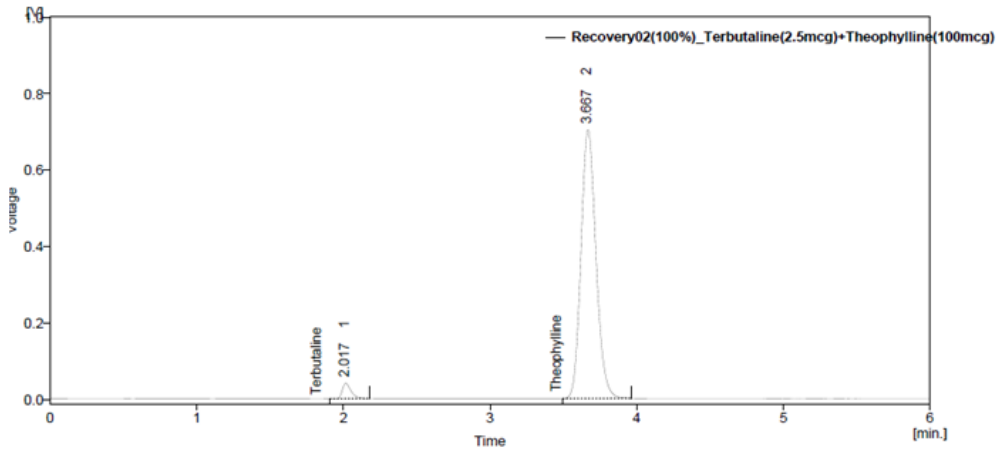


Fig. 28: Chromatogram of 100% recovery (injection 2)

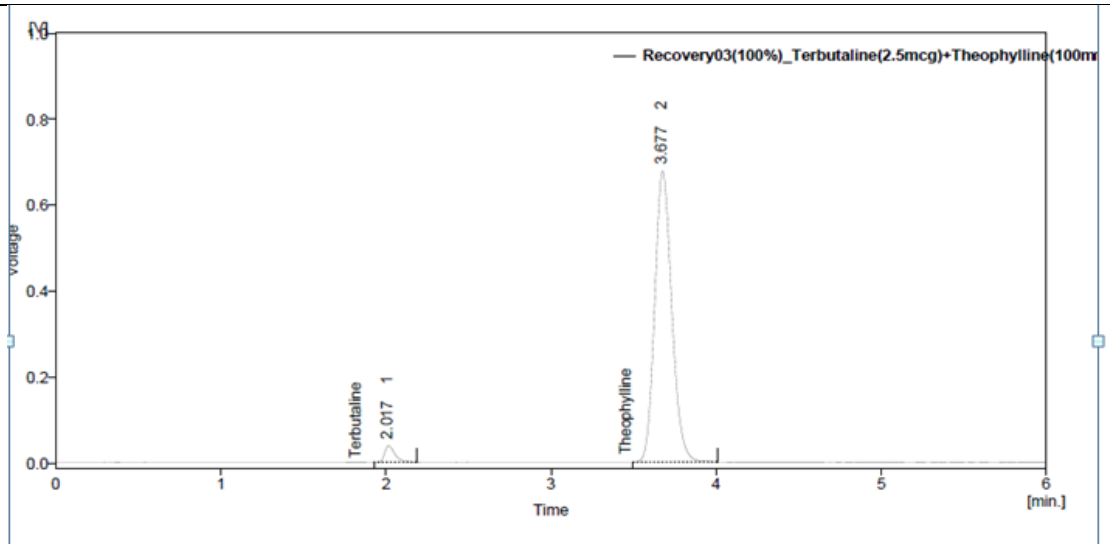


Fig.29: Chromatogram of 100% recovery (injection 3)

