International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

A VALIDATED AND STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TERBUTALINE SULPHATE, GUAIPHENESIN AND BROMHEXINE HCL IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A fast, rapid, sensitive and stability indicating high-performance thin-layer chromatographic (HPTLC) method is developed and validated for quantitative estimation of terbutaline sulphate (TS), Guaiphenasin (G) and Bromhexine hydrochloride (B.HCl) simultaneously in pharmaceutical formulation (Cough Syrup). The sample are chromatographed on silica gel $60F_{254}$- TLC plates, using solvent system Dichloromethane: Methanol: Acetic Acid (7.5: 1:0.5) and scanned at 254nm. The current method demonstrates good linearity over the range for TS was 200-1200ng/spot with r^2 of 0.999; G is 1.0-6.0mcg/spot with r^2 of 0.998 and B.HCl 500-3500ng/spot with r^2 of 0.997. The average recovery of the method is 100.47% for TS; 99.94% for G and 100.12% for B.HCl in formulation. The limit of detection and limit of quantification for TS, G and B.HCl were found to be 50-150ng/spot, 200-800ng/spot and 100-300ng/spot respectively. The developed method was successfully applied for the assay of market formulation. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameter and by changing analytical parameter operated proven that the method is robust.

Keywords: Guaiphenasin, Terbutaline sulphate, Bromhexine hydrochloride, HPTLC, Validation

INTRODUCTION

The Cough syrup is a combination of bronchodilator [1] mucolytic and expectorant for the control of productive cough in a palatable syrup dosage form. An extensive literature survey revealed UV [17, 18, and 19], HPLC [5, 20-25] and colorimetric determination for TS, G and B.HCl simultaneously or in combination with other drugs. But there is no method which describes the simultaneous determination of TS, G and B.HCl simultaneously from liquid oral pharmaceutical dosage form. The objective of this investigation was to develop simple, precise accurate and economical procedures for simultaneous estimation of terbutaline sulphate (TS), Guaiphenasin (G) [1, 5] and Bromhexine hydrochloride (B.HCl) from pharmaceutical preparation.

DRUG PROFILES [9, 7-12, 14-16]

Terbutaline is a selective beta 2-adrenergic causing bronchodilation increase in mucociliary clearance.

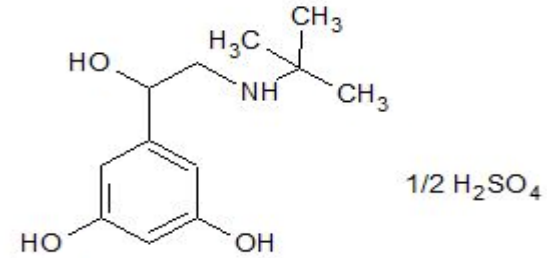

Figure 1: Chemical structure of Terbutaline Sulphate.

TS is *(RS)*-2-(*tert*-butylamino)-1-(3, 5-dihydroxyphenyl) ethanol sulphate also known as carbamothioic acid methyl (3-methylphenyl)-O-2-naphthalenyl ether. The molecular formula and

weight is (C12 H19 N O3)2H2SO4 and 548.65 respectively. It is white or almost white, crystalline powder; odorless or almost odorless. It is freely soluble in water; slightly soluble in ethanol (95%); practically insoluble in chloroform and in ether. Increases the volume and decreases the viscosity of bronchial secretions.

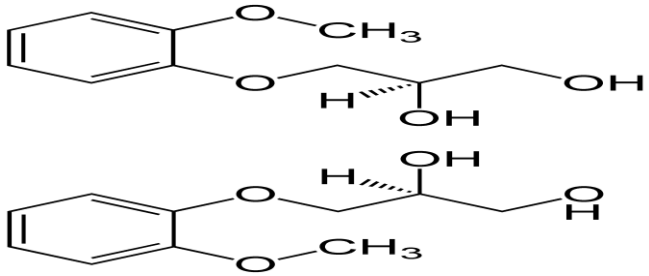

Figure 2: Chemical structure of Guaiphenasin.

Guaiphenasin [7 - 12] is *(RS)*-3-(2-methoxyphenoxy)-1, 2- propanediol having molecular weight 198.22 and formula as $C_{10}H_{14}O_4$. It is white or almost white, crystalline powder; odourless or with a slight characteristic odour. It is Soluble in ethanol *(95%)* and in chloroform; sparingly soluble in water; slightly soluble in ether.

Bromhexine [14, 16] is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucous.

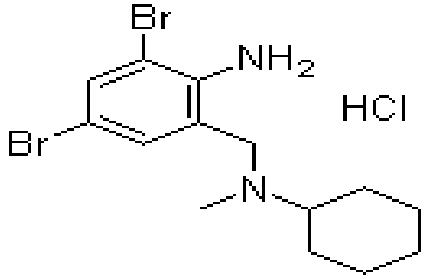

Figure 3: Chemical structure of Bromhexine Hydrochloride.

