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Simultaneous determination of Terbutaline and Bromhexine in Combined Pharmaceutical Dosage Form by RP-HPLC Method

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Abstract: A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Terbutaline and Bromhexine in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter with a mixture of methanol: acetonitrile: ortho phosphoric acid in the ratio of 80:10:10 (v/v/v) as mobile phase. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The percentage recovery was found to be 99.6 and 99.7 for Terbutaline and Bromhexine respectively. The proposed method is highly sensitive, precise and accurate, which was evident from the LOD value of 0.02 and 0.03 ppm for Terbutaline and Bromhexine respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of Terbutaline and Bromhexine.
Key words: Terbutaline, Bromhexine, RP-HPLC, Method validation, pharmaceutical formulation.

INTRODUCTION

Terbutaline, a selective β_2 agonist, is used as a bronchodilator for reversible airway obstruction. It is preferred to control nocturnal asthma. Its molecular formula is $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ and molecular weight is 548.7. Chemically it is bis[(1RS)-1-(3,5-dihydroxyphenyl)-2-[1,1-dimethyl ethyl amino] ethanol]sulphate.

Bromhexine, a synthetic benzyl amine derivative of vasicine, is a mucolytic agent rendering the sputum less viscous thereby facilitating easy expulsion of it from the respiratory tract. According to IUPAC it is 2, 4-dibromo-6-[[cyclohexyl(methyl)amino]methyl] aniline hydrochloride with a molecular weight of 412.6 and molecular formula $C_{14}H_{20}Br_2N_2 \cdot HCl$.

A number of methods have been developed for the estimation of Terbutaline and Bromhexine individually and also in combined forms along with other drugs which include HPLC [1-11], Spectrophotometry [12-24], Fluorimetry [25], Chemiluminescence [26], Capillary electrophoresis [27]. However no suitable HPLC method is available for the estimation of Terbutaline and Bromhexine alone in combined form. Hence we report a new simple, rapid and precise RP-HPLC method for the simultaneous determination of

Terbutaline and Bromhexine in combination dosage form.

Fig 1: Structure of Terbutaline sulphate

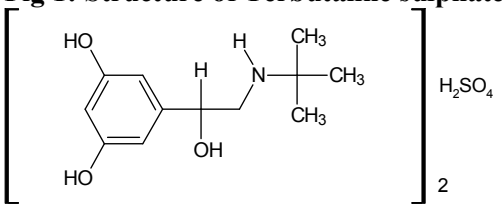
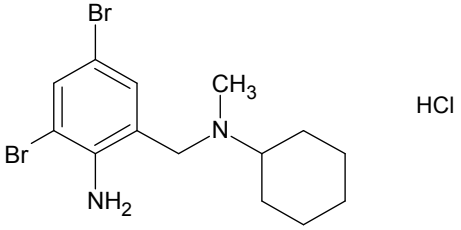


Fig 2: Structure of Bromhexine hydrochloride



EXPERIMENTAL

Chemicals and Reagents

Terbutaline and Bromhexine as pure standard reference drugs were purchased from Reddy's Laboratory, Hyderabad and pharmaceutical formulation from local market were used for this present study. Water, acetonitrile, methanol and orthophosphoric acid (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Bromhexine and Terbutaline, an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. UV-2306 Spectrophotometer was used for wavelength checking. Denver analytical Balance was used to weigh the drug.

Experimental Condition

Flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2^{oc}. For analysis the most suitable mobile phase was found to be methanol, acetonitrile and orthophosphoric acid in the ratio of 80:10:10. Detection was carried out at wavelength of 270 nm.

Preparation of Mobile Phase

For the preparation of mobile phase suitable for the present determination methanol, acetonitrile and orthophosphoric acid of HPLC grade were mixed, filtered and degassed in such a way that the final volume consisted of these in the ratio 80:10:10 respectively whose pH was found to be 5.1.

Preparation of mixed standard solution

Terbutaline and Bromhexine (1mg/ml) standard stock solutions were prepared using methanol as a solvent. Aliquots of mixed standard solutions of Terbutaline and Bromhexine were diluted in methanol to get a final concentration of 10, 9, 8, 7, 6, 5 and 4 ppm.

Preparation of sample solution of pharmaceutical formulation

Pharmaceutical form containing 1.5 mg/ml of Terbutaline and 4 mg/ml of Bromhexine was weighed and dissolved in 25 ml of methanol and sonicated for 15 min. Using methanol the volume was made up to 50 ml and filtered through 0.45µ membrane filter. The final mixed sample solution corresponding to 4.5 ppm of Terbutaline and 12 ppm of Bromhexine was prepared.

Recording of chromatograms

After stabilization of the base line with the optimized chromatographic conditions, standard solutions containing 4-10 ppm of Terbutaline and Bromhexine were injected and the corresponding chromatograms were recorded. Retention time of Terbutaline and Bromhexine were found to be 1.7 and 2.7 mins respectively. Likewise for sample solution chromatograms were recorded. Calibration curves were plotted using peak area retentions of standard drug peaks against concentration of corresponding standard solutions.

