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Original Article

Spectrophotometric Determination of Furosemide Using Pyrogallol Reagent in Pharmaceutical Preparations

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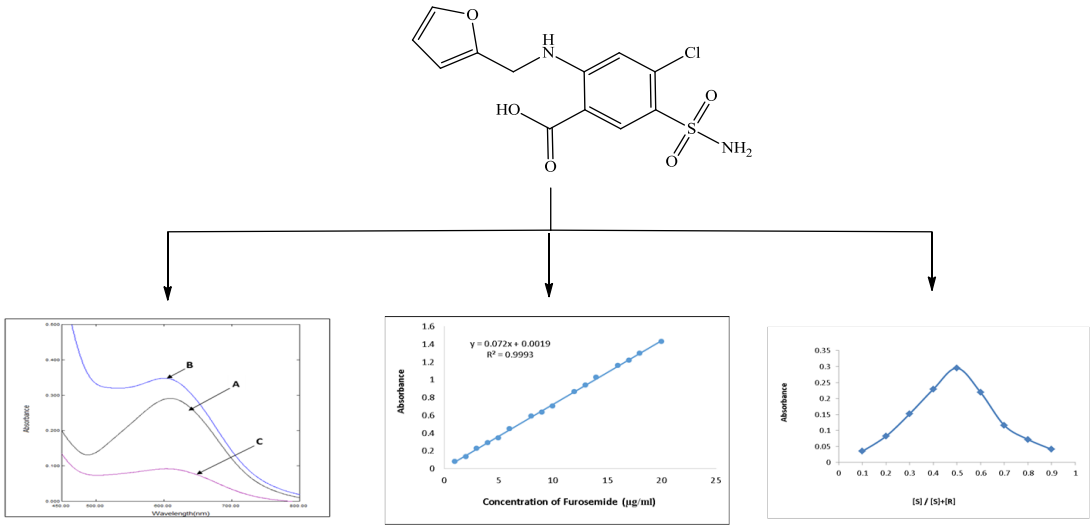
ABSTRACT

This research presents a rapid and accurate spectrophotometric method for furosemide determination. This method depended on charge transfer reaction of furosemide with pyrogallol reagent using sodium carbonate. It was observed that a product with a bluish-green color was formed after completing the addition and gave the highest absorption intensity at the wavelength of 610 nm. Following Beer's law, the straight standard curve was obtained in the concentration range of (1-20 µg/mL. The statistical results showed that the method has good accuracy and agreement. The molar absorptivity value was 2.3813×10^4 l/mol.cm and sensitivity of Sandell's was 0.0138 µg/cm^2. The relative standard deviation (RSD%) values ranged from 0.18 to 0.71%, relying on the concentration level. For the furosemide estimation, the suggested method has been successfully applied in its pharmaceutical preparations and pure form.

GRAPHICAL ABSTRACT



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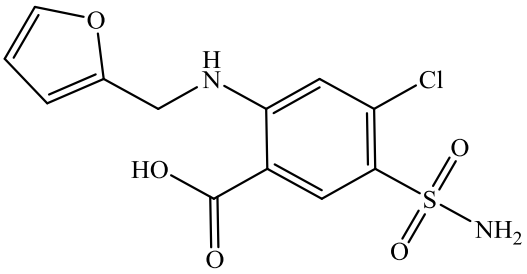
Introduction

Furosemide has several chemical names: 4-cliloro-*N*-(2-furylmethyl-5-sulfamoylanthranilic acid, 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid, 4-chloro-2-furfurylamino-5-sulphamoyl benzoic acid, and 5-(amino sulfonyl)-4-chloro-2-[(2-furanylmethy1) amino] benzoic acid [1]. Furosemide is white crystalline powder, molecular weight (330.7) g/mol, and molecular formula $C_{12}H_{11}ClN_2O_5S$ soluble in acetone and in dilute solutions of alkali hydroxides, slightly soluble in ethanol, and insoluble in water and methylene chloride [2]. It has the following structural formula (Scheme 1):



Scheme 1: Structural formula of Furosemide

Furosemide has several commercial names, most notably: lasix, Aisemide, Beronald, Desdimin, Frusemide, Fursemide, Lasilix, etc. It is considered a diuretic that prevents the body from absorbing an excessive amount of salt, and this salt is excreted through the urine. Furosemide is mainly used to treat fluid retention in body tissues or edema caused by heart failure, pulmonary edema, and liver and kidney disease; it is particularly effective in treating people with impaired kidney function who do not respond well to thiazide diuretics. It is also used to treat high blood pressure [3-6].

