Estimation of Furosemide Spectrophotometrically in Pharmaceutical preparations by Oxidative Coupling Reaction

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method was described for the determination of Furosemide (FUR). The method is based on the oxidative coupling reaction between Furosemide and 1-Naphthylamine-4-sulfonic acid in the presence of potassium permanganate to form a yellow colored product with maximum absorptions at 465nm, which is soluble in water. Beer's law is obeyed in the concentration range of 3 to 23㎍.ml⁻¹ with a molar absorptivity of 1.065×10⁴ L.mol⁻¹.cm⁻¹, and Sandell's sensitivity of 0.0310㎍.cm²⁻¹, respectively. The correlation coefficient of 0.9994, with recovery average % of 99.948. Limit of detection (LOD), limit of quantification (LOQ) of 0.971㎍.ml⁻¹ and 3.237 ㎍.ml⁻¹ and a relative standard deviation (RSD) % of 0.641 to 0.812. The proposed method has been used to successfully determine furosemide in pharmaceutical formulations (Tablet).

1. Introduction

Furosemide is a loop diuretic that is used to treat fluid retention in the body caused by heart failure, liver scarring, or renal illness, as well as to treat high blood pressure [1]. (FUR) is an abbreviation for furosemide, which is also known as frusemide [2]. In 1959, furosemide was patented, and in 1964, it was licensed for medicinal usage [3]. There are several chemical names for furosemide [5-(aminosulfonyl)-4-chloro-2-[(2-furylmethyl) amino] benzoic acid, 4-chloro-N-furfuryl-5-sulfamoylanthralnic acid, 4-chloro-N-(2-Furylethyl-5-sulfamoylanthranilic acid, 4-chloro-2-furfurylamino-5- sulphamoyl benzoic acid] (See Fig. 1). Chemical formula is C₁₂H₁₁ClN₂O₅S and molecular weight is 330.745 g/mole it's a low soluble in water, chloroform, and ether but it is soluble in acetone, methanol, and dimethyl formamide [4]. FUR is a crystalline powder that is white to slightly yellow in color and has a melting point of 206°C [5]. Furosemide has potential interactions with these medications: Aspirin and other salicylates, other diuretics (e.g.ethacrynic Acid, hydrochlorothiazide), Synergistic effects with other antihypertensives (e.g.doxazosin), and Sucralfate [6]. For the determination of furosemide, various analytical approaches have been used, spectrophotometry [7-10], HPLC [11-14], and other methods [15-20].

Fig.1: Chemical Structure of Furosemide

The purpose of this study is to develop and validate a simple, sensitive, and specific spectrophotometric method for estimating furosemide in pharmaceutical formulations.

2. Experimental Part

2.1. Instrumentation Used:
Spectrophotometric measurements were made using UV-visible double beam a type (T92+ Spectrophotometer, China), with using 1cm of matching quartz cells.
2.2. Materials and Solution of the Used: 
The substances employed in this study were all extremely pure equipped by my company (Fluka, bdh, SDI), and throughout the tests, methanol and distilled water were employed as solvents to preparing solutions. 

**Furosemide standard solution (1000 µg.ml⁻¹)**
It was prepared by dissolving 0.1000 g of furosemide powder in an amount of methanol and then completed the volume to the mark in a 100 ml volumetric flask, and Concentration 250µg.ml⁻¹ was prepared by taking 25 ml of the standard solution (1000 µg.ml⁻¹) and easing it in a volumetric flask of 100 ml and filled the volume to the mark with the distilled water.

**Potassium hydroxide of Solution (0.1 Molar)**
It was prepared by dissolving 0.561g of the substance in a little distilled water and completed the volume to the mark with the same solvent in a volumetric flask of 100 ml.

**Solution of potassium permanganate (1x10⁻² Molar)**
It was prepared by dissolving 0.158g of potassium permanganate in an amount of distilled water (D.W) and filled the volume to the mark with the same solvent in a volumetric flask of 100 ml.

**Solution of 1-Naphthylamine-4-sulfonic acid (2x10⁻⁵ Molar)**
It was prepared by dissolving 0.446 g of the reagent in an amount of distilled water (D.W), and then filled the volume to the mark with the same solvent in a volumetric flask of 100 ml capacity.

