A validated high performance liquid chromatographic method for estimation of bromhexine and terbutaline in bulk and tablet dosage forms

Abstract

Introduction: Bromhexine (BH) is a mucolytic agent used in the treatment of respiratory disorders marketed in combination with terbutaline (TB), a β2-adrenergic receptor agonist used as a fast-acting bronchodilator. **Materials and Methods:** BH and TB were estimated at 270 nm by using ODS C_8 column (length 250 mm and internal diameter 4.6 mm) as a stationary phase and a premix of phosphate buffer (0.05 M, pH 3): Acetonitrile (70:30 v/v) as a mobile phase. The total run time of this method was less than 20 min and the retention time for BH was found to be at 15.50 min while that of TB was 9.85 min at a flow rate of 1.0 ml/min, respectively. **Results:** Percentage label claim of tablet formulation using this method was found to be 99.35% for BH and 99.70% for TB, respectively. The standard deviation was found to be 0.225–0.351 for BH and 0.0.236–0.264 for TB for two different batches of tablet formulation. **Conclusion:** The results of analysis of two drugs from their tablet formulation using a developed method were found close to 100%. The low values of standard deviation indicate accuracy and reproducibility of the method. Thus developed methods can be used for the routine analysis of two drugs from a combined dosage form.

Key words: Bromhexine, terbutaline, high performance liquid chromatography, simultaneous validation

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INTRODUCTION

Bromhexine (BH) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus and is chemically known as 2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzylamine hydrochloride and *N*-(2-amino-3,5-dibromobenzyl)-*N*-methylcyclohexylamine hydrochloride. The drug is official in Merck Index,[1] BP,[2] and IP.[3] Terbutaline (TB) is a β2-adrenergic receptor agonist. Terbutaline is used as a fast-acting bronchodilator and as a tocolytic to delay premature labor. It is chemically known as 1,3-benzenediol, 5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-sulfate (2:1) (salt) and (±)-[(*tert*-Butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate. Terbutaline is official in Merck Index,[4] BP,[5] IP[6] and USP.[7] A literature survey reveals various high performance liquid chromatography (HPLC)[8-12] and spectrophotometric[13-17] methods for the determination of BH and TB in their single and combined dosage forms with other drugs.

According to the literature survey, there is no reported method for simultaneous estimation of BH and TB in combined dosage forms. The objective of present work is the development and validation of a method for the estimation of BH and TB in bulk and tablet dosage forms.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation of drugs was performed using Shimadzu LC-AHT 2010 High Performance Liquid Chromatography from Shimadzu Analytical (India) Pvt. Ltd., Mumbai.

HPLC condition

HPLC was performed on an ODS C_8 column (250× 4.6 mm i.d.; 5 μm particle size). The mobile phase consisted of phosphate buffer (0.05 M, pH 3): acetonitrile (70:30 v/v). The mobile phase was filtered through a nylon 0.45 μm, 47 mm membrane filter and was degassed before use. The flow rate was 1.0 ml/min. The determination was carried out at 270 nm, and the injection volume was 20 μL. The total run time was 10 min. The data were analyzed by Integrated LC software.

Chemicals and reagents

HPLC-grade phosphate buffer and acetonitrile were procured from S.D. Fine Chemicals Limited, Mumbai, India. A gift sample of BH and TB were provided by Ind Swift Ltd., Chandigarh, India.

Selection of detection wavelength

Both BT and TB are known to absorb in the ultraviolet region, hence a UV detector was used for their simultaneous estimation. Wavelength selected for simultaneous estimation of two drugs was 270 nm. The column was saturated with the mobile phase for about an hour at a flow rate of 1.0 ml/min, monitoring the eluent at 270 nm so as to obtain a steady base line. After the chromatographic conditions were set and the instrument was stabilized to obtain a steady baseline, 20 μL of standard drug solution each of BH (25 μg/ml) and TB (25 μg/ml) made in the mobile phase were loaded into the injection port of the instrument and injected after filtration through a 0.2 μm membrane filter. The injection was repeated three times. This was done to check retention times of the individual drugs. The mean retention time for BT and TB were found to be 15.50 min and 9.85 min, respectively [Figure 1].

