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Batch and Reverse-Flow Injection Spectrophotometric Determination of Hyoscine Butylbromide in Pharmaceutical Formulations

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ABSTRACT

Hyoscine butyl bromide (HBB), also known as scopolamine butyl bromide, is an anticholinergic medication used to treat bladder spasms, renal colic, esophageal spasms, and crampy abdominal pain. A fast and simple spectrophotometric approach is developed for the determination of hyoscine butyl bromide in pure and pharmaceutical formulations using batch and flow injection methods. The proposed approach is based on the reduction of ferrate ion to ferrous ion by HBB and the subsequent formation of excess Fe(III)-thiocyanate complex which gave maximum absorbance at 478 nm. The experimental conditions of both methods for the assay were studied and optimized. The absorbance was found to decrease linearly with the drug concentration to give a calibration curve which obeys Beer's law in the range of 0.5-200, and 5.0-200 µg/mL with a linear regression coefficient of 0.9964, and 0.9909 with detection limits of 0.14, and 2.10 µg/mL for both batch and rFIA methods, respectively. The proposed approaches were successively applied for the determination of the studied drugs in their different pharmaceutical dosage forms and gave an excellent per cent of recovery compared with the official international pharmacopoeia method.

1. Introduction

Scopolamine N-butyl bromide (hyoscine N-butylbromide, HBB) is an antispasmodic agent. It is used to treat the painful spasm of the intestines and stomach (including spasm associated with irritable bowel syndrome), urinary system and reproductive organs. It is sold under the trade name Buscopan [1]. Natural alkaloid hyoscine is found in Solanaceae species [2]. Hyoscine N butyl bromide (as shown in Fig 1) is not naturally occurring but is instead created when hyoscine reacts with butyl bromide in a hot, refluxing environment [3]. However, after burning cigarettes that had been reinforced with the drug's N-butyl derivative, the parent substance hyoscine was formed. Liquid chromatographic-mass spectrometry study of the smoke and ashes in prisoner who had hallucinations after Buscopan smoked cigarettes supported these results [2]. Hyoscine butyl bromide is a well-known scopolamine derivative. It is a widely used antispasmodic medication with the brand name Buscopan. The molecular formula of hyoscine butylbromide is $C_{21}H_{30}BrNO_4$ within a molecular mass of 440.40 g/mol. It has a pale tint or appears as small, practically crystalline particles, is extensively soluble in dichloromethane and water and is only sporadically soluble in pure alcohol [3]. HBB's weak central action is caused by its inability to penetrate the blood-brain barrier and inadequate absorption from the gastrointestinal tract (GIT). Hyoscine butyl bromide has a variety of therapeutic applications besides antispasmodic action [4]. The unusually low volatility, high polarity, and alkalinity of hyoscine butyl bromide make it difficult to create simple analytical procedures for it [5].Because hyoscine butyl bromide determination in biological fluids and pharmaceutical formulations is so crucial, it has been determined in pharmaceutical formulations using several methods including electrochemical methods [6, 7], titrimetric methods [8], gas-liquid chromatography [9], capillary electrophoresis (CE) [10, 11], high-performance liquid chromatography (HPLC) [12-15], and spectrophotometric method [16-23].

Flow injection analysis (FIA) is based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode

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potential, or other physical parameter as it continuously changes due to the passage of the sample material through the flow cell [24]. In reverse flow-injection analysis (rFIA), the reagent is injected into a continuous flowing stream of the sample. The progress in rFIA applications can be traced from the number of different publications [25].

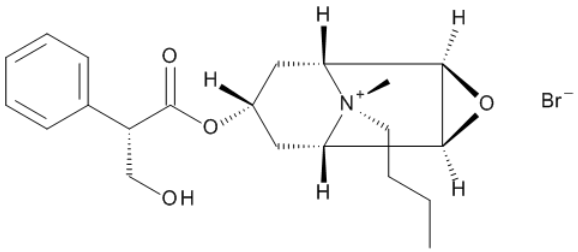


Fig 1. Hyoscine butyl bromide (HBB) chemical structure.

