JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Bromhexine and Phenylephrine HCL in its Combined Pharmaceutical Dosage Form

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ABSTRACT:

The present work describes various methods for the simultaneous estimation of Bromhexine and phenylephrine HCl in combined dosage form. A simple, accurate and reproducible RP-HPLC method have been development and validated for the simultaneous estimation of Bromhexine and Phenylephrine HCl in their marketed formulation. The RP-HPLC method utilizes isocratic elution technique with C-18 column (25cm x 0.46cm, 5µm particle size) with mobile phase consisting of Buffer (pH 5.0)-Acetonitrile-Triethylamine (80:20:0.25).The flow rate was 1.0ml/min and effluents were monitored at 225nm.The RP-HPLC method were validated by statically. The retention time of Bromhexine and Phenylephrine HCl were found to 3.66min and 5.29min respectively. Resolution of drug and the degradation products formed under different stress studies were successfully achieved. Linearity was evaluated over the concentration range of 4-12µg/ml for Bromhexine and 5-15µg/ml for Phenylephrine HCl by RP-HPLC method. The correlation coefficient and accuracy of both the method were found to be within the range. The %RSD of precision was found to be NMT 2% for HPLC method. The proposed methods were found to be robust when slight but deliberate changes were made in analytical condition. Simple, accurate and reproducible RP-HPLC method were developed and validated for the simultaneous estimation of Bromhexine and Phenylephrine HCl in their marketed formulation and it can be applied for the routine analysis in quality control laboratory.

KEY WORDS: Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Bromhexine (BH) and Phenylephrine hydrochloride (HCl)

Article history:

Received 14April 2016
Revised 20 May 2016
Accepted 5June2016
Available online 01 July2016

Citation:

Jivani N. P., Vekariya. H., Rajput H. P. Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Bromhexine and Phenylephrine HCL in its Combined Pharmaceutical Dosage Form. **J Pharm Sci Bioscientific Res**. 20166(4):523-528

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INTRODUCTION:

Bromhexine is a 2, 4 dibromo6 {[cyclohexyl (methyl) amino] methyl} aniline represent the class of mucolytic agent category which dissolves thick mucus and is usually used to help relieve respiratory difficulties. It does so by dissolving various chemical bonds within secretions, which in turn can lower the viscosity by altering the mucincontaining components. Phenylephrine HCl is benzenemethano,3-hydroxy-alpha-[(methylamino) methyl]-, hydrochloride represent the nasal decongestant. Phenylephrine is a sympathomimetic amine that acts predominantly on α-adrenergic receptors. It is mainly used to treat nasal congestion, but may also be useful in treating hypotension and shock, hypotension during spinal anasthesia, prolongation of spinal anasthesia, paroxysmal supraventricular tachycardia, symptomatic relief of external or internal hemorrhoids, and to increase blood pressure as an aid in the diagnosis of heart murmurs. Literature Review related that few analytical Methods have been reported for BH with other combination like UV

Spectrophotometry [17-18], HPLC [19-20]. Literature Review related that few analytical methods have been reported for PHE with other combination like UV Spectrophotometry HPLC. The Present work describes a validated reverse phase HPLC Method for Simultaneous determination of these two combined drugs in tablet.

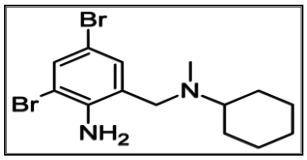


Fig 1 Structure of Bromhexine

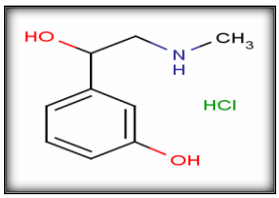


Fig 2 Structure of Phenylephrine HCl

MATERIALS AND METHODS

Instrument and Apparatus

RP-HPLC Instrument: Model: YL 9100, Column: C18 (25 cm × 0.46 cm) Hypersil BDS, Injector: 20µL fixed loop, Detector: SPD-20, Software: Spinchrom Software, Analytical balance: Electronic analytical balance (Shimadzu AUX220), Corning volumetric flasks and pipettes, UV Spectrophotometer: Shimadzu 1800PH Meter: WELLTRONIX, digital ph meter PM110, Glass Wares: Borosile, Ultrasonicator: Life care equipment Pvt. Ltd. Mumbai.