Standard stock solutions of pure drugs were made separately in the mobile phase containing 100 μg/ml of BH and TB, filtered through a 0.2 μm membrane filter. In a 10 ml volumetric flask, 2.5 ml standard stock solution of BH with 2.5 ml standard stock solution of TB was taken and volume made to the mark with the mobile phase. This mixed standard solution was loaded in the injector port of the instrument. The solution was injected and a chromatogram was recorded. This was done to check the resolution of two drugs. The two drugs were found to be perfectly resolved.

Calibration curve

In a series of a 10 ml volumetric flask, several dilutions of BH (15–55 μg/ml) and TB (6–36 μg/ml) were prepared in the mobile phase. Each solution was injected and a chromatogram was recorded. The peak area of a drug was calculated for each concentration level of two drugs and a graph was plotted between drug concentrations against the peak area. The linearity was observed in the concentration range of 15–55 μg/ml for BH and 6–36 μg/ml for TB.

Method validation

Linearity

A series of standard curves were prepared over a concentration range of 15–55 μg/ml for BH and 6–36 μg/ml for TB from a stock solution of BH and TB (100 μg/ml) in the mobile phase. Dilutions were prepared in the mobile phase, phosphate buffer: acetonitrile (70:30% v/v). The data from peak area vs. drug concentration plots were treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility. The experiment was performed in triplicate [Table 1].

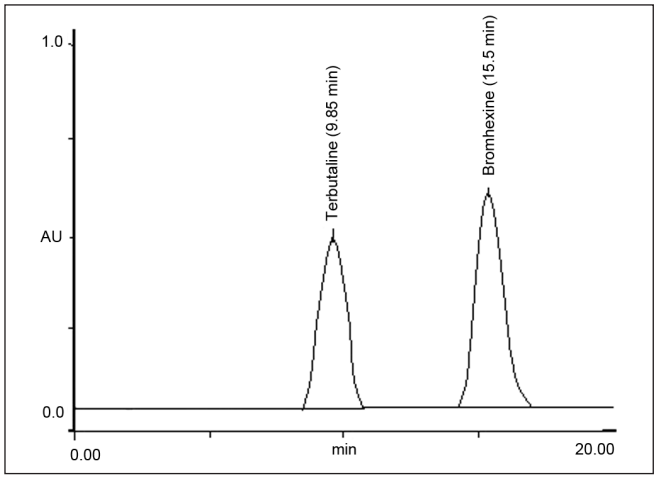

Figure 1: Chromatogram of BT and TB

Table 1: Validation parameters

Parameters	Results for BH	Results for TB
Linearity range (μg/ml)	15–55	6–36
Correlation coefficient	0.9999	0.9997
Slope	12040	9036.8
Intercept	199,633	144,988
Retention time (min)	15.50	9.85
LOQ (μg/ml)	14.50	5.75
LOD (μg/ml)	4.70	1.68

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of BH and TB were spiked with known amounts of BH and TB standard drugs and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate.

Precision

The intermediate precision and reproducibility, in accordance with ICH[18-20] (International Conference on Harmonization) recommendations, were determined as follows: Analysis repeatability was obtained by determining the relative standard deviation (RSD) of replicate samples of the intermediate precision and reproducibility study.

Intermediate precision (Inter-day and intra-day variation)

Measurements of inter-day and intra-day variation of BH and TB solutions were observed in triplicate on three consecutive days determined the intermediate precision [Tables 2 and 3].

Reproducibility

The reproducibility of the method was checked by determining precision on the same instrument, but by a different analyst. For both intra-day and inter-day variation, solutions of BH and TB at concentrations (25 μg/ml) were analyzed in triplicate.

LOD and LOQ

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as a noise level. The LOD was calculated as three times the noise level while ten times the noise value gave the LOQ.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of BH and TB. Robustness was determined by using reagents from two different lots and two different manufacturers.

Table 2: Intermediate precision (inter-day)

Concentration (μg/ml)		SD		% RSD	
BH	TB	BH	TB	BH	TB
30	20	0.135	0.290	0.136	0.292

Table 3: Intermediate precision (intra-day)

Day	Intra-day precision			
	SD		% RSD	
	BH	TB	BH	TB
1	0.149	0.245	0.149	0.246
2	0.177	0.249	0.178	0.250
3	0.151	0.205	0.151	0.206

Sample solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 72 h under laboratory bench conditions (25 ± 1°C) and under refrigeration (8 ± 0.5°C). The solution was subjected to HPLC analysis immediately and after a period of 24, 48, and 72 h.