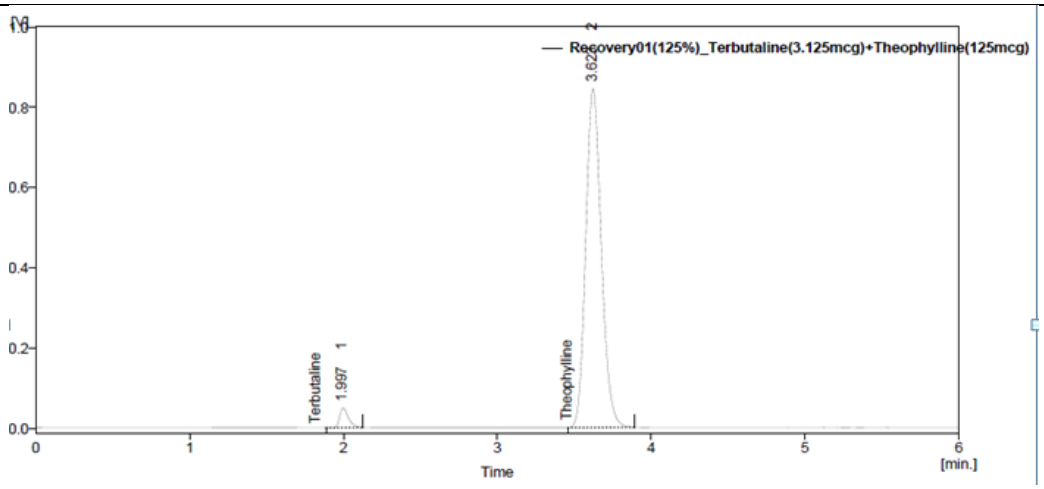


Fig. 30: Chromatogram of 125% recovery(injection 1)

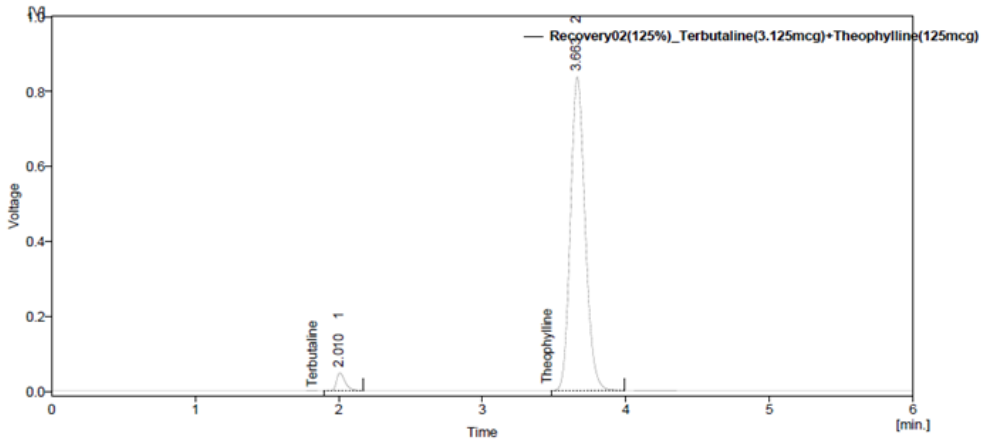


Fig. 31: Chromatogram of 125% recovery (injection 2)

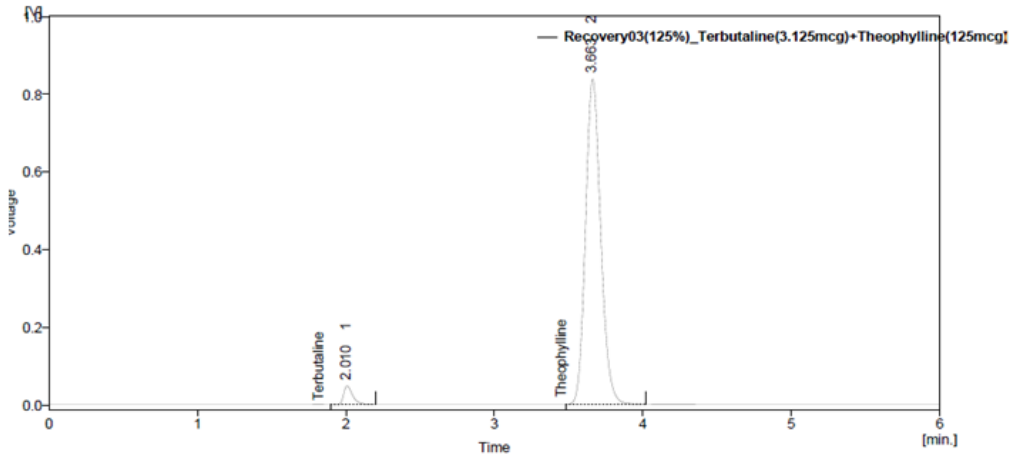


Fig. 32: Chromatogram of 125% recovery (injection 3)

Acceptance criteria

The % recovery of Terbutaline and Theophylline should lie between 98% and 102%.

Table 5 : Recovery results for Terbutaline

Recovery level	**Accuracy** Terbutaline					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
75%	1.87	92.288	95.500	1.86	99.01	**99.49%**
	1.875	95.414				
	1.875	98.797				
100%	2.5	166.024	166.230	2.47	98.98	
	2.5	165.944				
	2.5	166.723				
125%	3.125	190.241				

	3.125	197.059	195.503	3.14	100.48
	3.125	199.208			

Table 6 : Recovery results for Theophylline

Recovery level	**Accuracy** Theophylline					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
75%	75	3190.245				
	75	3270.81	3198.010	73.90	98.54	
	75	3132.976				
100%	100	5293.994				
	100	4982.116	5042.760	98.52	98.52	
	100	4852.17				**99.12%**
125%	125	5941.213				
	125	5944.656	5944.582	125.40	100.32	
	125	5947.876				

Observation

The percentage mean recovery of Terbutaline and Theophylline is 99.49% and 99.12% respectively.