B.HCl is white or almost white, crystalline powder; odourless or almost odourless. B.HCl is 2-amino-3, 5-dibromobenzyl- (cyclohexyl) methylamine hydrochloride. The molecular formula and weight is $C_{14}H_{20}Br_2N_2$, HCl and 412.59 respectively. It is sparingly soluble in ethanol (95%) and in methanol; slightly soluble in chloroform; practically insoluble in water.

MATERIAL AND METHODS

TS, G and B.HCl were of USP grade. The entire reagents used were of analytical grade. Water was deionised and double distilled. Pharmaceutical formulation containing TS, G and B.HCl was of our in house formulation.

***TLC Conditions* [6]:** The TLC plates were 20x 10cm, Precoated with silica gel F_{254} (E.Merck) (0.2mm thickness); spotting device was Camag Linomat V sample applicator, Camag (Muttenz, Switzerland); syringe was a 100µl (Hamilton); developing solvent is (Dichloromethane: Methanol: Acetic Acid 7.5: 1:0.5); developing chamber was a CAMAG glass twin trough chamber (20x 10cm); densitometer was CAMAG TLC Scanner 3 linked to winCATS software.

Preparation of standard solution:

Standard Stock Solution for Terbutaline Sulphate: Weigh accurately 31.2mg TS and transfer to 25.0ml volumetric flask, add 2.0ml distilled water and allow it to dissolved with sonication and make up the volume with methanol to 25.0ml. Take 1.0ml stock solution in 50.0ml volumetric flask and dilute with methanol up to the mark. (Solution A)

Standard Stock solution for Guaiphenasin: Weigh accurately 50.0mg G and transfer it in to 50.0ml volumetric flask, add about 30.0ml of methanol to dissolve and make up the volume to 50.0ml with methanol.

Standard Stock solution for Bromhexine HCl: Weigh accurately 20.0mg of B.HCl and transfer it into 25.0ml volumetric flask, add about 10.0ml of methanol and make up the volume to 25.0ml with methanol. Take 2.0 ml from the above and dilute it to get the concentration of 0.08mg/ml. (Solution B)

Combine Standard: Weigh accurately 50.0mg of G in 50.0ml volumetric flask and add 1.0 ml of solution A and 5.0ml of solution B and dilute up to the mark with methanol.

Sample Solution: Take 5ml of the formulation in 50.0ml volumetric flask add 30.0ml of methanol and make up the volume to 50.0ml with methanol.

MOBILE PHASE: Dichloromethane: Methanol: Acetic Acid (7.5**:** 1**:**0.5)

CHROMATOGRAPHY: HPTLC [6] was performed on 20x10cm TLC aluminium plates Precoated with a 200µm thick layer of silica gel 60F254. Samples were applied as an 8mm band width using CAMAG 100µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator CAMAG under a flow of nitrogen gas. A range of mobile phases with varying composition according to polarities were tested. Linear ascending development was carried out with Dichloromethane: Methanol: Acetic Acid (7.5: 1:0.5)

for TS, G and B.HCl in a CAMAG twin trough chamber (20x10cm). The chamber was previously developed at a distance of approximately 80.0mm from the point of application. After development, plates were dried at TLC plate heater III at a temperature of 60°C for 5minutes and scanning was performed using a TLC Scanner3 (CAMAG Switzerland) at λ_{max} 254nm in UV absorbance mode 4x0.45mm and the scanning speed was 100mm/sec. The plates were observed under UV 254nm and 366nm. The R_f values of resolved bands were noted. The identity of the bands of TS, G and B.HCl in the sample track was confirmed by overlying their UV absorption spectra with those of the respective standards using a Camag TLC Scanner 3 with winCATS software. The purity of each of these separated bands in the sample extract track was check by comparing the absorption spectra recorded at start, middle, and end positions of each of the band.

TLC DENSITOMETRIC QUANTIFICATION OF TS, G AND B.HCl:

Sample solution: sample solution described under the previous section was used for simultaneous quantification TS, G and B.HCl. (Figure 1 & 2).

