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CFIA-ISNAG fluorimeter for the Determination of Bromhexine-HCl in Drugs *via* the Measurement Scattered Light at ± 90˚

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Abstract

A precise, simple, and accurate continuous flow injection technique was used for the instantaneous estimation of bromhexine hydrochloride (BH-HCl) in tablet dosage form. The chemical and physical parameters of the reaction of BH-HCl with tetraphenylborate to produce a yellowish-white precipitate were determined using an ISNAG fluorimeter analyzer and diverging light at 90°. The calibration curve for BH-HCl was linear with correlation coefficients of 0.9994 and linearity percentage r^2% = 99.87 over a concentration range of 0.01-20 mmol/L, L.O.D = 0.3610 µg/125µL (0.007 mmol/L), and RSD% less than 0.3% for 3 and 13 mmol/L (five replicates). This approach was efficiently used to estimate the levels of BH-HCl in two distinct pharmaceutical companies. When the newly developed method was compared with the turbidimetric method using a t-test and standard addition, it was discovered that there had not been any significant difference between the two approaches at the 95% level of confidence.

Keywords: Flow injection, Bromhexine hydrochloride, Scattered light, Turbidity, Tetraphenylborate.

الحقن الجرياني المستمر – ازناكَ فلوروميتر لتقدير بروموهكسين هايدروكلوريد في العقاقير عن طريق قياس بعثرة الضوء عند ±90˚

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الخلاصة

استعملت طريقة بسيطة، سريعة ودقيقة لتقدير البروموهكسين هايدروكلوريد باستخدام الحقن الجرياني المستمر في المستحضرات الصيدلانية. تم دراسة كافة المتغيرات الكيميائية والفيزيائية لتفاعل بروموهكسين هايدروكلوريد مع رباعي فنيل بورات الصوديوم لانتاج راسب ابيض مصفر باستخدام جهاز ISNAG فلورميتر و بعثرة الضوء بزاوية 90˚. مدى الخطية لمنحني المعايرة تمتد من 0.01–20 ملي مول لتر$^{-1}$ بمعامل ارتباط 0.9994 بينما النسبة المئوية للخطية 99.87 %. حدود الكشف 0.3610 مايكروغرام / 125 مايكرولتر (0.007 ملي مول لتر$^{-1}$)، مع انحراف قياسي نسبي مئوي اقل من 0.3% لتركيز (3 و 13) ملي مول لتر$^{-1}$ (تكرارية خمس مرات) من بروموهكسين هايدروكلوريد، الطريقة طبقت بنجاح لتقدير البروموهكسين هايدروكلوريد في شركاتان مختلفتان. اجريت المقارنة بين الطريقة المستحدثة للتحليل والطريقة

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التقليدية لقياس التعكرية باستخدام الاضافات القياسية بوساطة اختبار t، لوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعة 95% .

1. Introduction

Bromhexine-HCl, also known as 2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzene methanamine hydrochloride (Figure 1), has the molecular formula $C_{14}H_{20}Br_2N_2$, HCl, and is a white or nearly white, crystalline powder that is insoluble in alcohol, water, and methylene chloride [1]. Bromhexine-HCl is one of the mucolytic agents that is utilized for treating respiratory problems that are caused by viscid or excessive mucus [2,3]. The mechanism of action of this agent is to increase the formation of serous mucus in the respiratory tract, resulting in thinner phlegm with a lower viscosity. Furthermore, BH-HCl is one of the mucous-modifying drugs that aid expectoration by improving the flow characteristics of bronchial mucous [3,4]. A literature review indicated the determination of bromhexine hydrochloride in dosage form, either in combined form or alone, by spectrophotometric [5-11], potentiometric [12], voltammetric [13], colorimetric [14], chemiluminescence [15,16], TLC [17], HPLC [18-25], RRLC-MS/MS [26], and HPTLC-HPLC [27]. This study presents a new method to determine BH-HCl in pharmaceutical formulations using tetraphenylborate as a precipitating reagent to form a yellowish-white precipitate with the use of a homemade instrument. The ISNAG fluorimeter detects diverged scattered light (i.e., visible light) at 2 x 90˚ using a multi-solar cell that covers 2x 100 mm of path length with a low-pressure mercury lamp tube (ultraviolet light) [28].

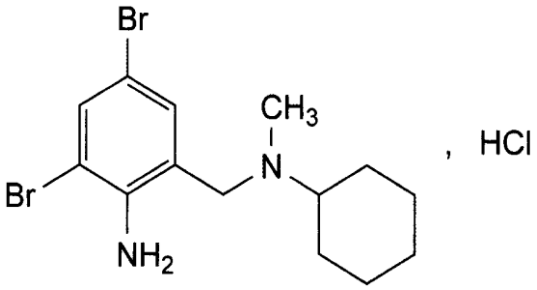


Figure 1: The structure of BH-HCl

2. Materials and methods

2.1. Standard drugs and reagents

A standard solution of BH-HCl (0.1 mol/L) (SDI-Iraq) with the molecular formula $C_{14}H_{20}Br_2N_2$.HCl with a molecular weight of 412.6 g/mol was prepared by dissolving BH-HCl (4.126 g) in distilled water (100 mL). A stock solution of sodium tetraphenylborate (0.05 mol/L) (Fluka), with the molecular formula $C_{24}H_{20}BNa$, and a molecular weight of 342.22 g/mol, was prepared by dissolving sodium tetraphenylborate (8.5555 g) in distilled water (500 mL).