Several techniques and different analytical methods have been used for the furosemide determination such as spectrophotometric methods [7-14], flow injection [15, 16], high-performance liquid chromatography methods [17-19], gas chromatography/mass spectrometry (GC/MS) [20, 21], electrochemical methods [22-24], potentiometric method [25], and atomic absorption spectroscopy [26].

The aim of this study is to develop method rapid, accurate, and simple for furosemide estimation with pyrogallol using charge transfer complex reaction, and also to apply the suggested method to several pharmaceutical formations.

Materials and Methods

Shimadzu UV-Visible-1800 dual beam with identical 1 cm cells was used to perform all spectral and absorbance measurements, Philips PW 9420 pH meter was used for the pH measurements. The chemicals used were all of a high degree of purity.

Working furosemide solution (100 µg/mL)

It was prepared by dissolving (0.01 g) of pure furosemide in 5 mL of ethanol, and then its 100 ml diluted in volumetric flask with distilled water.

Pyrogallol solution (0.1%)

It was prepared by dissolving (0.1 g) pyrogallol with ethanol and completing the volume to 100 ml in volumetric flask with absolute ethanol.

Sodium carbonate solution (0.1 M)

It was prepared weight (1.06 g) of Na_2CO_3 and dissolved with distilled water in 100 mL of volumetric flask.

Procedure for dosage forms

Three types of furosemide pharmaceutical preparations were used from different companies. Two types were in the form of tablets. They were prepared by weighing 5 tablets after grinding and mixing them well. One tablet (contains 40 mg of furosemide) was dissolved in 5 ml of ethanol and quantity of distilled water, and then the solution was filtered using filter paper and the volume was completed to 100 ml with distilled water. The third type was in the form of a syringe, three injections of (Diasix 20 mg /2 mL Lincoln, Gujarat, India) were taken and 5 mL were withdrawn from it. After that, 5 ml of ethanol was added to it and placed in a volumetric flask of 50 mL capacity and supplemented to the mark with distilled water. Next, the sample solution of three types was prepared by diluting the required volume with

distilled water in 100 mL of volumetric flask to obtain a 100 µg/mL solution.

Results and Discussion

Study of optimum reaction conditions

Different conditions and their effects on the intensity of absorption colored solution were studied through the reaction of furosemide with pyrogallol in the aqueous solution.

Study the effect of base type and amount

The effect of different types of strong and weak bases, displayed in Figure 1, was studied by adding fixed quantities (0.5 mL) at a concentration (0.1 M) of each of them separately. Based on the results, it was noted that sodium carbonate is the best to give it the highest absorption intensity. Table 1 indicates that the use of 1 mL volume gave the highest absorption intensity. Therefore, it was used in the subsequent tests.

