

## Article

# Using Safranin Dye for Indirect Spectrophotometric Determination of Furosemide in the Presence N-bromosuccinamide

Maha M. Al-Tae<sup>1</sup>, Mohammed Salim Al-Enizzi<sup>2</sup>

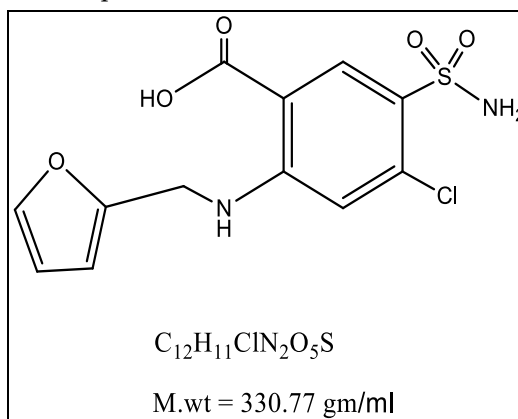
1. Chemistry Department, College of education for Girls, Mosul University, Mosul, Iraq  
\* Correspondence: [maha.ge1336@student.uomosul.edu.iq](mailto:maha.ge1336@student.uomosul.edu.iq)
2. Chemistry Department, College of education for Girls, Mosul University, Mosul, Iraq  
\* Correspondence: [mohammed.salim@uomosul.edu.iq](mailto:mohammed.salim@uomosul.edu.iq)

**Abstract:** A sensitive and accurate indirect spectroscopic method has been proposed for the determination of furosemide in acidic media. The method includes oxidation of safranin dye in the presence of N-bromosuccinimide as an oxidizing agent to form a pink-colored product that can be measured spectrophotometrically at a wavelength of 526 nm. The molar absorptance and Sandel sensitivity were (2.4×10<sup>4</sup> L/mol.cm) and (0.0135 μg/cm) respectively, the correlation coefficient was 0.9995 with a recovery rate of 97.24%, a standard deviation of 0.867%, 0.0966 μg/ml for (LOD) and 0.322 μg/ml for (LOQ). The technique was applied to determine the purity of furosemide and the medicinal forms of it.

**Keywords:** safranin dye, furosemide, N-bromosuccinamide

## 1. Introduction

Furosemide chemically name is [(2-furanyl methyl) amino] benzoic acid] 5-(amino-sulfonyl)-4-chloro-2 (Figure 1) [1] is a crystalline powder that is white to slightly yellow in color and has a melting point of 206°C [2], is an effective loop diuretic that speeds up the production of urine and the excretion of sodium by blocking the kidneys' ability to absorb chloride and sodium [3]. Because furosemide can conceal other drugs, it is on the World Anti-Doping Agency's list of prohibited substances.



**Figure 1.** Furosemide structure

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Furosemide is used to alleviate the body's fluid retention brought on by heart failure, liver scarring, or renal illness. It can be used to treat high blood pressure as well [4]. In 1959, furosemide was patented, and in 1964, it was licensed for medicinal usage [5]. It's a Water-soluble to a slight degree, chloroform, and ether, but it dissolves in dimethyl formamide, acetone, and methanol [6]. There could be interactions between furosemide and the following medications: Other salicylates, such as aspirin [7]. Numerous analytical techniques are available to detect furosemide in biological and pharmacological materials. There are also numerous known spectrophotometric methods for furosemide [8-17] HPLC [18-23] and other methods [24-33].

## 2. Materials and Methods

Apparatus is UV-Visible Shimadzu model 1800 Double-beam spectrophotometric with 1 cm thickness. The preparation of method for this research following:

### 1) Furosemide (100 $\mu\text{g/ml}$ )

In a 100 ml volumetric bottle, 0.01g of the medication was dissolved in 2 ml of sodium hydroxide, and then 100 ml of pure water were added.

### 2) Safranin (100 $\mu\text{g/ml}$ )

0.01g of dye dissolved in 100ml distilled water.

### 3) N-bromosuccinimide (NBS) ( $1 \times 10^{-2}$ M)

0.1779g of NBS dissolved in 100 ml distilled water, and another oxidizing agents prepared in the same concentrations.

