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New stability indicating RP-HPLC method for the simultaneous estimation of terbutaline sulphate and bromhexine hydrochloride in tablet dosage form

Sreenivasa Charan Archakam*[1], Sridhar Chenchugari[2], Chandrasekhar Kothapalli Bannoth[3]

[1]Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Anantapuramu-515002, Andhra Pradesh, India
[2]Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati – 517503, Andhra Pradesh, India
[3]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapuramu-515002, Andhra Pradesh, India

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ABSTRACT

Bromhexine hydrochloride in combination with Terbutaline Sulphate is used as a fast-acting bronchodilator. New stability indicating RP- HPLC method was developed for the simultaneous estimation of Terbutaline Sulphate and Bromhexine Hydrochloride in Tablet dosage form. A Shimadzu Prominence liquid chromatograph with LC-20 AT pumping system and SPD-20, A UV-Vis Detector, was used for the proposed method. The chromatography was performed in reverse phase mode using a Phenomenex Luna C-18 column (4.5 x 150 mm, 5µm) as stationary phase and isocratic mobile phase constituting of 0.1% v/v Perchloric acid: Acetonitrile (60 vol :40 vol) at a flow rate of 1.5 ml/min with UV detection at 254 nm. The developed RP- HPLC method demonstrated good linearity in the concentration range of 10-60µg/ml for Terbutaline Sulphate with R^2=0.9991 and 20-100µg/ml for Bromhexine Hydrochloride with R^2=0.9992. The retention time of Terbutaline Sulphate was found to be around 2.1 min and for Bromhexine Hydrochloride it was found to be around 8.0 min. The precision, accuracy and robustness results were found to be satisfactory and within limits. The assay was found to be 96.7% (w/w) for Terbutaline sulphate and 97.7%(w/w) for Bromhexine Hydrochloride. Specificity experiments revealed the absence of interference from the mobile phase. The forced degradation studies like acidic, alkaline, oxidation and thermal degradation studies were performed and the degradation products were not interfering with the retention times of both the drugs. Thus, the developed method could be used in the routine analysis of the drugs in tablet dosage form.

* Corresponding Author

Name: Sreenivasa Charan Archakam
Phone: +91-9490844432
Email: charan4ma@gmail.com

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INTRODUCTION

Bromhexine hydrochloride and Terbutaline sulphate are used in combination for the treatment of respiratory tract disorders. This combination is mainly used as a bronchodilator. The chemical structures of Terbutaline and Bromhexine were shown in Figure 1 and 2 respectively. Only two analytical methods were reported for simultaneous determination of Terbutaline and Bromhexine in the combined dosage form of these two drugs (Kumar, A. and Nanda, S., 2011; Satyanarayana P.V.V. *et al.,* 2012). However, there are analytical works reported on Terbutaline, Bromhexine and Guai-

phenesin (Gangwal S. and Trivedi, P, 1999), Terbutaline with other combinations (Samanthula G *et al.,* 2013; Giriraj P *et al.,* 2012; Patel J *et al.,* 2015;) and Bromhexine with other combinations (Rele R, 2015; Jayalakshmi B *et al.,* 2010; Ankit B *et al.,* 2015). Since very few methods based on HPLC were reported in the literature, the proposed work aims to develop a simple, precise, accurate and stability indicating RP-HPLC method for the simultaneous estimation of Terbutaline sulphate and Bromhexine Hydrochloride in tablets.

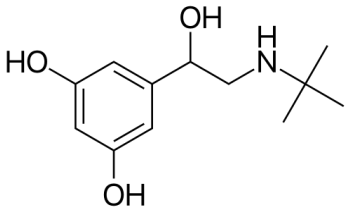

Figure 1: Chemical Structure of Terbutaline

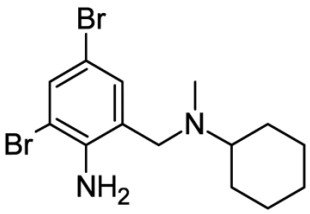

Figure 2: Chemical Structure of Bromhexine

MATERIALS AND METHODS

Instruments, reagents and chemicals

Shimadzu Prominence LC system equipped with LC- 20 AT pump, SPD- 20 A UV-Vis Detector, Rheodyne injection valve and Phenomenex Luna C-18 column (4.5 x 150 mm, 5µ) was used for the chromatographic separations. Sodium Hydroxide, Hydrochloric acid, Perchloric acid, Potassium dihydrogen orthophosphate, HPLC grade Acetonitrile, HPLC grade Methanol and HPLC grade Water were purchased from Merck Pvt Ltd (Mumbai, India). Terbutaline Sulphate and Bromhexine Hydrochloride were procured from Raffles Pharmaceuticals (Tirupati, India).