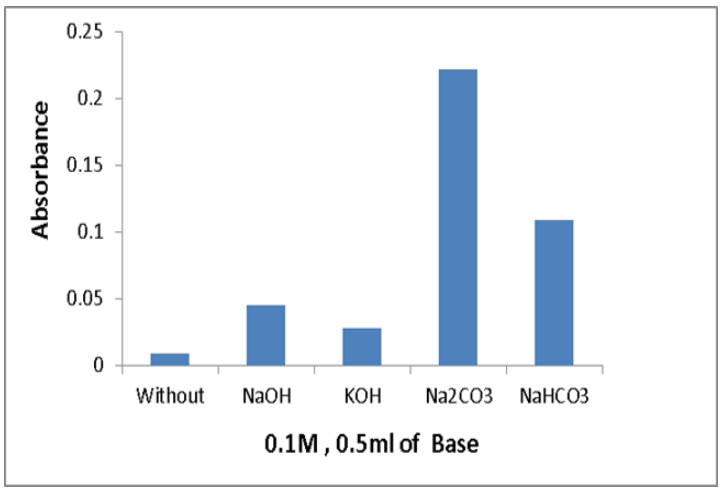


Figure 1: Types of base

Table 1: The amount of Na_2CO_3

mL of Na_2CO_3 (0.1 M)	0.25	0.5	1.0	1.5	2.0
Absorbance	0.090	0.223	0.291	0.243	0.195

Study the effect of buffer solutions

The pH of the solution was measured before this study and it was found as 4.8. Therefore, the types of different buffer solutions [27] with an acidic function of 4.8 were prepared and their effect on the absorption intensity was studied. Figure 2 illustrates that the use of buffer solutions leads to a decrease in the absorption that's why they were excluded in subsequent experiments.

Study the amount effect of pyrogallol reagent

The effect of the reagent quantity was studied by taking different volumes (0.25-2.5 mL) of pyrogallol (0.1%) and Table 2 demonstrates that 1 mL of the reagent solution was the best because it gave the highest absorption. Therefore, it was chosen in the subsequent tests.

Study the effect of surfactants

To show the surfactants effect on the absorption intensity of the formed product, different types of surfactants were selected (cetyltrimethylammonium bromide, cetylpyridinium chloride as cationic and sodium dodecyl sulfate as an anionic and non-ionic Triton X-100). It was clear from the results, shown in Figure 3, that their use had a negative effect on the absorption intensity of the product, and thus they were excluded in the subsequent studies.

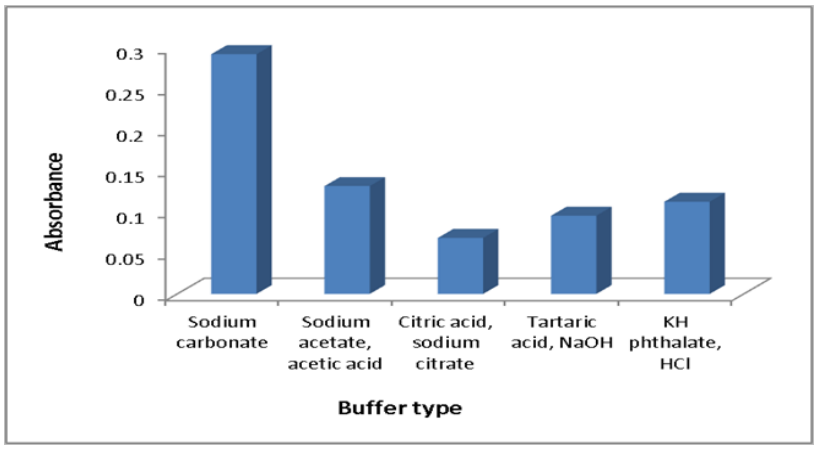


Figure 2: Buffer solution types

Table 2: The amount effect of pyrogallol

mL of pyrogallol solution (0.1%)	0.25	0.5	1.0	1.5	2.0	2.5
Absorbance	0.079	0.154	0.293	0.258	0.163	0.135

