Visible spectrophotometric Determination of ceftriaxone in pharmaceutical dosage forms via its Complexation with Fe (III)

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Abstract

A simple, rapid, efficient and inexpensive method was developed for the determination of ceftriaxone in pure and pharmaceutical dosage form with good accuracy and precision. The method is depend upon the reaction of ceftriaxone (CF) and ferric chloride at specific pH value of 2.5 to give orange-yellowish complex of CF- Fe(III) with maximum absorption at 485 nm. The complex was highly stable and The Lambert-Beer’s law was obeyed in the concentration range of 5–120 μg/ml. The proposed method was applied for the determination of CF in the drug Triaxon by both direct and standard additions procedures and found to be 989 and 978.5 respectively compared with the stated value of 1 gm per unit.

Keyword: ceftriaxone, antibiotic, Fe(III), spectrophotometry.

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1. INTRODUCTION

Ceftriaxone (CF) (6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-y1thio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid is a third-generation cephalosporin antibiotic[1]. It has broad spectrum activity against Gram negative and Gram positive bacteria [2]. CF is often used (in combination with macrolide and/or amino glycoside antibiotics) for the treatment of community-acquired pneumonia [3]. It is also used as a routine prophylactic antibiotic for the patients undergoing orthopedic surgery [4].

![Chemical structure of ceftriaxone sodium](image)

Fig1. Chemical structure of ceftriaxone sodium

Literature review showed various methods for the determination of cephalosporins. These methods include spectrophotometry [5-8], atomic absorption spectroscopy [9], fluorometry [10-11], liquid chromatography [12-14], chemiluminescence [15-17], polarography [18-19], HPLC [20-22]. Availability of ultra violate-visible spectroscopy apparatus in many laboratories and the simplicity of analytical procedures make the technique very attractive for wide range of applications such as drugs and medicaments. The literature is still poor in analytical procedure based complexation of drug with metal ion, especially for determination of drug in commercial dosage forms. This attractive avenue encouraged the author to develop a simple, rapid, inexpensive and reliable method for the detection of CF as CF-Fe(III) complex in pharmaceutical samples.
2- EXPERIMENTAL

2.1. Apparatus
Ultraviolet/Visible spectrophotometer (Analytik jena specord 40 USA) with matched 1 cm quartz cell was used for all measurements. Infrared spectrum for the produced complex was recorded on Shimadzu Fourier Transform Infrared model FT-IR8000. for pH measurement its used pH meter Hana ( microprocessor pH meter pH210).

2.2. Reagents and Chemicals
All chemicals used were of analytical reagent grade; distilled water was used for diluting reagents and samples. A pure Cefotaxime and Triaxone drug were purchased from the local market, ferric chloride and hydrochloric acid (36.4%) were purchased from BDH.
Ceftriaxone standard solution (1000 µg ml⁻¹) was prepared by dissolving 0.1 g of pure CF in sufficient water and diluted to 100 ml into a volumetric flask. A 100 µg ml⁻¹ of working ferric chloride standard was prepared by dissolving of 0.2900 gm of ferric chloride powder in sufficient water and diluted to 100 ml into a volumetric flask.

2.3 General Procedure and Analytical Curves

2.3.1 Direct Calibration Method
Aliquots of (0.25–4.5 ml) of stock standard solution of Ceftriaxone (100 µg l⁻¹ ) were transferred into seven of 5 ml volumetric flask and (0.45- 0.6 ml) of stock solution of Ceftriaxone (1000 µg l⁻¹ ) were transferred into four of 5 volumetric flask, then 0.5 ml of 100 µg Fe ml⁻¹ was added to each flask followed by adjusting the pH of all solution to 2.5 using dilute HCl or NaOH solution. The solutions were set aside for 2.5 min, and then diluted to 5 ml with water. These solutions were corresponding to (5-120 µg CF ml⁻¹).the absorbance were measured at 485 nm. The analytical curve was obtained by plotting absorbance against CF concentration and the corresponding linear regression equation was used to convert absorbance into CF concentration, for all analyzed Triaxone samples.

2.2.3 Preparation of Drug Triaxone vial,
10 vials of Triaxone were mixed in a clean agate mortar, A quantity of 0.100 g of fine powder was dissolved in sufficient water with continuous shaking, then was transferred into 100 ml volumetric flask and dilutes to mark with water.