There were no significant changes in the analyte composition over a period of 72 h. The mean RSD between peak areas, for the sample stored under refrigeration (8 ± 0.5°C) and at a laboratory temperature (25 ± 1°C), was found to be 0.159% and 0.234% for BH and TB, respectively. The method suggesting that drug solution can be stored without any degradation over the time interval suggested.

Specificity/selectivity

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well-shaped peaks indicate the specificity of the method. Better resolution was found for the drug peak with no interference proved that the method was found to be selective to the drug.

System suitability tests

The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The injection repeatability for the principal peak was the parameters tested on a 25 μg/ml sample of BH and TB to assist the accuracy and precision of the developed HPLC system.

Procedure of analysis for tablet formulation

Twenty tablets of BH and TB were weighed accurately and the average weight per tablet was determined. The tablet was finely powdered and powder equivalent to 100 mg of BH was accurately weighed transferred to a 100 ml volumetric flask containing about 75 ml of the mobile phase. The powder mixture was dissolved in the mobile phase with the aid of ultrasonication. The solution was filtered through Whatman filter paper no. 41 into another 100 ml volumetric flask. Washed the filter paper with the

mobile phase and added the washings to the filtrate. Volume of filtrate was made up to the mark with the mobile phase. To another 10 ml volumetric flask, 1 ml of this solution was transferred and the volume was made up to the mark with the mobile phase. To another 10 ml volumetric flask, 4.0 ml of this solution was transferred and the volume was made up to the mark with the mobile phase. This solution was filtered through a 0.2 μm membrane filter.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 μL sample loop of the injection port of the instrument, and the peak areas were recorded. A representative chromatogram has been given in Figure 1. The peak areas of each of the drug were recorded and the amount of each drug present per tablet was estimated from the respective calibration curves. The procedure of analysis was repeated five times with two different tablet formulations [Table 4].

Recovery studies

Recovery studies were carried out for formulation by addition of known amounts of standard drug solution to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed method. The results of recovery studies were found to be satisfactory [Table 5].

Table 4: Results of analysis of commercial formulation*

Brand name	Label claim (mg/ tablet)		% Label claim estimated		SD		Coefficient of variance	
					BH	TB	BH	TB
	BH	TB	BH	TB				
Efelin-X	8	2.5	99.19	99.60	0.351	0.236	0.354	0.237
Grilinctus-BM	8	2.5	99.50	99.79	0.225	0.264	0.226	0.265

*Each value is an average of five determinations

Table 5: Results of recovery studies

Brand name	Label claim (mg/ tablet)		Amount added to final dilution (μg/ml)		Amount recovered (μg/ml)		% Recovery	
					BM	TB	BM	TB
	BM	TB	BM	TB				
Efelin-X	8	2.5	2	2	1.98	1.96	99.00	98.00
			4	4	3.97	3.99	99.25	99.75
			6	6	5.95	5.98	99.16	99.66
Grilinctus-BM	8	2.5	2	2	1.97	1.98	98.50	99.00
			4	4	3.98	3.97	99.50	99.25
			6	6	5.97	6.01	99.50	100.17

RESULTS AND DISCUSSION

In this work the HPLC method has been developed for simultaneous estimation. The developed HPLC method for simultaneous estimation of BH and TB make use of a C_8 column. The mobile phase used for this method was phosphate buffer:acetonitrile (70:30) and detection of eluent was carried out at 270.0 nm. The total run time of this method was less than 20 min and the retention time for BH was found to be at 15.50 min while that of TB was 9.85 min at a flow rate of 1.0 ml/min, respectively. Percentage label claim of tablet formulation using this method was found to be 99.35% for BH and 99.70% for TB, respectively. Standard deviation was found to be 0.225–0.351 for BH and 0.0.236–0.264 for TB for two different batch of tablet formulation.

CONCLUSION

The developed HPLC method was found to be simple, specific, precise, accurate, and reproducible for the routine analysis of two drugs from a combined dosage form available in the market.

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