Most of the published spectrophotometric techniques for the determination of HBB required multi reagents, tedious, and multi-steps reactions were time-consuming [22]. Despite the high sensitivity of spectrophotometric techniques based on the production of ion-pair complexes [17, 20, 26-29]; they continue to have problems due to the laborious process, time-consuming extraction stage, and usage of dangerous chemical solvents. The described charge transfer formation reaction-based techniques were less sensitive and used a lot of organic solvents [30, 31].
Ragaa et al. [28] reported an extractive spectrophotometric analysis of HBB in the presence of sertraline hydrogen chloride and losartan-K salt in both their pharmaceuticals and powders via ion-pair complexation between thiocyanate rhodanide anion and both Cobalt(II) or Molybdenum(V). The approaches are based on the cobalt(II)–SCN formation (first approach) and molybdenum (V)–SCN ions (second method), with the medicines to generate undissociated complexes that could be extracted using a mixture of methylene chloride:n-butanol (6.5:3.5 V/V) and at 625 nm the blue-colored complex was measured. However, the orange-red color generated when the complex was extracted with dichloromethane was measurable at 478 nm.
The aim of the current work is to use ferrate(III) – thiocyanate complex reaction as a method to create a simple, fast, and inexpensive spectrophotometric approach for the determination of hyoscine butylbromide in pharmaceutical formulations using batch and reverse flow injection methods.

2. Experimental

2.1. Instrumentations

All measurements of absorbance were performed using an ultraviolet-visible (UV-Vis) spectrophotometer (JENWAY 6405) with a matching 1.0 cm glass cell.
The schematic diagram of the flow injection spectrophotometric system used in this work is shown in Fig 2. It consists of a peristaltic pump (DESAGA Heidelberg, with 6 channels and variable speed up to 10 ml/min) to deliver flow streams. The silicon rubber pump tubes with (1.4 mm i.d) were used to transport the solutions. A rotary valve (Rheodyne U.S.A.) with variable sample volumes was used to inject the reagent into the flowing carrier stream. The valve was made of a polytetra-flouroethylene (PTFE) with good resistance against the corrosion of chemicals. It contain grooves with internal diameter of (0.5 mm). A Y-shaped perspex piece was used to mix two streams of reagents. The flow cell that used for the present work is 1.0 cm and 100 µL volume.

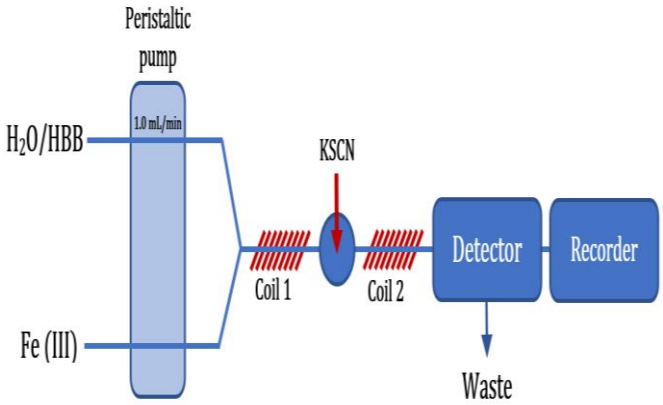


Fig 2. Schematic diagram of the rFIA-spectrophotometric manifold used for the determination of HBB.

2.2. Chemicals

All used chemicals were of analytical reagent grades. Ferric nitrate $Fe(NO_3)_3.9H_2O$ (Riedel-De Haen AG, Hannover), potassium thiocyanate (KSCN) (Merck KGaA, Germany), hydrochloric acid (Sigma-Aldrich, China), hyoscine butyl bromide ($C_{21}H_{30}BrNO_4$) (Boehringer Ingelheim, Germany). Throughout the experiment, deionized water was used for the preparations and dilutions of working solutions.
Solution of hydrochloric acid (1.00 mol/L) was prepared by diluting 8.3 mL of concentrated HCl (37%, sp.gr. 1.19) in 100 mL volumetric flask with deionized water. A 50 mmol/L of ferric nitrate ($Fe(NO_3)_3.6H_2O$) was prepared by dissolving 3.7134 g of the salt in a 5.0 mL, 1.0 mol/L HCl and little amount of deionized water; the solution was completed with deionized water to the mark using 250 mL volumetric flask. Potassium thiocyanate (250 mmol/L) solution was prepared by dissolving 6.074 g of potassium thiocyanate in a little deionized water and the solution was completed to the mark with deionized water in a 250 mL volumetric flask.
A stock solution of 1000 µg/mL of standard HBB was prepared by dissolving 0.10 g of pure HBB in a little amount of deionized water then the solution was completed to the mark with deionized water in a 100 mL volumetric flask. The solution was stored in a dark place and working solutions were prepared by subsequent dilution of this stock solution.