2.2. Sample preparation

Twenty tablets of BH-HCl (each tablet contains 8 mg of solvodine from Iraq and bisolven from Giza) were crushed and ground after being weighted (0.6042 g and 0.6370 g, equal to 0.04126 g of the active ingredient, 1 mmol/L), respectively. Each of the two types of samples was dissolved in distilled water. For removing any insoluble materials, the solution was filtered, the residue was rinsed with distilled water, and the volume was increased to 100 mL using the same solvent.

2.3. Apparatus

ISNAG fluorimeter analyzer: A homemade ISNAG (low-pressure mercury lamp) was used to measure the response, which is characterized by two lambdas (184.9 and 253.7 nm). A solar cell measuring 2[4× 2.5cm] was employed as the detector.

The flow system: Figure 2 shows the flow system to determine bromhexine-HCl. It consists of a peristaltic pump with two channels and variable speed (Ismatec, Switzerland). Valve 6: medium pressure injection valve with sample loop (IDEX Co., US) (1 mm i.d. Teflon, variable length); as a detector, two [4 × 2.5 cm] solar cells were used to collect signals *via* sample travel over a 100 mm line of 2 mm optical aperture.

Potentiometric recorder: Recorded the output signals (Siemens, Germany) (1V- 5V, 1000mV-5000mV).

Turbidimeter: The turbidity readings under batch conditions have been manufactured by the Hanna Company (US).

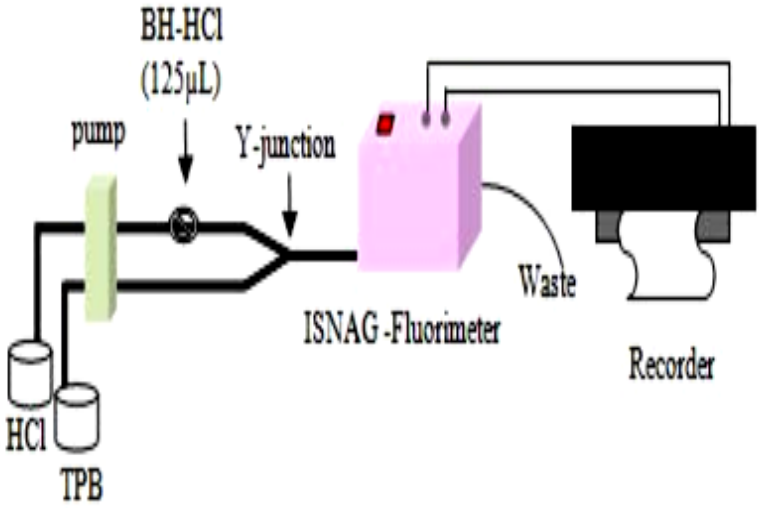
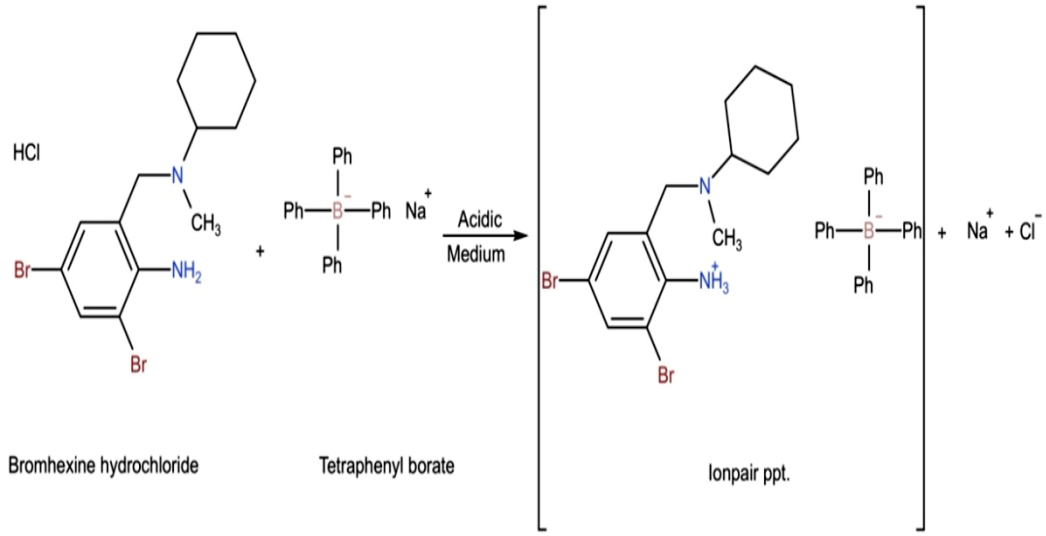


Figure 2: A flow injection diagram system of instrument analysis for determining BH-HCl

2.4. Methodology

A flow injection manifold was employed, along with a two-line homemade ISNAG fluorimeter (Figure 2). The first line represents a carrier stream (HCl, 30 mmol.L^{-1}, 1.5 mL/min.) that introduces the sample segment BH-HCl (10 mmol.L^{-1}, 125 µL) into the reaction stream, which is combined with the second line (TPB, 0.04 mmol.L^{-1}, 1.5 mL/min.) to form a yellowish-white precipitate as an ion pair complex at the Y-junction, with an outlet for the reactant's ion pair product. The precipitate was determined using a low-pressure mercury lamp (ISNAG-fluorimeter), which gives 253.7 and 184.9 nm, respectively. Because of their high frequency, the two lines readily diverge. The divergence of such an incident light beam will be detected at 90^{o} using a 2.0 mm path length flow cell that spans 100 mm and a 2 [4 × 2.5 cm] solar cell. Scheme 1 depicts a proposed mechanism for the BH-HCl-tetraphenylborate reaction [29].



