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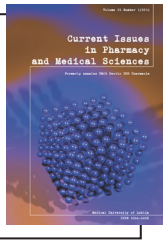

Modern kinetic spectrophotometric procedure for estimation of furosemide drug as bulk form and in pharmaceuticals preparations

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ABSTRACT

A simple, rapid, sensitive, inexpensive and easy to perform kinetic spectrophotometric procedure for the investigation of trace quantities of the drug, furosemide (FRO), as bulk and in the pharmaceutical preparations, has been improved upon. The enhanced method was depended on the fashioning of the Schiff's base by the reaction of the aldehyde group present in the 5-sulfo salicylaldehyde reagent, and the primary amino group present in furosemide. The latter acts as a ligand for the formation of an intense colored complex with Co(II) in an acidic medium, with maximum absorption at 608 nm. In the work, kinetic spectrophotometrics were established through the fixed time method. Moreover, Beer's law was applied on the range of concentration between 5-100 ppm, while the molar absorptivity and the Sandell sensitivity were 3.9295×104 $l.mol^{-1}cm^{-1}$, 0.008 $\mu g.cm^{-2}$, respectively. The detection limit (LOD) was 2.133 $\mu g/ml^{-1}$, and LOQ was 1.105 $\mu g/ml^{-1}$. Ideal circumstances for all colour improvement were seen, and the suggested procedure has been effectively employed in investigating amounts of furosemide (FRO) in bulk forms and in pharmaceutical preparations (tablets, injection sample). Additives and general excipient materials did not affect the studied method. A statistical comparison between the results that were obtained from the reference method gave good agreement.

INTRODUCTION

Furosemide is a sulphonamide derivative [1] widely used in medicine as a diuretic [1] of a potency similar to the thiazides, but more effective [5]. Preparations of FRO are either in injectable or tablet forms [6]. Furosemide's chemical name is 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl)amino)benzoic acid] (Fig. 1) [2]. It has the following generic names: Frusemide, Fursemide, Aisemide, Beronald, Desdimin, Lasilix, and others. The empirical formula is $C_{12}H_{11}ClN_2O_5S$, and it holds a molecular weight of 330.77. Furosemide is a white to slightly yellow, odorless, almost tasteless crystalline powder, slightly soluble in water, chloroform and ether [3], and soluble in acetone, methanol and dimethyl formamide [2], as well as in alkali hydroxides solutions [3]. Its melting point is about 210°C, with decomposition. In addition, it will crumble when exposed to air or light [4], and the pH of the aqueous solution is in the range 8.9 to 9.3. The UV spectrum of furosemide (0.01 mg/ml) in (0.1N) (NaOH), when scanned from 190 to 400 nm, using DMS solvent spectrophotometer, exhibits two maxima absorption at 226 and 272 nm [2].

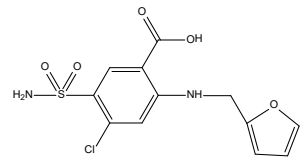

Figure 1. The chemical structure of Furosemide (FRO)

Most methods employed in investigating FRO are dependent upon HPLC [7-12]. However, a number of spectrophotometric-based approaches have been mentioned in literature [13,16], and for related drugs [17,18]. Simple kinetic spectrophotometric investigation is a new rapid-sensitive procedure for investigating trace quantities of FRO in bulk or pharmaceutical forms. This approach is dependent upon the Schiff's base formed by the primary amino group found

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in Furosemide and the aldehyde group found in the 5-sulfo salicylaldehyde reagent which acts as a ligand for the formation of an intense colored complex with Co(II) in an acidic medium. In our work, we investigated various kinds of pharmaceutical preparations and compared the results with standards. Such work revealed high accuracy and precision.

MATERIALS AND METHODS

Apparatus

- All absorbance and spectral estimations were performed on a double-beam applied UV-Visible 160 digital recording spectrometer (Japan).
- Heating-cooling water bath (Haake, Fe3).
- Analytical balance (Sartorius BL 210S).
- pH meter (Jenway 3020).