2.3. Real sample solutions

Ten tablets of the commercial sample were weighed, then it was crushed, powdered, and carefully mixed to create sample solutions of various tablets that were bought from local medicine stores. A stock solution was prepared accurately by dissolving the average weight of one tablet in 10 mL of deionized water inside a beaker, sonicated for 10 minutes and then filtered. The residue was washed with 5 mL of deionized water, then the filtrate was diluted to 25 mL with deionized water and kept in a dark volumetric flask.

2.4. Analytical procedure

A 2.0 mL volume of 1.0 M HCl solution and 2.0 mL of 0.01 mol/L Fe(III) were added to a 25 mL volumetric flask. Then, hyoscine butyl bromide which had a final concentration of 40 µg/mL, was transferred in the proper volume to the volumetric flask. The mixture was left to react for 2.0 minutes at room temperature (25 °C). After that, 2.0 mL of 0.01 mol/L thiocyanate solution was added and the volume was completed to 25 mL with deionized water. At the maximum wavelength, the absorbance was measured. A blank experiment was conducted using the identical steps but without adding the HBB solution.

The rFIA system in Fig 2 was operated. The procedure includes the mixing of two main streams. In the first stream, deionized water (blank) and/or HBB (100 µg/mL) solution was moved that merged with the acidic ferrate ion solution (5.0 mmol/L) stream and Fe(III) was reduced to Fe(II) in the delay coil (coil 1). Before reaching the flow cell, a 100 µL of thiocyanate solution (75 mmol/L) is injected into the mixed streams which is then mixed in coil 2. The absorbance at 478 nm was measured. At least three injections were made for every sample solution. The concentration of the analyte was measured using the calibration curves of absorbance versus the concentration of HBB (µg/mL) obtained from reference solutions under the same working conditions.

3. Results and discussion

The proposed spectrophotometric method for the determination of hyoscine butyl bromide in pharmaceutical formulations depend on the oxidizing the drug. First, using an excess amount of ferrate ion, Fe(III) reduced to Fe(II) and the bromide ion of HBB is easily oxidized [32], then determining the residual oxidant (ferrate ion) spectrophotometrically through a complexometric reaction using thiocyanate as a reagent to form a red colored compound that gives maximum absorbance at 478 nm (Fig 3).

In proposed flow injection method, thiocyanate reagent was injected, due to instability in baseline when HBB injected, additionally, injection of thiocyanate decrease its consumption as a reagent.

Fe(III) (excess)/acidic medium + HBB → Fe(III) + Fe(II) + Oxidized HBB

$Fe(III) + 2\ SCN^- \rightleftharpoons [Fe(SCN)_2]^+$ (Red colored complex)

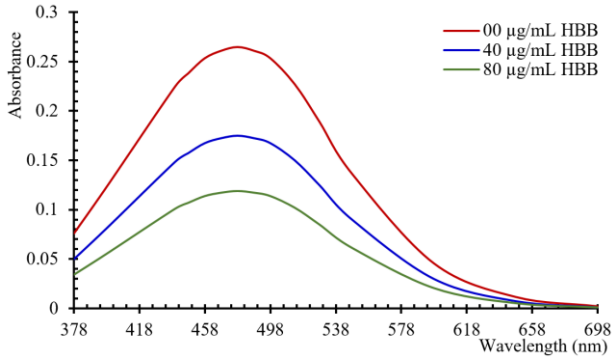


Fig 3. Absorption spectra of produced colored complex between Fe(III) and thiocyanate, and using 40 and 80 µg/mL HBB.