**Solution of pharmaceutical preparation (Tablets)**
Ten tablets are weighed (40 mg/ Tablet by company SDI), and the grains are crushed well, then a certain weight of the powder is taken, which is equivalent to 0.1g of furosemide, depending on the type of tablets used, and it is dissolved in a little of methanol and then filtered to separate the insoluble components, if any, then transferred to a volumetric flask of 100 ml capacity, and completed the volume to the mark with distilled water and then 25 ml is taken of this solution and transferred to a volumetric flask of capacity of 100 ml and filled the volume to the mark with distilled water to obtain a solution of 250 µg.ml⁻¹.

**The method's general principle**
Principle is the coupling of the reagent 1-naphthylamine-4-sulfonic acid with the drug furosemide and in the presence of the oxidizing agent potassium permanganate in an alkaline medium, a solution with a yellow color is formed, which gave the highest wavelength at 465 nm versus the blank solution.

3. Results and discussion
3.1. Preliminary study

* Studying the absorption spectrum of the drug only
The absorption spectrum of the 250 µg.ml⁻¹ of drug was recorded against the methanol as a reference. This was done by transferring 2 ml of the 250 µg.ml⁻¹ solution into a 25 ml volumetric flask and then filled the volume with methanol to the mark. The absorption spectrum was taken as in Figure (2), which shows that the drug gives absorption at the wavelength of 340 nm.

![Absorption spectrum of furosemide versus methanol](image)

* Studying the absorption spectrum of the drug with the reagent 1-Naphthylamine-4-sulfonic acid
Where 2 ml of a solution of the drug with a concentration of 250 µg.ml⁻¹ was mixed with 2 ml of a reagent solution of 2 x 10⁻⁵ M, 1 ml of potassium permanganate solution of 1 x 10⁻² M, and 1 ml of a solution of potassium hydroxide of 0.1 M in a volumetric flask with a capacity of 25 ml, then filled the volume to the mark with distilled water with shaking to homogenize the solutions. The absorption spectrum of the resulting yellow solution, was measured against the versus blank solution as a reference to obtain the wavelength and the highest value for the absorption was given at the wavelength of 465 nm, as in Figure (3).
the colored product formed at, as different volumes (0.3 ml of each solution were taken from the used (1.3–3) ml reagent with a concentration of 1×10−2 M, 2 ml of the furosemide solution were used and it was found that adding 2 ml of the reagent was the best to give it the highest absorption. Results are shown in the Table (2).

### 3.2. Choosing the best reagent

1 ml of each solution were taken from the used reagents, 2 ml of furosemide solution at a concentration of 250 µg.ml⁻¹, 1 ml of potassium permanganate solution as an oxidizing agent, and 1 ml of potassium hydroxide solution of 0.1 ml were used and it was found that 1 ml of potassium permanganate solution is the best which gives highest absorption. Results are in the Table (3).

### 3.3. Volume impact of the coupling reagent:

Impact of the volume of reagent 1-Naphthylamine-4-sulfonic acid on the intensity of absorption was studied. Where a series of volumes were taken from the used (1.3–3) ml reagent with a concentration of 2×10⁻² M, 2 ml of the furosemide solution of 250 µg.ml⁻¹, 1 ml of the oxidizing agent solution, and 1 ml of potassium hydroxide solution of 0.1 M. It was found that adding 2 ml of the reagent was the best to give it the highest absorption. Results are shown in the Table (2).

### 3.4. Choosing the best oxidizing agent:

Several experiments were carried out to find the best oxidizing agent to form the colored product. Several solutions of oxidizing agents were used of 1×10⁻² M, each with a volume of 1 ml, and 2 ml of 1-Naphthylamine-4-sulfonic acid solution and added 1 ml of the potassium permanganate solution were has a concentration of 1×10⁻² M in a volumetric flask with a capacity of 25 ml. It was noted that the best oxidizing agent was potassium permanganate, which gave a maximum absorption. Results are in the Table (3).

### 3.5. Volume effect of oxidizing agent:

effect of the volume of oxidizing agent solution with a concentration of 0.1 M on the intensity of absorption was studied, as different volumes (0.3–2) ml were used and it was found that 1 ml of potassium permanganate solution is the best which gives highest absorption. Results are in the Table (4).

### 3.6. Choosing the best base:

1 ml of different types of bases with a concentration of approximately 0.1 M were used and their impact...
on the intensity of absorption was studied. Results are shown in the Table (5).