Material and reagents

The Chemicals which were used in the procedure had high degree of purity. Preparation of solutions were as followed:

1. Furosemide (FRO) (500 ppm): The pure substance was purchased from SDI (the State company for Drug Industries and Medical Appliances), Samara, Iraq. The 500 ppm standard concentration stock solution was given by dissolving 0.05 gm of pure bulk substance in 100 mL methanol as solvent, utilizing a volumetric flask. It was then transferred to a dark flask. In such conditions, the solution stayed stable for more than one month.
2. Ethanolic sulphuric acid solution (2%): Herein, 2 ml of 98% concentrated sulphuric acid (GCC) was added to a 100 ml volumetric flask, with the volume completed through augmentation with 99% Absolute ethanol (BDH Chemicals Ltd).
3. 5-Sulfo salicylaldehyde (5SSA) (0.01) M: The reagent was generated by dissolving 0.202 gm of pure material (BDH Chemicals Ltd, reagent Laboratory) in 100 ml of 99.0% methanol (BDH Chemicals Ltd).
4. Cobalt chloride $CoCl_2 \cdot 6H_2O$ (0.003) M: Purchased from BDH Chemicals Ltd, reagent Laboratory, given by dissolving 0.071 g of pure substance in 100 mL of deionized water.

Suggested Procedure

In a progression of 25 ml flasks, 5 ml of 5SSA Reagent (0.01 M) was added to equal volumes of the standard solutions with concentrations of (10-100) ppm, and the volume were completed to 15 ml through augmentation with ethanolic sulphuric acid solution. The flasks were then heated and mixed in a boiling water bath at room temperature for 50 min so as to formulate the Schiff base. The solutions were left for ten minutes to cool, and were subsequently assayed. In so-doing, 1.5 mL of Co(II) solution was added, and the volumes completed with ethanol. Absorbance was obtained at (360 nm), individually against an ethanol solvent as blank. After 10 min, green colour absorbance was obtained at 608 nm against a blank reagent. The quantity of FRO in the samples was ascertained from the calibration curve.

ASSAY PROCEDURE FOR FUROSEMIDE (FRO) IN PHARMACEUTICAL

Preparations

A number of preparations containing Furosemide (FRO) as ingredient active were analyzed. These are summarized in Table 1.

Table 1. Studied pharmaceutical preparations

Pharmaceutical preparation	Declared composition	Company
Furosemide Tablets(5)	Per tablet 40 mg furosemide	Actavis, Branstaple company (U.K.)
Lasix, furosemide tablets(5)	Per tablet 40 mg furosemide	Sanofi-Aventis Deutschland, Sanofi Winthorp Industrie company (France)
Furosemide Tablets BP (5)	Per tablet 40 mg furosemide	Bristol Laboratories, Berkhamsted, Herts company (U.K.)
Furosemide bosi injection	Per 2 mL: ampoule 20 mg furosemide	Sanofi-aventis Guildford, Surrey company (U.K.)

Tablets Procedure

Five tablets from each sample product were taken and weighed, then powdered. This was subsequently reweighed so as to gain 0.05 gm of FRO. The sample amount was added to a 100 ml calibrated flask and made up to the last volume through the addition of methanol solvent. The sample was then analyzed so as to generate a calibration curve.

Injection Procedure

In this procedure, 5 ml ampoules of the sample product, containing 0.05 gm of FRO, were moved into 100 ml volumetric flasks and made up to the last volume with methanol solvent. The sample was then analyzed so as to generate a calibration curve.

RESULTS AND DISCUSSION

Effect of 5SSA Reagent Concentration

The intent was to study the reagent effect of 5SSA concentration on the absorbance. To do so, 2 ml of 500 ppm Furosemide (FRO) was transferred into a sequence of 25 mL volumetric flasks containing varying volumes for 5SSA of 0.01 M (0.5-8 ml). The volumes were then completed to 15 ml by addition of ethanolic sulphuric acid solution. Heating and mixing occurred in a boiling water bath for 50 min. Subsequently to form the Schiff base, 1.5 mL of Co(II) solution was added, and the volumes completed by utilizing ethanol. Herein, the most suitable volume of reagent so as to obtain the best absorbance was 5 mL, and this was utilized in further research (Fig. 2).

Figure 2. The volume effect of 5SSA (0.01) M addition

Acid Effect

To ascertain whether the type of acid utilized in the experiment has an effect on absorbance in the final product, a number of acids at 2% concentration, were investigate HCl, CH_3COO , H_2SO_4 and HNO_3). Of these, sulphuric acid demonstrated the better absorbance effect, hence this was utilized (as ethanolic sulphuric acid) in all experimental work.

Effect of Cobalt chloride concentration:

The effect of different volumes (0.1-3 mL) of Cobalt chloride (0.003 M) on the formation of the complex was also studied. This part of the on-going work revealed an expansion in the absorbance of the complex up to 1.5 mL, after which point, the effect remained constant. Therefore, 1.5 mL of 0.003 M Co(II) solution was used for the determination of drug, since it gave high sensitivity and minimum reagent blank (Fig. 3).

