SIMPLE COLORIMETRIC ASSAY FOR MICROGRAM DETERMINATION OF BROMHEXINE HYDROCHLORIDE WITH MBTH AND 2, 2' BIPYRIDYL

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ABSTRACT

The present work describes two new spectrophotometric methods for the determination of bromhexine hydrochloride in bulk and formulations. Method A is based on the reduction of Fe^{3+} into Fe^{2+} by the drug followed by the complex formation of Fe^{2+} with 2, 2' bipyridyl to form cherry red colored chromogen having maximum absorbance at 510nm and Method B is based on oxidation of 3-methylbenzothiazolinone-2-hydrazone (MBTH) by $FeCl_3$ followed by its coupling with the drug in acidic medium forming an intense green colored chromogen with absorbance maxima at 630nm. Beer's law is obeyed in the range of 5-25 µg/ml with molar absorptivity of 1.113 x 10^4 for method A and 2-10 µg/ml with molar absorptivity of 1.128 x 10^4 for method B. The results were favorably compared with those obtained by reference UV spectrophotometric method. The accuracy, precision, and the linearity ranges of the methods have been determined, and they have been validated by analyzing the title drug. No interference was observed from common pharmaceutical adjuvants. These two methods were successfully applied to the pharmaceutical formulations, capsules, tablets.

KEY WORDS

Molar Absorptivity, 3-Methylbenzothiazolinone-2-Hydrazone, Bromhexine Hydrochloride.

INTRODUCTION

Bromhexine, 2, 4-dibromo-6-{[cyclohexyl (methyl) amino] methyl} aniline, is widely used in medicine as a mucolytic drug. It works through decreasing the amount of respiratory tract fluid and reduces its viscosity by activating enzymes that hydrolyze mucopolysaccharides[1]. Several methods have been reported in the literature for analytical determination of this substance. It has been determined by different techniques including spectrophotometry [1-3], HPLC[3,4], Colorimetry[5], TLC[6], Flow-injection-spectrophotometry[7], Gas chromatography[8], Ion-Selective Electrode[9], Hybrid Linear Analysis[10], Capillary Isotachophoresis[11], Absorption Spectrophotometry and Electrophoresis[12] , Reverse phase liquid chromatography[13], Liquid Chromatography-Electro Spray Ionization-Mass Spectrometry[14], HPLC-ICP-MS compared with Radiochemical Detection[15], Flow Injection analysis of bromhexine using conventional and coated wire ion-selective electrodes[16]. In the present work, a simple, accurate and sensitive method for determining bromhexine hydrochloride in pure form and pharmaceutical formulations was introduced.

The literature survey on the analytical applications of methylbenzothiazolinone-2-hydrazone and 2**,** 2' bipyridyl indicates that these compounds have not been earlier reported as reagents for the spectrophotometric

determination of bromhexine hydrochloride in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop simple and rapid methods for the estimation of the sited drug in bulk and pharmaceutical formulations.

EXPERIMENTAL

Bromhexine hydrochloride was obtained in highly pure form (pharmaceutical grade) from the local pharmaceutical industry. Its pharmaceutical preparations obtained from different commercial sources. All other reagents were of analytical grade. Distilled water was used for preparation of all solutions. Ultraviolet and visible spectrophotometry were carried out through Elico UV – Visible Double beam spectrophotometer model SL-159.

Standard drug solution:

Accurately weighed 100mg of bromhexine hydrochloride was dissolved in 100ml distilled water to give a concentration of 1mg/ml. The final concentration was brought to 100 µg/ml for Methods A and B.

Reagents:

Method A:

- 1M 2, 2' bipyridyl
- 1M Ferric chloride
- 0.2M Ortho phosphoric acid

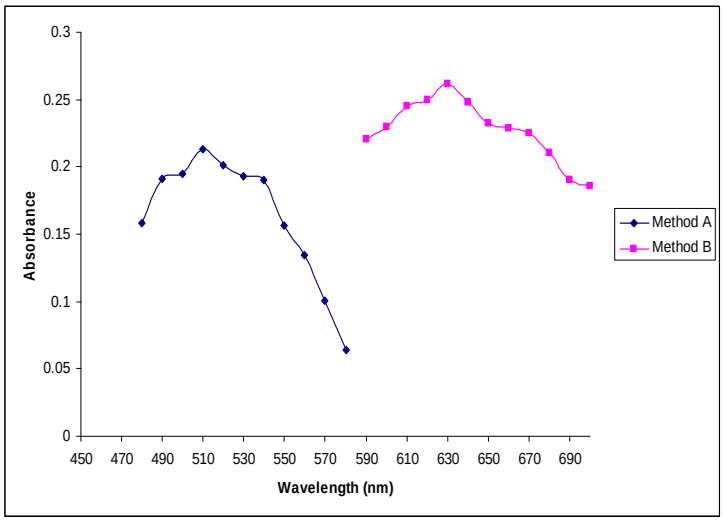
Method B:

