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Azo Coupling Reaction for indirect Spectrophotometric Determination of Furosemide using Resorcinol as a Reagent

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Abstract. A simple and accurate method to determinate furosemide (FUR) based on converting the secondary amine to primary amine with acidic hydrolysis then azotization by nitrous acid and coupled with resorcinol as a coupling agent in aqueous medium at pH 13. The optical characteristic like beers law limit found to be (0.25-2.5) µg.ml^{-1}, detection and quantification limits (0.0196) (0.0654) µg.ml^{-1}respectivly and Sandel sensitivity was 0.006738 µg.cm^{-2}. The least-square method was used to evaluate the regression equation and the correlation coefficient. The resulted azo dye has a maximum absorbance at 430 nm with light orange color. The developed method was successfully applied to determinate FUR in its formulation with 84-105 % as a recovery with a relative standard deviation not more 2% and less than 5% relative error. To validate the proposed method, the standard addition method was applied to evaluate FUR in different formulation sources.

1. Introduction

Furosemide is the most commonly used diuretics with high-potency loops in medical care figure 1, it is an organic acid and highly protein-bound that enters the proximal, it is an organic acid and highly protein-bound that enters the proximal tubular epithelial cells and is secreted by the anion transporter into the tubular lumen inactive, free form. [1]. The Food and Drug Administration (FDA) has approved loop diuretics for the treatment of edema disorders associated with congestive heart failure, liver cirrhosis, and renal disease including the nephrotic syndrome.[2] Furosemide has been linked with an increased risk of readmission from heart failure [3-4]. Furosemide might be a second-line agent in patients with symptoms of heart failure, and in patients with advanced kidney disease with an estimated glomerular filtration rate, loop diuretics Furosemide preferred to treat hypertension by less than 30 ml-1 per minute over thiazide diuretics [5]. Furosemide has an average bioavailability of 50%, while bumetanide and torsemide are closer to 80, Furosemide has 1.5 to 2 hours as half-life, but in those with renal/hepatic dysfunction or heart failure, it can be up to 2.6 hours. [6-8] Furosemide significantly increased sodium and potassium excretion, and significantly increased plasma renin production and concentration of aldosterone. [9] Chronic heart failure (CHF) patients have decreased water excretion capability and increased aquaporin-2 urinary excretion (U-AQP2). The natural and diuretic effects of furosemide are antagonized by increased sodium and water reabsorption in the collection ducts [10] Various analytical methods have been reported for determining of FUR including high-performance liquid chromatography [11-13], electrochemical sensor technique [14-16], and spectrophotometric determination in the different matrix [17-18]. This study aims to develop a selective, simple, and sensitive method for the determination of FUR in various samples and pharmaceutical forms.

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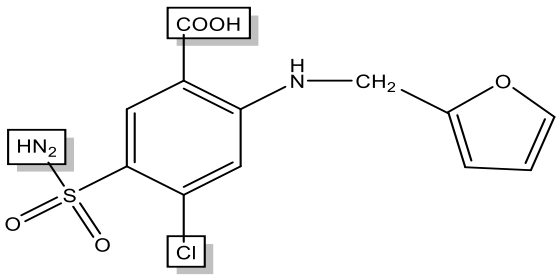


Figure 1 the chemical structure of furosemide

2. Experimental

2.1. Apparatus

This research was performed using Shimadzu 1800, Kyoto-Japan UV-Visible double beam spectrophotometer supplied by a 10 mm quartz cell.

3. Reagent and materials

3.1. Standard drug solution ($3.023x10^{-4}M$)

The standard solution of FUR drug was prepared form FUR pure substances supplied from the General Company for Pharmaceutical Industries - Samarra, Iraq by dissolving 0.05 g in 60 mL solution mixture of 10ml methanol, 10 ml concentrated hydrochloric acid, and 40 ml of distilled water. The solution was heated at 50 ^{o}C until its color changed to a light brown.

The solution was left to cool down, then transferred to a 100 ml volumetric flask and completed the volume to the mark, after which 40 ml of the prepared solution was transferred into a 200 ml volumetric flask and completed the volume to the mark with distilled water to obtain a solution at a concentration of 100 $\mu g.ml^{-1}$.