Scheme 1 : A probable proposed mechanism for the BH-HCl reaction with tetraphenylborate

3. Results and discussion

3.1. Chemical variations

3.1.1. The Influence of varying tetraphenyl borate concentration

At a flow rate of 1.3 mL/min (for both the reagent and carrier stream lines), a series of tetraphenyl borate (0.005-0.1 mmol/L) is utilized as a precipitating agent, BH-HCl (10 mmol/L), sample volume (85 µL), and open valve mode (time in seconds to load the manifold with the sample of the analyte). It can be shown that increasing the concentration of the reagent up to 0.04 mmol/L improves sensitivity, which is expressed as the peak response (Figure 3A), resulting in more precipitate particulates being formed, which might increase divergent light towards the solar cell. Above 0.04 mmol L^{-1}, the diverged light intensity decreased, which could be due to irregular agglomerate formation of precipitate particulate that prevented the diverged light from reaching the solar cell at 0-90˚ or a massive blockage that blocked the passage and reflected most of the incident light that was not detected by the solar cell. Table 1 shows all of the obtained results. Figure 3 shows that the obtained concentration of TPB is 0.04 mmol/L. On such a basis, HCl (50 mmol/L) was utilized as a carrier stream.

Table 1: Effects of the concentration of the TPB upon the function of the response, which is represented as the average peak height

[TPB] mM	$\bar{Y}_{iz\ (mV)}$ (n=3)	R.S.D%	C.I $\bar{Y}_{iz\ (mV)} \pm t_{0.025,\ 2}\sigma n\text{-}1/\sqrt{n}$
0.005	338	0.07	338± 0.59
0.02	478	0.1	478±1.19
0.04	745	0	745±0
0.08	623	0.13	623±2.01
0.1	510	0.23	510±9.91

C.I: Confidence interval

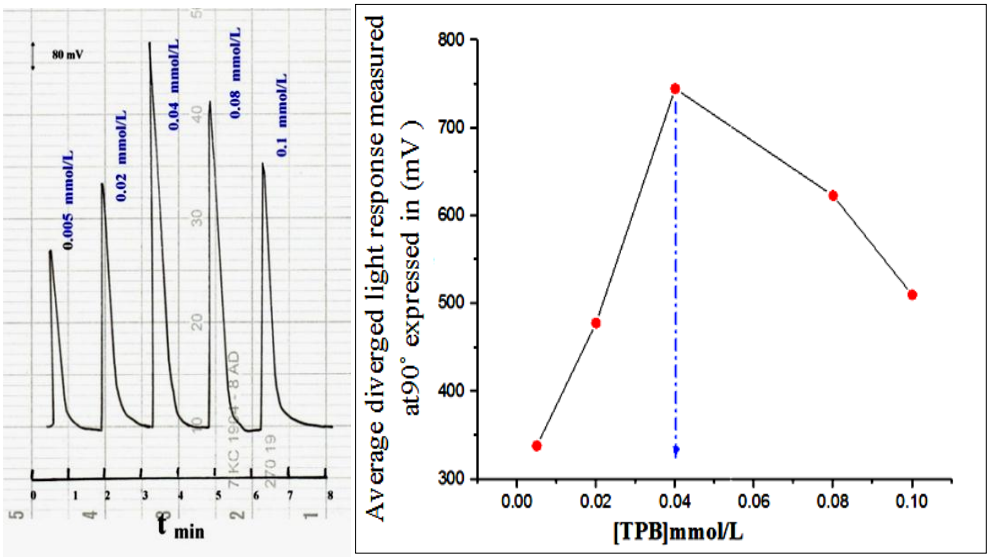


Figure 3: A. Response profile of bromhexine-HCl with various tetraphenyl borate concentrations B. Average peak heights *vs*. concentration of tetraphenylborate (TPB)

3.1.2. Effect of acidic media

Various acid solutions (50 mmol/L) for each of HCl, HNO_3, H_3PO_4, H_2SO_4, and CH_3COOH, along with H_2O, were prepared to determine the suitable medium employed in the precipitation of BH-HCl (10 mmol/L) by TPB (0.04 mmol/L), which was used as the carrier stream in Figure 4. Because of the compactness of precipitated particles, an increase in the response sensitivity of the HCl medium as the carrier stream results in an increase in the divergence of light at 90 degrees. As a result, HCl was chosen as the best medium for this experiment, and all results are displayed in Table 2.

