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PURELY CLEAR KINETIC SPECTROPHOTOMETRIC PROCESS FOR BROMHEXINE HYDROCHLORIDE EVALUATION IN PHARMACEUTICAL PREPARATIONS

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Abstract: For the evaluation of Bromhexine hydrochloride (BRO) in pure drugs as well as their pharmaceutical preparations, a successful, sensitivity spectrophotometric method has been proved as well as checked. The methodology is focused on the creation of the Schiff base by p-dimethylaminobenzaldehyde (PDMB), giving a bright yellow color to the reaction of the drug with the reagent . The yellow colored absorbance of species was assessed at its maximum absorption λ_{max} of 420 nm. The Beer's law was obeyed in the 10-60 µg/ml as the concentration range. The optical values were measured to be 8.693 $x10^3$ (L/mol/cm), 0.0005 (µg/cm2), Molar absorption coefficient as well as Sandell sensitivity respectively. The LOD and the LOQ for the suggested methodology were measured 3.280µg/ml, 4.114 µg/ml, respectively. To maximize the reaction conditions, all the variables were studied. The interference for the suggested method was identified in the existence of traditional excipients of pharmaceutics. By testing BRO in its pharmaceutical formulations, the validity of the approach was checked and objectively tested by statistical tests for its accuracy. Strong recoveries were obtained by the method developed; the results obtained in its pharmaceutical dosage forms were critically evaluated and used effectively for determining BRO.

***Keywords*:** Purely Clear, Kinetic Spectrophotometric, Bromhexine Hydrochloride Evaluation, Pharmaceutical Preparations.

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Introduction

Bromhexine hydrochloride (BRO) is a mucolytic agent seen in the prevention of viscous or prolonged mucus-associated pulmonary disorders. The mechanism of this agent is to stimulate the development of obviously serious mucus in the respiratory tract, allowing the phlegm thinner but less viscous. BRO is a medicine for mucous alteration that helps improve the flow characteristics of bronchial mucous membranes as well as promotes expectoration.[1,2]

It is chemically referred in IP and BP as 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl benzyl amine hydrochloride or 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl benzene methane amine hydrochloride Figure 1.[3,4]

The medicine has been analyzed by taking advantage from its chemical properties[5,6] as well as physical properties because of its physiological relevance. Inductively Coupled Plasma Mass Spectrometry,[7] Ion Selective Electrodes with Flow Injection Analysis[8] involves the various analytical processes utilized to measure the medicine as an unique active pharmaceutical ingredient, Glassy Carbon Electrode Electrochemical Oxidation,[9] Electro Kinetic Chromatography,[10] Gas Chromatography[11] with MS detection, Liquid Chromatography[12] as well as Voltammetry.[13] The medicine was also analyzed using Gas Chromatography and HPLC[14-17] direct and derivative UV Spectrophotometry in its combined formulations.[18-22] Such techniques require complex, scarcely available machinery and repeated experimentation. Easy, accurate spectrophotometry procedures have also been based purely on the development of chromosphere by the combination of the medication with an analytical reagent, including chromogenic reagent.[23-25] For pharmaceutical research, the availability of a large number of analytical reagents to operate as chromogens also leaves a great deal of

scope. A systematic literature review revealed that the quantification methods of our attention have not yet been established for the quantification of bromhexine HCl in pure and pharmaceutical dosage forms. We report a quantification method developed and validated in the present communication. Efforts have been made in the current research paper to establish and validate a clear determination of BRO in bulk and pharmaceutical formulations by spectrophotometry while several highly advanced instrumental methods have been written. Time of analysis, cost per analysis, complexity and most of the time, complexity as well as most obviously, the study is the qualified analyst to manage the resources.