3.2. Resorcinol Reagent Solution ($2.73x10^{-3}$ M)

Prepared by dissolving 0.03g in a 100 mL flask by 5 ml methanol, then complete the volume to the mark with distilled water.

3.3. $NaNO_2$ solution ($1.45x10^{-3}M$)

Dissolve 0.01g of sodium nitrite in a small amount of distilled water and transfer it to a 100 ml volumetric flask and complete the volume to the mark.

3.4. Sodium hydroxide solution ≈ M 1

4 gm of NaOH was dissolved in a small amount of distilled water, then the solution was transferred to a 100 ml volumetric container and the volume was adjusted to the mark with distilled water.

3.5. Buffer solution

The buffer solutions were prepared by mixing 50 ml of 0.2 M KCl solution (prepared by dissolving 1.49g of KCl in 50 ml distilled water) and 172 ml of 0.2M NaOH (prepared by dissolving 2 g NaOH by distilled water in 250 ml volumetric flask) to obtain pH 13 solutions.

4. Recommended procedure

Different aliquant 0.025-0.25 ml of 100 $\mu g.ml^{-1}$ of a hydrolyzed drug form solution were transferred to 10 mL volumetric flask and 0.4 ml of $1.45x10^{-3}$ M $NaNO_2$ were added and placed in an ice bath for 10 minutes, after which 0.8ml of resorcinol reagent (2.73×10^{-3} M) was added and 1.3 ml of pH13 buffer solution, absorbance was measured at 430 nm wavelength against the blank solution.

4.1. Procedure for tablet 40 mg

10 tablets of the FUR were weighed accurately, grinding them well, a weight of 0.1589 g Lasix (SDI) Iraqi, 0.1829 g Furosemide, Actavis (England), 0.1615 g Lasix, Sanofi (French), and 0.2115 g Furosemide/Brawn (Indian) was taken equivalent to the weight of 0.04g of FUR to make a sample solution, other solutions can be prepared serial dilution to prepare 100 $\mu g.ml^{-1}$, Then applied the proposed method to the quantitative determination of 1 $\mu g.ml^{-1}$.

4.2. Procedure for ampule 20 mg

Five ampoules formulation each one containing 20 mg was taken from different sources as it showed in Table 4, 20 ml methanol and 15 ml of HCL were added and heated for 30 minutes at 50 °C. After that, the solution was transferred to a volumetric flask of 100 ml, make a serial dilution to prepare the working solution, and applied the procedure to determinate FUR in different three concentrations.

5. Result and discussion

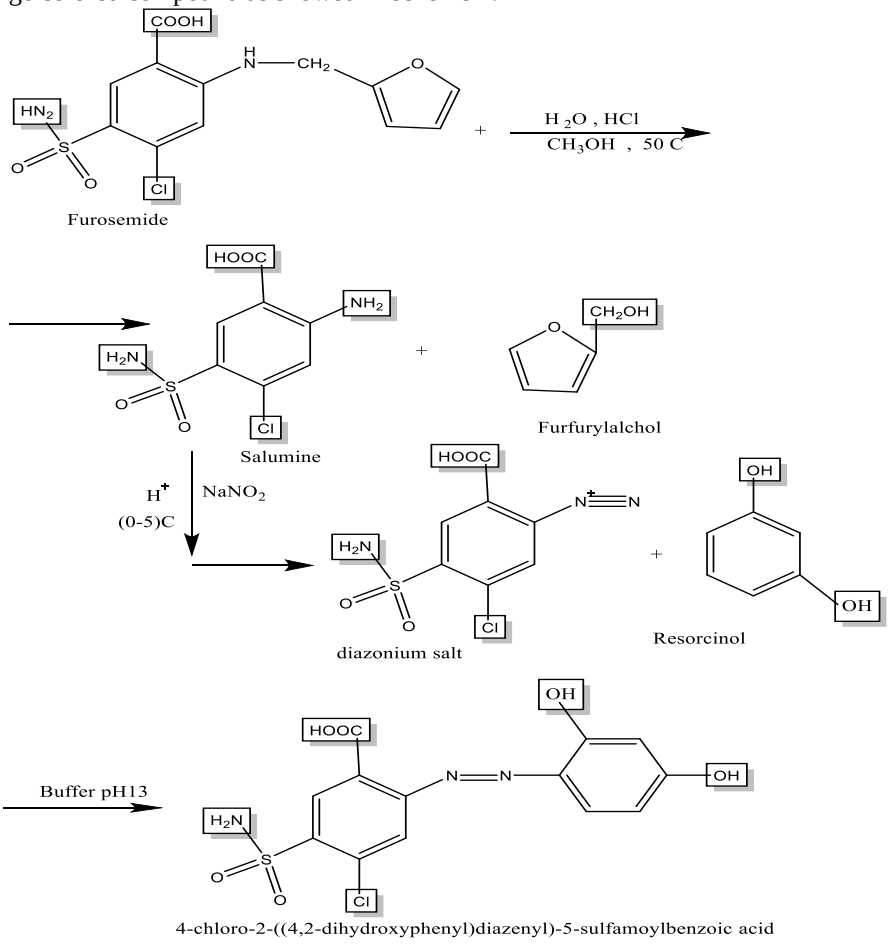
The optimum conditions for color development were performed by varying the parameters one at a time and fixing the others to observe the effect created on the absorption signals of the colored product. Preliminary analysis found that after diazotization in an alkaline medium the light orange substance had resulted in the treatment of FUR solution with resorcinol.