Acceptance criteria

The % Relative standard deviation of Assay preparations of Terbutaline and Theophylline should be not more than 2.0%.

PRECISION

Method precision

Prepared sample preparations of Terbutaline and Theophylline as per test method and injected 6 times in to the column.

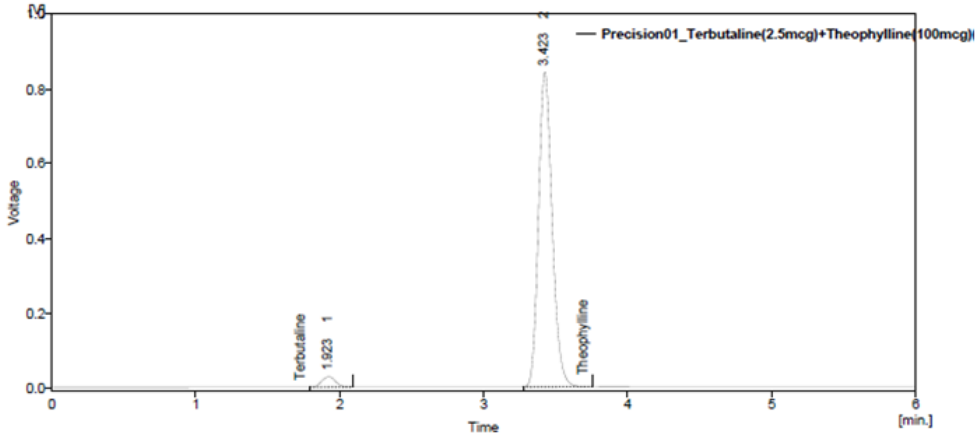


Fig. 33: Chromatogram of precision injection 1

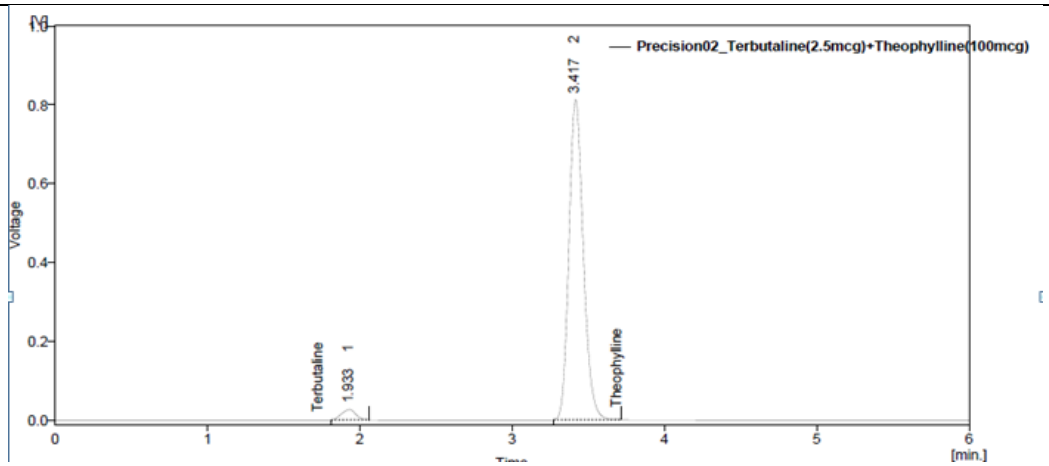


Fig. 34: Chromatogram of precision injection 2

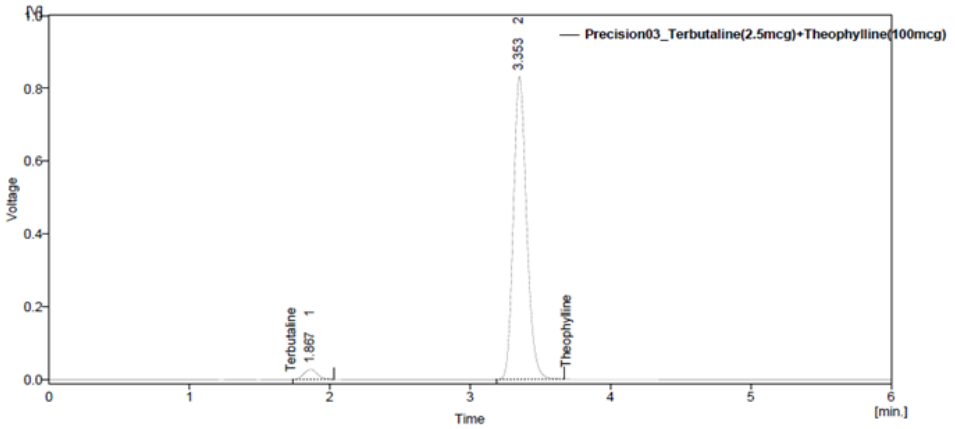


Fig. 35: Chromatogram of precision injection 3

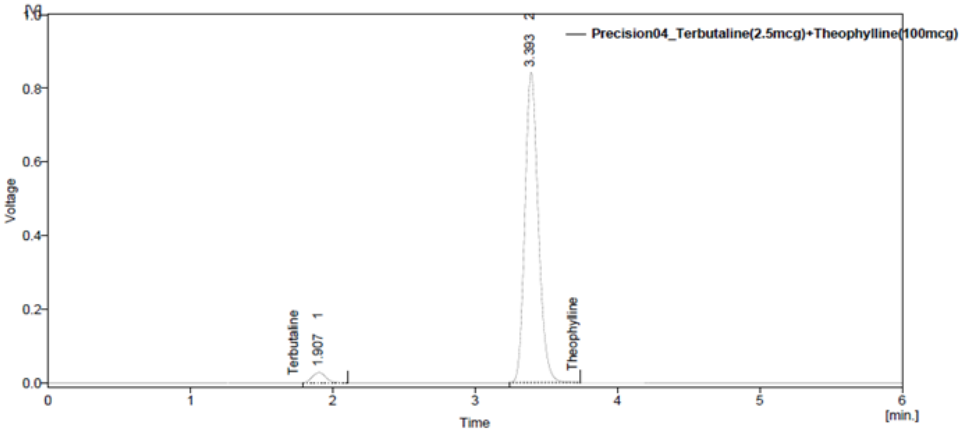