PREPARATION OF CALIBRATION CURVE:

Standard solutions of TS, G and B.HCl were prepared in combination and were applied (band width: 8mm, distance between the tracks: 12mm) in triplicates on a TLC plate using automatic sample spotter. The plates were developed in a twin trough chambers (20 x10 cm) up to a distance of 8cm using a solvent system of Dichloromethane: Methanol: Acetic Acid (7.5: 1:0.5). The plates were dried at TLC plate heater III and scanned at 254nm in absorbance mode using deuterium lamp. The areas of the resolved peaks were recorded. Calibration curve of TS, G and B.HCl was obtained by plotting peak areas vs. applied concentrations of TS, G and B.HCl respectively. (Figure 3)

SIMULTANEOUS QUANTIFICATION OF TS, G AND B.HCL IN SAMPLE: The solvent system was optimized to Dichloromethane: Methanol: Acetic Acid (7.5: 1:0.5). 4µl each of suitably used sample solution along with the standard solution were applied in triplicate on a TLC plates. The plate was developed in the given solvent system and scanned as mentioned above. The peak areas and absorption spectra were recorded and the amounts of TS, G and B.HCl were calculated using their respective calibration curves. **(TABLE 1)**

VALIDATION OF THE METHOD [2]:

ICH guidelines were followed for the validation of the analytical method developed for precision, accuracy and repeatability.

Precision: Three sets of three different concentration standard solutions were prepared. The intra day and inter day precision of the developed TLC method was determined by preparing the samples of the same batch in nine determinations with three concentrations and three replicate each on the same day. The inter-day precision was also determined by assaying the sample in triplicate per day for consecutive three days.

Instrumental precision: Instrumental precision was checked by repeated scanning of the same spot of TS, G and B.HCl and expressed as relative standard deviation (% RSD).

Specificity: Specificity of the proposed method was evaluated by comparing the R_f value of standard with that of sample. The peak purity of standards was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot**. (Figure 6 (a) (b) (c))**

Linearity: For linearity study standard solution was applied over plate in increasing concentration. The intercept, correlation coefficient, standard deviation was calculated from regression equation.

Accuracy (% Recovery): The recovery study was carried out by estimating the marker compounds in the pre analyzed sample of the product spiked with marker compound respectively at three concentration levels. Three replicate estimations were carried out for each concentration level**. (TABLE 3)**

Precision: (Table 4)
System Precision: System precision was calculated by analyzing one conc. level of standards 6 times. **(Table 5)**

Limits of detection (LOD) and Limits of quantification (LOQ): Limits of detection and limits of quantification were determined by calculation of the signal-to-noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit respectively. **(Table 6)**

Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters

such as mobile phase composition, saturation time and scanning time. **(Table 7)**

CONCLUSION

From the experimental data and results obtained, it can be concluded that the HPTLC method was found to be simple, precise, specific, sensitive and accurate for the qualitative and quantitative estimation of the Terbutaline Sulphate, Guaiphenasin and Bromhexine Hydrochloride in pharmaceutical liquid oral preparation. The method gave good resolution for all the drugs with a short analysis time below 10minutes. The developed method was validated. It was found to be novel, simple, precise accurate and sensitive. The good % recovery in formulation has no interference in the determination. The %RSD was also less than 2.0% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of Terbutaline Sulphate, Guaiphenasin and Bromhexine Hydrochloride in combined dosage form. It can be also used in the quality control in bulk manufacturing.