3.1. Reaction conditions optimization

According to the published research [33], a suitable acid for the medium was selected, obviously, the system must be acidic to prevent ferrate ion hydrolysis, therefore the following acids (HNO_3, $HClO_4$, H_2SO_4, CH_3COOH, and HCl) were tested, together with the three thiocyanate salts (NaSCN, KSCN and NH_4SCN). It shows that, although the results are very close to each. Hydrochloric acid as a medium and again KSCN are the most logical mixture to be used in all subsequent research.

The variation of HCl concentration with absorbance was studied. Solutions of 1.0 mol/L HCl were chosen for the initial observation using different volumes ranging from 0.5 to 6.0 mL for the batch method, and HCl solutions ranging from 0.01 to 0.22 mol/L HCl for rFIA were tested. It was observed that the acidic medium resulted in the formation of better color for the complex as shown in Fig 4. At very low acid concentrations the change in color intensity has a very low value because of iron hydrolysis [33]. It was observed that 2.0 mL of 1.0 mol/L HCl (the final concentration of the acid in the total volume of 25 mL is 0.08 mol/L) and 0.1 mol/L for batch and rFIA, respectively gave the best absorbance for the colored complex. Further increase in volume had little effect on the change in absorbance.

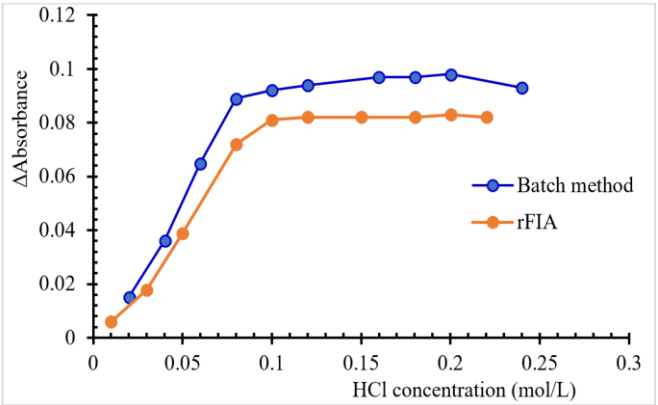


Fig 4. Optimization of the hydrochloric acid concentration.

The effect of ferrate ion concentration on the absorbance of the formed complex was studied, Fe(III) concentration was varied from 0.2 to 4.0 mmol/L using different volumes of 25 mmol/L Fe(III) for batch method, and from 1.0 to 14.0 mmol/L for rFIA method, while holding other parameters constant. It was observed that the peak intensities increased with increasing ferrate ion concentration, but the change in absorbance give a higher value at 1.4 mmol/L Fe(III) (3.5 mL, 0.01 mol/L), and 10 mmol/L for batch and rFIA methods respectively. The further increase gave lower values of absorbance. Therefore, these concentrations were taken as the optimum (Fig 5).

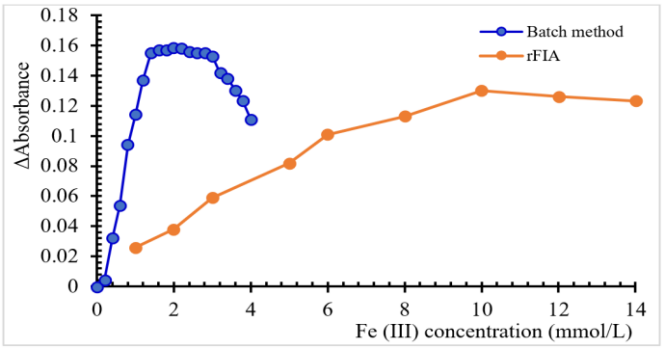


Fig 5. Optimization of the Fe(III) concentration.

Thiocyanate was used as the complexing agent for iron(III) ion. The effect of thiocyanate concentration was studied using different volumes in the range of 0 – 10 mL of 0.01 mol/L for batch method, and from 1.0 to 160 mmol/L for rFIA method, while other parameters were kept constant. Firstly, by increasing the concentration of thiocyanate the peak height (change in absorbance value) is increased from 0 to 1.6 mmol/L, then, the change in intensity decreased by increasing the concentration of thiocyanate as shown in Fig 6; for this reason, 1.6 mmol/L was selected as the optimum concentration for batch method, and 125 mmol/L was chosen as the optimum concentration for rFIA method.