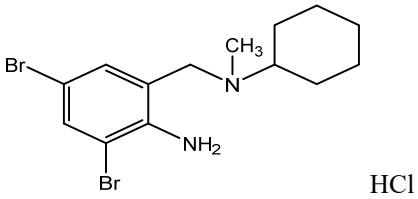


Figure 1. Chemical structure of Bromhexine hydrochloride.[4]

Instrumentation

303 PD UV-Visible Spectrophotometer, Apel, Japan (Single beam) and T80 UV-Visible Spectrophotometer, PG Instruments Ltd. (Double beam) were utilized for all absorption spectra recording using a matched pair of 10 mm path length Quartz cells. For measuring the reagents, a high precision analytical balance was used. Model vs-1205 wl, Science CO, LTD pH meter, Spinbot, Thephaw, Shaking water bath

Reagents and Materials

The analytical grade of all the chemicals was used. Every one the solutions were freshly fully prepared as well as deionized water was utilized for the entire test . Pure medication was derived from (SDI) bromhexine

hydrochloride. Samara-Iraq (State Drug As well as Clinical Appliances Organization). Certified to contain 99.5% purity as the active ingredient was used as the reference material, as obtained without further cleansing. p-dimethylaminobenzaldehyde was procured from (BDH) Chemicals Ltd, reagent Laboratory) company with 99.5% purity and HCl was acquired from (BDH) Chemicals Ltd 35% purity.

Preparation of Standard Stock Solutions

Weighed accurately in a one hundred ml volumetric flask near 0.05 g (50 mg) of the reference standard; swirled for mixing and put with deionized water on the label. An apparent concentration of 500 µg/ml was achieved. Further step-by-step dilutions were made to obtain working standard stock solution 50 µg/ml.

An alcoholic solution 0.04 M was prepared by dissociating 0.149 g of p-dimethylaminobenzaldehyde (PDMB) reagent in 25 ml of absolute ethanol as solvent in volumetric flasks by (BDH Chemicals Ltd, percent 99.9).

1M hydrochloric acid was prepared by diluting with deionized water a sufficient volume of concentrated hydrochloric acid to 100 ml.

Procedure for the Pharmaceutical Preparations assay of Bromhexine BRO

A variety of preparations containing, and including the following, Bromhexine BRO was taken as active ingredient.

1 - Solvodin tablets (8 mg Bromhexine HCl): - They were supported with the state, Samara-Iraq Company for Pharmacy Industries and Medical Equipment (SDI).

2 - Solvodin Syrup (4 mg Bromhexine HCl/ 5 ml BP): - they were founded from the state company for Pharmacy industries and medical appliances Samara –Iraq (SDI).

3 - Solvexin Syrup (4 mg Bromhexine HCl/ 5 ml): - they were provided from The United Pharmaceutical Mfg. (Amman- Jordan) Co. Ltd.

4 - Mucolyte Syrup (4 mg Bromhexine HCl/ 5 ml): - they were subsidized from Julphar Gulf Pharmaceutical Industries, Ras Al Kamiah, U.A.E.

Forms of pharmaceutical formulations Technique

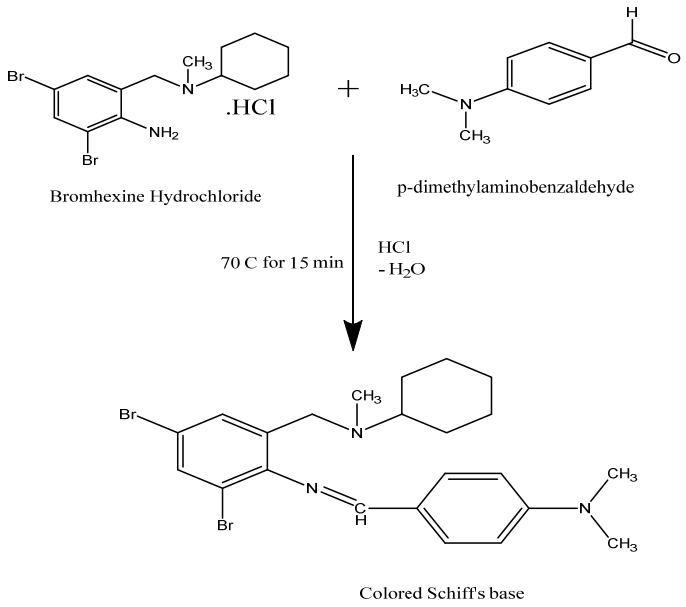
Tablet tests*:* 20 Tablets finely powdered and weighted from tablet form (every one tablet comprising of Bromhexine BRO 8 mg). The quantity from the powder equivalent to 0.05 g of every one BRO was specifically weighted as well as conveyed into a 25 ml beaker. The actual amount was dissolved with deionized water, the filtration of solution was generated utilizing Whatmann (41) filter paper. After extracting the components with a sutiable solvent, they vanish. It was transported in the one hundred ml volumetric flask by distilled water and palliated to the most recent capacity.. A liquid drug solution was evaluated as typical approach was investigated.[26]