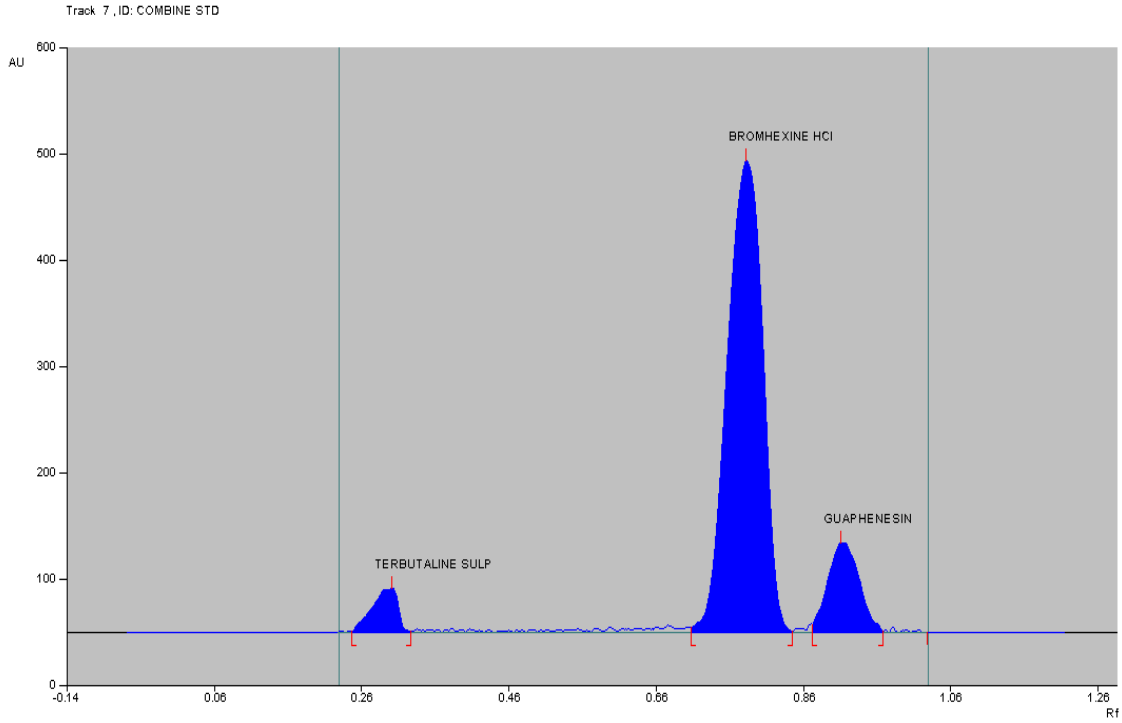

Figure 1: Shows densitogram of standard showing TS, G and B.HCl simultaneously.

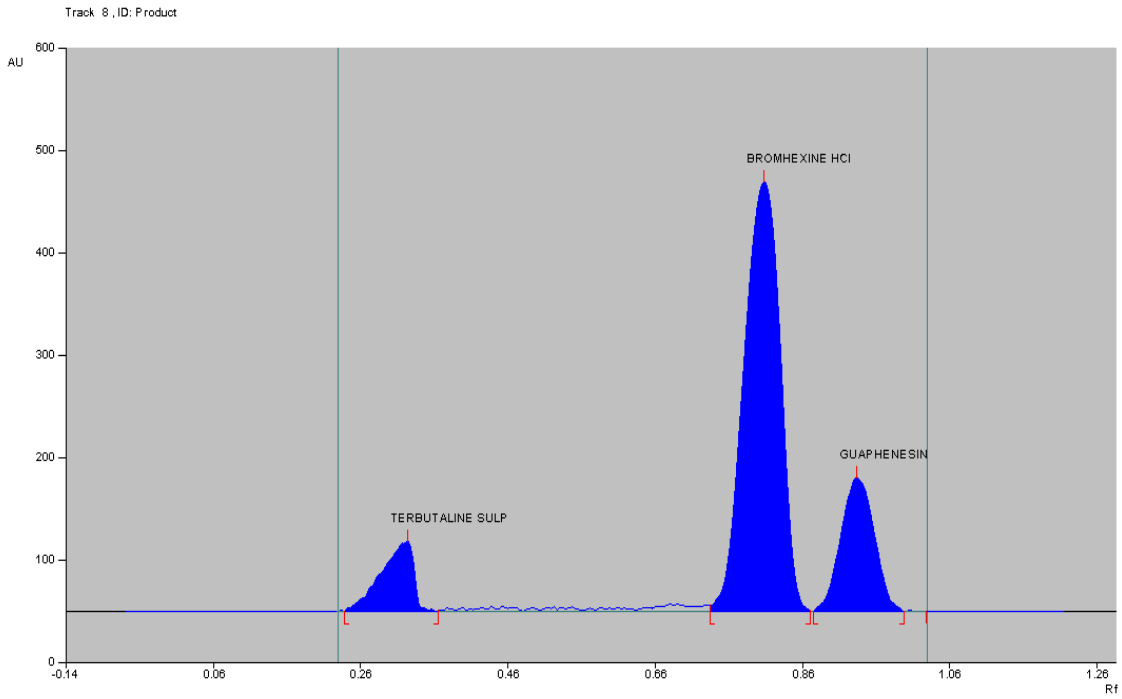

Figure 2: Shows densitogram of product showing TS, G and B.HCl simultaneously.

