OXIDATIVE COUPLING REACTION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF FUROSEMIDE USING CHLORPROMAZINE HYDROCHLORIDE AS A REAGENT

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Abstract: *A simple, economical, and rapid method has been developed for the determination of the pharmaceutical compound (furosemide) in its pure forms and several pharmaceutical formulations using oxidative coupling reactions. This method focuses on the reaction of the drug compounds with chlorpromazine hydrochloride as a reagent in an acidic medium in the presence of the appropriate oxidizing compound. The absorbance of the colored product was measured at 525 nm and molar absorptivity was 138891 L/mol.cm, compatible with Beer law in concentrations (2-32) µg/ml, the detection, and the quantification limits were (0.471, 1.571) µg/ml respectively, with a recovery rate of 101.15% and the relative standard deviation was less than 0.5%. The great correlation coefficient value (R^2 = 0.9953) and insignificant values of intercept (0.067) validated the good linearity of the calibration curve and agreement with Beer's law. The created method was applied successfully to determine furosemide in pharmaceutical formulation, and the method was in agreement with the standard addition method.*
Keywords: *Spectroscopic Determination, Furosemide, Chlorpromazine Hydrochloride.*
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1. Introduction

Furosemide (FA), scientifically named "5-(aminosulfonyl)-4-chloro-2-[(2 furanylmethyl) amino]benzoic acid". The FA has a chemical formula as $C_{12}H_{11}ClN_2O_5S$, with a molecular weight of 330.7 g/mol. It is a white, slightly yellowish crystalline powder [1, 2]. It is considered a diuretic and used to treat liver, high blood pressure, and kidney diseases and is taken orally, either alone or with other blood pressure medications [3-5]. Furosemide was estimated by several methods, including spectrophotometric [6-10], electrical [11, 12] and chromatographic [13, 14]. Figure 1 shows the chemical structure of furosemide.

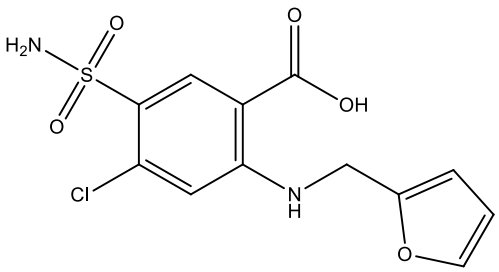

Fig. 1. Furosemide Chemical Structure

In this research, furosemide was estimated spectrophotometrically by oxidative coupling interaction with chlorpromazine hydrochloride as a reagent and in the existence of an appropriate oxidizing agent. The method was successfully applied to pharmaceutical preparations.

The current study aims to create a modest and reproducible approach for the selective spectrophotometric determination of FA in tablet and injection using a simple UV-VIS technique that can be conducted for the quality control process (CIP) of the drug or determine the pure FA in different drug formulations.

2. Experimental part

Instruments. The spectral measurements were carried out using Shimadzu UV-1800 double-beam spectrophotometry using glass cells with a width of 1 cm.

Chemicals and Reagents. Reagents solution: All chemicals used were of analytical purity grade. Detail information are given in Tables 1 and 2.

Table 1. Preparing the chemical compounds involved in the experiments

Chemicals	Cons.	Preparation	Final dilution with water
Furosemide	(100 µg/ml)	prepared freshly by dissolving 10 mg of the pure substance in 5 ml of Abs. ethanol	100 mL
Chlorpromazine hydrochloride	(0.1%)	prepared by dissolving 10 mg of the pure substance in pure water	100 mL
Potassium dichromate	(5×10^{-5} M)	prepared by dissolving 1.4709 mg of the pure substance in pure water	100 mL
Copper sulfate pentahydrate	(1×10^{-2} M)	prepared by dissolving 249.685 mg of the pure substance in pure water	100 mL
Hydrochloric acid	(1×10^{-1} M)	prepared by diluting the concentrated acid with pure water	100 mL

Table 2. Preparation of drug solution (Furosemide) from the pharmaceutical preparation

Injection	A 2ml vial containing 20mg of FA was added into a precise volume and diluted up to the mark (1litter volumetric flask) with distilled water.
Tablets	Ten tablets were weighed, and after grinding and mixing them well, the equivalent of the weight of one tablet was taken and dissolved well in an appropriate volume of ethanol, filtered then supplemented the volume to the mark (100 mL) with distilled water

3. Result and discussion

The absorption spectrum of the reagent and furosemide complex. The maximum absorbance of the colored product was 525 nm as shown in Fig. 2.