Fig. 36: Chromatogram of precision injection 4

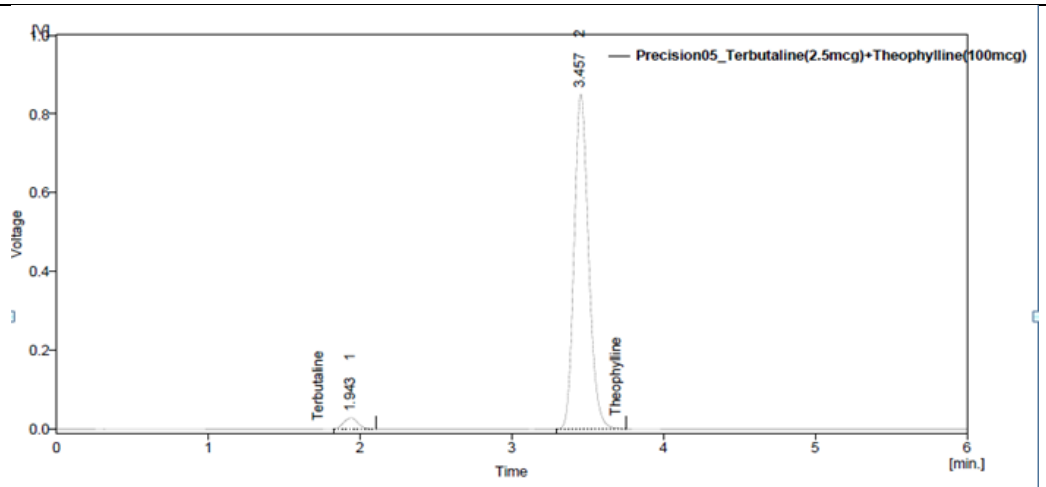


Fig. 37: Chromatogram of precision injection 5

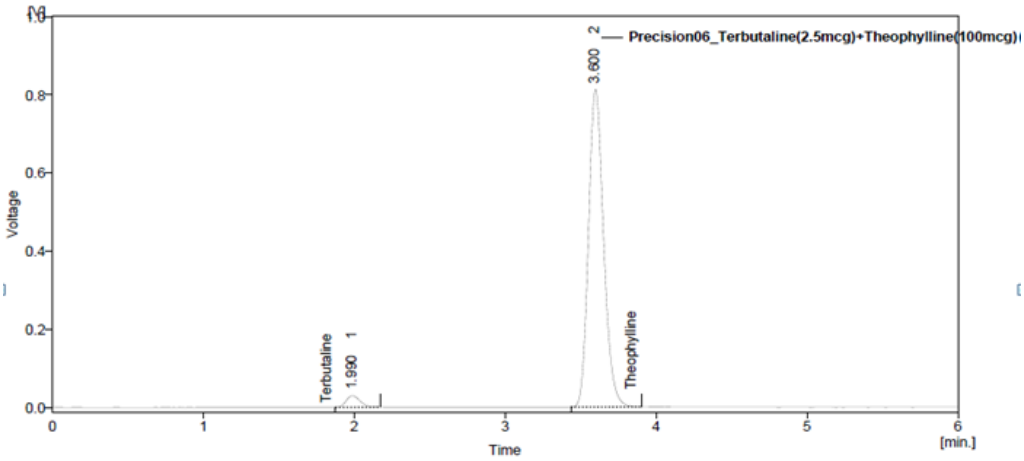


Fig. 38: Chromatogram of precision injection 6

Table 7: Results for Method precision of Terbutaline and Theophylline

Terbutaline			Theophylline		
S.No.	**Rt**	**Area**	**S.No.**	**Rt**	**Area**
1	1.923	182.723	1	3.423	5585.32
2	1.933	178.936	2	3.417	5418.603
3	1.897	178.962	3	3.353	5438.747
4	1.907	180.307	4	3.393	5499.970
5	1.943	181.926	5	3.457	5516.145
6	1.990	188.635	6	3.550	5645.393