Table 2: Effects of various acids as carrier stream on the response function that is expressed as an average peak height

Type of acidic medium (50mM)	$\bar{Y}_{iz\ (mV)}$ (n=3)	R.S.D%	C.I $\bar{Y}_{iz\ (mV)} \pm t_{0.025,\ 2}\sigma n\text{-}1/\sqrt{n}$
H_2O	237	0	237±0
HNO_3	532	0.11	532±1.45
HCl	624	0.06	624±0.093
H_2SO_4	498	0.17	498±2.10
H_3PO_4	372	0.09	372±0.83
CH_3COOH	282	0.08	282±0.56

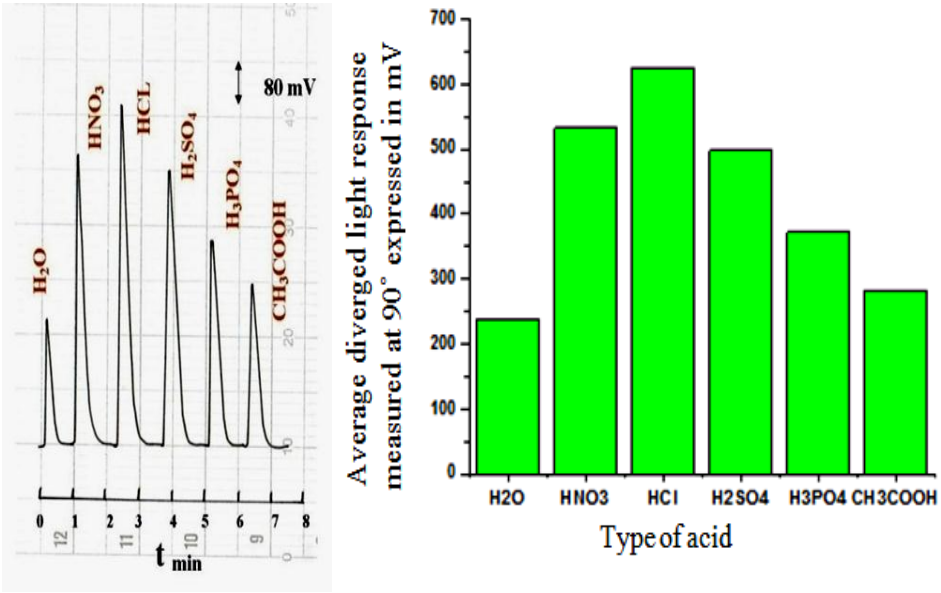


Figure 4: A. Effects of different acids that are utilized as carriers on the response profile

B. Average peak heights *vs*. type of acid

3.1.3. Effects of hydrochloric acid concentration

A series of diluted solutions of HCl (5-50 mmol/L) were prepared with a sample volume of 85 µL, and a 1.3 mL/min flow rate for the carrier stream and reagent (TPB 0.04 mmol/L). Figure 5B depicts an increase in diverged light with increasing HCl concentrations ranging from 5 to 30 mmol/L. It was noticed from Table 3 above 30 mmol/L that there was a decrease in response, which might be attributed to the dissociation of some precipitate particles. Therefore, 30mmol/L has been selected as the optimal concentration as a medium for precipitate formation. Figure 5A shows the response profile for variation in hydrochloric acid.

Table 3: Effect of HCl concentration on the response function that is represented as an average peak height

HCl (mM)	$\bar{Y}_{iz\,(mV)}$ (n=3)	R.S.D%	C.I $\bar{Y}_{iz\,(mV)} \pm t_{0.025,\,2}\sigma n\text{-}1/\sqrt{n}$
5	597	0.19	597±2.82
10	635	0.03	632±0.47
20	722	0	722±0
30	894	0	894±0
40	732	0.15	732±2.73
50	624	0.17	624±2.64

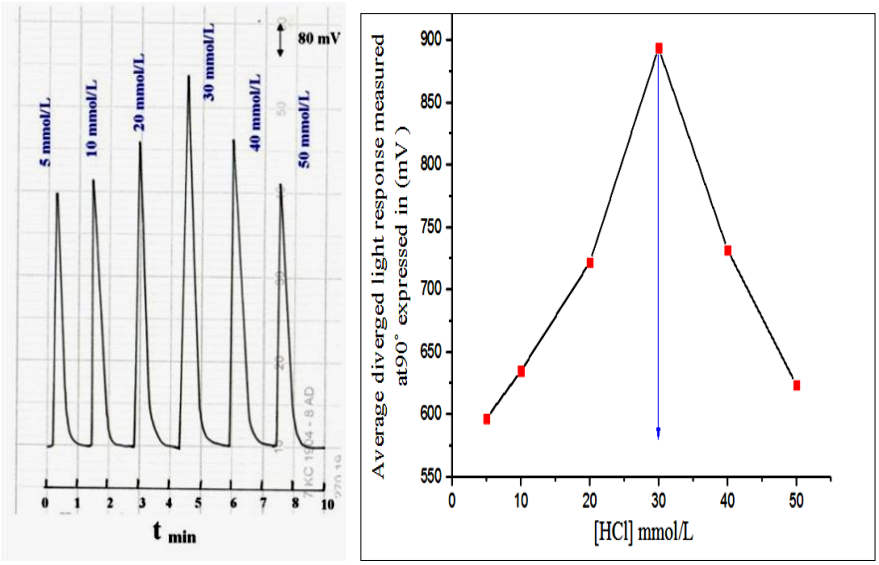


Figure 5: A. Effects of the hydrochloric acid that is utilized as acarrier stream on the response profile B. Average peak heights *vs*. concentration of hydrochloric acid