Preparation of Standard stock solutions

Standard Terbutaline Sulphate (TS) and Bromhexine Hydrochloride (BH) of 25 mg each were weighed and transferred in 25 ml of volumetric flask and 0.1% v/v Perchloric acid was added. Appropriate dilutions were made with 0.1% v/v Perchloric acid to get a final concentration of 100 µg/ml TS and BH.

Preparation of Test solution

Ten tablets were weighed and their contents were mixed thoroughly. An accurately weighed sample analogous to 2.5mg of TS and 8mg of BH was transferred into a 50ml volumetric flask and the volume was made up to the mark with 0.1% v/v Perchloric acid. From the above stock solution, 5ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with 0.1% v/v Perchloric acid, to obtain the concentration of 25 µg/ml of TS and 80 µg/ml of BH. Then filter through 0.45-micron membrane filter.

Chromatographic conditions

The chromatography was performed in reverse phase mode using a Phenomenex Luna C18 column (4.5 x 150 mm, 5µm) as stationary phase and isocratic mobile phase constituting of 0.1% v/v Perchloric acid: Acetonitrile (60 vol:40 vol) at a flow rate of 1.5 ml/ min with UV detection at 254 nm. The injection was done by using a Rheodyne injection valve system and the injection volume was 20µL. The runtime was set to 15 min. The peaks were characterised by their retention times. The optimised chromatogram was shown in Fig. 3.

RESULTS AND DISCUSSION

Method development and Optimization

A series of trials were conducted using phosphate buffer, methanol and acetonitrile, 0.1% Perchloric acid having different pH and different composition in the mobile phase to separate the two components. After reviewing the results, 0.1% v/v Perchloric acid: acetonitrile (60 vol:40 vol) was selected as the mobile phase and drug is soluble in Perchloric acid. Phenomenex, C18 is selected as the column as it is one of the most robust, reproducible and reliable RP-HPLC columns. This column was found to be stable at the desired pH and temperature. It offers good peak symmetry. Two wavelengths were selected for the chromatographic separation of the analytes. One of the detection wavelengths is 278nm; λ_{max} of other is universal detection wavelength 254nm. However, the results were good at 254nm. Hence it was fixed as detection wavelength. The flow rate of 1.5 mL/min was selected based on the separation of components, peak symmetries, the resolution between the peaks, column back pressure and retention times. The optimised chromatographic parameters for the proposed method was represented in Table 1. The retention times of TS was at around 2.133 min and for BH it was at 8.066 min. Single injections confirmed the peaks. The chromatograms were shown in Fig. 3,4 and 5. The system suitability parameters were acceptable and were represented in Table 2.

Method Validation

The developed method was validated as per ICH guidelines (ICH Q2(R1)) for various method parameters.

