Indirect Spectrophotometric Determination of Furosemide using Eriochrome Black-T Dye

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A simple, economical and sensitive indirect spectrophotometric method for the determination of furosemide in its pure form and in pharmaceutical preparations is described. The method is based on the oxidation of furosemide by permanganate in acidic medium, then the reaction of the remaining potassium permanganate to oxidize the dye Eriochrome Black-T at a wavelength of 535 nm. The method follows Beer's law within the range (0.25-10) μg mL^{-1} and the molar absorbance is 1.5×10^{4} L mol^{-1} cm^{-1}. The value of the determination coefficient was 0.9984, the recovery rate was 100.53% and the relative error value was 1.12%, while the value of the limit of detection (LOD) and limit of quantification was (LOQ) 0.118 and 0.395 μg mL^{-1} respectively. It was found that the method does not suffer from interactions, and the method can be applied to pharmaceutical preparations (ampoules and tablets).

Keywords: spectrophotometry, furosemide, oxidation, eriochrome black-T dye

Introduction

Furosemide is a diuretic used to treat fluid retention in the body caused by heart failure, liver scarring, or kidney disease. It can be used in cases of high blood pressure as well [1]. It is an antibacterial drug. However, the intense and rapid decomposition produced by this drug has led to the expansion of its application as a strong acid diuretic for various treatments in human and veterinary medicine [2]. It was first discovered in 1960 and quickly became known [3]. It has the chemical formula (4-chloro-n-furfuyl-5-sulfamyl-anthranilic acid) and its abbreviation is (FUR). Its molecular formula is $C_{12}H_{11}ClN_2O_5S$ and its molecular weight is 330.77 g/mol [4,5]. Furosemide is a white to slightly yellow crystalline powder, odorless and almost tasteless, slightly soluble in water, chloroform, and ether, Soluble in acetone, methanol, dimethylformamide and in alkali hydroxide solutions. Its melting point is 206°C, The pH of the aqueous solution is in the range of 8.9 to 9.3 [4,6]. Furosemide has potential interactions with these medications: aspirin, other salicylates, and other diuretics such as: ethacrynic acid and hydrochlorothiazide, and synergistic effects with antihypertensive agents such as: doxazosin [7], Analytical procedures for fluorosemide determination include spectroscopic methods [8-13]. Other methods are high-performance liquid chromatography (HPLC) [14-16], Flow injection [17], voltage measurement [18], Fluorometry [19-20], GC [21], TLC [22]. The present method describes a new simple, economical, accurate and sensitive spectrophotometric method for the determination of furosemide in pharmaceutical samples.

Eriochrome Black-T Dye: A crystalline solid, it is considered an azo dye and is found in the form of a black powder with a chemical formula of $C_{20}H_{12}N_3O_7SNa$, Its IUPAC name: Sodium 1-[1-hydroxynaphthylazo]-6-nitro-2-naphthol-4-sulfonate, and it is a soluble substance in water and insoluble in ethanol, acetone, and butanol. It is used as an indicator in the titration of complexes. This substance is carcinogenic, so it must be handled with caution. The color of the eriochrome Black-T solution is originally a dark blue color, and when it reacts with calcium or magnesium, it gives a complex that is red in color and turns at the end point into a blue color. It has the following chemical composition [24].

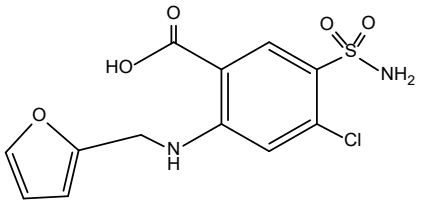

Fig.1. Chemical structure of furosemide.

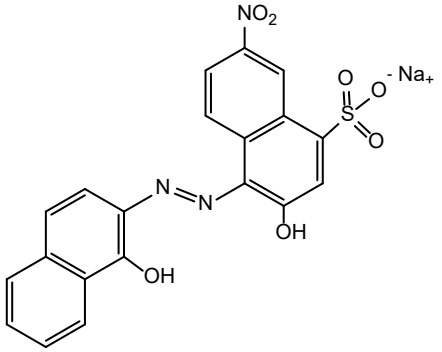

Fig.2. Chemical structure of Eriochrome Black-T dye.

Experimental part

Apparatus

Shimadzu UV-1800 Double-beam spectrophotometric was used for absorbance measurements with 1cm matched glass cells, use a sensitive balance type ae ADAM and use an elektro-mag water bath to perform the heating process.