avg	1.9322	181.915	**avg**	3.432	5517.363
stdev	0.0329	3.633	**stdev**	0.067	86.311
%RSD	**1.70**	**2.00**	**%RSD**	**1.96**	**1.56**

Observation

Test results for Terbutaline and Theophylline are showing that the %RSD of Assay results are within limits. The results were shown in table Table 7.

ROBUSTNESS

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

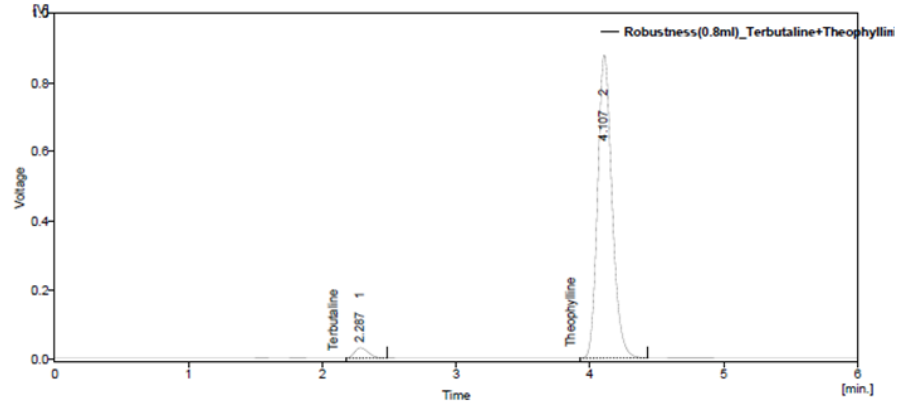


Fig. 39: Chromatogram of Terbutaline and Theophylline Robustness (0.8 ml/min)

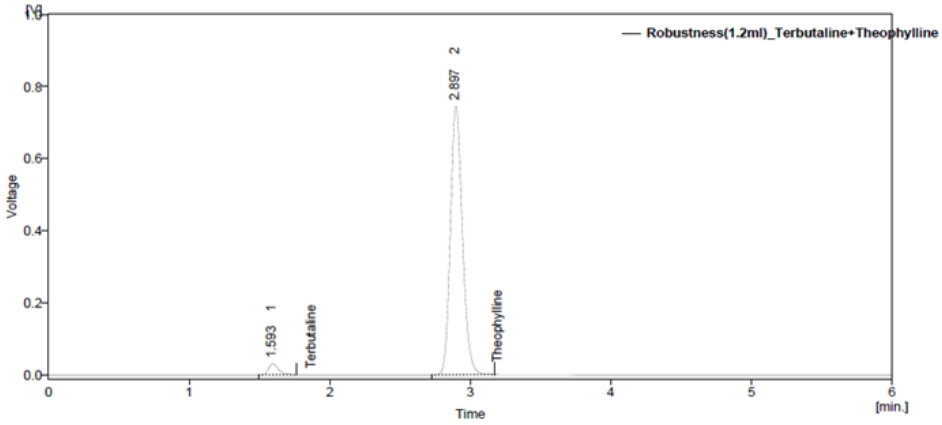


Fig. 40: Chromatogram of Terbutaline and Theophylline for Robustness (1.2 ml/min)