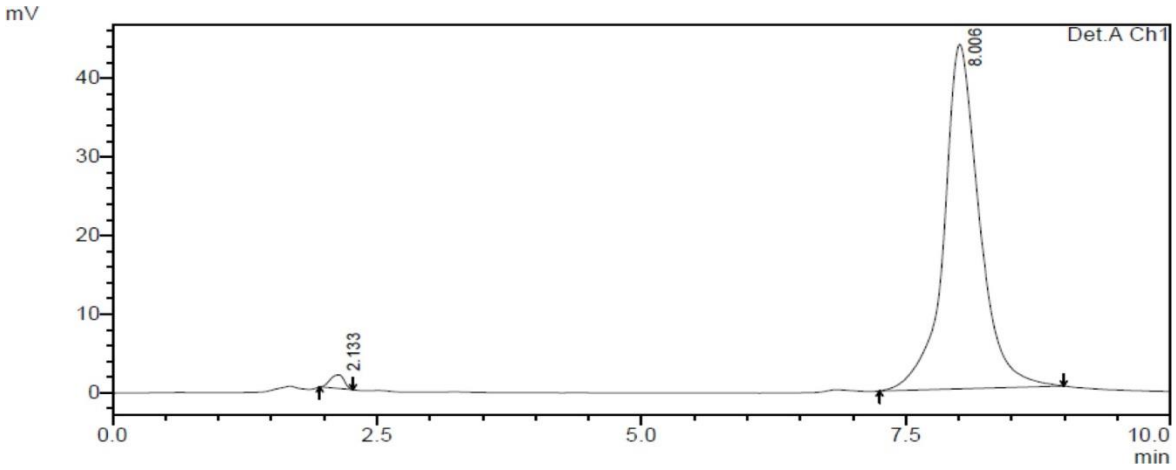

Figure 3: Optimized chromatogram of TS & BH

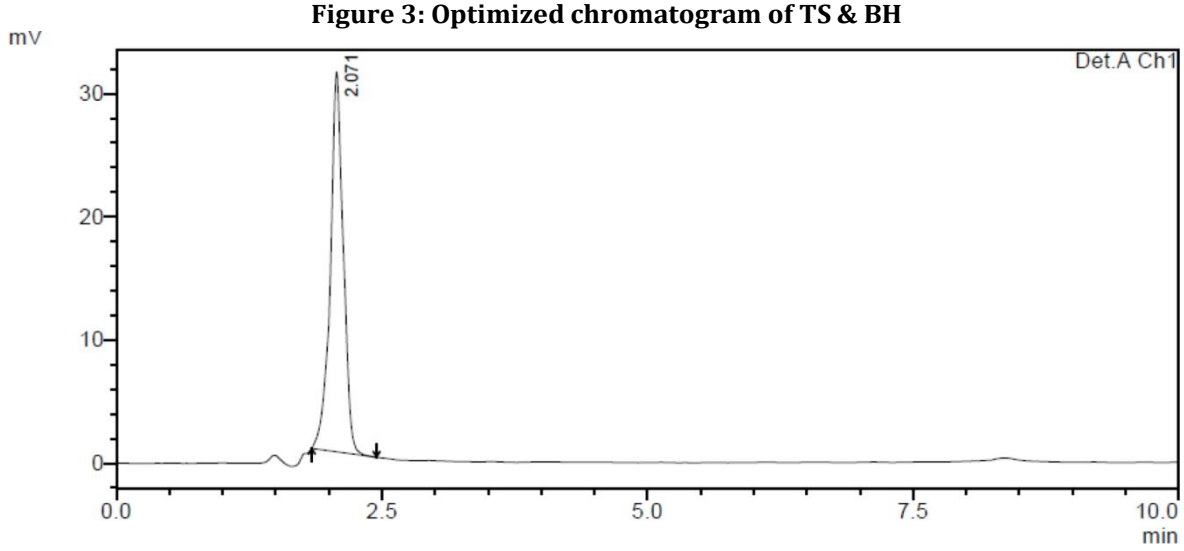

Figure 4: Confirmation HPLC peak of TS

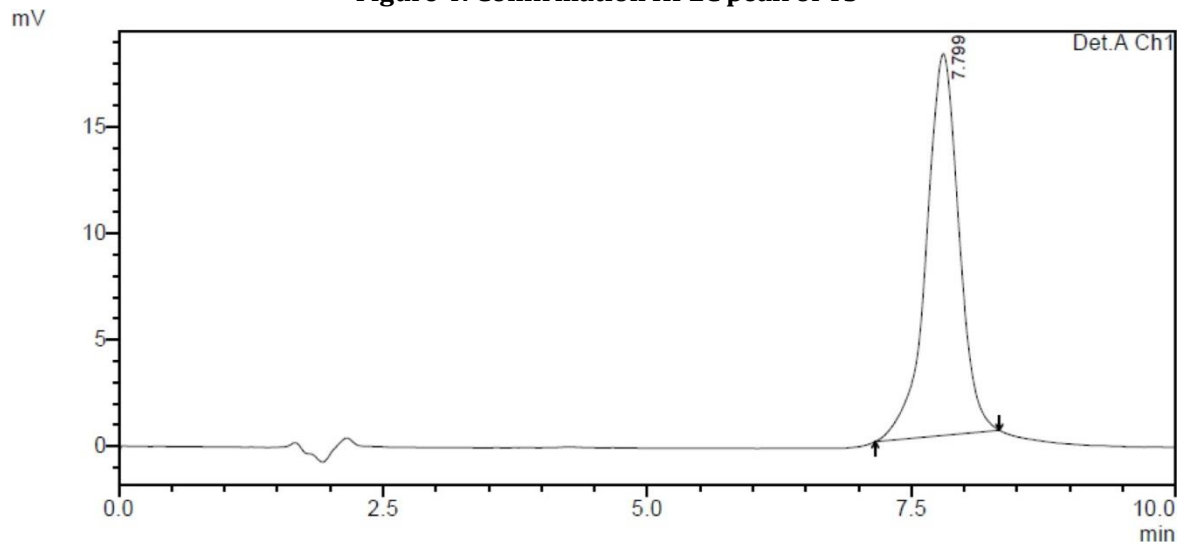

Figure 5: Confirmation HPLC peak of BH

Linearity

For linearity, the concentration range 10-50µg/ml and 20-100µg/ml was established for TS and BH respectively. A linearity curve was established with concentrations on the X-axis and corresponding peak area on Y- axis the linearity was determined. The specified range was derived from linearity studies by determining the difference between highest and lowest concentrations. The coefficient of regression values were 0.9991 for TS and 0.9992 for BH respectively. The linearity curves were shown in Fig. 6 and 7.