Table 1: Optimized chromatographic conditions for estimation of Bromhexine and Terbutaline

Mobile phase	OPA : Methanol : Acetonitrile 10:80:10 (v/v/v)
Pump mode	Isocratic
A.P.I Conc.	Terbutaline - 7 PPM, Bromhexine- 7 PPM
pH	5.1
Diluents	Mobile phase
Column	C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	270nm
Injection Volume	20 µL
Flow rate	1.0 mL/min
Run time	10 minutes
Retention Time	Terbutaline - 1.7 min, Bromhexine - 2.7 min
Theoretical plates	Terbutaline - 6645, Bromhexine - 16348
Tailing factor	Terbutaline -1.46, Bromhexine - 1.54

Fig 3: Calibration Plot for Terbutaline

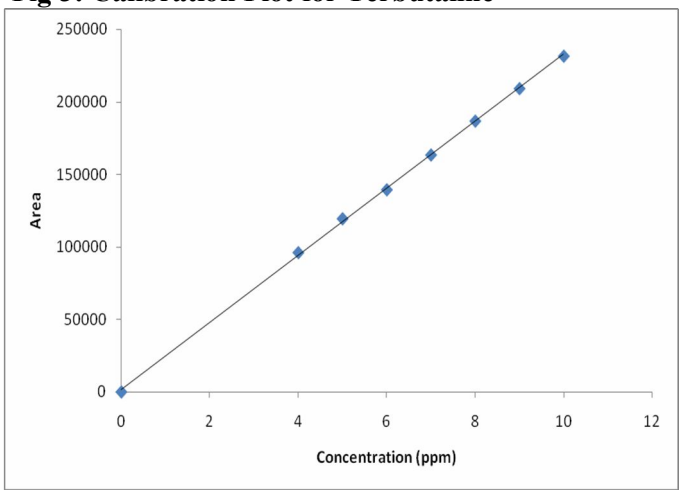


Fig 4: Calibration Plot for Bromhexine

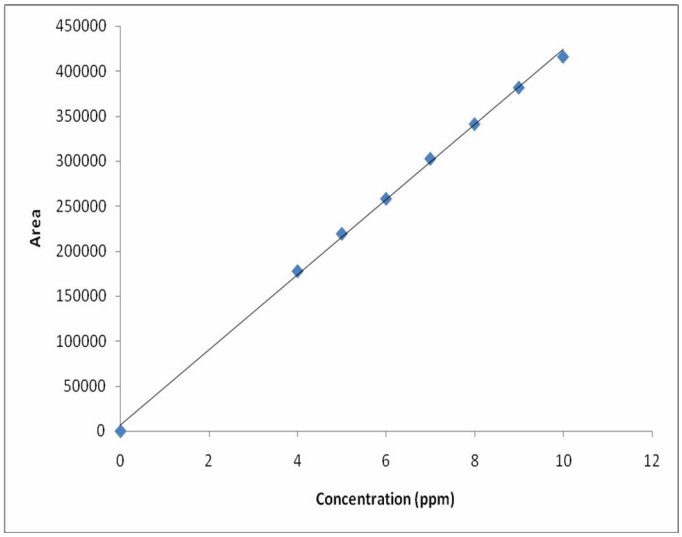
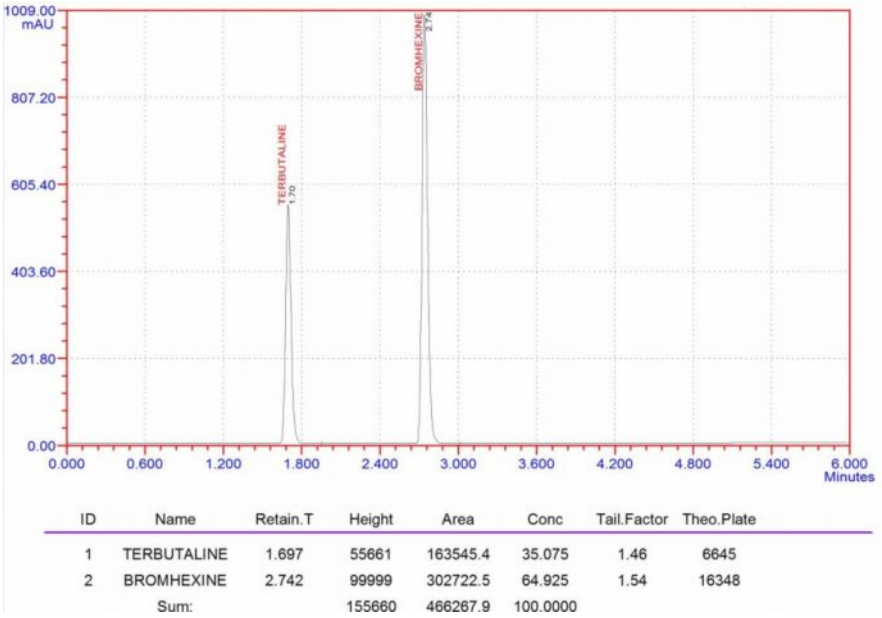