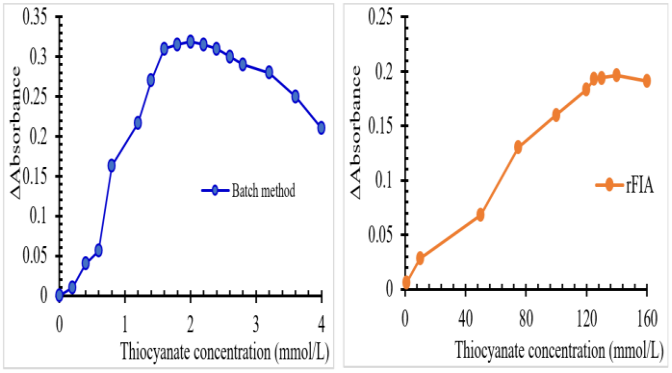


Fig6. Optimization of thiocyanate concentration.

In this work effects of time and temperature were studied. The time was studied at a different range from 0 to 5 min and temperature in the range of 20 to 35 °C for the batch method. The change in absorbance increases gradually with a change in time. While keeping other experimental parameters constant and gradually increasing the temperature of the reaction, the study showed that there was no significant change in the rate of reduction of iron III to iron II and its subsequent complexation after 30 °C as shown in the absorbance studied. Three minutes reaction time at 30 °C gave best change in absorbance for the proposed method, and then selected as optimum values (Fig 7).

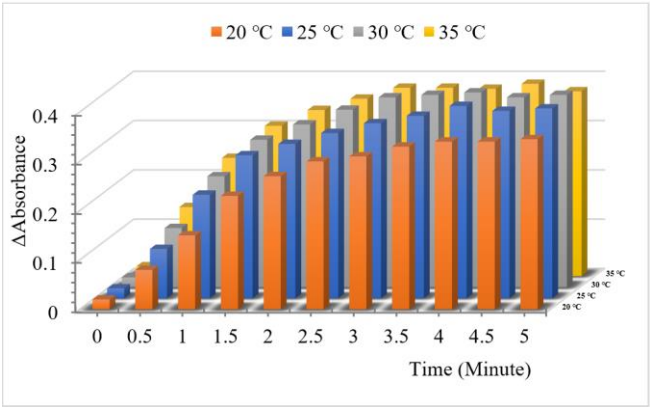


Fig 7. Effect of temperature and time on the proposed method.

The effect of reaction coil length (coil 1) in the range of (5 – 50 cm) for the reaction of HBB with Fe(III) was studied with a constant internal diameter of 0.8 mm. Fig 8 shows a continuous increase in absorbance change intensity as the length of the mixing coil increase from 5 to 25 cm, this indicates that the reaction needs time. Reaction coil 2 (for the reaction of ferrate ion and thiocyanate) gives maximum change in absorbance at 10 cm coil length. The effect of the flow rate on the intensity of the change in absorbance was studied in the range 0.5–4.0 mL/min. The change in absorbance value decreases with increasing flow rate because increasing flow rate leads to decreasing HBB oxidation reaction time, this leads to decrease in absorbance change value. At very low flow rate (0.5 mL/min) unstable results obtained by repeating the analysis. The results in Fig 9 shows best result at 0.8 mL/min, therefore, a flow rate of 0.8 mL/min was chosen for further studies.

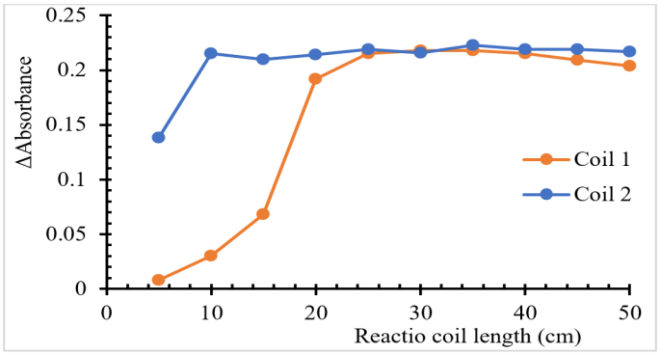


Fig 8. Effect of reaction coil length on the change in absorbance.