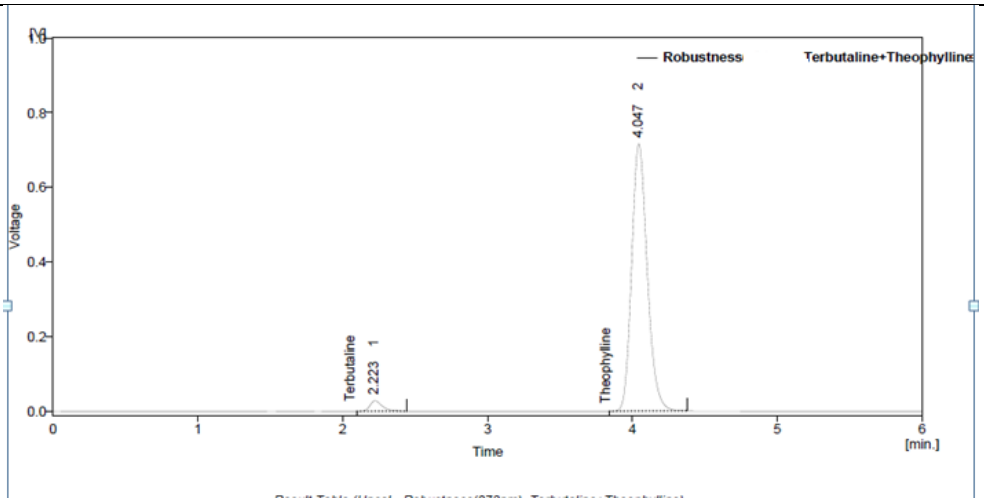


Fig. 41: Chromatogram of Terbutaline and Theophylline for Robustness (249nm)

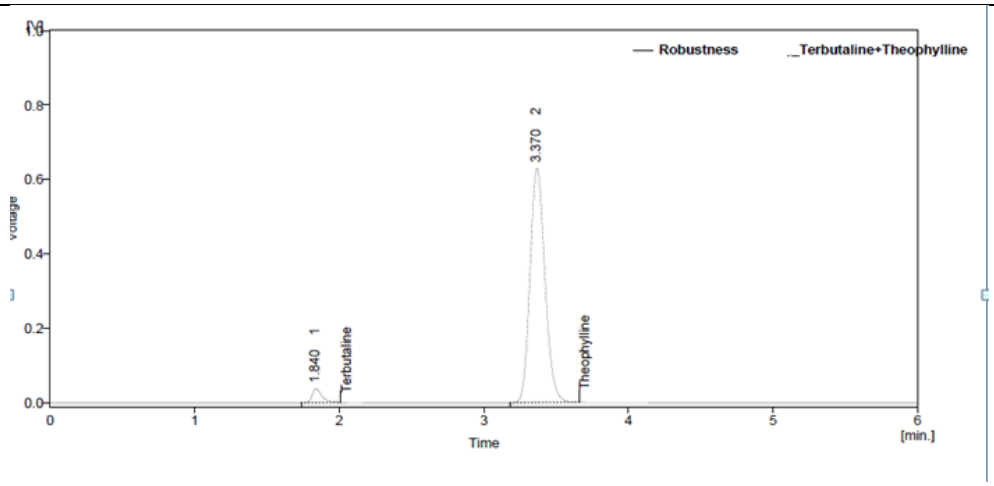


Fig. 42: Chromatogram of Terbutaline and Theophylline for Robustness (250nm)

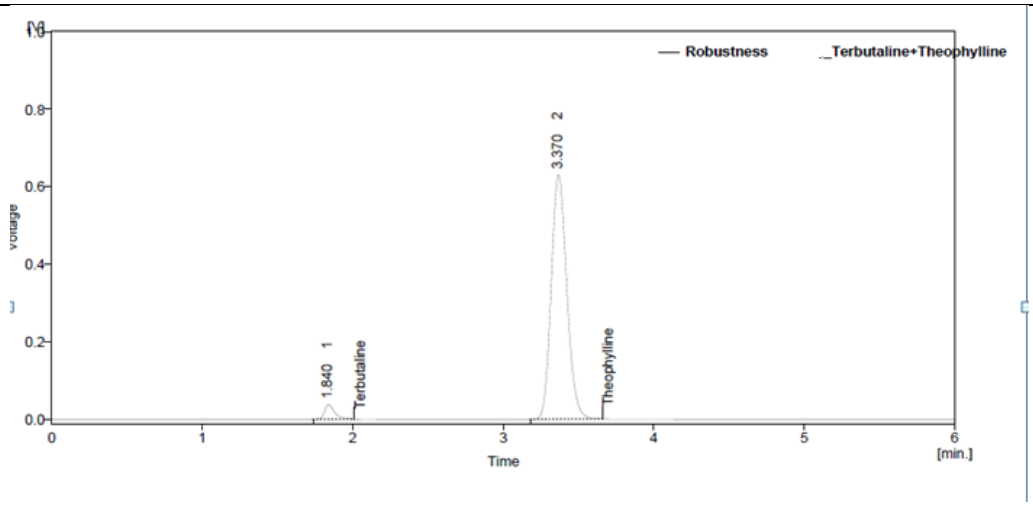


Fig. 43: Chromatogram of Terbutaline and Theophylline for Robustness (251nm

Table 8: Result of Robustness study

Parameter	Terbutaline		Theophylline	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow				
0.8ml/min	2.817	1.73	4.860	1.585
1.0 ml/min	2.32	1.732	4.035	1.586
1.2ml/min	2.022	1.72	3.487	1.57
Wavelength				
249nm	2.367	1.722	4.080	1.571
250nm	2.320	1.732	4.035	1.586
251nm	2.367	1.741	4.082	1.585