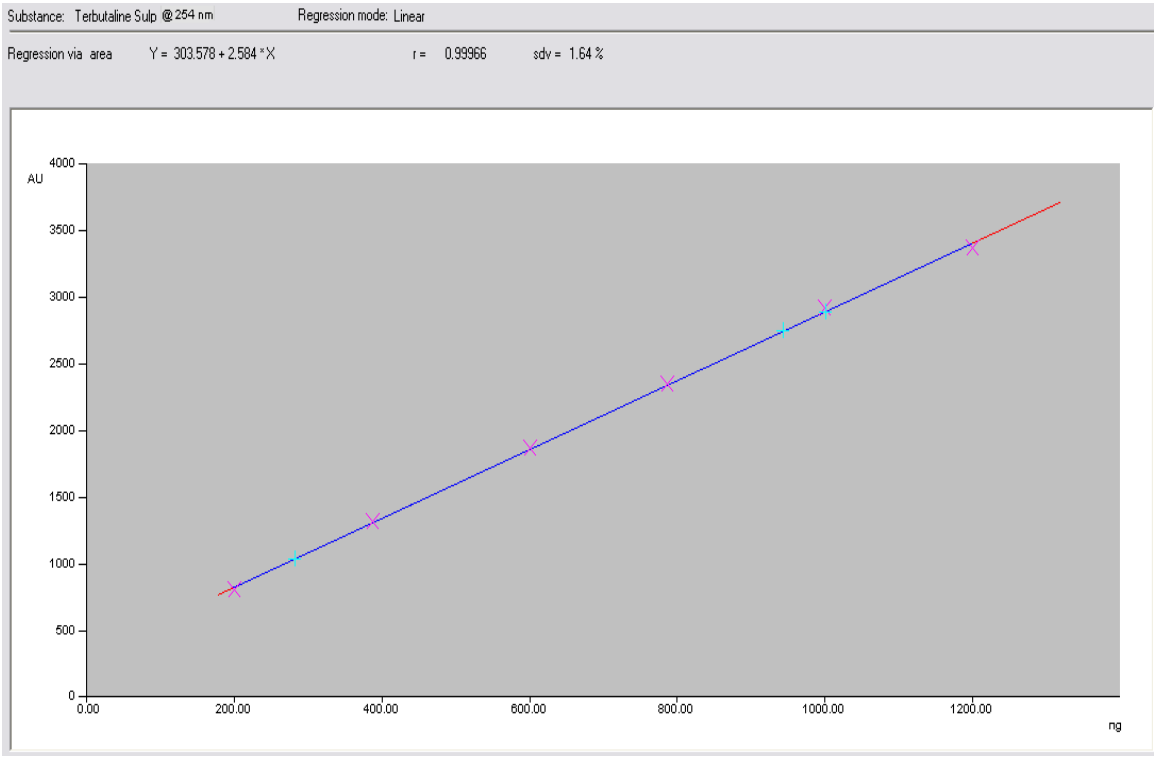

Figure 3: calibration curve of Terbutaline Sulphate.

