Spectrophotometric Determination of Bromhexine Using Charge Transfer Complex Reaction

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ABSTRACT

Three simple, sensitive, and rapid spectrophotometric methods were developed for the estimation of bromhexine (BRH) in pure and its commercial dosage forms. These methods were based on the formation of charge-transfer reaction between the drug, an n-electron donor and π-acceptors, 2, 3-dichloro-5, 6-dicyano-p-benzoquinone, chloranilic acid, and 1-chloro-2, 4-dinitrobenzene. The absorbance of the formed charge-transfer complexes was measured and utilized for the determination of BRH in its pure and commercial dosage forms. The developed methods were evaluated in terms of standard deviation, relative standard deviation, correlation coefficient, limit of detection, and limit of quantitation. Molar absorptivity and Sandell's sensitivity were calculated at the optimum experimental conditions. The validity of the proposed methods was ascertained by recovery studies which indicated that the present methods can be successfully applied for the determination of BRH in pure and commercial dosage forms.

Key words: Spectrophotometric methods, Bromhexine, Charge-transfer reaction, 3-dichloro-5, 6-dicyano-p-benzoquinone, Chloranilic acid, 1-chloro-2, 4-dinitrobenzene.

1. INTRODUCTION

Bromhexine hydrochloride (BRH) is a mucolytic agent and used in the treatment of respiratory disorders. It is chemically known as 2, 4-dibromo-6-([cyclohexyl (methyl) amino] methyl) aniline hydrochloride [1,2].

Various official methods were developed for the determination of BRH in pure and its formulations. Literature survey revealed that apart from official methods [3,4], different analytical techniques were developed such as UV spectrophotometry [5-7], and high-performance liquid chromatography [8-10] for the estimation of BRH in pure and dosage forms. Few methods were also reported based on the reaction schemes such as an ion pair and charge transfer complex formation [11-12] between the BRH and various analytical reagents. However, no methods were reported for estimation of BRH in biological fluids. As the reported methods suffer from disadvantages such as liquid - liquid extraction steps, longer time, pH control and narrow linear range, there is a need to develop sensitive, simple, fast, and cost effective methods for the determination of BRH in pure and dosage forms.

Hence, in the present work, three methods were developed which were based on the formation of charge-transfer complex between BRH and 3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ), chloranilic acid (CAA) and 1-chloro-2,4-dinitrobenzene (CDNB) (Figure 1).

2. EXPERIMENTAL PROCEDURE

2.1. Instrumentation

All measurements were carried out using a Shimadzu UV-Visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

2.2. Materials and Reagents

BRH hydrochloride is procured from M/s Malladi Drugs and Pharmaceuticals Limited, India as a gift sample. Bromex, BRH, and Bisolvon were purchased in the local market, Tirupati. All the chemicals used were of analytical reagent grade. Double distilled water is used throughout the experiment.

2.2.1. Preparation of standard solutions

A stock solution of BRH was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml of

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water and sonicated to get required concentration of 1 mg/ml. Further, it was diluted with double distilled water as required for the present investigation.

2.2.2. Preparation of Reagents
2.5% (w/v) of DDQ solution was prepared by dissolving 2.5 g of compound in 100 ml of acetonitrile for method A, 1.0% (w/v) of CAA solution was prepared by dissolving 1 g of compound in 100 ml of ethanol for method B and 3.0% (w/v) of CDNB solution was prepared by dissolving 3 g of compound in 100 ml of DMSO for method C.

2.3. Method Development
2.3.1. Method A (DDQ method)
Standard drug solution was transferred into a series of clean and dry volumetric flasks. To each flask, 3.2 ml of 0.2% DDQ solution was added and brought up to the volume with acetonitrile to get the required concentrations range of 8 µg/ml-44 µg/ml. A wine red color was developed, and absorbance was measured at 460 nm against the reagent blank.

2.3.2. Method B (CAA method)
Different volumes of standard drug solutions were prepared from stock solution in the concentrations range of 4 µg/ml-36 µg/ml in clean and dry volumetric flasks and 5.0 ml of 1.0% CAA solution was added to each flask. Maximum absorbance of produced pale yellow color solution was measured at 430 nm against the reagent blank.

2.3.3. Method C (CDNB method)
Into a series of volumetric flasks, various volumes of standard drug solution were transferred. To each flask, 3.6 ml of 3% CDNB solution was added and made up the solution in the concentration range of 10 µg/ml-50 µg/ml. Total contents were heated up to 98±2°C and cooled to room temperature. Maximum absorbance of each yellowish pink colored solution was measured at 435 nm against the reagent blank.

2.3.4. Procedure for analysis of pure drug
Accurately weighed amount of BRH was transferred into clean and dry volumetric flask, subsequently diluted with water to get the required concentration and analyzed by above mentioned the procedure.