Syrup tests*:* The content of two bottles of BRO syrup (Slvodine syrup) [for every 5ml of syrup includes 4 mg BRO] was adequately obtained and blended, after which (62.5 ml) was drawn from the syrup solution comprising 0.05 gm of BRO regular working solutions were established by adequate palliation from this solution, additionally the prescribed methodology was used of Bromhexine investigation.[4]

Quantitative strategy. The comparable approaches utilized HPLC that are defined in the British Pharmacopoeia[4] and the United States Pharmacopoeia.[15]

The Basic Procedure

By a volumetric flask sequence of 10 ml, variable aliquots of 0.2 ml - 1.2 ml BRO were transported. 1 ml of concentrated HCl was applied to each flask, the solution mixed mechanically followed by 1.5 ml alcoholic solution (PDMB) was used and the components have been warmed for 15 minutes at 70 ^{o}C in the water bath, then the yellow Schiff base was formed. At 420 nm averse to the blank agent solution, the yellow colored species absorbance was measured. The colored agents were stable for up to 3 hours. In Scheme 1, the development of the Schiff base was seen. The calibration graph was designed as well as the unknown concentration was taken from the calibration graph or computerized from the equation of regression generated from Beer's law equation by illustrating absorbance versus drug concentration. The colors have been discovered to be stable color for more than 3 days. In order to evaluate BRO in their pharmaceutical formulations using regression, the same protocol was followed.



Scheme 1. Formation of Schiff's base.[15]

Results and Discussion

Determination of Absorption Maxima (λmax)

1 ml of 500 μg.ml^{-1} of BRO was applied to a volumetric flask 10 ml as well as 1 ml of 1M solution of HCl to assess the λ_{max} of the colored species, mechanically combined the components and after that applied 1.5 ml of p-dimethylaminobenzaldehyde (PDMB) solution, heated the contents of the water bath at 70 °C for 15 min, then Schiff 's yellow base was created. The flasks can be cooled down to the room temperature as well as the solutions made with water highly level.

In the wide range of 200 nm to 800 nm against the reagent blank the colored specimens were calculated. The λ_{max} of the complex was estimated to be 420 nm. The spectrum of the planned system's absorption is seen in Figure 2. Each reagent blank showed negligible absorbance at the corresponding λ_{max} under lab conditions.

The optimal reagent concentrations required for the production of sensitive and quantitative colored products have been determined by varying the concentrations of one reagent and by fixing the concentrations of other reagents and by measuring their absorption effect at 420 nm.

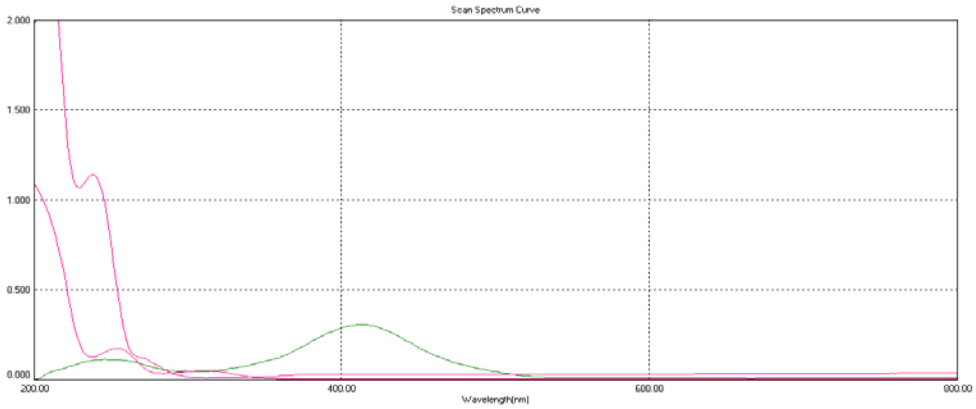


Figure 2. Absorption spectra of color product.