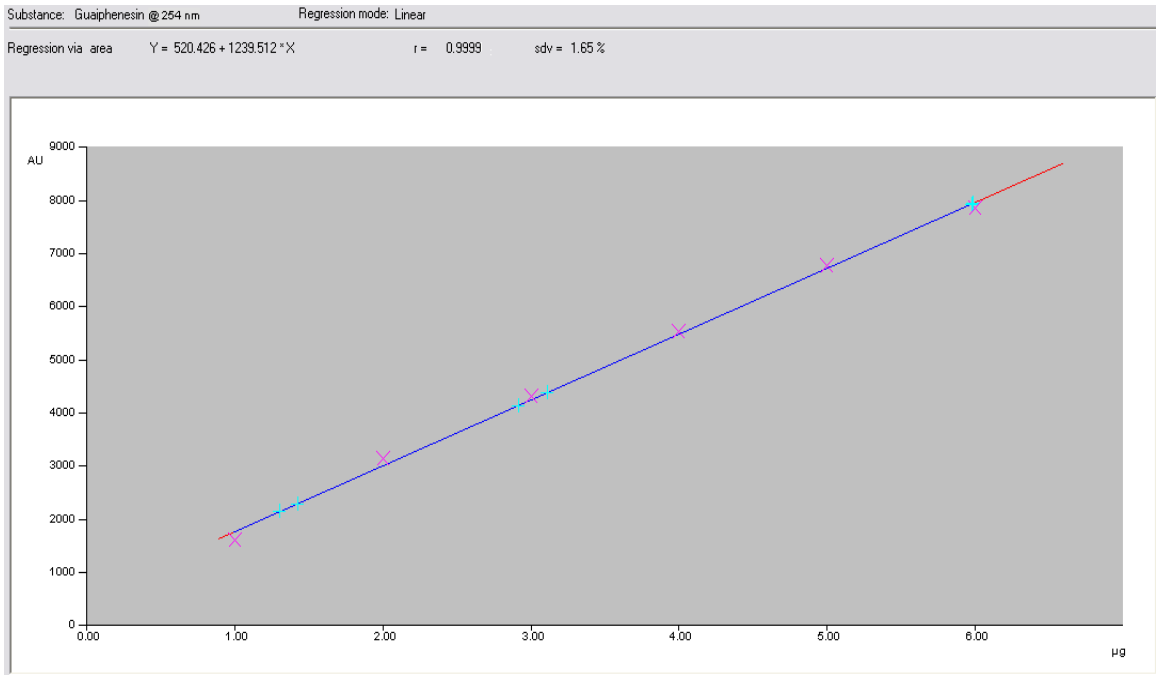

Figure 4: calibration curve of Guaiphenasin**.**

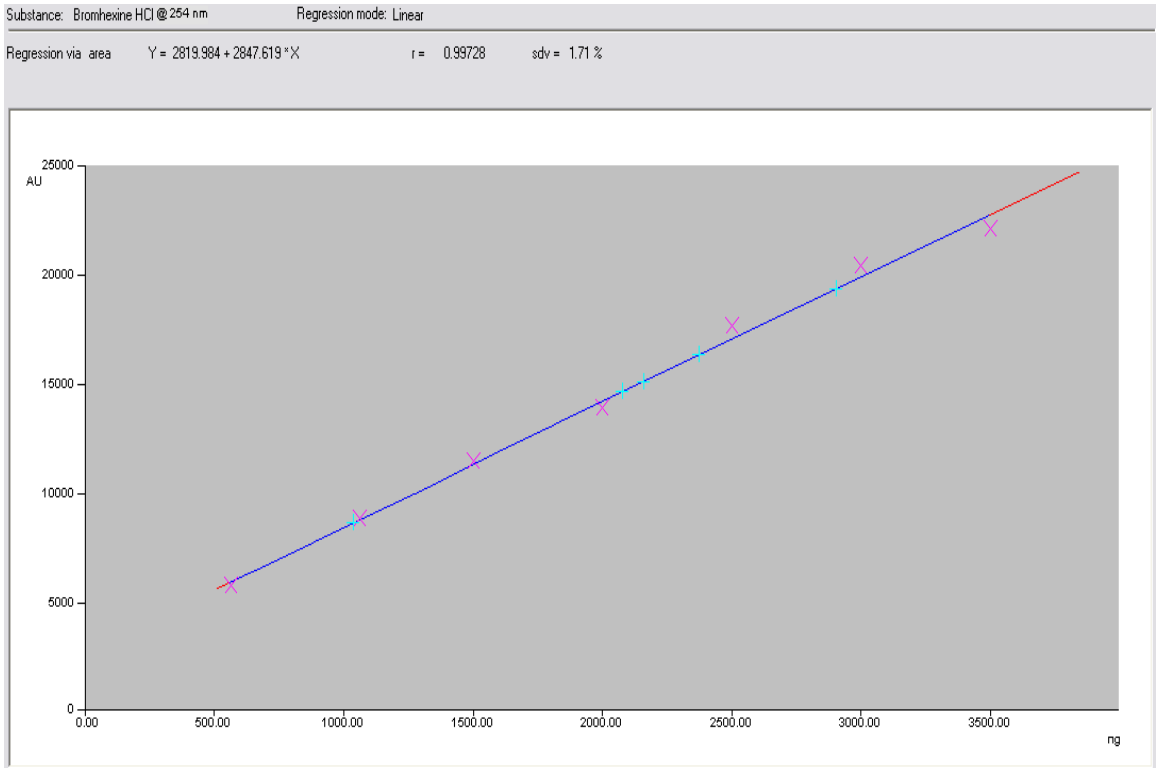

Figure 5: calibration curve of Bromhexine HCl.

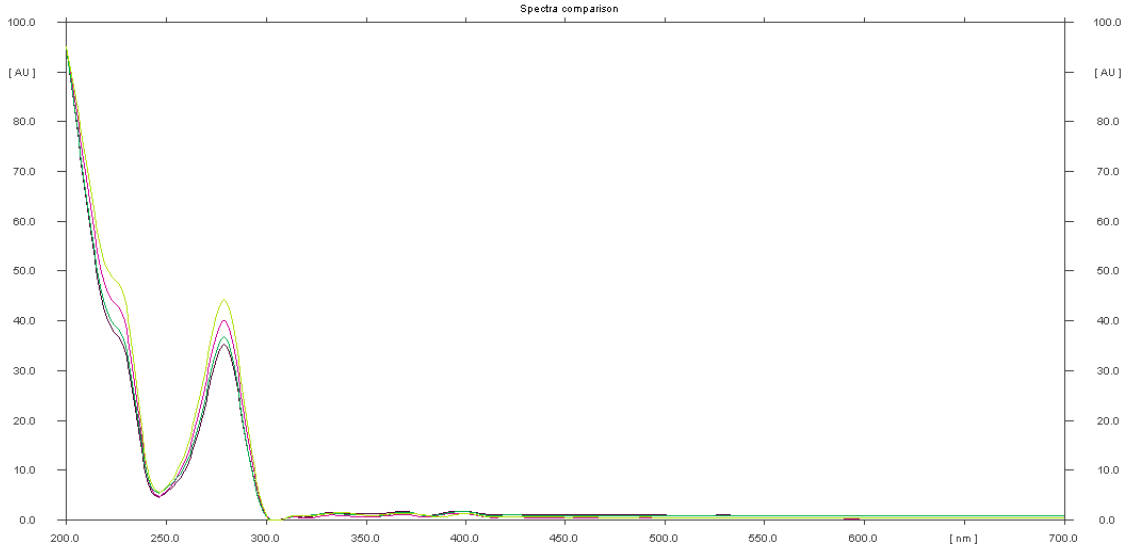

Figure 6 (a): Shows specificity of Terbutaline Sulphate in product at peak start, peak apex and peak end position.