2.3.5. Procedure for commercial dosage forms
The dosages of Bromex, BRH and Bisolvon containing BRH hydrochloride were purchased from the local market and analyzed by the developed methods. Twenty tablets of each formulation were weighed and grinded to make a fine powder. A quantity of grinded powder equivalent to 100 mg was taken into volumetric flask and analyzed as described above.

3. RESULTS AND DISCUSSION
In the present work, DDQ, CAA, and CDNB were π-acceptors and BRH as n-donor. Charge transfer complex was formed by electron is transfer from the donor to acceptor, which produce high intense color in the visible region of the electromagnetic spectrum.

3.1. Spectral Characteristics
3.1.1. Absorption spectrum
The reaction of DDQ, CAA, and CDNB with BRH results in the formation of wine red, pale yellow and yellowish pink complexes, respectively, which exhibit maximum absorbance at 460 nm, 430 nm and 435 nm, respectively (Figure 2).

3.1.2. The effect of reagent concentration
To measure the effect of concentration of the reagent on the formation of colored product at the chosen wavelength, various volumes of reagent were added to a fixed concentration of drug solution and absorbance was measured. It was found that 3.2 ml of 0.2% DDQ (method A), 5 ml of 1.0% CAA (method B) and 3.6 ml of 3% CDNB solutions (method C) were optimum for the production of high-intensity color and no change was observed after addition of few more ml of respective reagents.

3.1.3. Effect of the concentration drug
To study the effect of concentration of drug solution on the absorbance maximum, fixed volume of reagent i.e. DDQ, CAA, and CDNB was added to each volumetric flask containing different aliquots of drug solution was measured the absorbance at 460 nm, 430 nm, and 435 nm respectively against reagent blank. It was found that BRH obeyed Beer's law in the range of 8 µg/ml-44 µg/ml, 4 µg/ml-36 µg/ml, and 10 µg/ml-50 µg/ml with DDQ, CAA and CDNB, respectively.
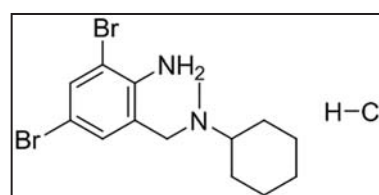

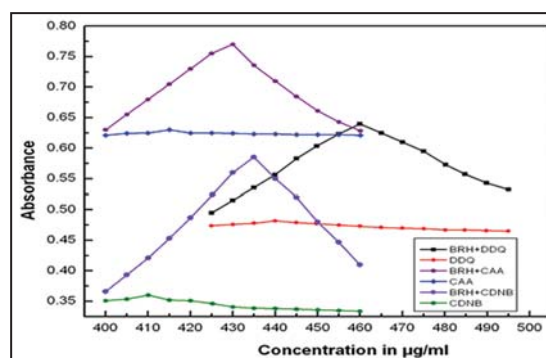**Figure 1:** Structure of bromhexine hydrochloride.

Figure 2: Absorption spectrum of bromhexine hydrochloride.

3.2. Analytical Method Validation

Validation is one of the important steps in analytical method evaluation [13]. The validation parameters, i.e. linearity, accuracy, precision, recovery, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness were evaluated to assess the method suitability.

3.2.1. Linearity

Linearity of the concentration drug solution for the developed methods was studied, and calibration plots were constructed (Figure 3). From the calibration plots, a linear correlation was calculated between the absorbance and the concentration. Beer's Law limit, Sandell's sensitivity, molar absorptivity were reported in Table 1.

3.2.2. Robustness and ruggedness

For the evaluation of robustness, some parameters like concentration of drug and reagent, wavelength range and shaking time were interchanged. The capacity remained unaffected by small changes in these parameters. Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical difference between
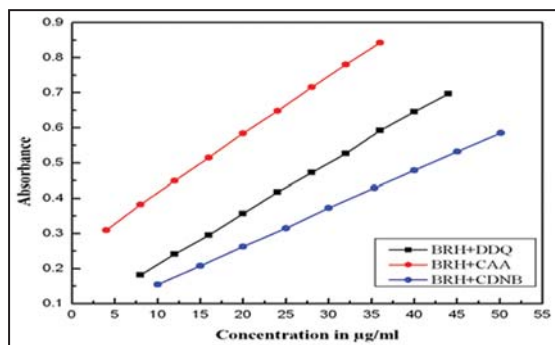

Figure 3: Calibration plot of bromhexine hydrochloride with analytical reagents.

different analysts and instruments suggesting that the developed methods were robust and rugged.

3.2.3. Accuracy, precision, and recovery

Accuracy of the proposed methods was proved by recovery studies (Tables 2 and 3). The recovery studies were carried out using the developed methods by adding known quantity of pure drug. The obtained results proved that the recovery values in drug and in dosages were within the acceptance limit.

Repeatability is determined by using different concentrations and studied the variances in intraday and interday using proposed analytical methods and found the % RSD less than 1.0, which indicated that the developed methods were precise.