Table 5: Choosing of best base

<table>
<thead>
<tr>
<th>Base Solution</th>
<th>KOH</th>
<th>1×10⁻¹ M</th>
<th>Na₂CO₃</th>
<th>Ca(OH)₂</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.782</td>
<td>0.665</td>
<td>0.570</td>
<td>0.427</td>
<td></td>
</tr>
</tbody>
</table>

From the above table, we note that the potassium hydroxide gave the highest absorption and therefore it was chosen in the subsequent experiments.

3.7. Volume impact of the base:
Different quantities of the base used were added of potassium hydroxide 0.1M to find out the optimum amount, it was found that 1 ml is the best which gives highest absorption. Results are shown in the Table (6).

Table 6: volume impact of the base

<table>
<thead>
<tr>
<th>1×10⁻¹ M of KOH, ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>SB</td>
</tr>
<tr>
<td>0.3</td>
<td>0.133</td>
</tr>
<tr>
<td>0.5</td>
<td>0.137</td>
</tr>
<tr>
<td>0.7</td>
<td>0.142</td>
</tr>
<tr>
<td>1</td>
<td>0.146</td>
</tr>
</tbody>
</table>

3.8. Sequence of additions Impact:
It was found that the best addition sequence which gives the highest absorption in (D+R+O+B). Results are shown in the Table (7).

Table 7: sequence of additions Impact

<table>
<thead>
<tr>
<th>No.</th>
<th>Order of Addition</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D+R+O+B</td>
<td>0.147</td>
</tr>
<tr>
<td>II</td>
<td>O+R+D+B</td>
<td>0.132</td>
</tr>
<tr>
<td>III</td>
<td>R+B+O+D</td>
<td>0.118</td>
</tr>
<tr>
<td>V1</td>
<td>B+O+D+E</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Potassium permanganate (O), reagent 1-Naphthylamine-4-sulfonic acid(R), Furosemide (D), and base (B).

3.9. Impact of time:
The time was studied by taking a series of volumetric flask with a capacity of 25 ml containing 2 ml of furosemide solution at a concentration of 250 µg.ml⁻¹, then adding to it 2 ml of the reagent solution of 1-Naphthylamine-4-sulfonic acid with a concentration of 2×10⁻⁵ M and then added 1 ml was from the solution potassium permanganate at a concentration of 1×10⁻² M then was added to it 1ml of potassium hydroxide solution a concentration of 0.1M, and the solutions were left for different periods of time after which they were diluted with distilled water to the mark. Then the absorbance of the solutions was measured at the wavelength of 465 nm against their blank solutions. The results are shown in the Table (8).

Table 8: The impact of time

<table>
<thead>
<tr>
<th>Time/min</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.781</td>
<td>0.672</td>
<td>0.548</td>
<td>0.530</td>
<td>0.518</td>
</tr>
</tbody>
</table>

The above table shows that 2 minutes is enough time to complete the oxidation and conjugation process, so this time was used in the following experiments.

3.10. Impact of temperature:
The formed product was studied using different temperatures, and it was found from the results that the absorbance remains stable within the range (15-70) degrees Celsius, and the absorbance decreases at high temperatures, and it was found that the colored product was gives the highest absorption at room temperature (25 °C). Results are shown in the Table (9).

Table 9: Impact of Temperature

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.573</td>
<td>0.642</td>
<td>0.782</td>
<td>0.760</td>
<td>0.731</td>
<td>0.701</td>
<td>0.684</td>
<td>0.652</td>
<td>0.625</td>
<td>0.572</td>
<td>0.548</td>
<td>0.492</td>
</tr>
</tbody>
</table>

3.11. The Product Stability:
The stability was studied using different time periods at room temperature (25°C) to know the stability of the formed product. This study was carried out by taking 2 ml of furosemide solution with a concentration of 250µg.ml⁻¹ representing concentration 20 µg.ml⁻¹ and adding 1 ml of solution potassium permanganate with a concentration of 1×10⁻² M, then add 2 ml of 1-Naphthylamine-4-sulfonic acid reagent solution with a concentration of 2 x 10⁻⁵ M, then add 1 ml of potassium hydroxide solution of 0.1 M in a volumetric flask of 25 ml, then complete the volume to the mark with distilled water. The absorption value of the formed colored product was observed to be stable for 60 minutes. Which is sufficient time to complete many of measurements. The results shown in Table (10).