### 4) Phosphoric acid (1M)

15.31ml was taken from the concentrated acid (density = 1.88g/ml, 85%) and diluted to 250 ml by distilled water.

### 5) Surfactants (0.1%)

In order to prepare surfactants, 0.1g of each material were dissolved in 100 ml of hot distilled water.

### 6) Furosemide tablet (40mg)

Five capsules of the pharmaceutical preparation were weighed and the equivalent of the weight of one capsule was taken and dissolved in 2 ml sodium hydroxide 1M. To get 400  $\mu\text{g.ml}^{-1}$ , to obtain a solution with a concentration of 100  $\mu\text{g.ml}^{-1}$ , the solution was filtered, and the volume was added to 100 ml of distilled water. After that, 25 ml of the final solution was removed, and different volumes (0.1, 0.3, 0.75) ml were taken to obtain concentrations (1, 3, 7.5) of furosemide. The results appear in Table 5.

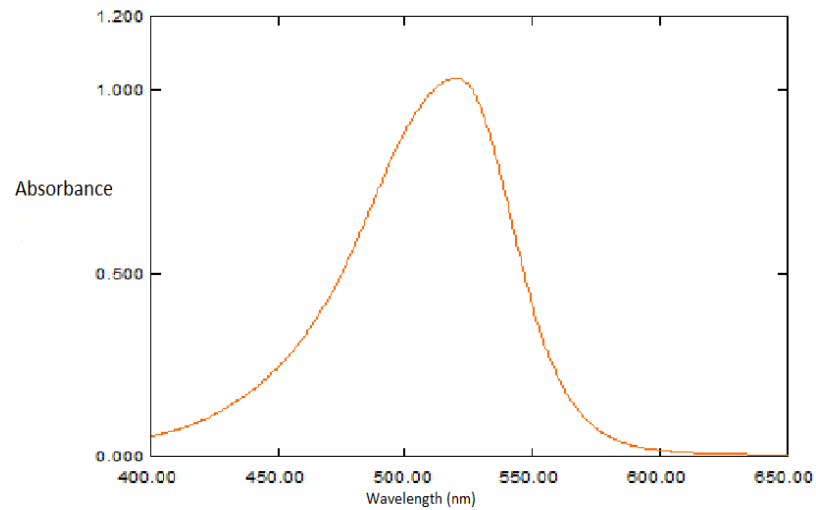
### 7) Furosemide Ampoule (20mg/2ml)

2ml taking of the drug solution and diluting it into 100 ml of distilled water to obtain a solution with a concentration of 200  $\mu\text{g.ml}^{-1}$ , from which 100  $\mu\text{g.ml}^{-1}$  were prepared different volumes (0.1, 0.3, 0.75) ml were taken to obtain concentrations (1, 3, 7.5)  $\mu\text{g.ml}^{-1}$  of furosemide, given that Table 5 displays the results.

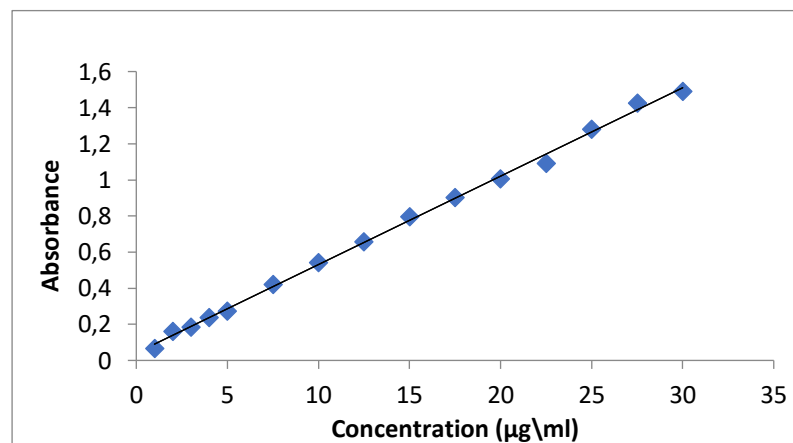
## 3. Results and Discussion

### 3.1. The absorption spectrum and the standard curve of the dye

The absorption spectrum of the dye at a concentration of 100  $\mu\text{g.ml}^{-1}$  was studied by taking increase sizes of dye (0.1-3.0) ml within a 10 ml volumetric flask to obtain the optimal amount that gives the best absorption at a wavelength of 526 nm, as seen in Figure 2.