- 0.2% MBTH
- 0.7% $FeCl_3$ in 0.5% hydrochloric acid

Assay procedure for the determination of Bromhexine hydrochloride

Method A: Aliquots of the standard solution containing 0.5 - 2.5ml (50-250µg) of Bromhexine hydrochloride, 1ml of ferric chloride solution and 1ml 2, 2' bipyridyl were added to heating tubes. The mixture was homogenized by shaking, immersed in a water bath at 100°C for 20 minutes, then cooled to room temperature then 1 ml of 0.2M Ortho phosphoric acid was added. The contents in all the tubes were transferred into a series of 10 ml volumetric flask then diluted up to the mark with distilled water and the absorbance was measured at 510nm (Fig:1) against reagent blank which shown nil absorbance at corresponding wave length. The calibration curve was prepared to calculate the amount of the drug.

Fig: 1 Absorption spectrum of colored chromogen in methods A and B



Method B: Aliquots of standard drug solution containing 0.2 -1.0 ml (20-100µg) was transferred into a series of graduated tubes. To each tube 1ml MBTH and 1.5 ml $FeCl_3$ were added shake well and transfer the contents of each tube in to a series of 10 ml volumetric flasks and made up the volume up to the mark with distilled water. The green color developed was measured at 630nm (Fig:1) at which the reagent blank has no absorbance and the calibration curve was prepared.

Assay of pharmaceutical formulations

Tablet powder equivalent to 100 mg was accurately weighed and dissolved in water and filtered. The filtrate was made up to 100 ml and appropriate aliquots of the drug solution were treated as described above and the results were tabulated. While for the syrup form, 1mM bromhexine solution was prepared through dissolving an appropriate volume of the syrup and then diluted by water using an appropriate volumetric flask.

RESULTS AND DISCUSSION

The method A is based on the reduction of Fe^{3+} into Fe^{2+} by the drug followed by chelation of Fe^{2+} with 2,2‘ bipyridyl to form a red colored chromogen. The colored chromogen has λ_{max} at 510nm. In method B, MBTH was oxidized by the ferric chloride in acidic medium followed by its coupling with the drug to form green colored complex having λ_{max} at 630nm.Actually, this is an iron catalysed oxidative coupling reaction of MBTH with the drug.Under reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling agent. This intermediate undergoes electrophilic substitution with the drug to form the colored product.

The optical characteristics such as absorption maxima, Beer’s law limits, Molar absorptivity and Sandell’s sensitivity for these methods are presented in Table-1.The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1

TABLE – 1 Optical Characteristics, Precision and Accuracy of Proposed Methods

Parameters	Method A	Method B
λ_{max} (nm)	510	630
Beer’s law limit (µg/ ml)	5- 25	2 - 10
Sandell’s Sensitivity (µg/cm^2/0.001 abs. unit)	0.033	0.033
Molar absorptivity(Litre.$mole^{-1}$.cm^{-1})	1.113 x 10^4	1.128 x 10^4
Stability of Color (hours)	10	5
Regression equation (Y)*		
Intercept (a)	0.2318	0.0048
Slope(b)	0.0045	0.0031
% RSD$	1.020	1.66
% Range of errors (95% confidence limits):		
0.05 significance level	0.852	1.387
0.01 significance level	1.261	1.999

Y = a + bx, where Y is the absorbance and x is the concentration of bromhexine hydrochloride in µg/ ml.

$ For six replicates

The accuracy of these methods was ascertained by comparing the results obtained with the proposed and reference methods in the case of formulation are presented in Table-2. As an additional check on the accuracy of these methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulation and percent recovery experiments were also done. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

TABLE - 2.Assay and Recovery of bromhexine hydrochloride in Pharmaceutical Formulations

Formulations	Labeled amount(mg)	Recovery by reference method*(%)	Recovery by proposed methods (%) **	
			Method A	Method B
Bromhexine	8	100	100.1	100.25
Barkasin	4	9.92	99.75	99.50

* Reference method was UV method developed in the laboratory.

** Recovery amount was the average of six determinants

CONCLUSION

It could be concluded that the developed methods for bromhexine hydrochloride assay is simple, sensitive (microgram amount can be determined), relatively precise, accurate and can be satisfactorily applied to the analysis of bromhexine hydrochloride in bulk and pharmaceutical formulations. The proposed methods are used for the routine analysis of the drug in the quality control.

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