5.1. Absorption spectra

The primary test of the present method involved diazotization of hydrolyzed FUR with sodium nitrate then reacted with resorcinol in basic medium to form colored azo dyes. The absorbance and ⅄ max of azo dye were measured against the reagent blank figure 2. The complex shows maximum absorbance at 430 nm.

5.2. Chemistry of reaction

The acidic decomposition of furosemide breaks the secondary amine bond with the methylation group to form the furfural alcohol compound while converting the secondary amine to primary amine, which can be easily azotized with nitrous acid to form a diazonium salt, and coupled with resorcinol in alkaline media to form a light orange colored compound as showed in scheme 1.



Scheme 1: A proposed chemical mechanism for the reaction

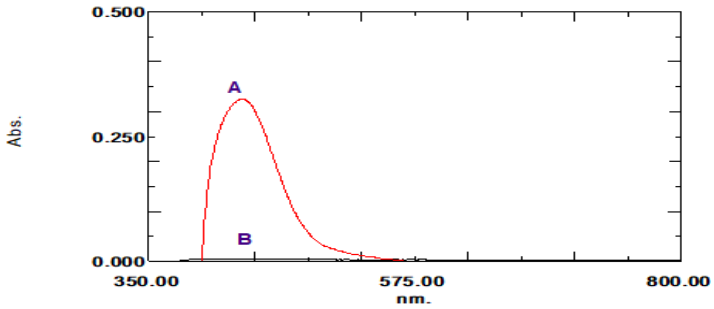


Figure 2. Absorption spectra of the colored complex for 2 µg.ml-1of FUR (A), against blank solution (B)

5.3. Effect of $NaNO_2$ concentration

The effect of $NaNO_2$ concentration on the absorbance of the colored complex was studied in the range of (0.1 -0.9) ml from$1.45x10^{-3}$ M $NaNO_2$, the maximum color intensity was achieved with o.4 ml (5.8×10^{-5}M). High concentration cause decreased intensity.

5.4. Effect of reagent concentration

The effect of adding different concentrations of resorcinol reagent for determinate 2 µg.ml-1 of FUR was investigated by choosing the best concentration that gives the maximum absorption intensity. The study showed that the concentration of 0.8 ml from $2.73x10^{-3}$ M resorcinol shows the best absorption increment so it was preferred for subsequent studies.