**Figure 2.** Safranin dye's absorption spectra



**Figure 3.** The standard calibration curve for dye

2 ml were taken, which is equivalent to  $20 \mu\text{g}\cdot\text{ml}^{-1}$  of dye and its use in subsequent studies falls within the standard calibration curve for dye.

### 3.2. Setting the optimal conditions

#### 3.2.1. Choosing the type of oxidizing agent

2 ml of safranin dye ( $100 \mu\text{g}\cdot\text{ml}^{-1}$ ) was taken, then 1 ml of the oxidizing agent  $1 \times 10^{-2}$  M and 1 ml of hydrochloric acid were added in a 10 ml volumetric flask. The solutions were diluted with distilled water and leave it for 10 minutes at room temperature. Next, at a wavelength of 526 nm, the absorption was measured in comparison to the blank solutions. The table shows that the best oxidizing agent is NBS. The results are shown in Figure 4.

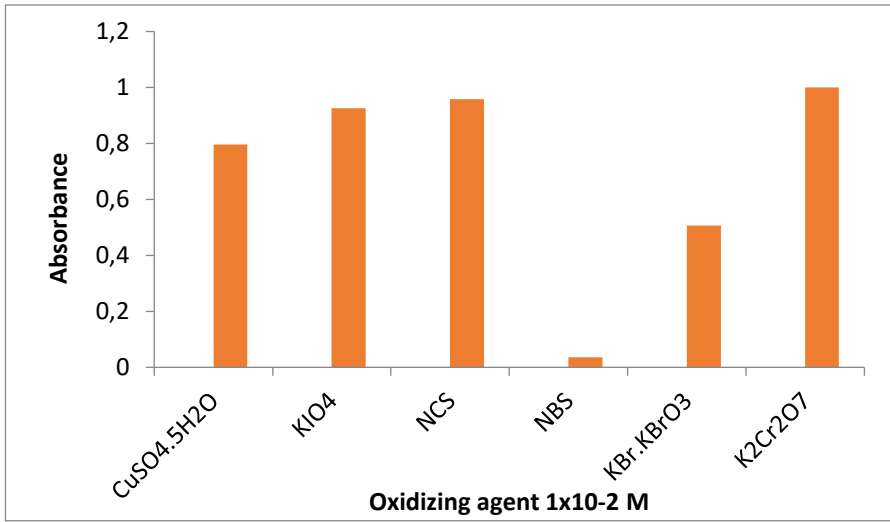


Figure 4. The effect of various oxidizing agent

3.2.2. Different volumes of the oxidizing agent

The effect of the volume of the oxidizing agent NBS  $1 \times 10^{-2}$  M was studied, different volumes of (0.0 - 1) ml were used, and it was found that 1 ml is the best volume, as it gives the best dye retention. The results are shown in Figure 5.