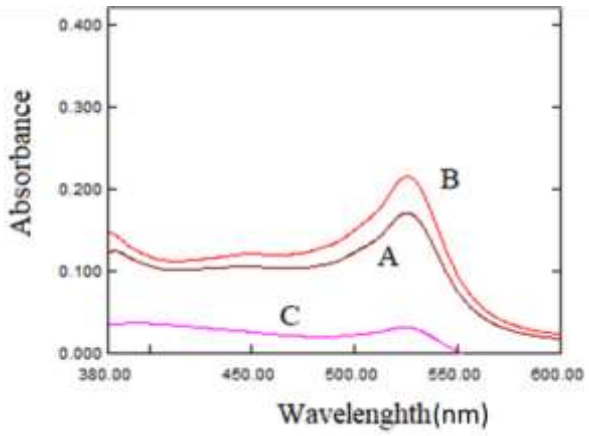

Fig. 2. Absorbance spectrum of 4 µg/ml of FA against blank (A); 4 µg/ml of FA against water (B); Blank against water (C)

Method Validation. Under the optimized conditions, Fig. 3 shows a linear correlation between absorbance and furosemide concentration in the range of (2-32) μg/ml. The correlation coefficient was 0.9953 the slope of the curve was 0.042. After that, a negative deviation from Beer's law occurred.

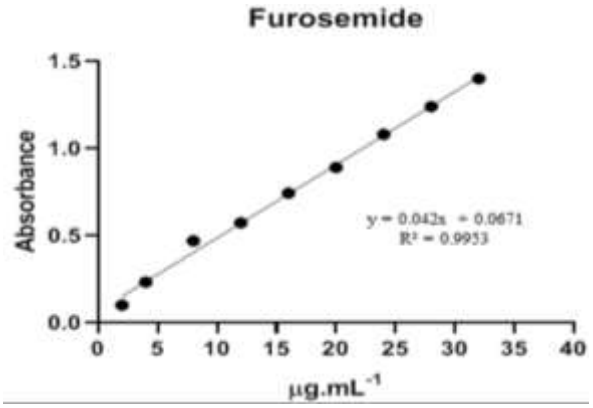

Fig. 3. Standard calibration curve for furosemide

Reagent Volume. Chlorpromazine reagent was prepared in a concentration of 0.1%. The effect of the reagent volume (0.25-2.5 mL) was examined. Fig. 4 shows the optimal amount of the reagent on the reaction product which gives the highest color intensity.

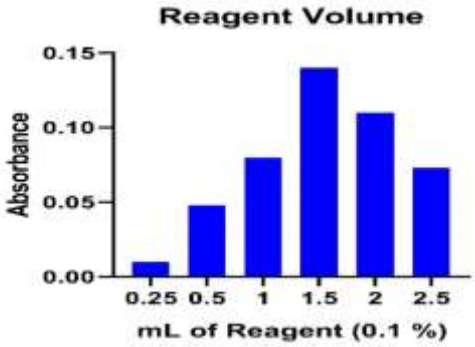

Fig. 4. The effect of Chlorpromazine volume (0.1%)

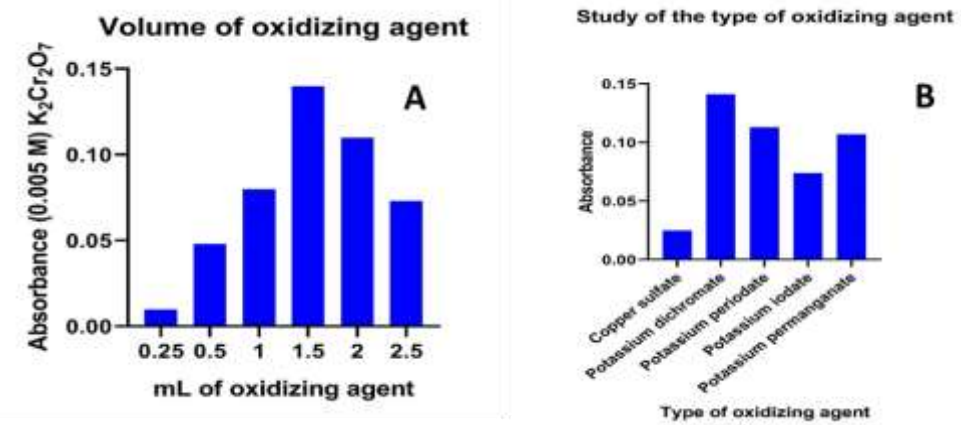

Fig. 5. Volume of oxidizing agent (A); Type of oxidizing agent (B)