Chemicals and Materials:

The regents used in present research work are Acetonitrile (Finar), Water (HPLC grade, Finar), Acetic acid (Rankem), Methanol (HPLC grade, Finar), Bromhexine (Suryen Pharma), Phenylephrine HCl (Suryen Pharma).

Chromatographic conditions:

Column: C18 (25 cm × 0.46 cm) Hypersil BDS, Mobile Phase: Buffer (pH 5.0)-Acetonitrile-Triethylamine (80:20:0.25), Flow Rate: 1.0 ml/min, Detection Wavelength: 225 nm, Run time: 7 min, Injection volume : 20.0 µl

Preparation of standard solutions:

(A) Phenylephrine HCl standard stock solution: (100µg/mL)

A 10 mg of Phenylephrine HCl was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

(B) Bromhexine standard stock solution: (80µg/mL)

A 8 mg of Bromhexine was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Phenylephrine HCl (10 µg/mL) and Bromhexine (8 µg/mL)

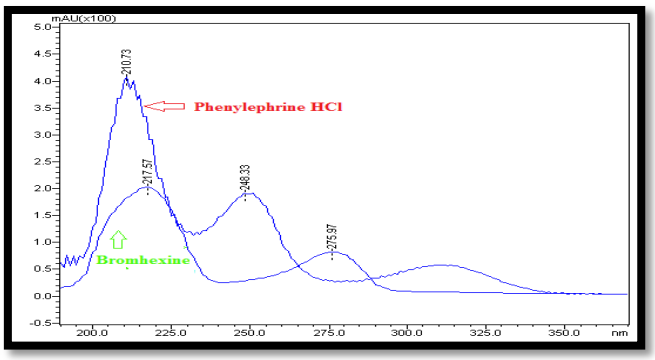
Take 1 mL from the Phenylephrine HCl stock solution and 1mL from Bromhexine stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Selection of wavelength:

Standard solution of Phenylephrine HCl (10 µg/mL) and Standard solution of Bromhexine (8 µg/mL) in Methanol were scanned between 200-400 nm using UV-visible spectrophotometer.

Both solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions. Both Phenylephrine HCl (10 ppm) and Bromhexine (8 ppm) in Methanol show reasonably good response at 225 nm.



Method Development

Standard solutions of 5-15 µg/ml of Phenylephrine HCl and 4-12 µg/ml of Bromhexine were injected in column with 20µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Buffer (pH 5.0)-Acetonitrile-Triethylamine (80:20:0.25).The detection was carried out at wavelength 225nm.The chromatogram was stopped after separation achieved completely. Data related to peak like area, height,

retention time, resolution etc were recorded using software.

Stability-Indicating Method

A. Acid degradation

Acid decomposition studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 2.5 hrs at 70°C. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 10µg/ml for Phenylephrine HCl and 8µg/ml for Bromhexine.

B. Base degradation

Basic decomposition studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 3 hrs at 70°C. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 10µg/ml for Phenylephrine HCl and 8µg/ml for Bromhexine

C. Oxidative degradation

Oxidative decomposition studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 1hrs at 70 ºC. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 10µg/ml for Phenylephrine HCl and 8µg/ml for Bromhexine

D. Photo Degradation

Photo Degradation studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was kept in presence of Sunlight for 1.5 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Phenylephrine HCl and 8µg/ml for Bromhexine

E. Thermal degradation

Thermal Degradation studies were performed One ml of stock solution was transferred in to 10ml of volumetric flask. The volumetric flask was stored in oven at 105°C for 4 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Phenylephrine HCl and 8µg/ml for Bromhexine

Validation of RP-HPLC method

1) Linearity

The linearity for Phenylephrine HCl and Bromhexine were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 4-12 µg/ml respectively,5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Phenylephrine HCl (100 µg/ml) and Bromhexine (80 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml and 4,6,8,10 and 12 µg/ml for Phenylephrine HCl and Bromhexine respectively In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

2) Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. Repeatability

Standard solution containing Phenylephrine HCl (20 µg/ml) and Bromhexine (8 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (5,10,15 µg/ml) of Phenylephrine HCl and (4,8,12 µg/ml) of Bromhexine were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (5,10,15 µg/ml) of Phenylephrine HCl and (4,8,12 µg/ml) of Bromhexine were analyzed three times on the different day and % R.S.D was calculated.