Chemicals

Furosemide (100 μg mL^{-1}): Prepared by dissolving 0.010 g of pure furosemide in 2 mL of sodium hydroxide base (1M), stirring well until the substance is dissolved, then transferring it to a 100 mL volumetric flask and completing the volume with distilled water to the mark.

Eriochrome Black-T dye (200 μg mL^{-1}): The dye was prepared by dissolving 0.05 g of the pure substance in a small amount of water, stirring to ensure dissolution, then transferring it to a 250 mL volumetric flask and supplementing it with distilled water to the mark.

Potassium permanganate (1×10^{-3} M): Prepare the oxidizing agent by dissolving 0.0158 g in beaker, stirring well to ensure the substance is dissolved, then transferring it to a 100 mL volumetric flask and placing it in an opaque bottle. The other oxidizing agents were prepared with the same concentration.

Acids (1 M): hydrochloric, sulfuric, phosphoric, and acetic were prepared by taking the appropriate volume of each acid and supplementing it with distilled water in volumetric flask of 100 mL.

Analysis of furosemide tablets (40 mg): Five tablets of the pharmaceutical preparation were weighed and ground well, then the equivalent of the weight of one tablet was taken and dissolved with 2 mL of sodium hydroxide base, then the solution was filtered and the volume was added to 100 mL with distilled water to obtain a concentration of 400 μg mL^{-1}.

Analysis of furosemide injection (20 mg/2 mL): 2 mL of the injection content was withdrawn and diluted to 100 mL using distilled water to obtain a concentration of 200 μg mL^{-1}. Then a concentration of 100 μg mL^{-1} was prepared by withdrawing 50 mL^{-1} of the solution into a 100 mL volumetric flask and completing the volume by distilled water to the mark, then dilute the solution to obtain the required concentrations.

Results and discussions

The preliminary study

The absorption spectrum of the dye was studied at a concentration of 200 μg mL^{-1} and it was found that the dye gives the highest absorption at 535 nm, as in Fig.3. The dye standard curve was created in order to obtain the ideal amount of dye that gives the highest absorption. Therefore, increasing volumes of dye (0.1-3.0) mL were taken in 10 mL volumetric flask, and the method follows the beer's law at concentrations (2-60) μg mL^{-1}. Calibration curve for dye described by equation y = 0.0172x + 0.0313; R^2 = 0.9978.

Effect of the type of oxidizing

We used 2 mL of dye with a concentration of 200 μg mL^{-1}, adding 1 mL of various oxidizing agents prepared (1×10^{-3} M), then adding 1 mL of hydrochloric acid, diluting to the mark in 10 mL volumetric flask, and measuring the absorbance at a wavelength of 535 nm Table 1 shows that potassium permanganate is the best in shortening the dye.

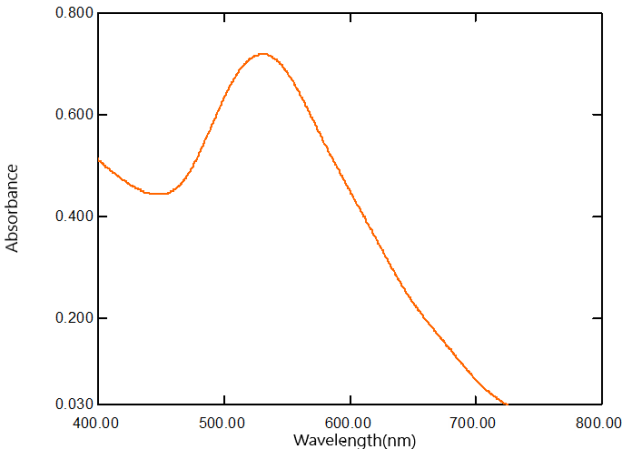

Fig.3. Absorption spectrum of Eriochrome Black-T dye (200 μg mL^{-1}).

Table 1. Different types of oxidizing agents.

Type of oxidizing agents 1×10^{-3} (M)	Absorbance
KIO_4	0.164
$KBrO_3$-KBr	0.119
$KMnO_4$	**0.045**
$CuSO_4.5H_2O$	0.582

Effect of volume of the oxidizing agent

Different and increasing volumes of potassium permanganate were taken, and the effect of volume on the solutions was observed, giving a maximum absorption of 1 mL. The solutions were measured at a wavelength of 535 nm. The results presented in Table 2.

Table 2. Different volumes of oxidizing agent.