Study the type & volume of oxidizing agent. The effect of different types of oxidizing agents on the adsorption of the resulting complex was studied by using potassium iodate

(KIO_3), aqueous copper sulfate ($CuSO_4.5H_2O$), potassium dichromate ($K_2Cr_2O_7$), potassium periodate (KIO_4) and Potassium permanganate ($KMnO_4$). The study showed that the best oxidizing agent is potassium dichromate (1.0 ml), as shown in Fig. 5. Potassium dichromate was chosen to be the powerful oxidizing agent due to its ability to readily donate oxygen atoms.

Studying the type & volume of acid. To select the best acid that can give the highest absorbance, different types of acids starting from HCl, H_2SO_4, and H_3PO_4 with a concentration (0.1 M) were tried and added to the volumetric flask. It was indicated that the use of HCl (2.5 ml) gives higher absorption as shown in Fig. 6. The effect of the base NaOH was also studied at a concentration of (0.1 M) and a decrease was observed in the absorption value, so the addition of the base was excluded. The choice of acid can indeed influence the intensity of the color produced. So, hydrochloric acid, being a strong acid, can effectively protonate leading to vivid color changes or intense hues. It indeed offers advantages in color intensity.

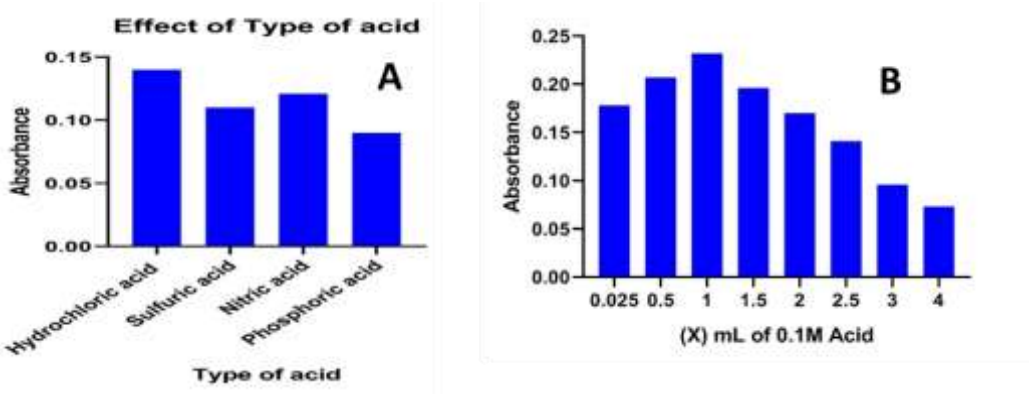

Fig. 6. Type of acid (A); Volume of acid (B)

Addition sequence effect. The effect of the addition sequence on the product intensity of absorption of the two colored solutions was separately studied with variable order additions. The result in Fig. 7 confirmed the best and most reliable sequence in subsequent measurements.

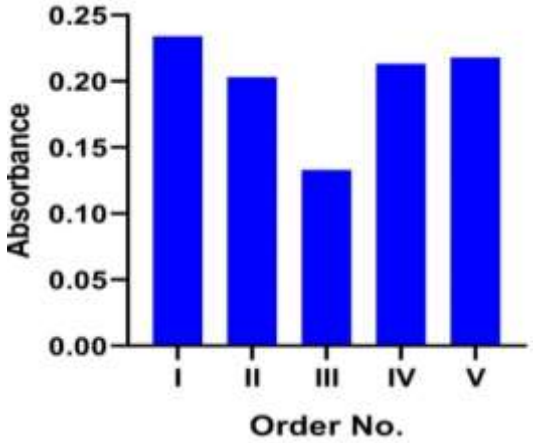

Fig. 7. Order of Addition

I	**Drug+ Reagent+ Oxidizing agent +Acid**
II	**Drug + Oxidizing agent +Acid+ Reagent**
III	**Drug + Acid+ Reagent+ Oxidizing agent**
IV	**Reagent + Oxidizing agent + Acid + Drug**
V	**Reagent + Acid + Oxidizing agent + Drug**

Effect of temperature and product stability. The effect of different temperatures ranging between 40 and 60 °C on the stability and absorption intensity of the resulting complex was studied using the optimal conditions obtained from previous experiments. Under optimum reaction conditions, the results shown in Fig. 8 indicated that the laboratory temperature (40°) was the best and it gives the highest absorption value. In addition, the complex remains stable for 90 minutes (Table 3).

