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].

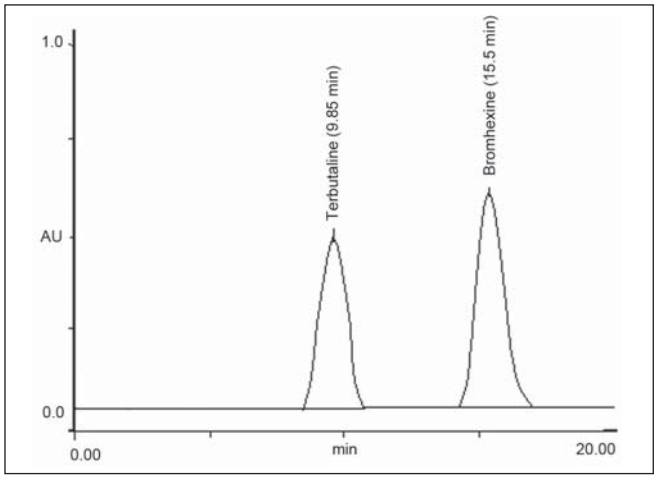

Figure 1: Chromatogram of BT and TB

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