Table 1: Spectral characteristics of the drug with reagent.

Parameter	Method A	Method B	Method C
λ_{max} (nm)	460	430	435
Beer's law limit (μg/ml)	8-44	4-36	10-50
Molar absorbance (L.mol^{-1} cm^{-1})	5.3423	6.3587	4.8210
Sandell's sensitivity (μg.cm^{-2}/0.001 AU)	0.001545	0.001298	0.0017
Correlation coefficient (r^2)	0.9961	0.9890	0.9995
Slope (m)	0.0115	0.0120	0.0104
Intercept (c)	0.0681	0.2137	0.0523
% RSD	0.6758	0.6678	0.7591
Color	Wine red	Pale yellow	Yellowish pink
LOD	0.8005	1.0533	0.8766
LOQ	2.4260	3.1921	2.6564

LOD: Limit of detection, LOQ: Limit of quantification

Table 2: Evaluation of accuracy and precision of the proposed method in bulk form.

Method	Taken mg/ml	Intra day				Inter day			
		*Found	Recovery %	±SD	% RSD	*Found	Recovery %	±SD	% RSD
A	2	1.95	97.50	0.0154	0.7944	1.93	96.91	0.0172	0.8885
	4	3.93	98.17	0.0216	0.5501	3.96	98.88	0.0356	0.9010
	6	5.92	98.61	0.0436	0.7380	5.93	98.78	0.0408	0.6888
B	6	5.90	98.44	0.0344	0.5832	5.91	98.63	0.0213	0.3610
	8	7.90	98.69	0.0575	0.7287	7.91	98.88	0.0309	0.3917
	10	9.89	98.90	0.0737	0.7457	9.83	98.27	0.0752	0.7660
C	4	3.89	97.37	0.0251	0.6444	3.92	98.12	0.0258	0.6594
	6	5.88	97.92	0.0508	0.8662	5.89	98.08	0.0524	0.8910
	8	7.87	98.40	0.0688	0.8743	7.90	98.73	0.0725	0.9179

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

Table 3: Evaluation of accuracy and precision of the proposed method in pharmaceutical dosage forms.

Method	Pharmaceutical formulation	Taken mg/ml	Intra day				Inter day			
			*Found	Recovery %	±SD	% RSD	*Found	Recovery %	±SD	% RSD
A	Bromex	5	4.85	97.1	0.0393	0.8109	4.87	97.53	0.0432	0.8859
	Bromhexine	8	7.89	98.68	0.0484	0.6140	7.90	98.83	0.0686	0.8676
	Bisolvon	10	9.90	99.03	0.0320	0.3235	9.85	98.51	0.0893	0.9065
B	Bromex	5	4.86	97.26	0.0216	0.4441	4.88	97.73	0.0427	0.8746
	Bromhexine	8	7.89	98.64	0.0534	0.6772	7.92	99.08	0.0480	0.6059
	Bisolvon	10	9.86	98.68	0.0530	0.5378	9.74	97.41	0.0801	0.8222
C	Bromex	5	4.86	97.26	0.0432	0.8883	4.85	97.06	0.0427	0.8806
	Bromhexine	8	7.90	98.81	0.0592	0.7494	7.92	99.04	0.0659	0.8320
	Bisolvon	10	9.85	98.53	0.0861	0.8746	9.82	98.26	0.0765	0.7794

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

Table 4: Determination of bromhexine hydrochloride in the presence of excipients.

Excipients	Amount taken g/ml	*Found mg/ml	Recovery %	±SD	RSD %
Glucose	2	1.96	98.41	0.0147	0.7478
Sucrose	3	2.95	98.44	0.0250	0.8476
Lactose	4	3.93	98.33	0.0121	0.3078
Dextrose	2	1.97	98.5	0.0178	0.9080
Talc	3	2.96	98.77	0.0225	0.7595
Starch	25	24.94	99.74	0.023	0.094

*Average of six determinations, RSD: Relative standard deviation, SD: Standard deviation

3.2.4 Specificity and selectivity
To assess the specificity and selectivity of developed method, the effect excipients like starch, lactose, glucose, sugar, talc, etc., were studied. The results indicated (Table 4) that there was no effect of interference from the excipients on the developed methods.

3.2.5 LOD and LOQ
LOD and LOQ were calculated for the proposed methods by using the formula.

$$LOD = 3.3 \ s/S \text{ and } LOQ = 10 \ s/S$$

where s = standard deviation of the response, S = slope of the calibration curve.

4. CONCLUSIONS
In the present work, the developed methods were simple, sensitive, fast, cost-effective and do not require the extraction process and pH controls for estimation of BRH hydrochloride in pure and commercial dosage forms. The linearity, correlation coefficient, recovery results, LOD, LOQ, molar absorptivity, and Sandell's sensitivity values showed that current methods can be applied for the determination of BRH regular quality control analysis.

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Bibliographical Sketch

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