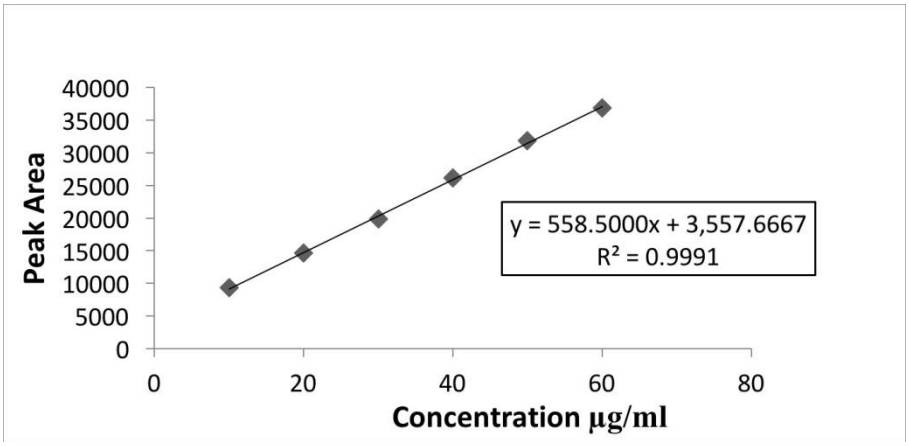

Figure 6: Linearity curve for Terbutaline Sulphate

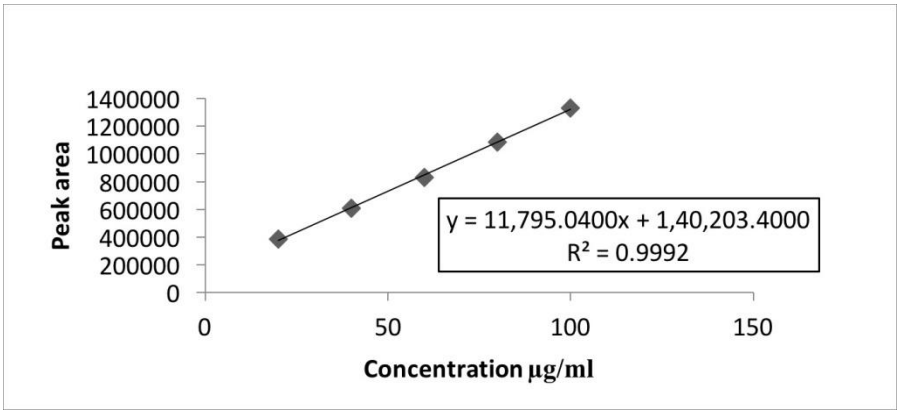

Figure 7: Linearity curve for Bromhexine Hydrochloride

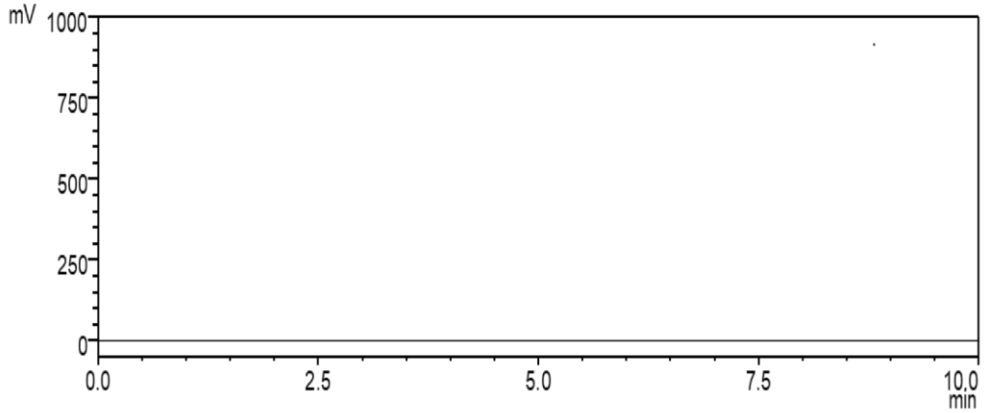

Figure 8: Chromatogram for Specificity with the mobile phase

Precision

The precision of the developed method was studied by system precision (injection reproducibility) and the method precision. All the values were obtained within the acceptable range of < 2% RSD.