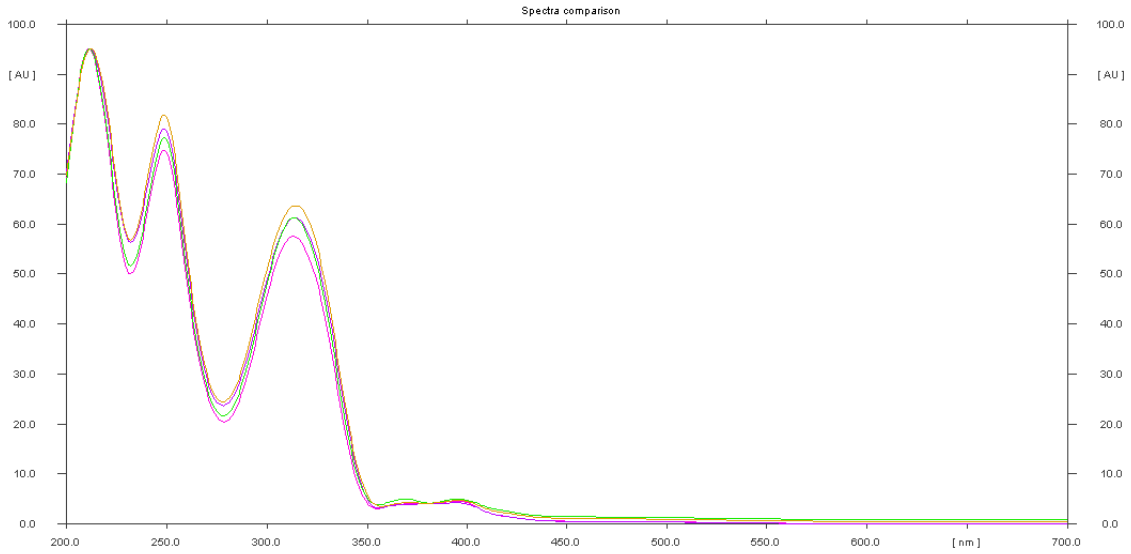

Figure 6 (b): Shows specificity of Bromhexine HCl in product at peak start, peak apex and peak end position.

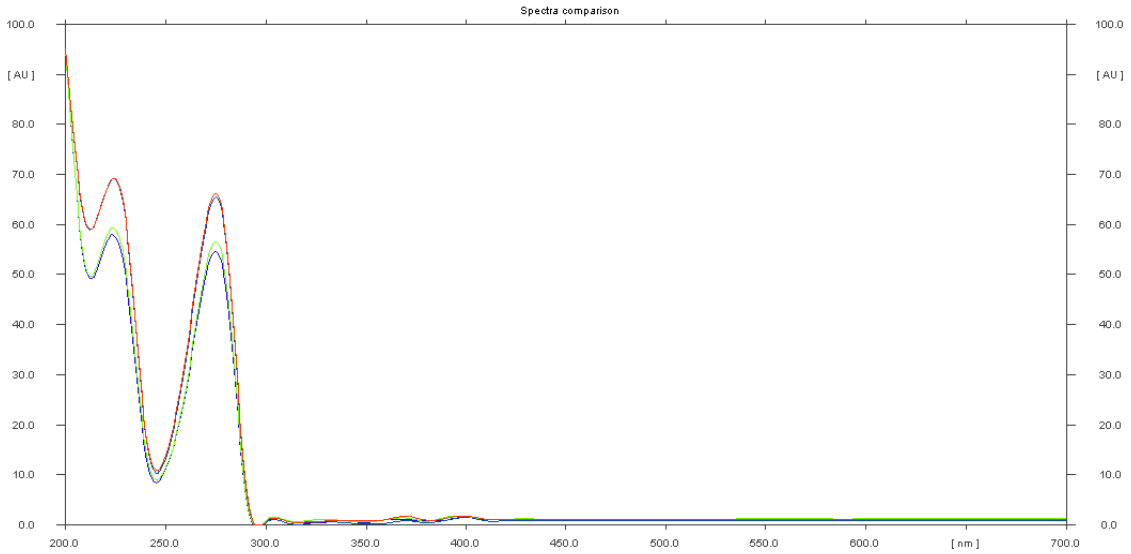

Figure 6(c): Shows specificity of Guaiphenasin in product at peak start, peak apex and peak end position.

Table 1: Shows results of Simultaneous Quantitative Study

Compound	Quantity in Product/5ml	Quantity in Product (%)
Terbutaline Sulphate	1.263mg	101.04
Guaiphenasin	50.325mg	100.65
Bromhexine HCl	4.045mg	101.13

Table 2: Data obtained for linearity of TS, Guaiphenasin and B.HCl.