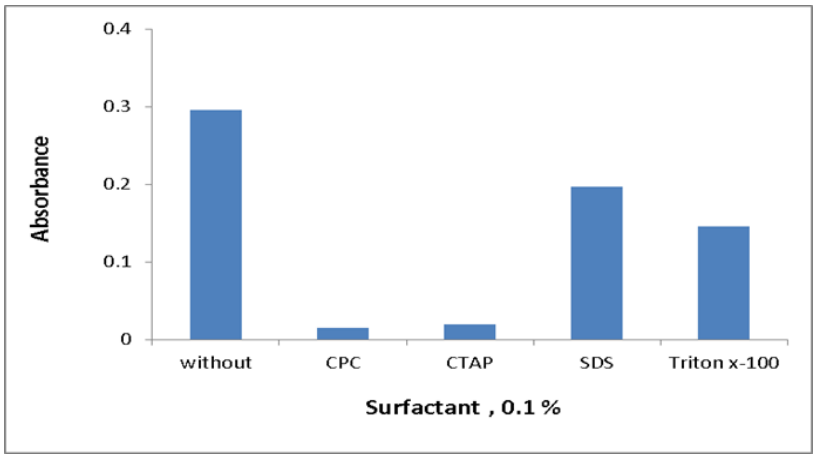


Figure 3: Effect of surfactant

Study the effect of addition sequence

The effect of different sequences was studied to choose the best sequence for the reactants. Based on Table 3, it was noted that the following sequence (S+B+R) gave the highest absorption intensity. Therefore, it continued to be adopted in the subsequent experiments.

Stability of reaction product

The time effect on the absorption of the colored solution was studied with different time periods, and the absorption was measured against the blank solution at 610 nm, as the stability of the colored product for three different amounts of furosemide was studied. The results indicate that the reaction takes place five minutes after completing the additions and is stable with the highest absorption for at least 50 min.

Final absorption spectrum

Under the previously established optimal conditions, the final absorption spectrum of the product formed by reacting furosemide in the presence of sodium carbonate with pyrogallol reagent was studied. The absorption spectrogram showed the highest absorption intensity of the product formed at the wavelength 610 nm, as represented in Figure 4.

Table 3: Order effect of addition

Addition Sequence	S+B+R	S+R+B	R+S+B	R+B+S	B+S+R	B+R+S
Absorbance	0.296	0.218	0.080	0.088	0.095	0.091

S=Furosemide; B=Na_2CO_3; R=Pyrogallol

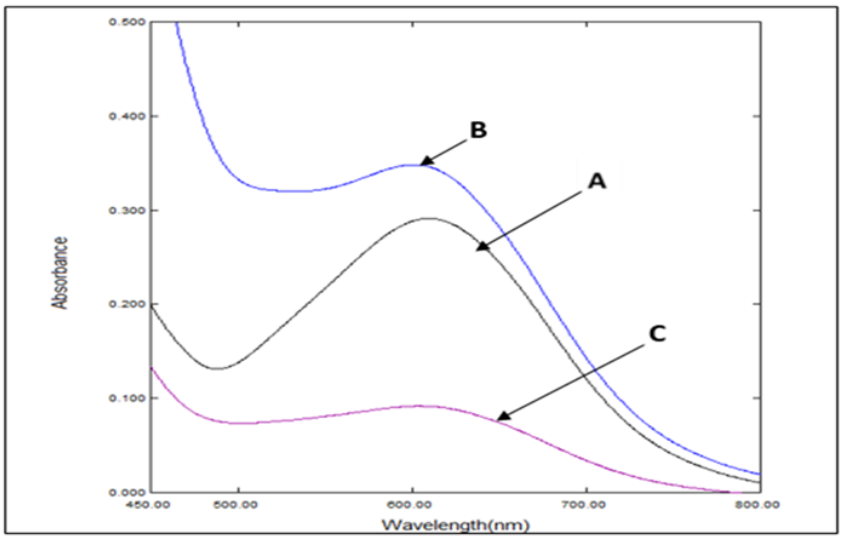


Figure 4: Final absorption spectrum of 100 µg of furosemide measured against (A) blank, (B) distilled water, and (C) blank measured against distilled water.

Approved working method and standard curve

After fixing the optimal conditions for furosemide determination, the standard curve for the working method was prepared as follows: The increasing volumes of 100 µg.ml^{-1} from concentration furosemide solution were added. Then, 1 mL of sodium carbonate at a concentration of (0.1 M) and 1 mL of pyrogallol solution at a concentration of (0.1%) were added and diluted with distilled water to 25 mL, and then the absorbance of the solutions was measured at 610 nm against the blank solution. Figure 5 represents the straight standard curve following Beer's law with concentrations ranging from 1.0 to 20 µg/mL. The value of the estimation factor indicates that the linear specifications of the standard curve are excellent. The molar absorptivity was 2.3813×10^4 l/mol/cm, Sandell's sensitivity was 0.01388 µg/cm^2, and also the values of the detection limit (LOD) and the limit of quantification (LOQ) for the method were 0.093 and 0.310 µg/mL, respectively [28].