5.5. Buffer effect

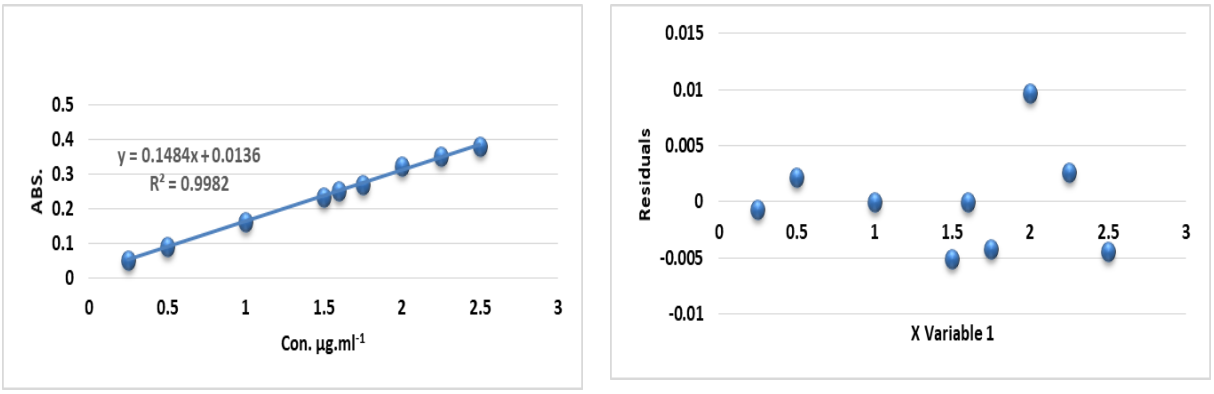
The colored compound was formed in basic conditions, hence we studied via a basic buffer solution, which was marked by the fact that the colored compound was more stable by using the buffer rather than the NaOH base alone. The effect of buffer solution was studded after determinate the best pH value for the best absorption value. Different volumes of the prepared buffer solution were studied to select the most appropriate volume, the study showed that the volume of 1.3 ml of buffer solution was the most appropriate to give the highest absorption intensity and therefore it was fixed for subsequent studies. The colored compound was formed in a basic environment, and therefore we experimented with using a buffer solution, which was distinguished by the fact that the colored compound was more stable when using the buffer than using the base NaOH only.

5.6. Effect of different solvent

The effects of different solvents on the absorption signal of the color product formed were studied using the following solvents (water (430 nm), methanol (423nm), ethanol (425 nm), acetone (423nm), and 2-propanol (425nm) the study showed that the best solvent is the water, which gives the highest absorption value.

6. Calibration curve and linearity

The optimal experimental conditions for the evaluation of the drug compound were applied, and a linear calibration curve was obtained in the concentration range 0.25-2.5 µg.ml^{-1}. Some optical properties of the formed colored compound were measured as shown in Table 1. Plotting the residual of the standard concentrations with absorption value figure 4b represent that the residual was distributed randomly or uniformly around the average of its values and equal to zero and random distribution around the average values, indicating that there are no systematic errors in the proposed working method.



(a) (b)

Figure 3: calibration curve (a) and residual value (b)

TABLE 1: Optical characterization and quantitative parameter of suggested study

Parameter	**value**
λmax (nm)	430
Colour	Light orange
Linear range (µg.ml^{-1})	0.25-2.5
Regression equation	y = 0.1484x + 0.0136
Slop	0.1484
Intercept	0.0136
Molar absorptivity (L.mol^{-1}.cm^{-1})	49083.5
Correlation coefficient	0.9982
Detection Limit (µg.ml^{-1})	0.0196
Quantitative limit(µg.ml^{-1})	0.0654
Sandell's sensitivity (µg.cm^{-2})	0.0067

7. Accuracy and precision

The accuracy and compatibility of the proposed method were studied by measuring both the relative error (RE) and the relative standard deviation (RSD). Five determinations for three different concentrations of the drug were selected, which are within a linear range. The RE did not exceed 5% while RSD, was within limits (≤ 2%) as shown in Table 2, which indicates the possibility of the assessment process with good accuracy and compatibility.

TABLE 2: precision and accuracy data

Furosemide. Conc. (µg.ml^{-1})		Relative Error %	RSD %
Taken	Found*		
1.6	1.65	3.12	1.49
2.1	2.15	2.38	0.23
2.4	2.37	-1.25	0.22

** Five replicate*

8. Stoichiometry study

Prepare equal concentration from FUR and resorcinol (3× 10^{-4}M) to study the stoichiometric ratio of the azotized drug compound and resorcinol reagent at pH 13 (figure 4), the molar ratios and continuous variation methods were applied for this purpose. The study showed that the ratio of the drug compound to the coupling reagent is 1: 1.