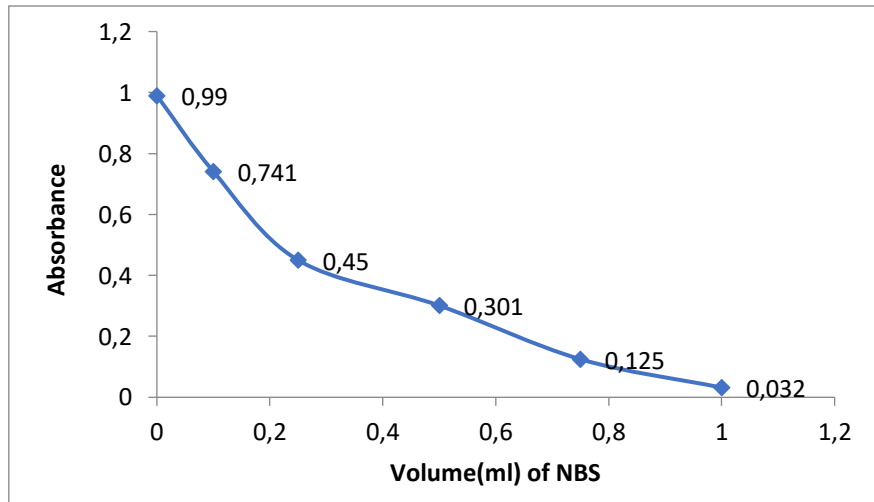
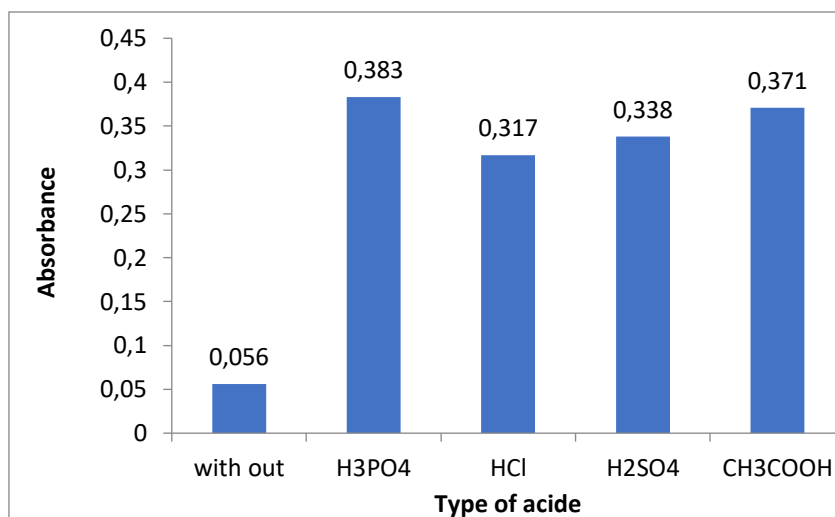


Figure 5. Effect of different amounts of oxidizing agent

3.2.3. Different types of acids

1 ml of different types of acids (1 M) was used added to 1 ml of the drug compound with 1 ml oxidizing agent (NBS) and left for 5 minutes, 2 ml dye was added and left for 10 minutes, then the absorbance was measured at 526 nm. The results appear in Figure 6.



**Figure 6.** Effect of different types of acids

#### 3.2.4. Different amounts of acid (H<sub>3</sub>PO<sub>4</sub>)

Different amounts (0.1-1.5) ml of the acid used H<sub>3</sub>PO<sub>4</sub> were added in order to find out the optimal amount. It was found that 0.5 ml is the best and gives the highest absorption, as seen in Table 1.

**Table 1.** Different amounts of phosphoric acid

Volume (ml) of H <sub>3</sub> PO <sub>4</sub> (1M)	Absorbance
0.1	0.263
0.25	0.384
<b>0.5</b>	<b>0.39</b>
0.75	0.383
1	0.381
1.25	0.363
1.5	0.360

#### 3.2.5. Oxidation time

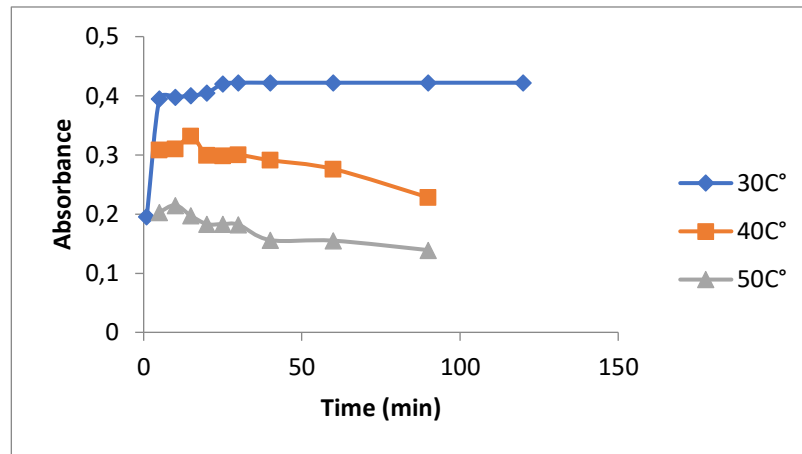
The time required for oxidation was studied by leaving the solutions after dissolving the 1ml of drug compound (100 µg.ml<sup>-1</sup>), then adding 1 ml of the oxidizing agent (1×10<sup>-2</sup> M) and 0.5 ml of phosphoric acid (1M), then the solutions were left for different periods of time, then adding 2 ml of safranin dye 100 µg.ml<sup>-1</sup>. After that, it was diluted with distilled water to the mark, then the solution has been measured against its blank solutions at a wavelength of 526 nm. The outcomes are displayed in Table 2.