3.2. Physical variation

3.2.1. Flow rate

The BH-HCl (10 mmol/L)-TPB (0.04 mmol/L) system was used, and the sample volume was 85 µL. Different flow rates (0.7-2.0 mL/min) for the carrier stream and precipitating agent, respectively. Figure 6 shows that at the low flow rates, peak height increased up to 1.5mL/min, respectively, for the carrier stream and reagent, with wider peak widths, possibly because of dispersion and dilution, followed by a constant and subsequently a drop in peak height at flow rates higher than 1.5 mL/min. To get a regular response, narrow (ΔtB), and limit reaction solution consumption, the optimal flow rate for a complete reaction between BH-HCl and TPB was 1.5 mL/min. Table 4 shows all of the obtained results.

Table 4: Effects of flow rate on response function that is represented as an average height of the peak

Flow rate for both line	$\bar{Y}_{iz\ (mV)}$ (n=3)	R.S.D%	C.I $\bar{Y}_{iz\ (mV)} \pm t_{0.025,\ 2}\sigma n\text{-}1/\sqrt{n}$	Δt_B (Sec)
0.7	687	0.16	687±2.73	66
1.0	720	0	720±0	55
1.3	894	0.04	894±0.89	30
1.5	933	0.08	933±1.85	25
1.7	930	0	930±0	23
2.0	897	0.10	897±2.22	20

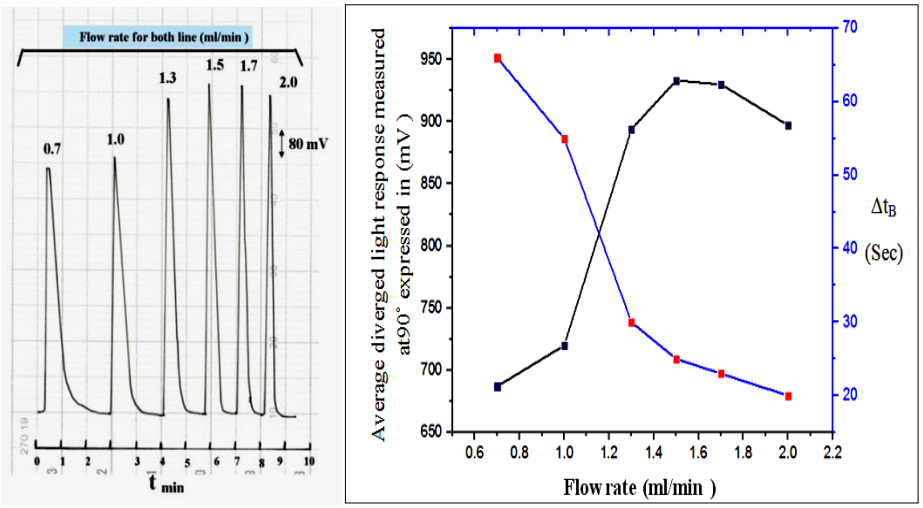


Figure 6: A. Diverged light versus time profile with the use of the variable flow rate
B. Plot average peak heights *vs*. flow rate

3.2.2. Effect of different sample volumes

The BH-HCl (10 mmol/L)-[TPB] (0.04 mmol/L) system was used. Flow rate of 1.5 mL/min for carrier stream (HCl) and reagent in open valve mode. The injection volume was varied from 75-200 µL. Figure 7A and Table 5 show any increases in a sample volume of as high as 125 µL increased in the height of the responses, followed by a modest rise or a nearly constant response. This could be due to the elongation of the period leading up to the detection point because of the larger sample volume, along with the size or weight of the produced precipitate particles, which could result in a slight delay in the weight. As a result, 125 µl was the ideal sample volume.

Table 5: Effects of sample volume upon the response function expressed as an average peak height

Sample volume (µL) (0.5 mm)	$\bar{Y}_{iz\ (mV)}$ (n=3)	R.S.D%	C.I $\bar{Y}_{iz\ (mV)} \pm t_{0.025}, {}_2\sigma n\text{-}1/\sqrt{n}$	Δt_B (Sec)
75	591	0	591±0	20
85	935	0.3	935±6.97	25
125	1080	0.15	1080±4.02	30
150	1085	0.09	1085±2.43	44
200	1078	0.33	1078±8.84	68