Table 10: The Stability of the Reaction Product

<table>
<thead>
<tr>
<th>20 µg.ml⁻¹ of EUR</th>
<th>Time (min)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.697</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.729</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.761</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.782</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.781</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.782</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.781</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.781</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.767</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>0.749</td>
<td></td>
</tr>
</tbody>
</table>

3.12. Spectrum of ultimate absorption:
The final absorption spectrum was measured after fixing the optimal conditions using 2 ml of furosemide solution at a concentration of 250 µg.ml⁻¹, 2ml of reagent solution of 1-Naphthylamine-4-sulfonic acid at a concentration of 2x10⁻⁵ M, 1ml of potassium permanganate at a concentration of 1×10⁻² M, and 1ml of potassium hydroxide at a
concentration of 0.1M, and complete the volume to the mark in a 25ml volumetric flask with distilled water, then final absorption spectrum of the yellow product was measured against the blank solution, it was found that it gives the highest absorption at the wavelength 465 nm. As shown in Figure (4).

![Absorption Spectrum](image)

**Fig. 4: Final absorption spectrum for the determination of furosemide**

SB: It symbolizes the absorption spectrum of Furosemide solution versus the blank solution.

SW: It symbolizes the absorption spectrum of Furosemide solution versus the distilled water.

BW: It symbolizes the absorption spectrum of the blank solution versus distilled water.

The optimal conditions for the determination of furosemide are summarized in the Table (11).

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>( \lambda_{\text{max}} )</th>
<th>465 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount ml of 1 \times 10^{-2}M potassium permanganate</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>Amount ml of 2 \times 10^{-2}M 1-Naphthylamine-4-sulfonic acid</td>
<td>2 ml</td>
<td></td>
</tr>
<tr>
<td>Potassium hydroxide of 0.1M</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °C</td>
<td></td>
</tr>
</tbody>
</table>

**Table 11: Summary of optimum condition**

3.13. Calibration Curve:
Increasing volumes (0.3-2.3 ml) of Furosemide solution at a concentration of 250 \( \mu \text{g/mL} \) were added to volumetric flask 25 ml capacity containing 2 ml of 1-Naphthylamine-4-sulfonic acid of 2 \times 10^{-2} \text{ M}, 1 ml of potassium permanganate solution of 1 \times 10^{-2} \text{M}, and 1 ml of potassium hydroxide with a concentration of 0.1M. Then the absorbance of all solutions was measured against the blank solution at a wavelength of 465 nm. Results indicated in Figure (5) that follows law Beer’s within the limits of concentration from (3 – 23 \( \mu \text{g/mL} \)) of furosemide solution, and the molar absorption coefficient was calculated its value was 1.065 \times 10^{4} \text{L.mol}^{-1}.\text{cm}^{-1} \text{and Sandell’s sensitivity was calculated and found to be equal to 0.0310 } \mu \text{g}.\text{cm}^{-2}, \text{and the correlation coefficient is 0.9994}.

![Calibration Curve](image)

**Fig. 5: Calibration curve for the determination of furosemide when reacted with 1-Naphthylamine-4-sulfonic acid in the presence of oxidizing agent potassium permanganate**

3.14. Method validation:
Three different concentrations of furosemide with a concentration of 250 \( \mu \text{g/mL} \) were used to verify the accuracy and precision of the method represented by Relative Error RE%, Recovery percentage (R %), and Relative standard Deviation RSD%. By taking an average of six readings for each of them, the recovery rate was 99.999% and the relative standard deviation (0.641-0.812%), and results in the Table (12) are shown, meaning that the method is of high accuracy and has good agreement.

<table>
<thead>
<tr>
<th>Diuremid 40 mg/Tablets</th>
<th>Diuremid 40mg/ Tablets Measured</th>
<th>Recovery, R %</th>
<th>Average of Recovery, R %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.01</td>
<td>100.333</td>
<td>99.948</td>
<td>0.641</td>
</tr>
<tr>
<td>9</td>
<td>8.9</td>
<td>.98.888</td>
<td>580</td>
<td>0.580</td>
</tr>
<tr>
<td>16</td>
<td>16.1</td>
<td>100.625</td>
<td>812</td>
<td>0.812</td>
</tr>
</tbody>
</table>

3.15. Detection Limit (LOD) and quantitation limit (LOQ):
Detection limit and quantitation limit were studied and found that the LOD (3s.d/slope) and the LOQ (10s.d/slope) were 0.971 and 3.237 \( \mu \text{g/mL} \), respectively.