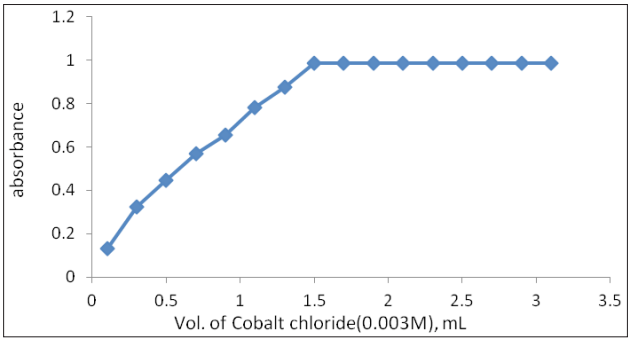

Figure 3. The volume effect of cobalt chloride (0.003 M)

Effect of temperature

The temperature effect was examined for the range of 20-120°C. This part of the study showed that the color intensity expanded with increase in temperature up to 25°C, after which, minimal improvement was seen. In this work, the highest absorbance was at 100°C, however, room temperature produced a sufficient colour intensity for the experimental work, hence, to simplify the experimental work, the boiling bath temperature, utilized to obtain the Schiff base formation, was set to room temperature.

Effect of addition sequence

To ascertain whether difference in the order of reagent addition had an effect on the outcome of the work, the experiment was repeated with the sequence of addition varied (Table 2). The results of this work indicate that (No. 1): FRO (drug) – 5SSA (reagent) – ethanolic sulphuric acid (acid) – Co(II) solution (M) gave the highest intensity and was used in the subsequent experiments (Table 2).

Table 2. Effect of addition sequencer on absorbance

No.	Order of addition	Abs.
1	D+R+A+M	0.788
2	R+M+D+A	0.566
3	M+D+A+R	0.493
4	A+R+M+D	0.202

(where D=Drug, R=Reagent, M=Co(II), A=Acid)

Effect of time of Schiff base reaction completion

To ascertain the effect of time of completion for generation of the Schiff base reaction, a series of time trials were undertaken. Of these, the best effect (at room temperature) was achieved at 50 minutes of boiling bath immersion. This time was utilized in all further experimental work.

Reaction Time Effect

In this part of the work, it was seen that the colour intensity reached a peak and stabilized (over a 24 hour period) when the drug (FRO) responded with the Schiff base and Co(II) solution after 10 minutes of procedural time. This exposure interval was then utilized in all further experimentation.

Absorption Spectra

A variety of wave lengths were tested so as to achieve the best spectral effect for the colour reaction resulting from the FRO-Schiff base and Co(II) solution. This was first tested against a blank solution of ethanol solvent. Such work found the maximum absorption to occur at 608 nm (Fig. 4).

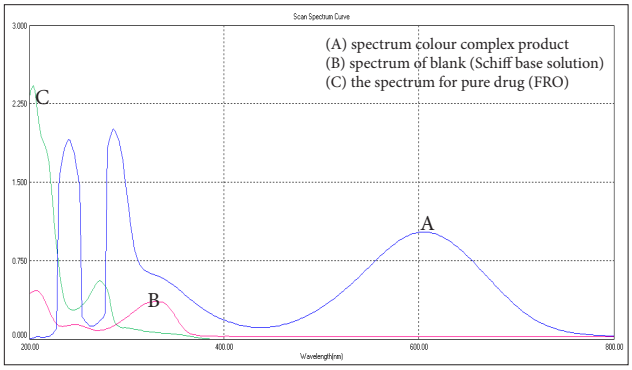

Figure 4. The spectra of colour complex formed by the reaction between the FRO-Schiff base and Co(II) solution against blank (Schiff base solution). Herein, the absorption was maximum at 608 nm, where (A) - The colour post-reaction (40 ppm FRO); (B) - Blank (Schiff base solution alone); (C) - Pure drug (FRO)

The Kinetic study and Calibration curve

In this procedure, to ascertain the Fixed Time Kinetic Spectrophotometric procedure absorbance for the reaction solutions with varying quantities of FRO, pre-choice fixed time was set at intervals of 2 min. The absorbance between the times t_1 (2 min) and t_2 (4, 6, 8, 10, 12, 14, 16 and 18 min) was recorded and plotted against the drug concentration. The corresponding linear regression equations with values of r^2 are summarized in Table 3. The result of such work revealed that the slope expands by the time, and the most satisfactory values of r^2 and the intercept were achieved at 10 min (Table 3).