Regression equation	Linearity range	Standard deviation	Regression coefficient
TS			
Y = 303.578+8.2584* X	200-1200 ng	Sdv = 1.64 %	r = 0.999
G			
Y = 520.426 + 1239.512* X	500-3500 ng	Sdv = 1.65 %	r = 0.999
B.HCl			
Y = 2819.984 + 5.695* X	1.0-8.0µg	Sdv = 1.71 %	r = 0.997

Table 3: Statistical data of accuracy of TS, G and B.HCl:

Terbutaline Sulphate					
Sr.no.	**TS in sample (µg)**	**Std. added (µg)**	**Total added conc.**	**Found conc.**	**% Recovery**
1	25.0	20.0	45.0	45.61	101.35
2	25.0	20.0	45.0	45.01	100.02

3	25.0	20.0	45.0	44.99	99.97
4	25.0	25.0	50.0	50.56	101.12
5	25.0	25.0	50.0	50.12	100.24
6	25.0	25.0	50.0	50.57	101.14
7	25.0	30.0	55.0	54.48	99.05
8	25.0	30.0	55.0	55.69	101.25
9	25.0	30.0	55.0	55.09	100.16
Average					100.47
Guaiphenasin					
S.No.	**G in sample (µg)**	**Std. added (µg)**	**Total added conc.**	**Found conc.**	**% Recovery**
1	1000.0	800.0	1800	1800.24	100.01
2	1000.0	800.0	1800	1800.95	100.05
3	1000.0	800.0	1800	1790.25	99.45
4	1000.0	1000.0	2000	2000.99	100.04
5	1000.0	1000.0	2000	2000.68	100.03
6	1000.0	1000.0	2000	1999.33	99.96
7	1000.0	1200.0	2200	2200.69	100.03
8	1000.0	1200.0	2200	2198.62	99.93
9	1000.0	1200.0	2200	2200.63	100.02
Average					99.94
Bromhexine HCl					
S.No.	**B.HCl in sample (µg)**	**Std. added (µg)**	**Total added conc.**	**Found conc.**	**% Recovery**
1	80.0	64.0	144.0	143.97	99.97
2	80.0	64.0	144.0	144.78	100.54
3	80.0	64.0	144.0	144.01	99.56
4	80.0	80.0	160.0	159.99	100.00
5	80.0	80.0	160.0	160.65	100.40
6	80.0	80.0	160.0	160.06	100.03
7	80.0	96.0	176.0	176.39	100.22
8	80.0	96.0	176.0	176.24	100.14
9	80.0	96.0	176.0	176.47	100.26
Average					100.12

Table 4: Statistical data of Precision of TS, G and B.HCl:

S. No.	**Application of sample (µl)**	**Concentration obtained**		
		TS (µg)	**G (µg)**	**B.HCl (µg)**
1	2	1388.91	3522.6	10750.8
2	2	1369.09	3595	10933.6
3	2	1400.25	3510.1	10996.3
Average		1386.08	3542.56	3542.56
% RSD		1.137	1.293	1.293
1	4	2876.8	7165.9	21111.5
2	4	2869.3	7259.6	212138.0
3	4	2790.4	7061.2	212122.6
Average		2845.5	7162.23	7162.23
% RSD		1.68	1.38	1.38

1	6	4166.7	10577.2	31655.3
2	6	4221.8	10766.6	32006.9
3	6	4170.5	10625.6	32659.4
Average		4186.33	10656.46	10656.46
% RSD		0.73	0.92	0.92

Table 5: Statistical data of system precision of TS, G and B.HCl:

S. No.	Application of sample (µl)	Area of standard TS	Area of standard G	Area of standard B.HCl
1	6	1358.6	3621.2	10656.2
2	6	1356.7	3600.9	10766.6
3	6	1340.4	3599.9	10552.2
4	6	1342.7	3598.8	10665.9
5	6	1375.6	3611.1	10736.8
6	6	1353.0	3599.0	10598.7
Average		1354.5	3605.15	10662.73
% RSD		0.938	0.253	0.757

Table 6: Detection Limit (LOD) and Quantitation Limit (LOQ) of TS, Guaiphenasin and B.HCl - Based on Signal-to-Noise.

Name of the Active constituents	LOD (ng)	LOQ (ng)
TS	50ng/spot	150ng/spot
G	200ng/spot	800ng/spot
B.HCl	100ng/spot	300ng/spot

Table 7: Statistical data of robustness of TS, G and B.HCl

Parameter	Initial condition	Change in condition	Effect found
Mobile phase composition	Dichloromethane: Methanol: Acetic Acid (7.5: 1:0.5)	Dichloromethane: Methanol: Acetic Acid (7.3: 1.2:0.5)	No change
Development distance	8 cm	6cm	No Change
Saturation time	20 min.	30 min.	No change
Extraction time	20 min.	30 min.	No change

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