Volume (mL) of $KMnO_4$ 1×10^{-3} (M)	Absorbance
-	0.513
0.1	0.426
0.25	0.368
0.5	0.138
0.75	0.085
1.0	**0.045**
1.25	0.047
1.5	0.048

Study the effect of acid type

In order to choose the appropriate type of acid to obtain the highest absorption, solutions were prepared containing equal concentrations of furosemide at a concentration of 100 μg mL^{-1}, then 1 mL each of hydrochloric, sulfuric, phosphoric and acetic acid was added, then the oxidizing agent was added and waited for 5 minutes, then the dye was added, and the solutions were diluted with water to the limit of the mark was measured in 10 mL volumetric flask. The absorption was measured at 535 nm for each solution against its blank solutions. Fig.4. shows the results obtained that the highest absorption was in the case of using phosphoric acid, and it was adopted in subsequent experiments.

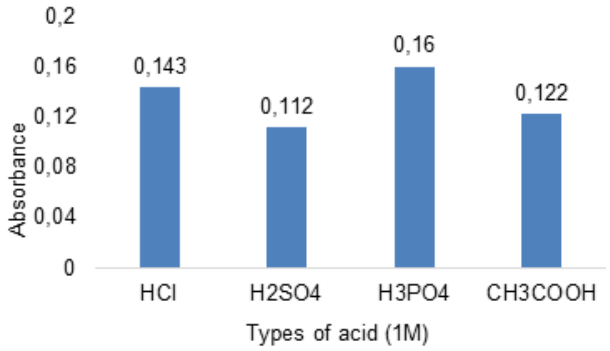

Fig. 4. Effect of different types of acids.

Study the effect of the amount of acid

Increasing volumes of phosphoric acid were added, ranging from 0-2 mL. It was noted that 1 mL given increased absorption, see Table 3.

Table 3. Effect of different amounts of acid.

Volume mL of H_3PO_4 (1M)	Absorbance
-	0.012
0.25	0.032
0.5	0.106
1.0	**0.163**
1.5	0.124
2.0	0.124

Study the effect of oxidation time

The effect of the time required to complete oxidation and the occurrence of shortening of the dye was studied during different times. It was noted in Table 4 that waiting 7 minutes after adding the oxidizing agent gives the best absorption.

Study the effect of temperature and stability time

The effect of different temperatures (30, 40, and 50 °C) on the dye shortening reaction was studied at different times and using the optimal conditions obtained from previous experiments. It is clear from Fig.5. that the highest absorption was at laboratory temperature and the sensitivity of the reaction decreased when the temperature was increased. A laboratory temperature of 30 °C was used in subsequent experiments.

Table 4. Effect of oxidation time.

Time (min)	Absorbance
1.0	0.062
3.0	0.105
5.0	0.160
7.0	**0.244**
10.0	0.229

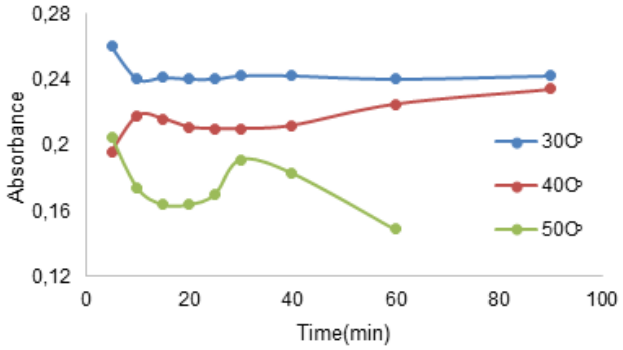

Fig.5. Effect of temperature on formation and stability time.

It can be seen from Fig.5 that the formation time of the product was 10 minutes, and its stability was for more than an hour at room temperature 30 °C.

Study the effect of addition sequence

The sequence of adding solutions to this reaction has a significant impact on the intensity of the color of the resulting compound. Therefore, a number of laboratory experiments were conducted with different addition sequences. It was noted from the results obtained in Table 5 that the order (I) has the highest absorbance. This order was followed in subsequent experiments.

Table 5. Effect of addition sequence.

Order of addition	No.	Absorbance.
D+A+O+EBT	I	0.242
D+O+A+EBT	II	0.048
EBT+D+O+A	III	0.015
EBT+A+O+D	IV	0.017

D = (Furosemide), A = Acid (H_3PO_4), O = Oxidizing agent ($KMnO_4$), EBT=dye (eriochrome black T).