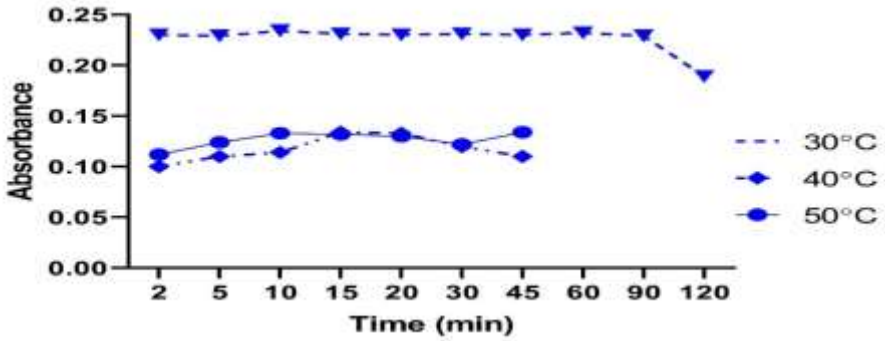

Fig. 8. Effect temperature and time stability

Table 3. Optimal conditions and regression parameters of the created method for furosemide determination

λ_{max} (nm)	**525**
Reagent 0.1 % (ml)	**1.5**
Oxidizing agent ($K_2Cr_2O_7$) (5×10^{-3} M)	**1.0 mL**
HCl 0.1 M (ml)	**2.5**
Temperature (°C)	**40**
Development Time (min.)	**5**
Product Stability Time (min.)	**90**
Method Linearity range (µg /mL)	**2 – 32**
Detection Limit Value (LOD) (µg /mL)	**0.471**
Quantitation Limit Value (LOQ) (µg /mL)	**1.571**
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	**13889**

The accuracy and precision test for the suggested method. The accuracy and precision of the suggested approach were evaluated by calculating the recovery rate and relative standard deviation (R%, RSD) using 5 replicates for 3 different concentrations of each of the medicinal compounds, as shown in Table 4.

Table 4. Method accuracy and precision

Added quantity ($\mu g.ml^{-1}$)	Recovery* (%)	Average recovery (%)	RSD* (%)
8	**96.29**	**101.15**	**0.20**
16	**104.00**		**0.10**
24	**103.17**		**0.45**

***Average of Five Determination**

Study the nature of the resulting complex. The continuous changes method was applied to find out the stoichiometry ratio between furosemide and chlorpromazine in the presence of the oxidizing agent in the acidic medium [15]. The results in Fig. 9a indicate that

the ratio was 1:1 for the compound with the react. To confirm the composition ratio of the product, the Job method [16] has been applied as well. The results were compatible with the previous result which was calculated by the continuous changes method. Based on the results, a reaction mechanism was suggested and the formation of the color product probably occurs as follows [17].