**Table 2.** Time of oxidation's effect

Time/ min	1.0	3.0	<b>5.0</b>	7.0	10
Absorbance	0.287	0.384	<b>0.395</b>	0.367	0.309

#### 3.2.6. The effect of temperature and stabilization time

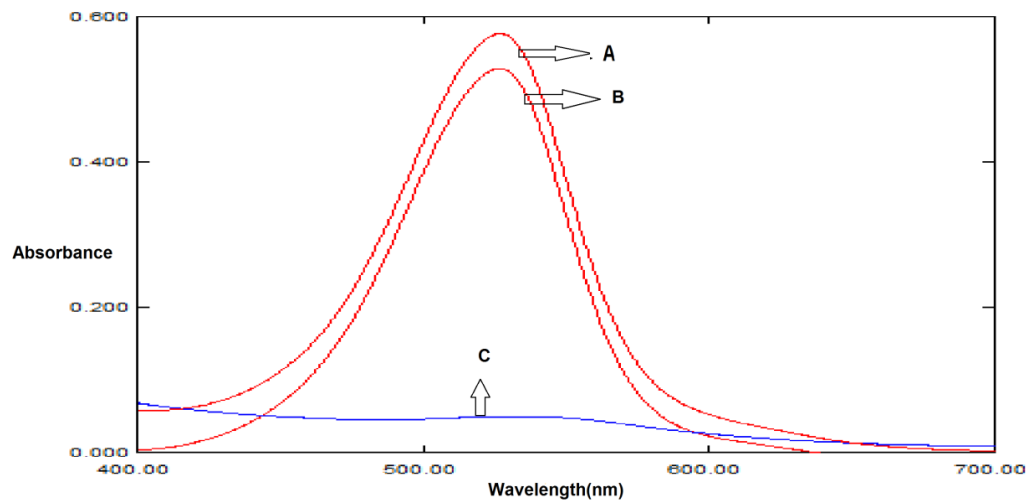
Different temperatures were studied on the formation and stability of the product. It was found that the absorbance decreases at high temperatures. The formation time of the product is 25 minutes for more than 100 minutes. It was found that the colored product it gives the best absorption at a temperature of 30 C° and the results are shown in Figure 7.



**Figure 7.** Effect of temperature and stabilization time

### 3.2.7. Final absorption spectrum

After adjusting the reaction's ideal conditions, as shown in Figure 8, the absorption spectrum was obtained.



**Figure 8.** Final absorption spectra used to determine furosemide

A: Absorption spectrum of  $6 \mu\text{g}\cdot\text{ml}^{-1}$  of furosemide versus distilled water.

B: Absorption spectrum of  $6 \mu\text{g}\cdot\text{ml}^{-1}$  of furosemide versus blank solution.

C: Absorption spectrum blank solution versus distilled water.

### 3.2.8. The optimal conditions

The optimal conditions are listed in Table 3.

**Table 3.** An overview of optimal conditions

Optimal condition	
$\lambda_{\text{max}}$ (nm)	526
Amount of NBS $1 \times 10^{-2}$ M (ml)	1
Amount of $\text{H}_3\text{PO}_4$ 1M (ml)	0.5
Safranin $100 \mu\text{g}/\text{ml}$ (ml)	2
Temperature $^{\circ}\text{C}$	30

### 3.3. Calibration Curve

Increasing amounts (0.05-1) ml of furosemide ( $100 \mu\text{g}\cdot\text{ml}^{-1}$ ) were put into volumetric flasks measuring 10 ml, then 1 ml of oxidizing agent ( $1 \times 10^{-2} \text{ M}$ ), and 0.5 ml of phosphoric acid (1 M), then the solutions were left for 5 minutes, then add 2 ml of safranin dye ( $100 \mu\text{g}\cdot\text{ml}^{-1}$ ), and complete the volume to the mark with distilled water. Following dilution, the solutions were kept at  $30 \text{ C}^\circ$  for twenty-five minutes. Thereafter, each solution was measured in relation to the blank solution at a wavelength of 526 nm, as in Figure 8. Follow the law of beer in concentration range ( $0.5\text{-}10 \mu\text{g}\cdot\text{ml}^{-1}$ ).