3.16. The nature of colored product:
Two continuous change approaches (Job’s method) and (molar ratio method) were used to determine the nature of the colored product formed and the source of the drug’s connection with the reagent.
In both methods, the concentration of the furosemide solution and the reagent solution 1-Naphthylamine-4-sulfonic acid is the same concentration 2 \times 10^{-2} \text{ M}. In the Job method, a series of volumetric bottles with a capacity of 25 ml was taken. Different volumes of the drug solution were placed, ranging from (1-9) ml to bottles containing decreasing volumes of the reagent (9-1 ml) and and diluted with distilled water to the limit of the mark, and measurement of the absorption of these solutions at 465 nm in comparison to their blank solutions. Figure (6) shows that the correlation ratio between the drug and the reagent is 1:1.
to ensure that the reaction ratio between the furosemide and the reagent is 1:1, the molar ratio method was used where 1 ml of the drug solution was placed in a series of 25 ml volumetric bottles, different volumes of the reagent (0.2-2.9 ml) were added to it, and the rest was supplemented with the addition of solutions in the optimal volumes, then completed with distilled water, diluted to the mark. The absorption of these solutions was measured at wavelength 465 nanometers against the blank solution for each of them. With the method of continual changes, it was discovered that the molar ratio agrees with the blank solution. As shown in figure (7).

4. Applications
The method can be used to test the following pharmaceutical formulations (Tablets), each of which contains 4mg of furosemide. Three different concentrations (5, 10, 15㎍/ml) of the preparations solution (Tablets) were taken, and then the absorptions was measured at a wavelength of 465 nm versus the blank solution by the same steps followed when preparing the calibration curve, and the average of five was calculated Measurements for each concentration, and results in the Table (13) was indicated.

Table 13: The direct method

<table>
<thead>
<tr>
<th>Furosemide 40mg (Tablet)</th>
<th>Furosemide 40mg (Tablets) easured</th>
<th>Relative Error, RE %</th>
<th>Recovery %</th>
<th>Average of Recovery %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.97</td>
<td>-0.6</td>
<td>99.400</td>
<td>98.18</td>
<td>0.552</td>
</tr>
<tr>
<td>10</td>
<td>9.80</td>
<td>-2</td>
<td>98.000</td>
<td>98.700</td>
<td>0.475</td>
</tr>
<tr>
<td>15</td>
<td>14.57</td>
<td>-2.9</td>
<td>97.130</td>
<td>97.330</td>
<td>0.361</td>
</tr>
</tbody>
</table>

Table (13) shows that the proposed method was successful in recognizing the furosemide-containing pharmaceutical product. The average recovery value was 98.18 percent in this case.

5. Conclusions
Many reagents were used as oxidative coupling for furosemide, as well as many methods were used to determine this drug in its free form and in its pharmaceutical preparation. But in this proposed method was used a suitable coupling reagent (1-Naphthylamine-4-sulfonic acid) in the presence of an oxidizing agent and in an alkaline medium for the purpose of using this method as a routine method for the determination of this drug. Where this method gave a linear relationship with a recovery rate of 99.948%, so it turns out that this method is a simple, fast and highly sensitive.
6. References


التقدير الطيفي للفوروسيميد في المستحضرات الصيدلانية بواسطة تفاعل الإفتران التأكسدي

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قسم الكيمياء، كلية التربية للعلوم النصية، جامعة كركوك، كركوك، العراق
قسم الكيمياء، كلية العلوم، جامعة كركوك، كركوك، العراق

الملخص

يتضمن البحث تطوير طريقة بسيطة وسريعة وحساسية لتقدير الفوروسيميد (FUR) في المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الإفتران التأكسدي بين فوروسيميد والكافتش-1-تقليل أمين-4-حمض سلفونيك وهو موجود برمكيدات البروتينات كعامل مؤكد لتكوين ناتج أصفر اللون قابل للذو في الماء لفصولها. ينخفض تقريبا في النسب 465 نانومتر. يعتمد تقريبا على النسب 3 إلى 23 مايكروغرام. لم. م. ا. م. ب. ومصادر مولارية 1065×10. شمل 1، ودالة ساندل 0.3104 مايكروغرام. م. م. الميتابول. الدائرة 99.948 %، معادل الصرف 0.99948 %، كمك الصرف 0.971 مايكروغرام، م. م. م. م. م. م. م. 0.0310 مايكروغرام. م. م. م. م. م. م. 0.3237 مايكروغرام. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. م. 0.812% تم تطبيق الطريقة المقترحة بنجاح لتقدير الفوروسيميد في المستحضرات الصيدلانية (أقران).