Accuracy

The method of standard addition determined accuracy and it is expressed as recovery (%). Test formulation was taken in the concentration of 25 µg/ml of TS and 80 µg/ml of BH and standard stock solutions were spiked at 80%, 100% and 120% levels. The obtained results were found to be satisfactory and were shown in Table 3.

Specificity

The blank was injected to check the interference of the blank with the chromatogram of TS and BH was checked by recording and comparing the chromatograms of blank and that of TS and BH. No interference was found in the obtained chromatogram. The chromatogram was represented in Fig. 8.

Solution Stability

Sample and Standard solutions are prepared at the method concentration (100 µg/ml) and are injected for 2, 4, 8, 12, 15, 20, 24h time interval and their peak areas were noted. The results showed that for all the injections the peaks characteristics

Table 1: Optimized method chromatographic conditions

Software	LC-solutions
Column	Phenomenex, C_{18},5µ (250mm×4.6)
Pump Mode	Isocratic
Injection volume	20 µl
Flow rate	1.5ml/min
Wavelength	254nm (UV Detector)
Runtime	10min
Mobile phase	0.1% v/v Perchloric acid: acetonitrile (60:40)

Table 2: System suitability parameters

Peak#	Drug	Ret. Time (min)	Area	Height	Theoretical Plate#	Tailing Factor
1	Terbutaline	2.111	545454	74251	2087.134	0.777
2	Bromhexine	8.015	110171	6118	5461.217	1.581

Table 3: Accuracy and % recovery studies

Terbutaline Sulphate			Bromhexine Hydrochloride		
% Level	Sample area*	Average % recovery	%level	Sample area*	Average % recovery
80%	28535	99.3%	80%	1802045	97.1%
100%	30569	96.7%	100%	2116456	104.6%
120%	32848	95.3%	120%	2201613	105.9%

*Results are mean of three samples

Table 4: Summary of Robustness

		Terbutaline Sulphate				Bromhexine Hydrochloride			
Parameter	Condition	System suitability parameters*			Condition	System suitability parameters*			
		RT	Theoretical plates	USP Tailing factor		RT	Theo-retical plates	USP Tailing factor	
Change in flow rate	1.4 ml/ min	2.27	2103.701	0.791	1.4ml/min	8.3	3674	0.975	
	1.6 ml/ min	2.0	1997.041	0.802	1.6ml/min	8.1	3479	1.083	
Change in Mobile phase composition	65:35 Perchlo-ric acid: ACN	2.17	2822.298	0.878	65:35 Perchloric acid: ACN	6.8	1892	1.189	
	55:45 Perchlo-ric acid: ACN	2.05	2171.211	0.971	55:45 Perchloric acid: ACN	7.5	5561	1.606	
Change in Detection Wave-length	252nm	2.1	2249.135	1.20	252nm	8.6	3623.	1.027	
	256nm	2.0	2322.850	0.928	256nm	7.5	4204	1.230	

*Results are mean of three samples

Table 5: Forced degradation studies

CONDITION*	Terbutaline Sulphate %DEGRADATION	Bromhexine Hydrochloride %DEGRADATION
Acidic	6.42	4.47
Basic	5.78	6.34
Thermal degradation	4.21	3.25
Peroxide	10.2	9.78

*Results are mean of three samples

were satisfactory with no significant degradation and thus both the solutions were stable at least up to 24 h after their preparation.

Ruggedness and Robustness

The robustness of a method is tested for parameters such as flow rate (± 0.2 mL/min), wavelength

(± 5 nm), mobile phase composition etc., and determining the effect (if any) on the results of the method. All the results were satisfactory. The results were represented in Table 4.

Assay

The assay for TS and BH were found to be 96.7%(w/w) and 97.7%(w/w) respectively.

Forced degradation studies

The forced degradation studies were also studied as per ICH guidelines in acid (0.1N Hydrochloric acid), alkali (0.1N Sodium hydroxide), oxidation (1% Hydrogen Peroxide) and thermal degradation (60^0C) environments. The drugs were found to stable under all the stress conditions. The results were shown in Table 5.

CONCLUSION

The developed stability indicating RP-HPLC method for the simultaneous estimation of Terbutaline Sulphate and Bromhexine Hydrochloride in tablet dosage form is simple, precise, accurate, specific, less time consuming for the analysis. Hence, this method can be routinely used in the quality control laboratories and their stability studies.

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