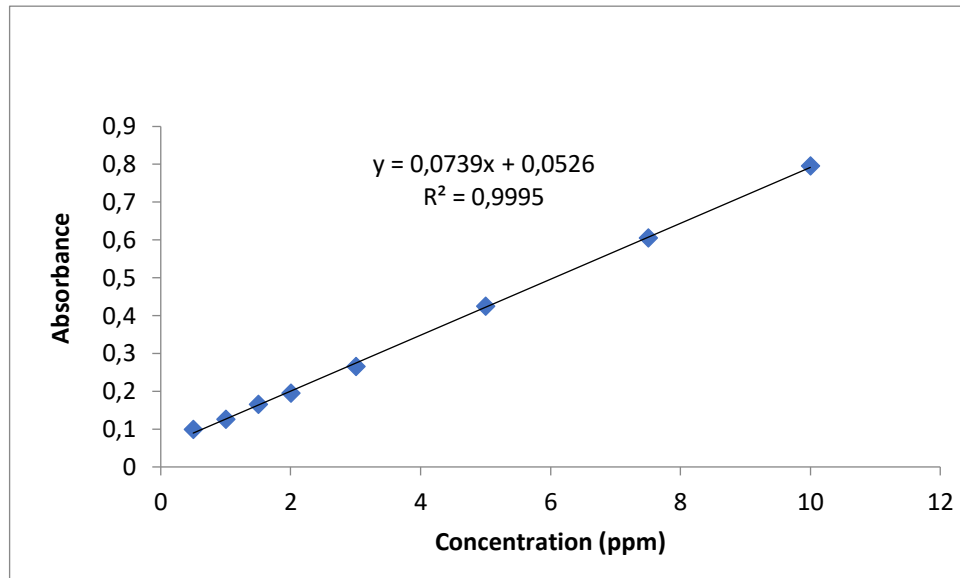
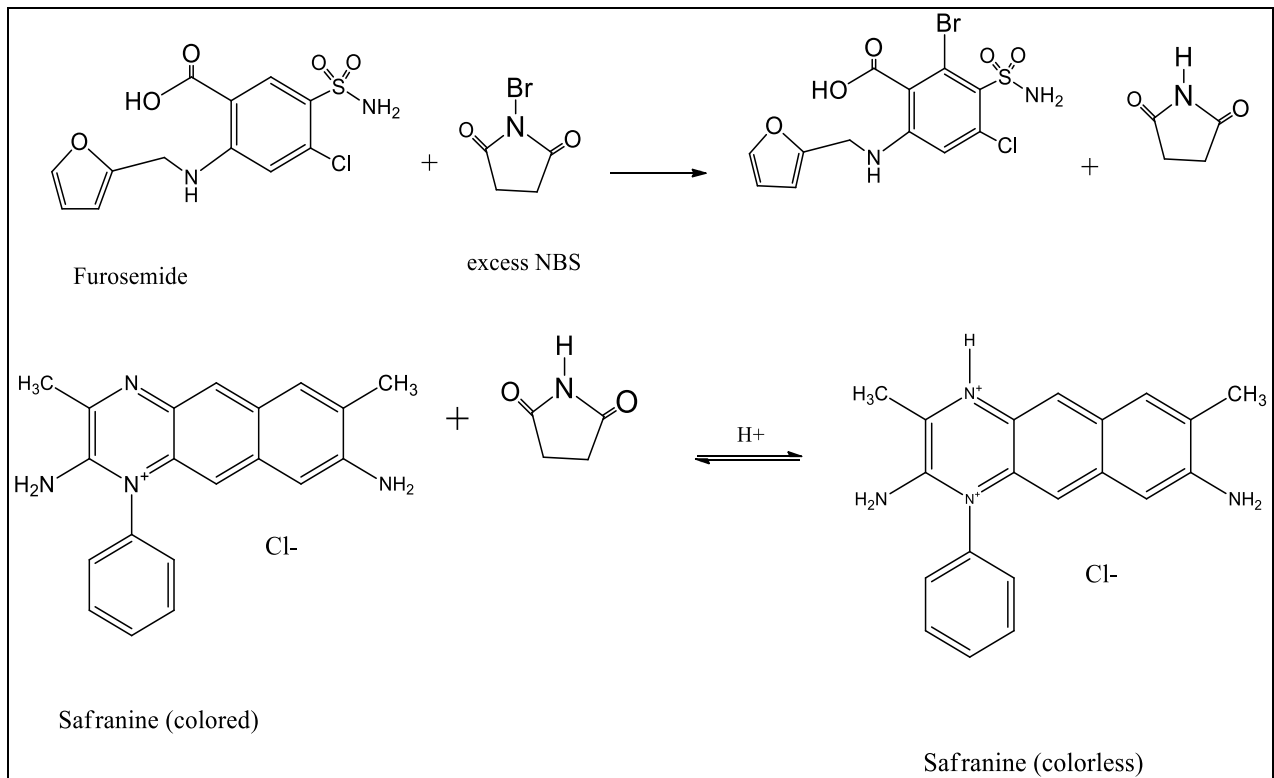