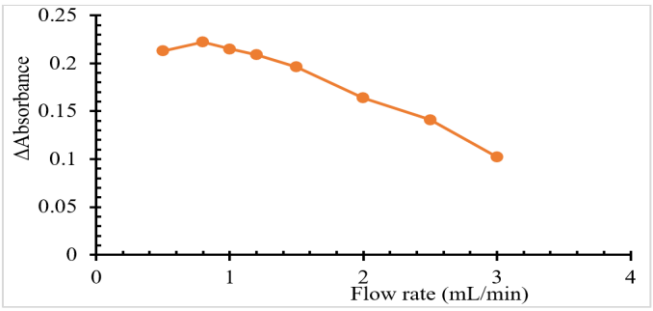


Fig 9. Effect of flow rate on the change in absorbance.

The stability of the reaction between ferric, thiocyanate, and HBB is taken place according to the previous method in optimum conditions and is then analyzed using UV-Visible spectrophotometry at a maximum wavelength of 478 nm and 30 ℃ and normal daylight. Under optimum conditions at the specific concentration of the analyte, the absorbance of the complex formed was measured as a function of time. It was discovered that the formed complex was stable for over 50 minutes (Fig 10). Table 1 illustrates optimum physical and chemical conditions were selected for the determination of HBB.

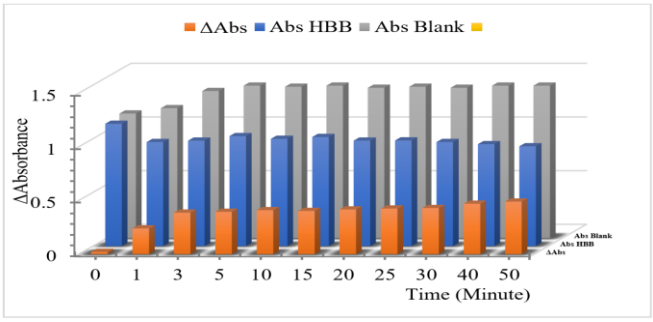


Fig 10. Stability of the formed coloured complex.

Table 1. Optimum physical and chemical conditions were selected for the determination of HBB.

Parameter	Optimum condition	
	Batch method	rFIA
HCl	0.08 mol/L	0.1 mol/L
Fe(III) concentration	1.4 mmol/L	10 mmol/L
KSCN concentration	1.6 mmol/L	125 mmol/L
Temperature	30 ℃	25 ℃
Reaction time	3.0 minutes	-
Stability of the color	More than 50 minutes	-
Reaction coil length	-	25 cm, 10 cm
Flow rate	-	0.8 mL/min
Injected reagent volume	-	100 µL
Wavelength	478 nm	478 nm

3.2. Calibration graph

Fig 11 shows the dependence of the absorbance on the HBB concentration. There is a linear relation between HBB concentration and absorbance. Beer-Lambert's law is obeyed by plotting the absorbance against the drug concentration in the range of 0.50 to 200, and 5.0 to 200 µg/mL for batch and rFIA methods, respectively. The calibration curve was typical of a straight line graph with the equation: Abs = b c + x where Abs= absorbance; b = slope; c = concentration of HBB in µg/mL and x = the intercept. The following calibration curve was obtained under optimized conditions that can be used for the analysis of unknown samples in this range. For samples with HBB concentration greater than 200 µg/mL, it is necessary to dilute them with water to shift them to this concentration range. The coefficient of determination R^2=0.9964, 0.9909 with the detection limit (DL) of 0.14, 2.1 µg/mL, limits of quantification of 0.42, 6.3 µg/mL, and standard deviation of 0.111, 0.028 for batch and rFIA methods, respectively.