Heating Time Influence

The components of the mix have been heated for more than 15 minutes at 70±1 °C to research the effect of heating time on the production of maximum color. The strength of the color was produced with deionized water following dilution to 10 ml. It is evident from the estimation that after 15 minutes of heating, the maximum intensity of color was achieved and steadily increased. Therefore, the optimum heating time was set at 15 minutes.

Reagent Concentration Influence

The effect on the related absorbance at the concentration values of PDMB solution and HCl has been studied. Different PDMB solution concentrations were checked 0.5-3.0 ml volumes of PDMB 0.04 M. Research has shown that maximum absorbance was provided by 1.5 ml of PDMB. (0.0024 M) With growing quantities of PDMB, there is no further improvement in the strength of the color. So the amount of 1.5 ml of PDMB was selected during the next experiments.

Effect of acid

The analysis indicated that the proximity of acid increased in the high absorption of the resulting product, so that a few acids, such as HCl, CH_3COOH, H_2SO_4 and HNO_3, were inspected at 1 M as the concentration they were given that each of the acids studied obtained the absorption of the color product, HCl was the best acid to obtain the main absorption that was chosen in the experiments that followed. Used for the following tests, so that in subsequent observations it was suggested at 1 ml.[19]

Influence of addition order

Different orders were carried out to add the reagents. From the results indicated in Table1, that the order (No.1), drug BRO, HCl acid additionally PDMB reagent solution, gave the largest strength as well as was chosen in the preceding experimentation. (A = Acid , D = Drug , R = Reagent).[22]

Table 1. Effect of order for addition.

No.	Addition order	Abs.
1	D+A+R	0.758
2	D+R+A	0.493
3	R+D+A	0.536
4	A+R+D	0.202

Quantitative analysis

The Kinetic study and Calibration curve

The constant time kinetic spectrophotometric drug accumulation methodology in this procedure, absorption was studied for reaction solutions with different quantities of BRO drug at a pre-choose specified time at 5 min increments. t_1 (5 min) and t_2 (10, 15, 20, 25, 30, 35, 40 and 45 min) were recorded for absorbance. The associated linear regression equations with r^2 values are tabulated in Table 2. and illustrated against the concentration of drug. Clearly, the slope extends by the time the satisfactory r^2 value was obtained and the intercept was obtained for the BRO for an altered duration of 15 minutes, which was then designated for the estimation as the most accepted time interval and showed broader quantification concentration ranges. For this reason, less time is needed on the basis of the expanded concentration range and study.[28-29]

Table 2. Fixed time processes at varying concentrations and time intervals were used to consider drug regression equations.

Reaction time (min)	Linear range(mg/l)	The equation of Regression	r^2
5	10 – 50	y= 0.0127 x + 0.4210	0.9910
10	10 - 50	y= 0.0111 x + 0.5120	0.9923
15	10 - 60	y= 0.0211 x + 0.0003	0.9987
20	10 - 60	y= 0.0279 x + 0.0712	0.9956
25	10 - 50	y= 0.0388 x + 0.0444	0.9944
30	10 - 50	y= 0.0191x + 0.1223	0.9938
35	10 - 50	y= 0.0153x + 0.0661	0.9880
40	10 - 40	y= 0.0045 x + 0.0132	0.9842
45	10 - 40	y= 0.0012 x + 0.0412	0.9811