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

RUGGEDNESS

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

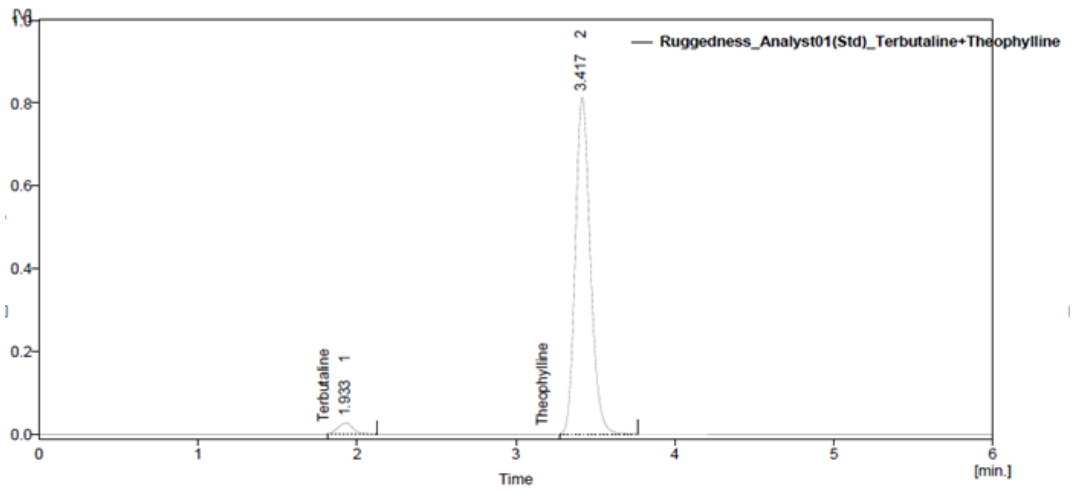


Fig. 44: Chromatogram of Analyst 01 standard preparation

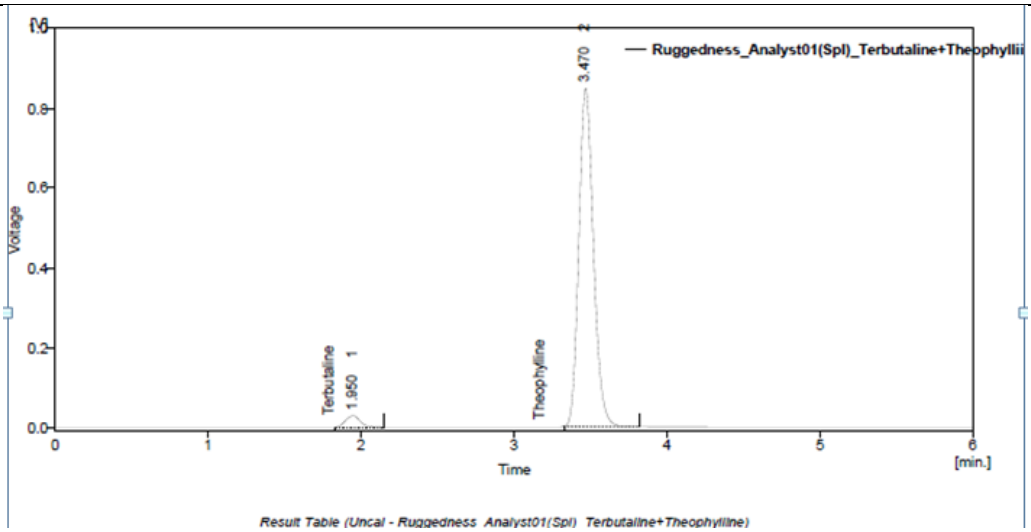


Fig. 45: Chromatogram of Analyst 01 sample preparation

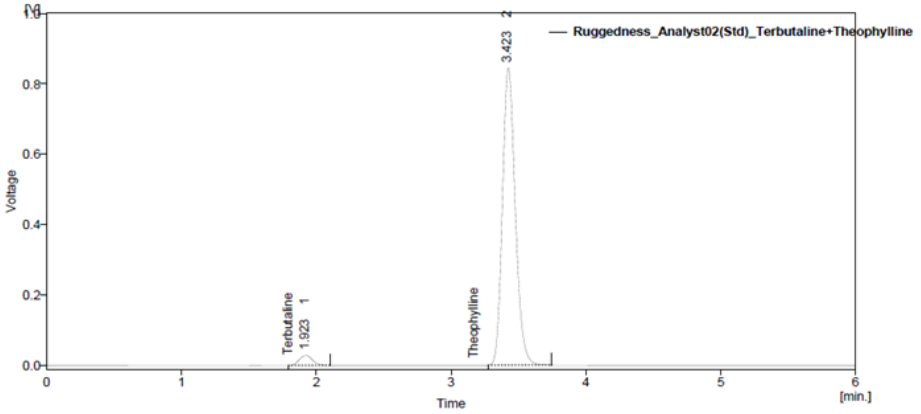


Fig. 46: Chromatogram of Analyst 02 standard preparation

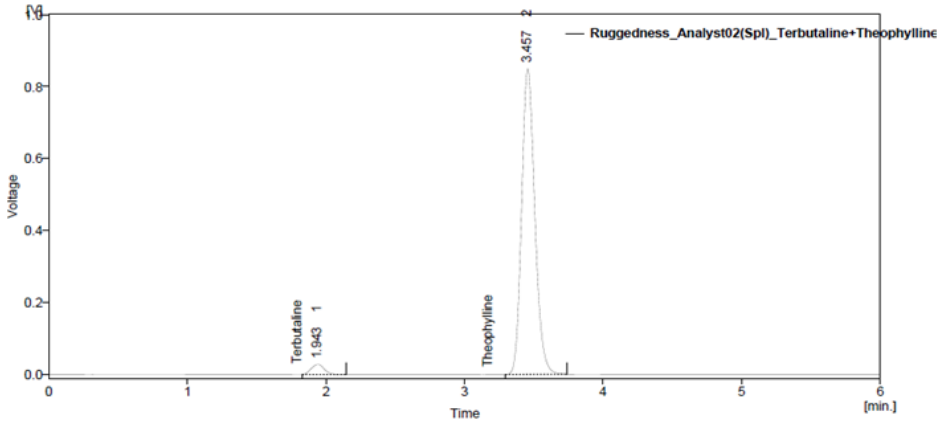


Fig. 47: Chromatogram of Analyst 02 sample preparation

Table 9: Results for Ruggedness

Terbutaline	%Assay	Theophylline	%Assay
Analyst 01	97.99	Analyst 01	99.96
Analyst 02	98.37	Analyst 02	97.59
%RSD	0.27	%RSD	1.69

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Terbutaline and Theophylline was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

BIBLIOGRAPHY

[1]. The Drugs and Cosmetics Act and Rules, 1940.
[2]. Methods of Analysis- http://www.pharmatutor.org/pharma-analysis
[3]. Douglas, A.; Skoog, F.; James, H.; Stanley, R. C. Liquid Chromatography. In *Instrumental Analysis*, 9th ed.; Cengage Learning India Pvt. Ltd.: New Delhi, 2007; 893 - 934.
[4]. Skoog**;** Holler**;** Crouch**;** Liquid Chromatography**.** In *Instrumental Analysis***,** Cengage Learning India**.:**New Delhi**.** 2011**;** 893**.**