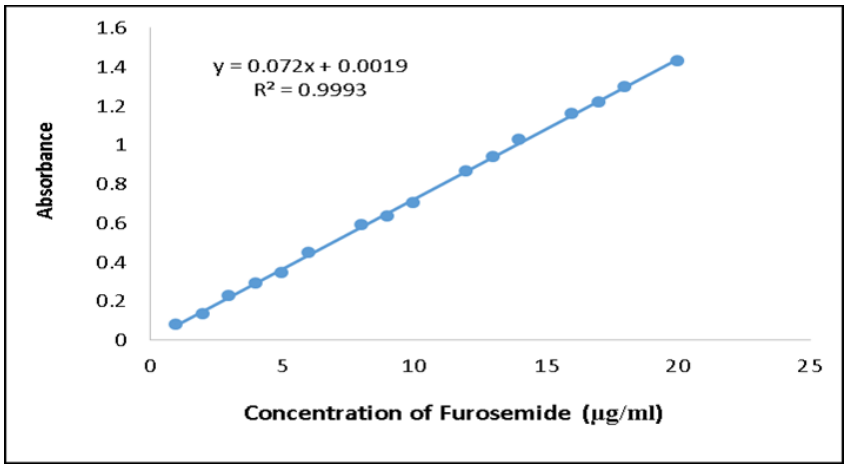


Figure 5: The standard curve of Furosemide

Accuracy and precision of the method

The accuracy and compatibility of the method were studied, where five replicates were measured for three different concentrations (16, 10, and 4) µg/mL of furosemide solution and treated using the approved method. Based on Table 4, it is clear that this method has good precision and accuracy.

Stoichiometric ratio of complex

The continuous changes "Job's method" [29] is used to find the interaction ratio between the drug compound furosemide and the reagent pyrogallol by following the method of work: A series of volumetric flask containing different volumes of solutions of the equal concentrations 3.023×10^{-4} M were prepared from each furosemide solution (0.5-4.5 mL) and the volumes were supplemented to 5.0 mL of the reagent solution, and then the other solutions were added under the optimal experimental conditions. The absorption of each sample was measured against its blank solution at 610 nm. Figure 6 displays the reaction ratio of furosemide with pyrogallol reagent is (1:1).

Accordingly, the proposed formula for the reaction of furosemide with pyrogallol is as shown in Figure 7 [12].

Interferences study

To examine the method's selectivity and its application for the pharmaceutical preparations, the effect of the presence of some interactions was studied on the furosemide estimation. The method depended on the addition of different amounts from (100, 500, and 1000) micrograms of the interfering materials to 25 mL volumetric flask containing 4 micrograms of furosemide. It was noted from Table 5 that the studied compounds do not affect the furosemide estimation using the suggested method.

Table 4: Method accuracy and precision

Conc. of Furosemide (µg/ml)	Recovery*(%)	R.E* (%)	RSD* (%)
4	99.86	-0.136	0.711±
10	99.74	-0.240	0.394±
16	100.27	0.274	0.185±