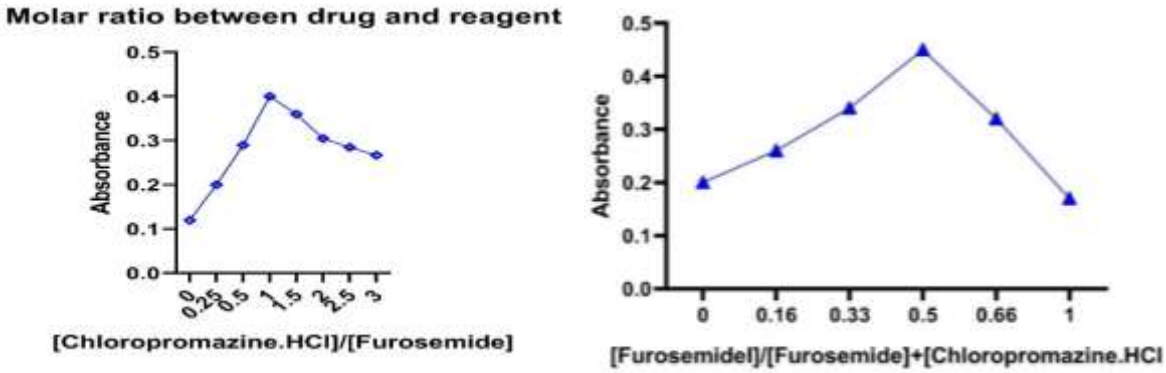

Fig. 9a. Mole and Job Methods

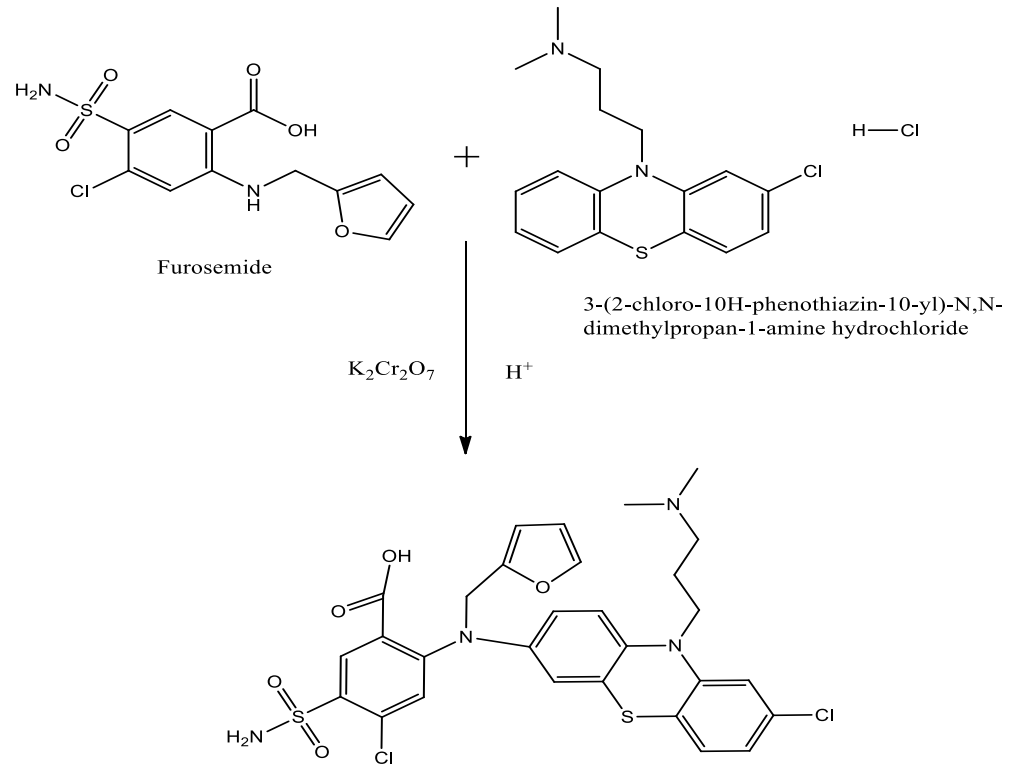

Fig. 9b. Suggested reaction

Complex stability constant. Based on the Job and continuous changes methods, the stability constant (K_{st}) of the formed product was calculated at a ratio of 1:1 by applying the following equation:

$$Kst = \frac{1-\alpha}{\alpha^2 C}$$

$$\alpha = \frac{\mathrm{Am} - \mathrm{As}}{\mathrm{Am}}$$

Where *Kst* is the stability constant, L/mol, α= the dissociation degree;
C is the concentration of the formed product which is the same concentration of Furosemide;
Am is the absorbance of the complexes at the optimum amount of *reagent (*chlorpromazine*)*;
As is the absorbance of the complexes at stoichiometric amounts of chlorpromazine reagent according to the 1:1 ratio under the optimum conditions reaction.

The results illustrated in Table 5 indicate that the complex (Drug:Reagent) has good stability of the colored product.

Table 5. The stability constant of the resulting complex

Conc.(mol·l^{-1})	Absorbance		α	Average K_{st} (l. mol^{-1})
	As	Am		
2×10^{-6}	0.017	0.025	0.320	**1.525×10^{6}**
4×10^{-6}	0.019	0.034	0.441	
6×10^{-6}	0.030	0.052	0.423	

Applications of Method. The proposed approach was conducted to estimate the FA in commercial dosage forms. The results in Table 6 show that the developed method has a very good accuracy value and is in good agreement with the original content in pharmaceutical dosage forms.