[5]. Chatwal**,** R**.** G**.;** Anand**,** K**.** S**.** High Performance Liquid Chromatography**.** In *Instrumental Methods Of Chemical Analysis***,** 5th ed**.;** Himalaya Publishers**.:**Mumbai, 2010**;** 2.570 - 2.629**.**
[6]. Sharma**,** B**.** K**.** High Performance Liquid Chromatography**.** In *Instrumental Methods Of Chemical Analysis***,** 24th ed**.;** Goel Publishers**.:** Meerut**,** 2005**;** 295 - 300**.**
[7]. Alfonso, R. G.; Ara, H. D. M.; Glen, R. H.; Thomas, M.; Nicholas, G. P.; Roger, L.S.; Steve, H. W. Chromatography. In *Remington: The Science and Practice of Pharmacy*, 20th ed.; Lippincott Williams & Wilkins: Philadelphia, 2000; 587
[8]. Adsorption Chromatography- http://www.separationprocesses.com/Adsorption/AD_Chp05a.htm
[9]. Adsorption Chromatography- http://cemca.org/andcollege/andcwebsite/subject01/CHEtext.pdf
[10]. Types of Chromatography- http://www.separationprocesses.com/Adsorption/AD_Chp05a.htm
[11]. Partition Chromatography – http://media.rsc.org/Modern%20chemical%20techniques/MCT5%20Chromatography.pdf
[12]. Ion Exchange Chromatography- http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-IN/products/ion-exchange-chromatography-iex/
[13]. Ion Exchange Chromatography- http://wolfson.huji.ac.il/purification/PDF/IonExchange/AMERSHAM_iIEXandChromatofocManual.pdf