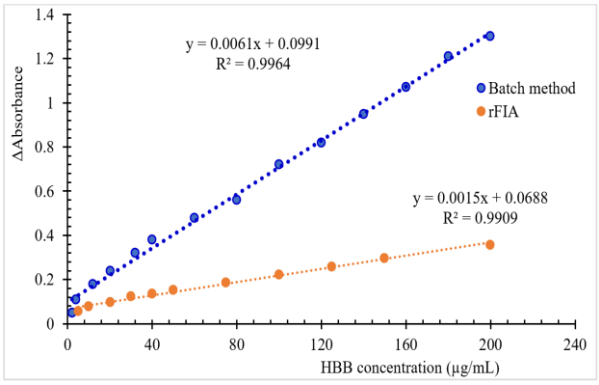


Fig 11. Calibration graph constructed between change in absorbance and HBB concentration.

3.3. Accuracy and precision

Five replicate measurements were done on the three different concentrations of standard HBB solutions to evaluate the proposed method's accuracy and precision. Recovery percent (R%) was used to assess accuracy, while relative standard deviation (RSD) of the same solutions was used to assess technique precision. Table 2 displays the findings, which demonstrate high accuracy and precision.

Table 2. Inter and intra-day analysis for the evaluation of the precision and accuracy of the proposed approach.

Method	**Conc. of HBB (µg/mL)**		**Recovery %**	**SD**	**RSD%**
	Added	**Found**			
Batch	50	49.080	98.160	0.032	0.064
	100	99.170	99.170	0.035	0.036
	150	149.020	99.347	0.136	0.091
rFIA	50	48.562	97.124	0.305	0.628
	100	98.688	98.688	0.233	0.236
	150	148.896	99.264	0.210	0.141

* the average value of five determinations.

3.4. Interferences

To evaluate the method's effectiveness, the impact of additives and excipients that frequently accompany HBB in its dosage forms was studied. The highest concentration of the foreign chemicals, which was assumed to be the tolerance limit, resulted in a recovery in the estimation of the HBB concentration (100 µg/mL) ranged from 98.02 to 101.02%, and 97.72 to 99.87%. According to Table 3, these foreign compounds had no discernible influence with the HBB determination.

3.5. Application

The developed spectrophotometric method was successfully applied to assay five commercially available brands of HBB in tablets (Buscopan, Bescoraze, HyosinAwa, Hyolab and Scopinal) purchased from local drug stores. The results obtained by the developed approach were compared with the standard method (UV method). The results obtained for both methods were in complete agreement with the amount labelled and to the results obtained from the UV method and. The data is shown in Table 4 showing no significant difference between the developed and reference method showing a good congruence between the two.

4. Conclusions

The proposed batch and rFIA methods are low cost, simple and fast. Like HPLC and other procedures, they don't require complicated steps or specialized knowledge. The proposed approaches has high analytical frequency (20 samples/h for batch, and 30 samples/h for rFIA). Additionally, the supplies and tools employed in the current investigation are low-cost and typical of most quality control labs.

HBB reacts with Fe(III) and then colored complex forming of excess Fe(III) with thiocyanate. The suggested spectrophotometric methods enable the determination of HBB in the ranges 0.50 to 200, and 5.0-200 µg/mL for batch and rFIA methods, respectively. Table 5 shows a comparison of the proposed methods with the existing methods. The process requires the fewest number of experiment variables and is devoid of time-consuming processes like heating or extraction, which is reflected in its excellent precision.

Table 3. Effect of interferences on the proposed method.

Interferences	Concentration (µg/mL)	HBB (µg/mL) / Batch method			HBB (µg/mL) / rFIA		
		Add	Found	Recovery%	Add	Found	Recovery%
HBB		100	99.74	99.74	100	99.13	99.13
Calcium hydrogen phosphate	150	100	98.02	98.02	100	97.87	97.87
Crospovidone	200	100	100.07	100.07	100	99.57	99.57
Lactose	200	100	101.02	101.02	100	99.49	99.49
Maize Starch	200	100	98.68	98.68	100	98.31	98.31
Magnesium stearate	200	100	99.02	99.02	100	98.79	98.79
Microcrystalline cellulose	200	100	99.26	99.26	100	99.01	99.01
Silicon dioxide	200	100	98.38	98.38	100	98.94	98.94
Sucrose	200	100	99.44	99.44	100	98.92	98.92
Talc	200	100	100.38	100.38	100	99.87	99.87
Tartaric acid	100	100	98.18	98.18	100	97.72	97.72