** Average of five measurements*

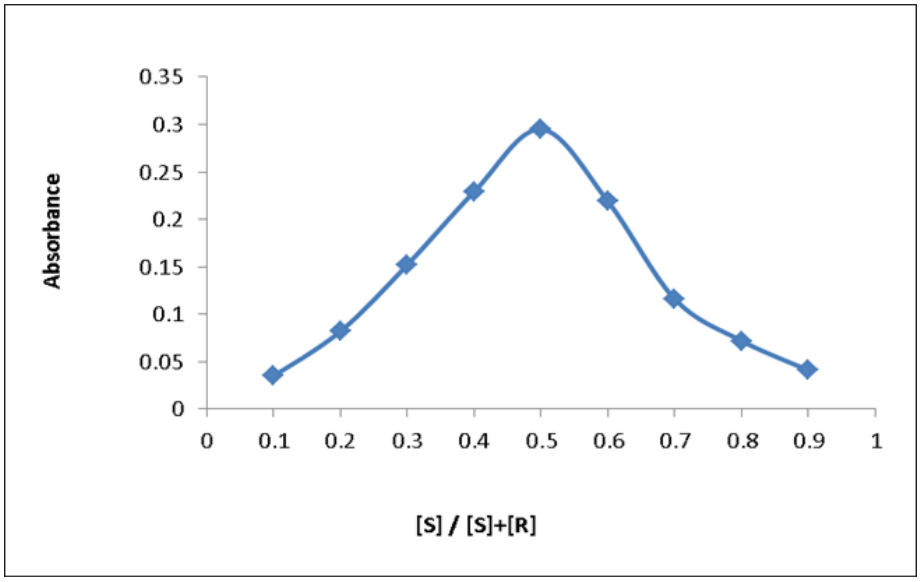


Figure 6: Continuous changes

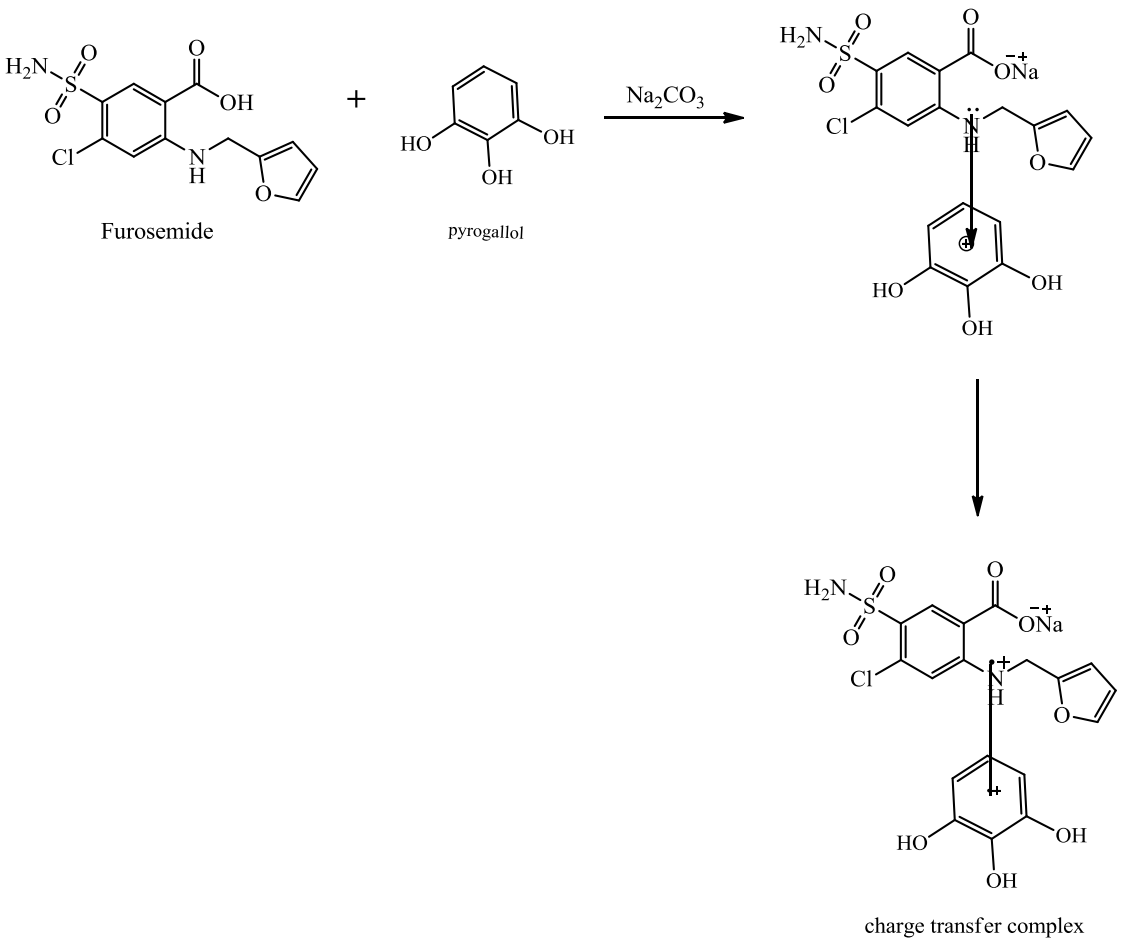


Figure 7: Mechanism of furosemide charge transfer complex formation reaction

Table 5: The effect of interferences

Foreign Compound	Recovery % of 100 µg of furosemide per µg foreign compound added		
	100	500	1000
Starch	101.02	99.31	98.97
Glucose	100.68	101.36	99.65
Fructose	99.31	97.60	100.34
Lactose	98.28	99.65	101.71
Sorbitol	97.26	98.63	96.91

Analytical application

The proposed method was applied to the pharmaceutical preparations of furosemide, which were in the form of tablets and injections, and from different origins, by taking three different concentrations of the solutions of the previously mentioned drug preparations. Table 6 represents the success of the suggested method for the furosemide determination in pharmaceutical preparations in the form of tablets and injections. The method had good accuracy and precision.

The proposed method was compared with the standard method approved [2] using t-test and F-test [30]. The results in Table 7 demonstrated that the calculated value of t-test and F-test for five degrees of freedom at a confidence level of 95%. This indicates that there is no variation between the suggested method and the method adopted in the literature, which illustrated the proposed method has a good application for different models of pharmaceutical preparations.

Table 6: Determination of furosemide in pharmaceutical formulations

Pharmaceutical preparation	Taken Amount (µg/mL)	Measured Amount (µg/mL)	Recovery* (%)	R.E* (%)	RSD*(%)	Drug content found (mg)	Values of t, F-tests*
Furosemide 40 mg\Tablets Actavis, Barnstape, EX32 8NS, UK	4	3.975	99.375	-0.625	1.212±	39.75	t= 0.71 F=3.14
Lasix 40 mg\Tablets Sonafi Winthrop Indusrie-France	4	03.93	98.25	-1.75	0.501±	39.30	t=1.98 F=3.42