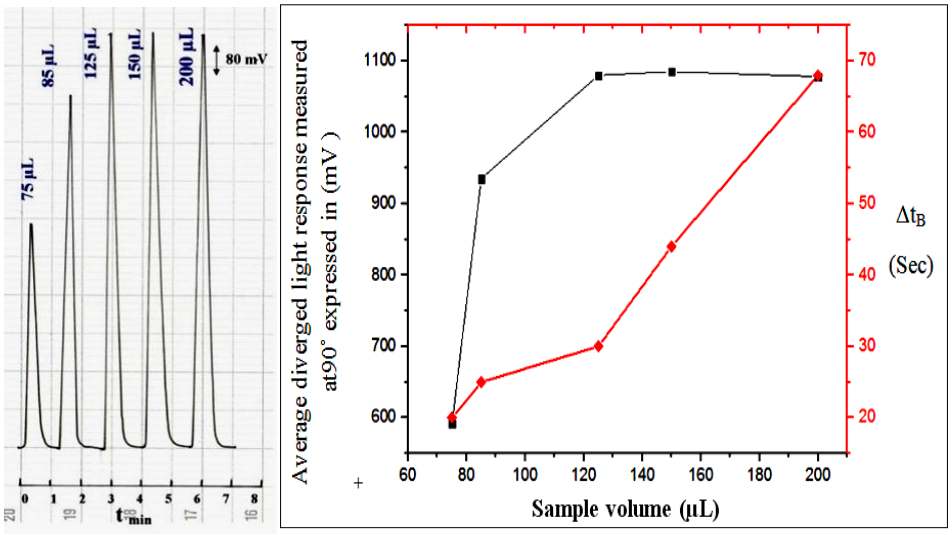


Figure 7: Effects of sample volume upon: A. Response profile B. Output of the diverged beam of incident light using the BH-HCl-TPB system

3.2.3. Calibration graph

A series of BH-HCl concentrations that range from 0.01 to 20 mmol/L were prepared under the stated optimum conditions for preparing a scatter plot diagram, which is followed by the selection of a calibration graph; Figure 8A displays the response profile for the present investigation. The variation of scattered divergent light utilizing an ISNAG fluorimeter with a BH-HCl concentration is shown in Figure 8B. The obtained results for the variation of measured responses with BH-HCl concentrations show a linear range between 0.01 and 20 mmol.L^{-1} with a coefficient of correlation, r = 0.9994, indicating that an increase in the BH-HCl concentration results in increasing the precipitate with a smooth surface, which acts as a reflecting mirror to the detector, which will be measured at 90 degrees according to instrument design. This approach was compared to the traditional method of measuring turbidity using turbidimeter equipment (HANA). Table 6 shows the obtained results.

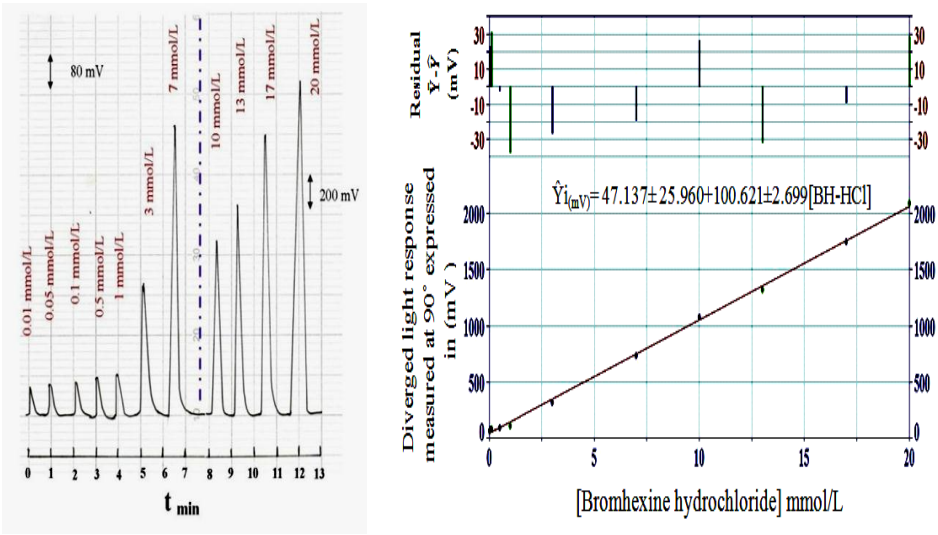


Figure 8 - Calibration graph for variations of the BH-HCl concentration on:
A. Response profile versus time B. Diverged light expressed by linear equation with the use of the ISNAG-fluorimeter. Residual (ȳi-Ŷi),ȳi: practical value, Ŷi: estimate value

Table 6: Summary of results for determining bromhexine hydrochloride using an ISNAG-fluorimeter

Method	[BH-HCl] mmol/L Linear range	r r^2	t-value at 95%, n-2		Linear regression equation at 95%, n-2 $\hat{Y}i_{(mV)} = a \pm s_a t + b \pm s_b t$ [x]
			t_{tab}	t_{cal}	
ISNAG-Fluorimeter	0.01-20	0.9994 0.9987	2.262 << 83.283		**47.137±25.960+100.621±2.699**

3.2.4. Repeatability and detection limit

Repeatability has been researched for determining BH-HCl through measurement of diverged light at 90˚ for the reaction of BH-HCl with TPB acid at the concentrations of 3 and 13 mmol.L^{-1}, as shown in Figure 9. The limit of BH-HCl detection is calculated by two methods. Gradual dilution of low concentrations in the graph of the calibration curve or based upon the slope's numerical value. Table 7 shows the obtained results.