Figure 9. Calibration curve for determination of furosemide



Scheme 1. Potential reaction mechanism

### 3.4. Accuracy and compatibility of the method

Three concentrations (1, 3, 7.5)  $\mu\text{g}\cdot\text{ml}^{-1}$  of furosemide ( $100 \mu\text{g}\cdot\text{ml}^{-1}$ ) were used to verify the accuracy and precision of the method by taking five readings for each of them as in Table 4, which means that the method has high accuracy and compatibility.

**Table 4.** Study of accuracy and precision

Compound	Amount added ( $\mu\text{g}/\text{ml}$ )		Recovery%	Average Recovery%	RSD%
	Taken	Found			
Furosemide	1	0.955	95.50	97.23	1.66
	3	2.88	96.00		0.703
	7.5	7.515	100.2		0.24

Using the recommended technique to identify the medication component in pharmaceutical preparations, as seen in Table 5.

**Table 5.** Determination of the medicinal ingredient in formulations for pharmaceutical use

Preparation of Pharmaceuticals	Validated Value	Amount Present ( $\mu\text{g}/\text{ml}$ )		Drug content found (mg)	Recovery (%)	Average Recovery (%)
		Taken	Found			
Tablet BRISTOL Laboratories.Ltd	40mg	1	0.982	39.28	98.20	97.59
		3	2.87	38.26	95.66	
		7.5	7.42	39.57	98.93	
Injection Syria Ibn Hayyan PHARMA	20mg \ 2 ml	1	0.966	19.32	96.60	97.43
		3	2.86	19.06	95.33	
		7.5	7.529	20.07	100.38	

Table 5 shows the extent of the efficiency and success of the developed method for its application to pharmaceutical preparations. The recovery in the Tablet ranged from (95.66-98.93)% and in the Injection ranged from (95.33-100.38)%.

### 3.5. The standard addition method to pharmaceutical preparations

To verify and prove the efficiency and success of the proposed method, The standard method of addition to pharmaceutical preparations was applied for furosemide in injection (ampoule) and tablet form. Figure (9,10) shows the application of the standard addition to pharmaceutical preparations when taking (1.0 and 2.0)  $\mu\text{g}\cdot\text{ml}^{-1}$  from ampoule and tablet.