Utilizing the circumstances depicted in the strategy, a linear calibration curve was acquired for Furosemide (FRO) (Fig. 5). In this, it is evident that Beer's Law is obeyed for concentrations between 5-100 ppm. Other Spectral and Statistical information for the investigation of FRO by way of the purposed procedure are summarized in Table 4.

Table 3. Regression equations for the considered drugs of various concentrations at various time intervals, using the fixed time procedure

Reaction time (min)	Linear range (ppm)	Regression equation	r^2
2	12-50	y = 0.0076x+0.321	0.9912
4	10-50	y = 0.0105x+0.211	0.9933
6	10-60	y = 0.1154x+0.123	0.9943
8	5-80	y = 0.1179x+0.093	0.9986
10	5-100	y = 0.1188x+0.071	0.9994
12	5-100	y = 0.1191x+0.128	0.9989
14	5-90	y = 0.1173x+0.099	0.9980
16	5-70	y = 0.1145x+0.137	0.9977
18	5-60	y = 0.1112x+0.154	0.9969

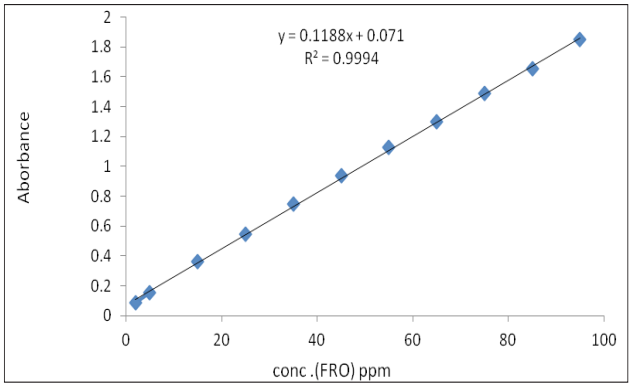

Figure 5. The FRO Calibration curve

Table 4. Analytical data for the time fixed procedure of the kinetic spectrophotometry for furosemide

Parameter	Value
λ_{max}, nm	608
Correlation coefficient, r^2	0.9994
Slope(b)	0.0188
The Molar absorptivity (L.mol^{-1}.cm^{-1})	3.9295×10^4
The law of Beer limits (µg/ml)	(5-100)
The sensitivity of Sandell (µg.cm^{-2})	0.008
Intercept (a)	0.071
The detection Limit (LOD) (µg/ml^{-1})	1.105
The quantification Limit (LOQ) (µg/ml^{-1})	2.336

Precision and Accuracy

The precision and accuracy for the studied kinetic Spectrophotometric procedure were investigated at three various FRO concentrations. The experiment was repeated five times. Percentage relative error (E%) as accuracy and Percentage relative standard deviation (RSD%) as precision with regard to the proposed procedure were computed (Table 5). The resulting low values of RSD%. and low values of E% evidence good accuracy and precision. These results demonstrate the repeatability and reproducibility of the proposed method.

Table 5. The precision and Accuracy of the studied method

Conc. of FRO ppm present	Conc. of FRO ppm Found	% Error	% Recovery	% R.S.D
10.00	9.85	- 1.50	98.50	0.89
40.00	39.88	- 0.30	99.70	0.56
80.00	80.15	0.18	100.18	0.21

Stoicheiometry of reaction

Literature states that the best Schiff base formation holds a FRO/5SSA mole ratio of 1:1. Such ratio forms a new ligand having low absorbance. However, the absorbance sensitivity, as seen in this experimental work, can be increased by further reaction with Co(II) so as to give an intense colored complex. The stoicheiometry of the reaction of FRO with 5SSA and Co(II) was performed by utilizing the mole ratio method, as well as the method suggested by Job. The methods were applied by utilizing 1×10^{-3} M solution of the drug, the reagent and Co(II) salt to determine the stoicheiometry of the complex product. Subsequently, the absorbance of the solutions were measured at maximum wave length of the colour product (608 nm) (Fig. 4). Results of this work reveal that a 2:1 ration of new ligand (Drug with 5SSA:Co(II) complex was formed at 608 nm. This was ethanol soluble. The reaction mechanism schematics based on the above reaction is shown in Figure 6.