Diasix 20 mg\ Injecting Lincoln,Gujarat-India	4	4.041	101.02	1.025	±1.163	20.205	t= 1.70 F=3.51

**Average of five determinations*

Table 7: Method's comparison

Analytical parameters	The suggested work	Literature Method [11]	Literature Method [12]
Types of reaction	Charge transfer	Charge transfer	Charge transfer (Method of C)
Reagent	pyrogallol	2, 3 –dichloro -5, 6-dicylano-1,4-benzoquinone)	Chromazurol S
λ_{max}(nm)	610	450	525
Range of Beer's law (µg.ml^{-1})	1.0-20	20-160	0.8-32
Molar the absorptivity (1.mol^{-1}.cm^{-1})	2.3813×10^4	2.0847	1.57×10^4
Correlation coefficient	0.9993	0.9997	0.9968
RSD (%)	±0.185 to ±0.711	0.20833	±0.2889 to ±0.2691
Color of the product	Blue	Reddish pink	Red
Applications	Pharmaceutical preparations	Pharmaceutical preparations	Pharmaceutical preparations

Method's Comparison

The suggested method has been compared with another spectroscopic method in the literature, and Table 7 indicates this comparison.

Conclusion

The proposed method is fast, easy, and accurate that has been developed for furosemide estimation. The method's principle depends on the reaction charge transfer complexation between furosemide as donor with pyrogallol

reagent as an acceptor in the presence of sodium carbonate form a product of greenish-blue color dissolved in the aqueous medium with the highest absorption at 610 nm. The method follows Beer's law for the range of concentrations 1-20 μg.mL^{-1}. The statistical results showed that this method has good accuracy and precision. The method has good sensitivity, as it does not need use the organic solvents, solvent extraction process, or does not require temperature control. The method succeeded in estimating furosemide in more than one drug.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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