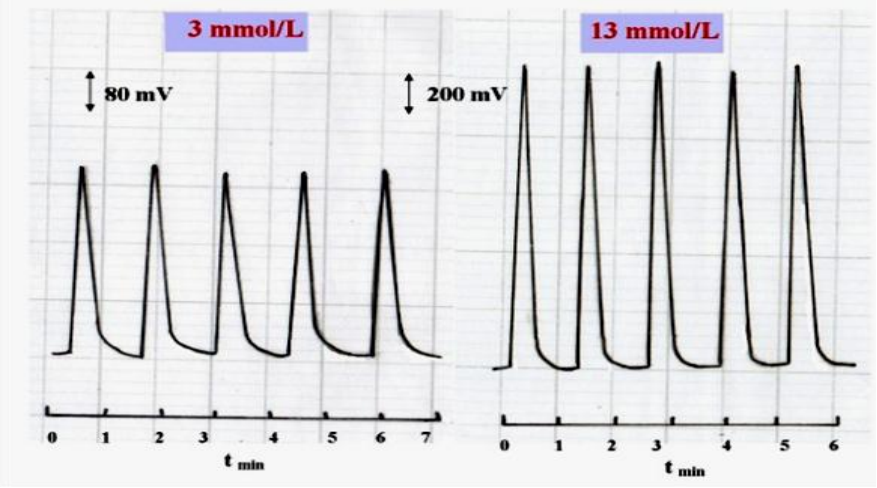


Figure 9: Response profile-time for five successive repeated BH-HCl concentration measurements (3 and 13 mmol/L) with using an ISNAG-fluorimeter.

Table 7: Repeatability and detection limit for BH-HCl at optimum parameters *via* divergence of light measured at 90˚ expressed in mV.

Detection limit		[BH-HCl] mmol.L^{-1}	Repeatability 95% atconfidence interval $\bar{Y}_{i(mV)} \pm t_{0.025, n-1} \sigma n-1/\sqrt{n}$ (n=5)	RSD%
Practically based upon gradual dilution for minimal concentration	Theoretical based upon slope value $X = 3S_B$/slope for n = 13			
0.3610 µg/125µL	0.5384µg/125µL	3	322 ±0.97	**0.24**
		13	1323±1.49	**0.090**

X = LOD value based upon the slope, S_B = standard deviation of blank repeated for 13

3.2.5. Application of a developed method for the determination of BH-HCl in pharmaceutical preparations

Continuous flow injection analysis using a homemade ISNAG fluorimeter has been utilized for the determination of BH-HCl in two different pharmaceutical preparations (solvodine and bisolvine). A set of solutions has been prepared for every one of the pharmaceutical drugs (1 mmol/L) through the transfer of 1 mL to each of the five volumetric flasks (10 mL). This was followed by the addition of gradual standard BH-HCl volumes (0,

0.01, 0.02, 0.03, and 0.04 mL) of 0.1 mol/L for obtaining 0, 0.1, 0.2, 0.3, and 0.4 mmol/L when using the ISNAG fluorimeter and turbidimeter (classical method). The measurements have been carried out using both approaches. Figure 10 exhibits the profile of the response for the present work and standard addition graphs of calibration with the use of the ISNAG-fluorimeter. The results have been solved mathematically with the standard addition approach. The results are listed in Table 8A, at a 95% confidence level, which shows practically concentration of BH-HCl in every one of the pharmaceutical drugs with the use of two different analysis approaches. Table 8B shows the practical content of the active ingredient at a 95% confidence level and the efficiency of determination, in addition to the paired t-test, which compares two different paths [30,31].

First: Individual t-test: Calculating the t-value to compare a newly developed approach (the ISNAG fluorimeter) to the quoted value (8 mg) listed in Table 8B column 6. It has been noted that the calculated t-value is less than the critical tabulated t-value in the case of using the ISNAG fluorimeter (developed method). This is an indication of the fact that there has not been a considerable difference between the quoted values of every one of the individual companies with t_{cal} at 95% interval of confidence.
Second: The paired t-test has been utilized for comparing the developed approach with using the ISNAG fluorimeter CFIA with the classical approach. The result that has been obtained indicates that clearly there has not been any considerable difference between the two methods, since the calculated t-value (-0.693) is less than t_{tab} (4.303) for determining BH-HCl in the pharmaceutical preparations at 95% level of confidence, as can be seen from Table 8B.