Using the circumstances mentioned in the process, for (BRO) a linear calibration curve is provided Figure 3. which appears that law of Beer is followed in the range of concentration between 10-60 ppm with a good value of linearity cofactor r^2 by the expected approach, others spectral including Statistical Information for the Investigation of (BRO). For the proposed process, the optical features such as law of Beer limitations, Sandell sensitivity as well as molar absorptivity were measured and the findings are described as summary in Table 2. As can seen in Figure 3, regression analysis of the law of Beer plot at its λ_{max} showed a strong correlation. We selected different concentration sets for the regression study, but the 10 to 60 μg/ml standard BRO concentration range, the right fit curve was built. We took a series of volumetric flasks 10 ml for the verification of Beer's law and applied the working standard solution 500 μg / ml) from 0.2 ml to 1.2 ml in sequence, prepared by adding of all reagents as described in the assay system. The regression equation y = bx + a

where y is the absorbance, b is the slope, x is the drug concentration in µg/ml and a is the intercept is defined by the plotted graph of absorbance versus concentration, obtained by the least square method. Table 3 summarizes the findings.

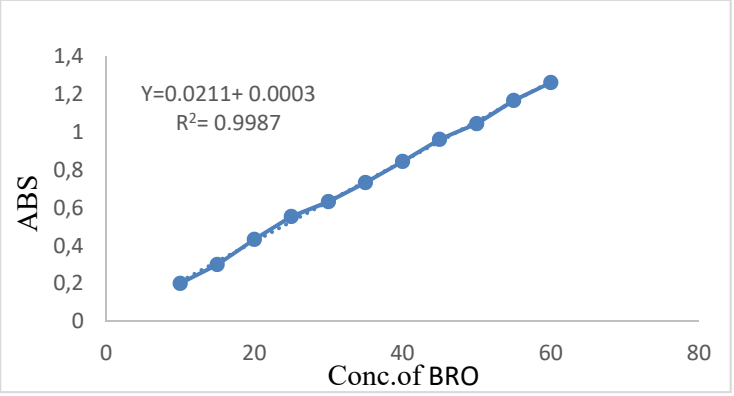


Figure 3. Calibration graph of formation for color product

Table 3. Optical as well as regression characteristics, reliability and validity of the suggested technique.

No.	Parameters	Value
1	λ_{max} (nm)	420
2	Beer's law limitation (µg/ml)	10 - 60
3	Sensitivity of Sandell (µg/cm^2/0.001 Abs. unit)	0.0005
4	Molar absorptivity (L mole^{-1}.cm^{-1})	8.693 x 10^3
5	Stability of Color (hours)	3
6	Regression equation	y = 0.0211 x + 0.0003
7	Correlation coefficient	0.9986
8	% RSD	0.822*
9	Recovery %	99.670*
10	Limit of detection (µg/ml)	3.280
11	Limit of quantification (µg/ml)	4.114

*** mean of three determinations**

Solvent effect

The sort of solvent that decomposes the medicine substances used influences both the overall absorption wavelength as well as strength. Table 4 shows the solvent effect, with water being the best solvent, which gives a very high maximum absorption strength when using PDMB as an aromatic aldehyde with drug. From an economic and economic point of view, water tends to be a strong solvent, with highly sensitivity.[28]

Table 4. Solvent influence on the absorbance.

Solvent	Absorbance	λmax (nm)	ε , $L \cdot mol^{-1} \cdot cm^{-1}$
Dimethyl sulphoxide	0.231	320	2.021×10^{2}
Methanol	0.651	360	1.321×10^{3}
Chloroform	0.512	390	4.988×10^{1}
Dichloromethane	0.451	320	3.911×10^{2}
Benzene	Turbid	Turbid	Turbid
2-propanol	0.250	330	2.451×10^{3}
Formic acid	0.390	340	2.781×10^{2}
Acetone	0.451	360	2.213×10^{2}
Acetonitrile	0.521	370	1.991×10^{1}
Dimethyl formamide	0.550	390	3.990×10^{1}
Tetra-butyl alcohol	0.230	290	1.001×10^{3}
Ethanol	0.521	370	4.721×10^{3}
Diethyl ether	0.421	390	1.541×10^{1}
Water	0.771	420	$7.425\ x10^{3}$

Studies of Interference

Recovery studies have been performed to study the possible interference of widely utilized excipients as well as another additives like lactose, Glucose, PVP, Mannitol, Sucrose, Acacia, Benzoic acid, Starch, Glycolate Sodium, Ascorbic acid ,Cellulose and Magnesium stearate. To a known quantity of drug under the experimental conditions used the

recovery calculations show that there was no major effect interference with the drug's estimation from the excipients.