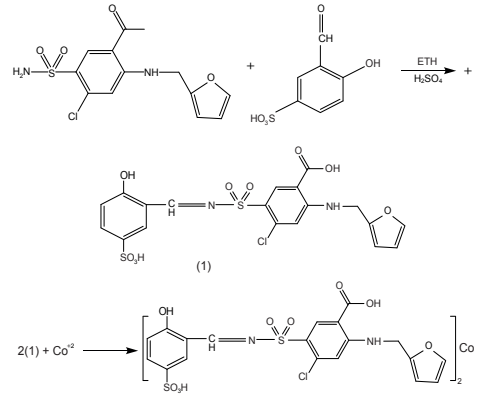

Figure 6. Probable reaction pathway for the formation of complexes of FRO with 5SSA and Co(II)

In the aforementioned work, the stability constant for the product complex was ascertained by measuring the absorbance of a solution which included the stoicheiometric measure of the new ligand (FRO with 5SSA and Co(II), against that of solution at 1 ml of 2×10^{-3} M and Co(II) ion at five times concentration. The calculated average stability constant for the colour result in ethanol under the characterized experimental circumstances was 2.38×10^{6} l^{2}·mol^{-2}.

Effect of solvent

The type of solvent used to dissolve the drug substance employed affects both the wavelength and intensity of maximum absorption. As evidenced in Table 6, methanol was seen to be the most effective solvent, giving very high intensity of maximum absorption when 5SSA is employed as the aromatic aldehyde. Methanol is also a decent solvent from the point perspective of economy and sensitivity.

Table 6. Spectrophotometric attributes of the colored product in different organic solvents

Solvent	λ_{max}, nm	ε, L.mol^{-1}.cm^{-1}
Acetone	580	3.176×10^3
Chloroform	530	1.3368×10^3
2-propanol	436	8.4271×10^2
Acetic acid	345	1.8710×10^2
Dimethyl sulphoxide	570	2.9010×10^2
CCl_4	510	Two layers
Dioxine	480	Two layers
Dimethyl formamide	510	1.0320×10^3
Ethanol	560	6.1010×10^3
Benzene	530	2.3210×10^2
Methanol	608	3.9295×10^4
Teri butyl alcohol	555	Two layers
Formic acid	430	2.8733×10^2
Pyridine	360	Turbid
Di ethyl ethe	420	1.8761×10^2

Interferences

We also assessed the specificity of the suggested kinetic spectrophotometric procedure (a fixed time procedure) for investigating FRO drugs in situations allied with common excipients such as talc, lactose, acacia, starch, glucose, sucrose, polyvinyl-pirrolidone (PVP), magnesium stearate and aspartate. This procedure was independently tested utilizing ten-times concentrations, with the designated amounts of FRO and excipients suggested by the discovered calibration curve (2 ml). Hence, 2 ml of 100 ppm FRO and 2 ml of every excipients was utilized upon dilution to 25 ml, at an error of max ± 2% with respect to the normal. In such work, after three independent runs, no obstructions were indicated (Table 7).

Table 7. Excipient effect at 400 ppm on the recovery of FRO at 40 ppm, utilizing the fixed time method

Interference	% Error	% Recovery
Talc	- 2.150	97.850
Lactose	+ 2.140	102.140
Acacia	+ 1.150	101.150
Starch	- 2.660	97.340
Glucose	- 4.100	95.900
Sucrose	+ 3.150	103.150
Magnesium stearate	- 2.200	97.800
Aspartate	+ 1.110	101.110
PVP	- 3.450	96.550

Procedure application

The procedure set out previously was subsequently applied for the assay of common FRO pharmaceutical preparations (Table 8).

Table 8. Furosemide as pure substance and in various dosage forms

Pharmaceutical preparations including FRO	Average recovery %	
	Proposed procedure	Standard procedure (4)
Pure FRO	98.410	98.230
Furosemide tablets (40) mg (FRO)	100.360	99.660
Lasix furosemide tablets (40)mg (FRO)	100.420	100.170
Furosemide tablets BP (40) mg (FRO)	101.220	99.450
Furosemide bosi injection (20) mg (FRO)	98.860	98.340

In this work, three independent determinations were made. The utilized strategy was that set out in British Pharmacopeia – 2009. The outcomes were replicable and the determination procedure of formulations was through Standard Practice.

CONCLUSION

The presented spectrophotometric procedure was rapid, easy, sensitive and precise. Moreover, it is relevant for the investigation of FRO in tablet and injectable forms. The proposed procedure is also independent of basic exploratory conditions, and the reagents utilized are freely available. These points of interest empower the utilization of the presented techniques in the quality routine control investigation of Furosemide (FRO) in pharmaceutical preparations.

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