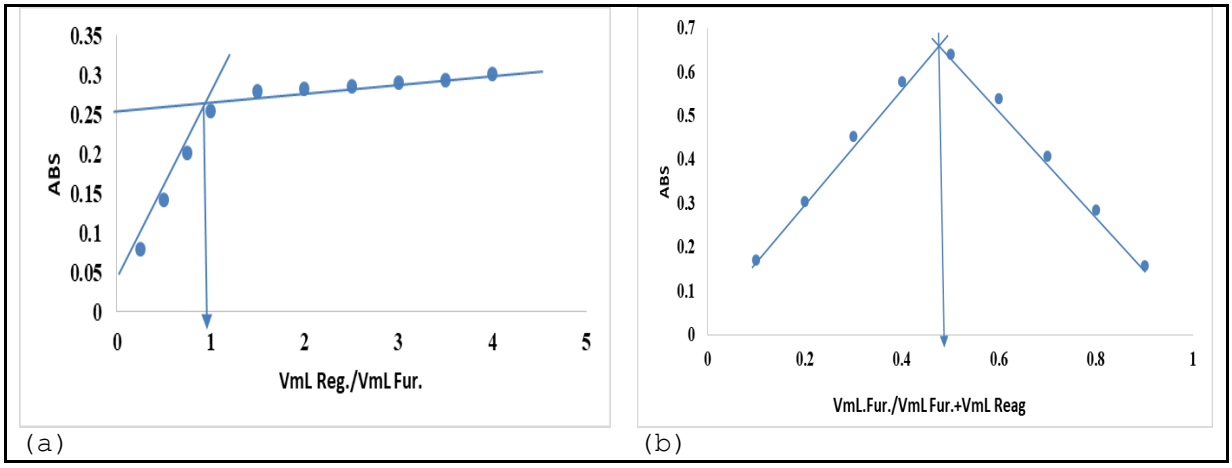
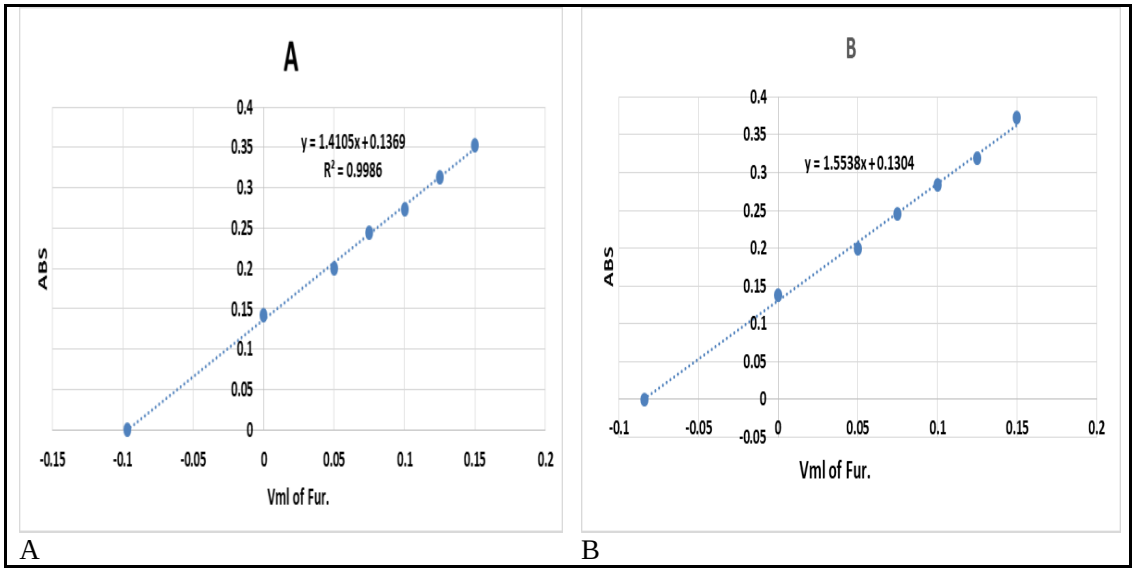


Figure 4: Stoichiometric study with mole ratio (a) Continuous variation (b)

9. Application in pharmaceutical formulation

9.1. Tablet 40mg

As shown in figure 5, the suggested method has been successfully applied to quantitative FUR produced by different companies using the standard addition methods. Table 3 shows the recovery value for determination 1 $\mu g.ml^{-1}$ FUR from different sources of pharmaceutical formulation.