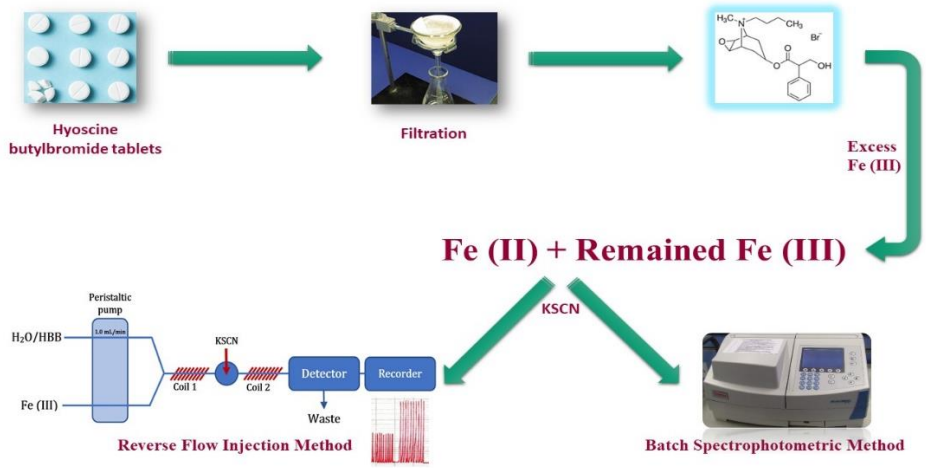
Table 4. Results of analysis of commercial drug tablets containing HBB by the presented method and standard ultraviolet spectrophotometric method.

Commercial name	Name of Drugs	Manufacture and Country	Labeled amount (mg/tablet)	Drugs found (mg / tablet)		Error %	
				Standard method*	Batch method*	rFIA method*	Batch	rFIA
Bescoraze	Scopolamine Butyl Bromide	Awamedica, Erbil, Iraq Under license for Razi Pharmaceutical Industries (Aleppo-Syria)	10.000	9.770	9.740	9.411	-0.307	-3.675
Buscopan	Hyoscine butylbromide	Delpharm Reims, France	10.000	9.750	9.860	9.690	1.128	-0.615
HyosinAwa	Hyoscine Butylbromide	Awamedica Co., P.O. Box 0116-49, Erbil, Iraq	10.000	9.510	9.610	9.422	1.052	-0.925
Hyolab	Hyoscine butylbromide	Khandelwal Laboratories Pvt Ltd. Wagle industrial estate, Thane- India	10.000	9.520	9.350	9.418	-1.786	-1.071
Scopinal	Hyoscine-N-butylbromide	Julphar- Gulf pharmaceutical industries, Ras Al Khaimah, U.A.E.	10.000	9.830	9.570	9.760	-2.645	-0.712

* Average of three replications.

Table 5. Comparison of the proposed method's analytical quality with a number of previously published studies that were used to determine HBB.

Detection system	Limit of detection (µg/ml)	Calibration range (µg/ml)	Samples	Reference
Derivative spectrophotometry	0.260	2.000 – 14.00	Pharmaceuticals	[34]
Potentiometric	1.980	4.400 – 4404	Pharmaceuticals	[35]
Spectrophotometric	0.260	1.000 – 20.00	Pharmaceuticals	[27]
Spectrophotometric	0.090	0.100 – 50.00	Pharmaceuticals	[23]
Spectrophotometric	0.092	1.000 – 10.00	Pharmaceuticals	[22]
Chemiluminescence	0.500	0.005 – 20.00	Pharmaceuticals	[36]
Chemiluminescence	0.001	0.005 – 15.00	Pharmaceuticals	[37]
Spectrophotometric	0.140	0.500 – 200.00	Pharmaceuticals	This work, Batch
Spectrophotometric	2.100	5.000 – 200.00	Pharmaceuticals	This work, rFIA



Scheme 1. Graphical abstract illustrate determination of HBB steps using proposed methods.

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