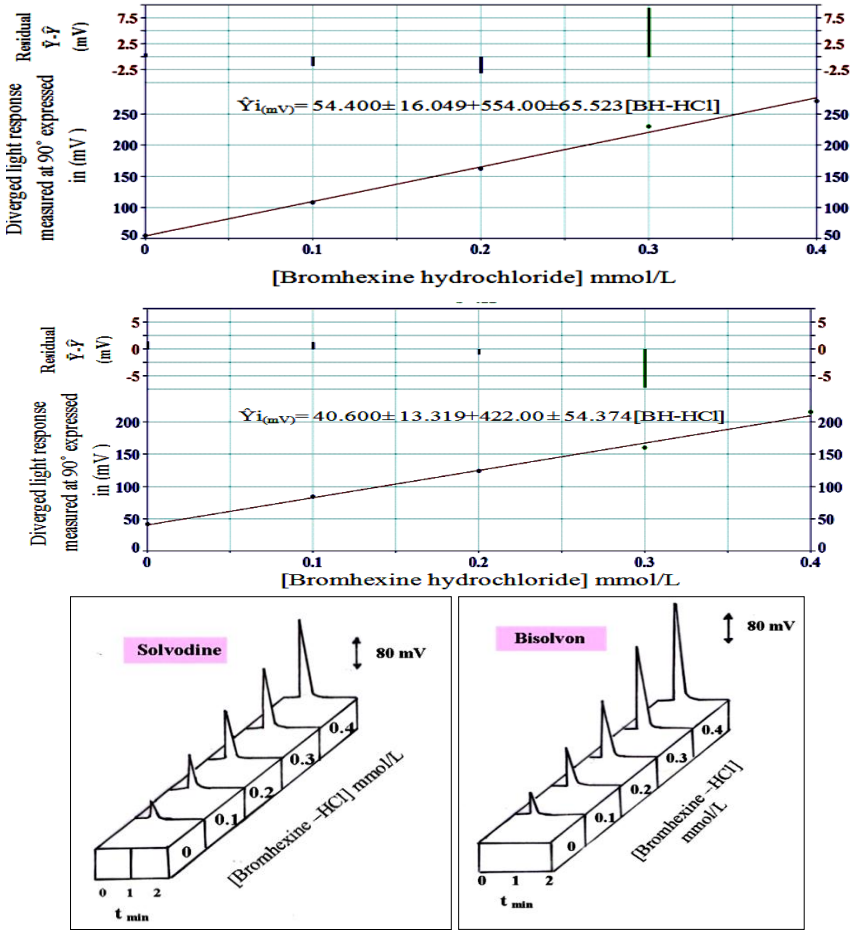


Figure 10: A. Standard addition graph of calibration and a response profile sample vs. time for two pharmaceutical preparations using an ISNAG fluorimeter, B.Solvodine, C. Bisolvon

Table 8A: The results of standard addition for determining BH-HCl in two pharmaceutical preparations

No. of samples	Commercial name, content company country	Sample weight equivalent to 0.04126 gm (1 mmol/L) of active ingredient (g)	New method using ISNAG fluorimeter							
			Turbidimeter							
			Bromhexin-HCl [mmol/L]					**Std.addition eq. at 95% for n-2** $\hat{Y}_{i(mV)}= a\pm s_a t+b\pm s_b t[x]$ $\hat{Y}_i= a\pm s_a t+b\pm s_b t[x]$	**r** r^2 **% r^2**	**Practical concentration (mmol/L)**
			0	0.1	0.2	0.3	0.4			10 mL
										100 mL
1	Solvodine (8 mg) SDI-Iraq	0.6042	42	84	124	160	215	40.600±13.3185+422.00±54.374[X]	0.9975 0.9951 99.51	0.0962 0.9620
			28	48	64	96	128	23.200±16.497+248.000±38.712[X]	0.9892 0.9786 97.86	0.0935 0.935
2	Bisolven (8 mg) The Nile Co. Egypt	0.6370	55	108	162	230	271	54.400±16.049+554.00±65.523[X]	0.9979 0.9959 99.59	0.0982 0.982
			34	60	92	118	150	32.800±4.676+290.000±19.092 [X]	0.9994 0.9987 99.87	0.11318 1.1318

$\hat{Y}$ = Estimated response in mV for ISNAG fluorimeter and turbidimetry (FTU), [x] = [BH-HCl] mmol/L.
r = Coefficient of correlation,r^2% = percentage of linearity.

Table 8B: Summary of the results for the paired t-test, efficiency, and practical content for determining BH-HCl in two pharmaceutical preparation samples

Sample number	**Confidence interval for mean wt. $\overline{wi}$ ±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95% (g)**	**Theoretical content of active ingredient at 95%(mg)**	Practical content $\overline{wi}$ ±4.303$\sigma_{n-1}/\sqrt{n}$ (mg) for (n = 3), at 95%	Determination Efficiency (Rec. %)	Individual comparisons $(\bar{x}-\mu)\sqrt{n}/\sigma_{n-1}$ ISNAG fluorimeter Analyzer with Quoted value $t_{0.05/2,2}$ = 4.303	Paired t-test Compared between two methods		
			ISNAC-Fluorimeter (newly method)			Xd	$\bar{x}$d (σ_{n-1})	$t_{cal}=\bar{x}_d\sqrt{n}/\sigma_{n-1}$ at 95 %
			Turbidimetry (classical approach)					
1	0.11715±0.0018	8 ± 0.1229	7.6960±0.523	96.20	/-2.478/< 4.3030	0.216	- 0.4916	/- 0.693/
			7.4800±0.423	93.50	/- 5.290/> 4.3030		1.0000	<< 12.706
2	0.1235±0.0017	8 ± 0.1101	7.8552±0.234	98.19	/- 2.665/< 4.303	- 1.1992		
			9.0544±0.512	113.18	8.861 > 4.303			

Xd: Difference between two methods,σ_{n-1}:Difference standard deviation, d: difference average value,μ: quoted value,n= no. of the samples = 2, $t_{0.025,\infty}$ = 1.96 at 95 %.

4. Conclusion

A new homemade instrument, ISNAG (low-pressure mercury lamp: 184.9 and 253.7 nm), was used for the estimation of bromhexine hydrochloride. The method works by measuring the precipitate formed by bromhexine hydrochloride and sodium tetraphenylborate when the drugs are pure and tablet dosage forms. When the newly developed method was compared to the traditional method (turbidimetric technique) using the t-test and standard addition method, it was discovered that there had not been any significant difference between the two approaches at the 95% level of confidence.

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