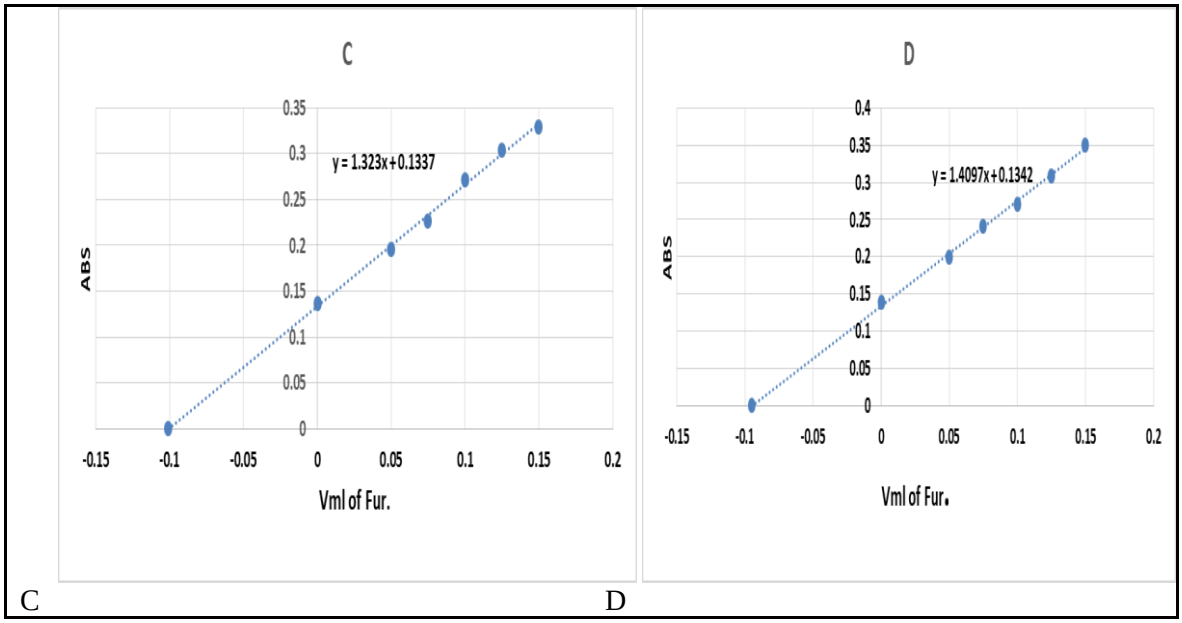


Figure 5: Determination of FUR in pharmaceutical preparations by the standard addition method (A) for the Lasix, Sanofi (French), B Lasix (SDI) Iraqi, (C) Furosemide/Brawn (Indian), (D) Actavis (England)

TABLE 3: Quantitative determination of 1µg.ml^{-1} of FUR from different origins

Drug	Company	Rec.%
Lasix, tab	SDI / Iraq	84.0
Lasix, tab	Sanofi / France	97.0
Apex, Tab	Ajanta / India	98.0
Furosemide, Tab	Actavis / England	105.0

9.2. Ampule 20mg

The approach has been tested with success to the quantitative determination with different three concentration of drug compound in its ampule form for different origins as indicated by the recoverability values in Table 4

TABLE 4: Quantitative determination of the different concentration of FUR from different origins

Pharmaceutical preparation	Labeled amount mg	Conc.taken* µg.ml^{-1}	Conc.found µg.ml^{-1}	Rec.%	RSD
Furosemide/brawn/India	20	1.6	1.39	86.87	0. 148
		0.66	0.54	81.81	0. 083
		0.33	0.28	84.84	0. 083
Lazine/Syria	20	1.6	1.54	96.25	0. 130
		0.66	0.65	98.48	0. 130
		0.33	0.31	93.93	0. 114
Lasix, Sanofi/France	20	1.6	1.70	106.25	0. 114
		0.66	0.65	98.48	0. 083
		0.33	0.36	109.09	0. 114
Lasixin, ncpc. China	20	1.6	1.47	91.87	4.500

		0.66	0.58	87.87	0.100
		0.33	0.29	87.87	0.054

** Five replicate*

10. Conclusion

The proposed method was characterized by its ease, speed, and accuracy in estimating the drug in an aqueous medium. The drug evaluation was possible when converting the secondary group of amine to a primary amine using the acid hydrolysis process with concentrated hydrochloric acid. The results showed good accuracy and compatibility when applied to estimate FUR in its pharmaceutical formulation.

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