Final absorption spectrum

After establishing the optimal conditions for the reaction, the absorption spectrum was taken as in Fig.6. The optimal conditions are summarized in Table 6.

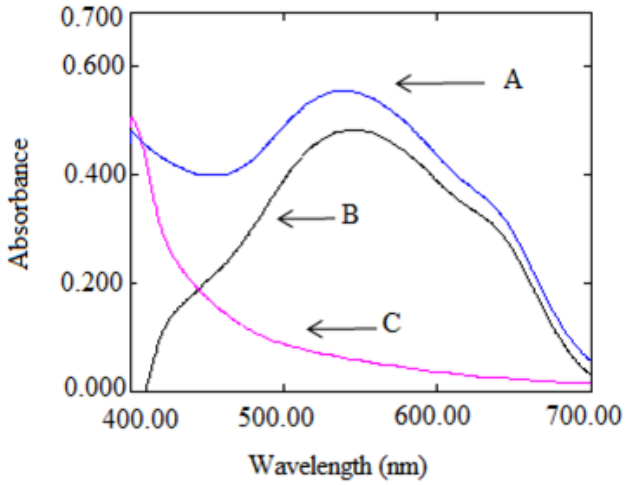

Fig. 6. The final absorption spectrum for the determination of furosemide; A: absorption spectrum of 7 μg mL^{-1} of furosemide against blank distilled water; B: absorption spectrum of 7 μg mL^{-1} of furosemide against blank solution; C: absorption spectrum of blank soiution against distilled water.

Table 6. Summary of optimal conditions.

Experimental condition	
λ, nm	535 nm
Color	Dark purple
Vol. of 1M H_3PO_4, mL	1
EBT (200 μg mL^{-1}) amount, mL	2
Conc. of $KMnO_4$ (M)	1×10^{-3}
Vol.of $KMnO_4$, mL)	1
Oxidizing time, min	7
Temp., °C	(30 °C)
Development time after dilution, min	10

Standard curve for determination of furosemide

Following the optimal conditions mentioned in Table 6, the standard curve was prepared for the determination of furosemide by taking increasing volumes (0.025-1.0) mL of furosemide solution with a concentration of 100 μg mL and 1 mL of phosphoric acid (1 M). Then adding 1 mL of potassium permanganate 1×10^{-3} M and left for 7 minutes, then 2 mL of dye with a concentration of 200 μg mL^{-1} is added and diluted with distilled water to the mark. The absorption intensity of the solutions is measured against the blank solution at a wavelength of 535 nm. The method follows the beer's law in the concentration range (0.25-10) μg mL^{-1}. Calibration curve for dye described by equation y = 0.046x + 0.0075; R^2 = 0.9987.

Study of accuracy and precision of the method

The accuracy and precision of the proposed method was studied by calculating the recall and relative standard deviation using five replicates for three different concentrations (1-7.5) μg mL^{-1} of furosemide. The results recorded in Table 7 show the accuracy and precision of the method.

Application of the proposed method to pharmaceutical preparations of furosemide

The proposed method was applied to the pharmaceutical preparations of furosemide, which were in the form of (tablets and ampoules), by taking three different concentrations of the mentioned pharmaceutical preparations. The solutions and work steps were applied according to the proposed method and in optimal conditions, and then the values of the relative error, the recovery rate, and the were calculated, and the results are shown. Table 8 shows the success of the method for determining furosemide in tablet and ampoule form.

Table 7. Study of accuracy and precision.

Compound	Amount added (μg mL^{-1})		Recovery (%)	Average Recovery (%)	RSD (%)
	Taken	Found			
Furosemide	1	0.98	98.00	100.53	1.31
	5	5.16	103.20		1.52
	7.5	7.53	100.40		0.54

Table 8. Application of furosemide in pharmaceutical preparations.

Pharmaceutical preparation	Certified value	Amount present(μg)		Drug content found (mg)	Recovery (%)*	Average Recovery (%)
		Taken	Found			
British Bristol Tablets	40 mg	1	0.95	38	95	97.11
		5	4.83	38.64	96.60	
		7.5	7.48	39.89	99.73	
Injection Syria (IBN HAYYAN PHARM)	20 mg/2mL	1	0.96	19.20	96	99.57
		5	5.07	20.28	101.4	
		7.5	7.60	20.26	101.33	

* Average of five determinations.