Table 2: Regression analysis of the calibration curve

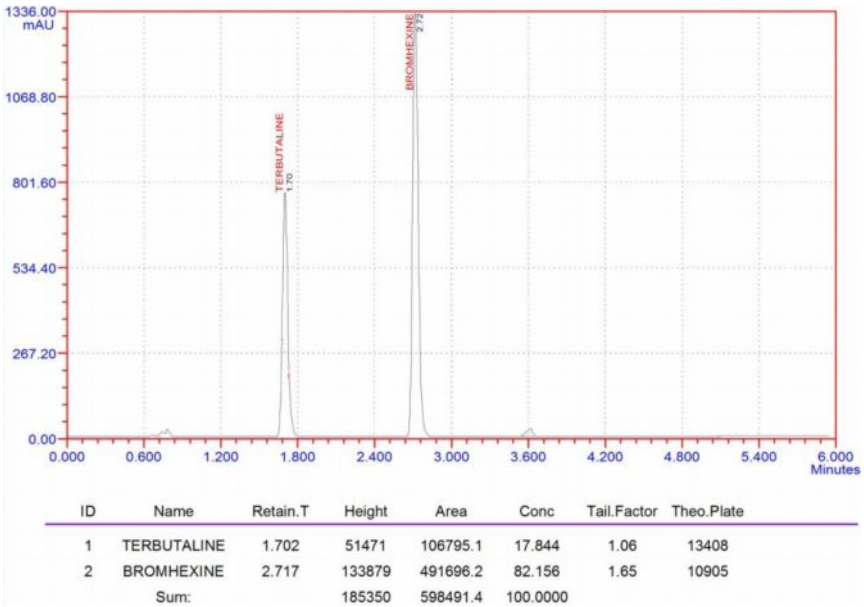
Parameters	Terbutaline	Bromhexine
Calibration range (ppm)	4-10 ppm	4-10 ppm
Slope	22637	40094
Intercept	5344	18933
Correlation coefficient (r^2)	0.9998	0.9996

Fig 5: Typical chromatogram of standard Terbutaline and Bromhexine mixture



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	TERBUTALINE	1.697	55661	163545.4	35.075	1.46	6645
2	BROMHEXINE	2.742	99999	302722.5	64.925	1.54	16348
	Sum:		155660	466267.9	100.0000		

Fig 6: Chromatogram of pharmaceutical dosage form



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	TERBUTALINE	1.702	51471	106795.1	17.844	1.06	13408
2	BROMHEXINE	2.717	133879	491696.2	82.156	1.65	10905
	Sum:		185350	598491.4	100.0000		

RESULTS AND DISCUSSION

Method validation

The method was validated by determining linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing 4-10 ppm of both Terbutaline and Bromhexine.

Linearity

The linearity of the response for Terbutaline and Bromhexine assay method was determined by preparing and injecting standard solutions of Terbutaline and Bromhexine. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied with correlation coefficient (r^2) value, slope and intercept as shown in Table 2.

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Terbutaline and Bromhexine combined test solution in the same equipment at a concentration value of 7 ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision as % RSD was reported.

Recovery

The recovery of the standard solutions was done by adding them to pre-analyzed sample solution at different levels i.e. 50%, 100%, and 150% separately to study the accuracy of the above method. The corresponding results were recorded.

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Terbutaline and Bromhexine. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Terbutaline and Bromhexine.

Table 3: Precision for Bromhexine and Terbutaline

Peak Area of Terbutaline	Peak Area of Bromhexine	RSD (Acceptance criteria ≤ 2.0%)
163419	307364	Terbutaline-1.251 Bromhexine-1.211
169626	307710	
165986	312786	
167927	302868	
166822	312422	
165948	310444	

Table 4: System suitability and validation parameters

Parameters	Terbutaline	Bromhexine
Theoretical plates (N)	6645	16348
Retention time (min)	1.7	2.7
Tailing factor	1.46	1.54
LOD (ppm)	0.03	0.02
LOQ (ppm)	0.15	0.08
Accuracy (%)	99.6	99.7
R.S.D. (%)	1.251	1.211

Table 5: Formulation: BROZEET

S. No.	Drug	Dosage	Sample Conc.	Area	Amount of drug estimated	% of drug estimated
1	TER	1.5mg/ml	4.5 ppm	106795	4.48mg/ml	99.55
2	BRX	4 mg/ml	12 ppm	491696	11.79mg/ml	98.25

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines and are shown in **Table 4.**

Analysis of marketed formulations

The validated HPLC method was adopted for the quantification of Terbutaline and Bromhexine in their combined pharmaceutical dosage form and the typical chromatograms of the formulation are shown in Fig 6. The results of analysis are given in **Table 4.** The contents of the pharmaceutical dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Terbutaline and Bromhexine in pharmaceutical dosage form.

CONCLUSION

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Terbutaline and Bromhexine in combined pharmaceutical dosage formulations.The method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Terbutaline and Bromhexine in the bulk as well as pharmaceutical dosage form. The percentage of recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

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