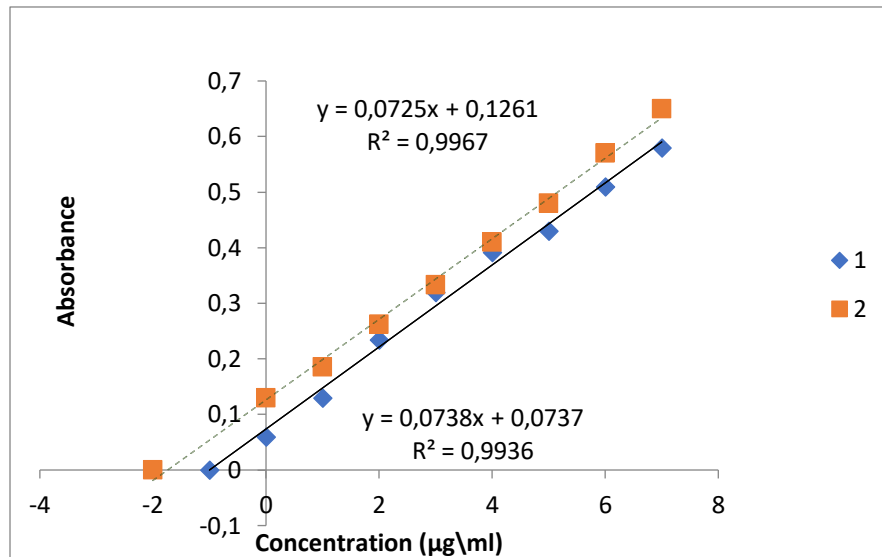


Figure 9. Standard addition to the pharmaceutical preparation Injection (Ampoule)

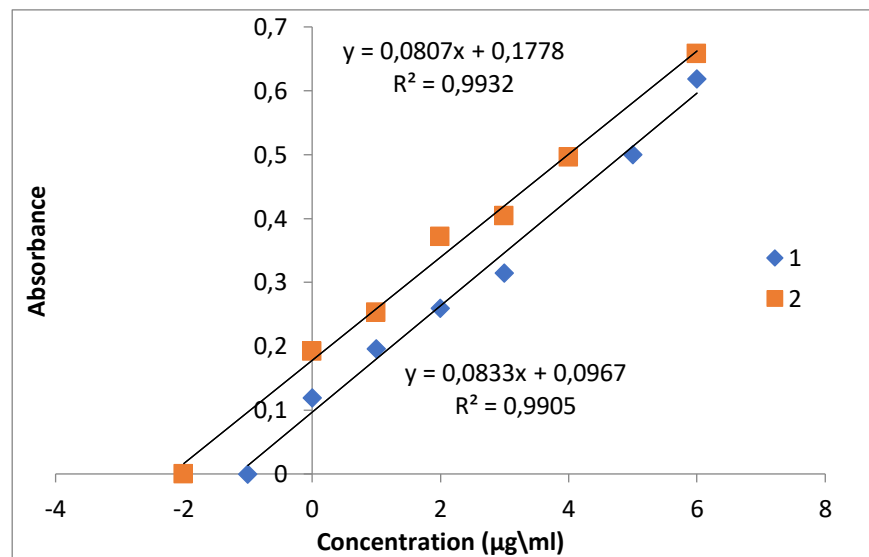


Figure 10. Standard addition to the pharmaceutical preparation (Tablet)

### 3.6. Compare the method with other method

The proposed method for determination furosemide was compared with other spectroscopic methods. Table 6 outlines how the suggested method was compared to existing spectroscopic techniques, with the findings showing that the suggested method was successful in determining furosemide in the preparation of pharmaceuticals.

Table 6. The method's comparison with alternative methods

Analytic parameters	Current method	Literature method <sup>(18)</sup>
$\lambda$ max (nm)	526	465
Molar absorptivity (L .mol <sup>-1</sup> . cm <sup>-1</sup> )	2.42×10 <sup>4</sup>	1.065×10 <sup>4</sup>
Sandell's sensitivity (µg.cm <sup>-2</sup> )	0.0135	0.0310
Beer's law Range (ppm)	0.5-10	3-23
RSD %	0.867	0.677

#### 4. Conclusion

The pharmaceutical compound furosemide was established in medicinal formulations as well as in its most pure form like tablets and injections. The method was sensitive and accurate, based on the oxidation of the safranin dye in the presence of the acid in the aqueous medium.

#### 5. Acknowledgments

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#### 6. Author's statement

We certify that this research or manuscript is an original work and has not been published anywhere or submitted for publication. All sources for this work have been duly cited. We take full responsibility for the content of this manuscript, including any errors or omissions, and to ensure that all changes are made by the author and that all contributors are properly registered. We realize that any violation of these data may result in this manuscript being removed from publication. Each of the individuals mentioned in the study contributed equally to the work and writing.

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