Application of the standard additive to pharmaceutical preparations

To prove the efficiency of the proposed method and its success in determination, the standard addition method was applied to the pharmaceutical preparations of furosemide in the form of tablets and ampoule, as shown in Fig. 7, 8, by taking concentrations of (1, 2) μg mL^{-1} of the tablets and ampoule solution.

Compare the proposed method with another method

The proposed method using Eriochrome Black-T dye was compared with the oxidative coupling reaction method using 2,4-dinitrophenylhydrazine in the same analytical variables for the determination of furosemide, as shown in Table 9.

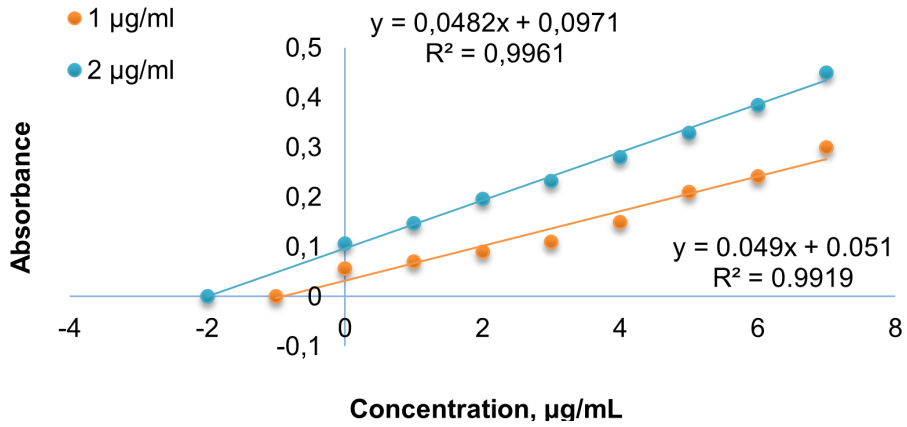

Fig. 7. Application of the standard addition to the pharmaceutical preparation (tablets).

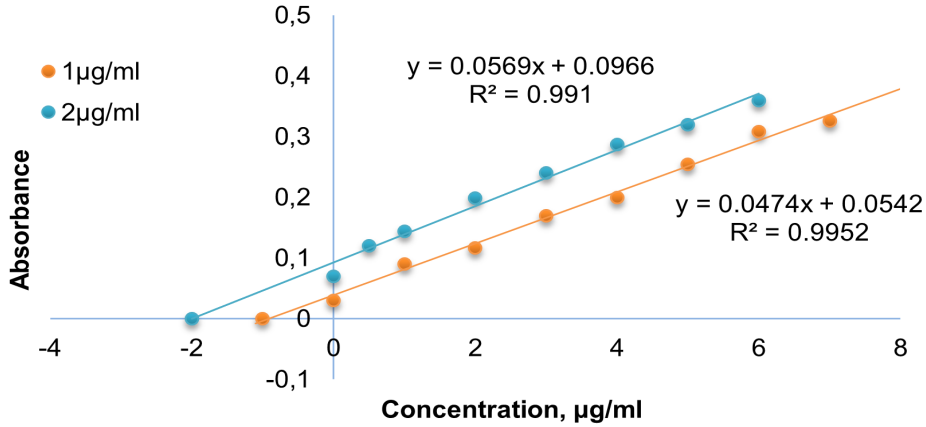

Fig. 8. Application of the standard addition to the pharmaceutical preparation (ampoule).

Table 9. Comparison of the proposed method with other spectroscopic methods.

Analytical parameters	Present method	Literature method		
		Oxidative coupling [25]	Diazotization [25]	Diazotization [26]
λ_{max}, nm	535	467	605	434
Reagent	Eriochrome black-T	2,4-Dinitrophenyl hydrazine	m-amino phenol	3,5-dimethyl phenol
Temperature, °C	30	25	20	-
Beer's law range, μg mL^{-1}	0.25-10	0.4-14	0.4-6	50-0.4
Colour of the product	Dark purple	Brown	Dark red	Orange
Molar absorptivity (L mol^{-1} cm^{-1})	1.5×10^4	1.184×10^4	1.0302×10^5	1.3899×10^4

Conclusion

Using the dye shortening reaction through oxidation with permanganate, which is important in an acidic medium, the drug compound was determinated in its pure form and in its pharmaceutical preparations in the form of tablets and ampoules, The results showed the validity of the method and the possibility of its determination. The method was sensitive, economical and simple.

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Author's statement

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Author's Contributions

Each of the individuals mentioned in the study contributed equally to the work and writing

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