Table 6. Determination of the drug compound in pharmaceutical preparations by the proposed method

Pharmaceutical dosage	Certified weight	Amount present (µg. ml^{-1})	Drug content found*(mg)	Recovery* (%)	Average recovery (%)
Furoject Injection Turkey	20 mg	2	20.85	104.25	106.56
		4	20.60	103.00	
		8	22.49	112.45	
LASIMEX Tablets S.D.I. - IRAQ	40 mg	2	41.93	104.82	105.28
		4	42.46	106.15	
		8	41.95	104.87	

Evaluate the results with the standard addition method (SAM). To prove the efficiency of the recommended method and its success in estimation and that it is free from the effect of the additive compound, the standard addition method was applied to estimate the drug compound (furosemide) and due to the lack of requirements for the standard method approved in the British Pharmacopoeia, it can be inferred from the results shown in Fig. 10 and Table 7 that the obtained method is compatible with the suggested method, which indicates that the method has good selectivity.

Table 7. Comparison of the accuracy of the suggested method for the micro-determination of furosemide in pharmaceutical dosage with the standard addition method

Pharmaceutical preparation	Certified value (mg)	Amount present (µg. ml^{-1})	Drug content found (mg)		Recovery (%) of standard addition procedure
			Present method*	Standard addition procedure	
Furoject Injection Turkey	20	2	20.85	20.93	104.65
		4	20.60	20.10	100.50

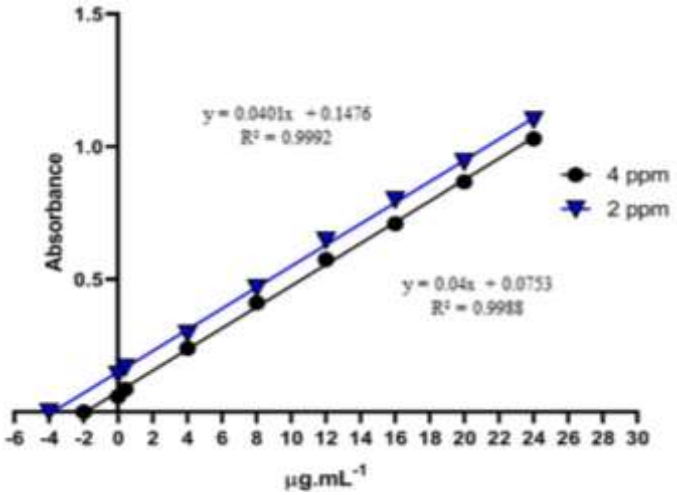

Fig. 10. Standard addition curve for the determination of furosemide in a pharmaceutical preparation

A comparison of the method. The developed technique was compared with other published UV-Vis spectrophotometric methods based on the same reaction (oxidative coupling reaction). The results confirmed that the developed method has a greater sensitivity compared with published papers. Furthermore, this method is straightforward and does not involve any extraction steps. In addition, the applications of the method are broader than those mentioned in the literature. It includes the determination of the drug compound in tablets and injections Table 8.

Table 8. Comparison of the suggested UV-Vis spectrophotometric methods with the published methods for Furosemide determination.

Parameter	Current method	Literature method [18]	Literature method [19]
Type of reaction	oxidative coupling reaction	oxidative coupling reaction	oxidative coupling reaction
Reagent	Chlorpromazine	1-Naphthylamine-4-sulfonic acid	2,4-di-nitrophenyl hydrazine
Maximum wavelength (nm)	525	465	467
Medium of reaction	Acidic	Alkaline	Basic
Beer's law (µg /ml)	2-32	3-23	0.4-14
Molar absorptivity (l/mol.cm)	138891	10650	11890
Application	Injection and Tablet	Tablet	Tablet

Conclusion

A rapid and simple spectrophotometric method was developed for the determination of furosemide based on the oxidative coupling reaction and measuring the product formed at 525 nm. The drug was estimated in the range of 2-36 µg/ml of furosemide with good accuracy, as the recovery rate was 101.15% and precision was less than 0.5%. The method was applied

successfully in the estimation of pharmaceutical preparations, as it was found from the study of the nature of the complex that the complex is formed in a ratio of 1:1 (drug:reagent). The developed method was characterized by ease, sensitivity, and the absence of the need for prior extraction

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