Recovery Accuracy and Precision calculations

The precision and accuracy of the recommended methodology was assessed by conducting five pure-form replicate BRO determinations at three different concentrations (30,35 and 40 µg/ml) with small-term (intra-day) accuracy, as indicated in Table 5. In the intra-day study for the proposed model, relative standard deviations (percent RSD) , the normal analytical errors, and recoveries acquired were found to be appropriate. The suggested technique is therefore successful in assessing the BRO by carrying out recovery observations via standard addition technique, the accuracy of the studied methodology was more tested. A known quantity of pure BRO has been applied to the analyzed dose forms for this reason and then calculated by the prescribed procedure *(For five determinations) as well as shown in Table 5. measurement of BRO in pharmaceutical formulation with standard addition technique.

Table 5. Determination of BRO in pharmaceutical formulation by standard addition technique.

Quantity of Drug before Addition (µg)	Quantity of medicine Added (µg)	Theoretical Amount (µg)	Mean Amount Recovered (µg) (n=5)	Mean % of Recovery (n=5)	RSD%
20	10	30	29.33	99.970	0.828
20	15	35	34.29	99.979	0.956
20	20	40	39.87	99.996	0.441

Procedure application

The suggested approach was applied to evaluate four different dosage forms containing BRO-HCl in order to assess the analytical utility of the spectrophotometric method. Based on three determinations, best outcomes have been achieved with good recovery including reproducibility for three separate concentrations of each pharmaceutical preparation Table.6. The suggested techniques have been successfully applied to the analysis. Finally, statistical analysis [29,30], F and t tests indicate that there is no major various in accuracy between the studied framework as well as the official model of the BP

Table 6. Determination of BRO in pharmaceutical formulation by studied approach and standard approach.

(BRO) pharmaceutical preparations	Proposed procedure		Standard procedure		Nominal Values (t),(F)
	RSD%	Recovery%	RSD%	Recovery%	
Pure (BRO)	0.822	99.670	0.811	100.230	
Solvodin tablets(8) mg (BRO) S.D.I Iraq	0.912	100.210	1.201	99.550	2.191 (F)Value
Solvodin Syrup (4mg/5ml) (BRO) S.D.I Iraq	0.542	100.310	1.321	98.920	1.027 (t)Value
Solvodin Syrup (4mg/5ml) (BRO) The United Pharmaceutical Mfg Jordan	0.771	99.540	1.021	99.550	
Solvodin Syrup (4mg/5ml) (BRO) Julphar Gulf Pharmaceutical U.A.E.	0.662	99.970	0.734	99.640	

*The sum of five determinations was the recovery number

**The tabulated t-value and the tabulated F- value from the tables are 2.31 and 6.39 at 95% level sequentially is the BRO material in the official system

The traditional method was also obtained from (2009) British Pharmacopeia, wherever it was. As shown in the results obtained in Table 6, the measured values (F, t) were lower from the tabulated values at the 95 percent trust mark for five degrees of freedom. This basically implies that the current technique, as well as\the reference standard, is accurate and reliable.

Conclusions

The preferred method is very easy and does not require any pre-treatment of the drug as well as a tedious extraction procedure. The method has a broader spectrum with reasonable precision and accuracy. Accordingly, the information provided in the work indicates that the proposed method was accurate, sequential, selective additionally offers advantages of reagent availability and stability, reduced and extremely responsive time usage. In the pharmaceutical industry, pharmacies as well as research laboratories, it can even be extended to routine BRO analysis. On the other hand, the UV-visible spectrophotometer process, unlike the LC/MS process and HPLC processes, is simple and not very expensive in simplicity and user-friendly compared to the previously described methods, the device could be considered superior. Furthermore the approach is free of interference by conventional additives and excipients.

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