3) **Accuracy**

For Phenylephrine HCl

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 225 nm. The amount of Phenylephrine HCl was calculated at each level and % recoveries were computed.

Bromhexine

4 µg/ml drug solution was taken in three different flask

label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 225 nm. The amount of Bromhexine was calculated at each level and % recoveries were computed.

4) LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

Result and Discussion

Selection of Mobile Phase

The mobile phase Buffer(pH 5.0) : Acetonitrile : TEA (80:20:0.25v/v) was selected because it was found to ideally resolve the peaks with retention time (RT) 3.663 min and 5.297 min for Phenylephrine HCl and Bromhexine respectively and the same is shown in fig. 6.22.

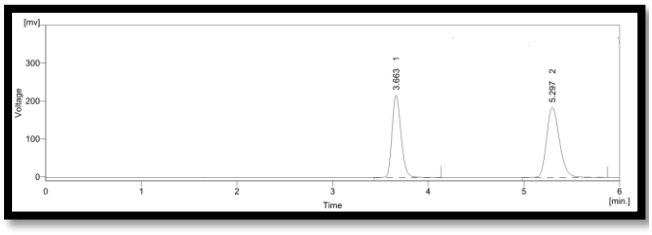


Fig 4 Chromatogram of Phenylephrine HCl-Bromhexine in Buffer (pH 5.0) : Acetonitrile : TEA (80:20:0.25v/v) (Flow rate-1.0 ml/min).

Observed values for system suitability test:

Table 1: Results for system suitability test.

Parameters	Data observed	
	Phenylephrine HCl	Bromhexine
Theoretical plates per column	6963	7225
Symmetry factor/Tailing factor	1.375	1.382
Resolution	7.689	

DISCUSSION

The development RP-HPLC method was found suitable for simultaneous estimation of Bromhexine and Phenylephrine HCl with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance indicated the repeatability of the proposed method. High correlation coefficient ranges for the 0.99 showed the stable linear detector response in different concentration ranges for the above drugs. The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for Bromhexine and Phenylephrine HCl are 99.66-99.78% and 99.76-100.01% respectively indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate, wave length and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of BH and PHE from the commercial combined tablet dosage form were in good agreement with respective to label claim indicating that there were no interferences from the

commonly used tablet excipients and buffer used in analysis.

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of Bromhexine and Phenylephrine HCL in combined tablet dosage form for routine quality control analysis.

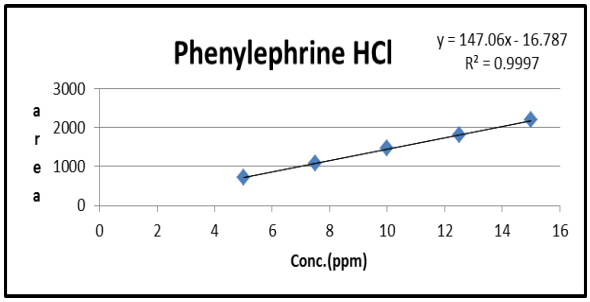


Fig 5: Calibration Curve of Phenylephrine HCl (5-15µg/ml)

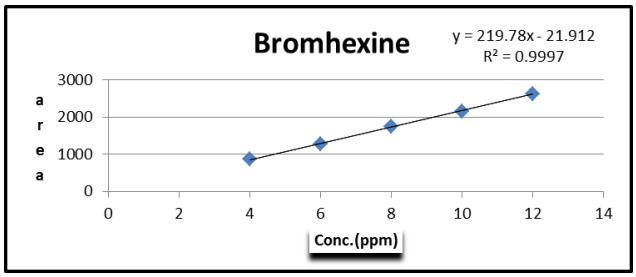


Fig 6: Calibration Curve of Bromhexine (4-12 µg/ml).

ACKNOWLEDGEMENT

The authors are thankful to Smt. R. B. Patel Mahila Pharmacy College, Atkot, Rajkot, India for providing necessary facilities to carry out the research work.

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