2.2.4 Standard Additions Method,
Aliquots of the above–prepared Triaxone sample solution were pipetted into seven of 5-ml calibrated flaks containing 0.000, 0.250, 0. 500, 1.000, 1.50, 2.000, 3.00 and 4.00ml of 100 µg ml⁻¹CF and into three of 5 ml calibrated flask containing 0.45, 0.50 and 0.55ml of 1000
μg ml⁻¹ CF then the same steps were proceeded according to the procedure previously mentioned under direct calibration method.

3. RESULTS and DISCUSSION

3.1 Absorption Spectra,
UV-Vis spectra of the pure CFX drug and its complex with Fe (III) were recorded using Ultraviolet/Visible spectrophotometer (Analytik jena specord 40 USA) with 1cm matched quartz cell for recording the spectra at 100 and 1000 mg l⁻¹ of CF standard solution and CF-Fe(III) to verify the formation of complex. the pure drug gave one absorption maxima at 485 nm and Iron (III) solution gave one absorption maxima at 290 nm (Figures not shown), while the spectrum of the pink chelate shows a new absorption maxima at 485 nm indicating the formation of complex between the drug CF and Fe(III) solution in aqueous medium.

3.2 Optimum Conditions
3.2.1 Effect of pH: It is evident from Fig. 2 that the absorbance increased gradually from pH 1 and reaches to maximum value at pH 2.5 and then the absorbance decreased by increasing pH because of the dissociation of complex, consequently the optimum pH of 2.5 was selected for complete formation of chelating complex.

![Fig. 2: effect of pH.](image)

3.2.2 Effect of Fe (III) concentration: It was found that the absorbance of CF-Fe (III) complex increases as the concentration of Iron (III) ion increases and then deviate towards the
Fe concentration axis Fig. 3. Consequently, the optimum concentration of Fe (III) of 10 μg ml⁻¹ was selected for complete formation of chelating complex.

3.2.3 Effect of Reaction Time: Fig. 4 shows the effect of reaction time on the formation of CF-Fe(III) complex, this step was achieved by stabilization of other parameters (concentration of Fe(III) and pH) and measuring the absorbance of the same sample in different periods (0.5, 1.0, 1.5, 2.0, 2.5, ……8.0) min. It shows that the absorbance increases rapidly with reaction time up to 2.5 min then reaches an equilibrium, which indicates that there is no advantages in going beyond 8 min.
3.3 Suggested Structure of the Complex,

spectroscopic techniques, such as FTIR, and mole-ratio procedure performed by UV-Vis spectrophotometry have been used to elucidate the probable structure of CF-Fe(III) complex produced at optimum conditions. Fig. 5 shows that the mole ratio between CF and Fe (III) was 1:1 complex. The stability constant was estimated by using the following equation [23]:

\[
K = \frac{(A_1 - A_3)(A_2 - A_1)}{(A_2 - A_1)^2 C}
\]

Where \( K \) is stability constant, \( A_1, A_2, A_3 \) refers to the absorbance of intersect point of the two slopes, at constant absorbance, first point absorbance on the Fig. 4, respectively and \( C \) is the molar concentration of complex vs. \( A_1 \). It was found to be \( 73.49 \times 10^4 \text{ M}^{-1} \) at \( \lambda_{\text{max}} \) 485 nm.

![Absorbance vs. VL/VM](image)

Fig. 5: Mole ratio method for CF-Fe(III) complex.

VL = volum of legand
VM = volum of metal

An FTIR spectra of ceftriaxone and its complex are similar and the main frequencies can be seen in (Fig. 6 and 7). The lactam (C=O) band appears at 1780 cm\(^{-1}\) in the spectrum of ceftriaxone while the overlapped amide and ester (C=O) bands appears at 1640 cm\(^{-1}\); the complexes show these bands at around 1720-1740 and 1630-1650 cm\(^{-1}\) ranges respectively. All this suggests that coordination of the ligand occurs through the oxygen atom from the
lactam carbonyl group rather than the amide and ester carbonyl groups. The lactam carbonyl bands were substantially shifted toward lower frequencies (40-60 cm$^{-1}$) relative to the value of the uncomplexed ceftriaxon while in the overlapped amide and ester carbonyl bands the shifting was not significant.

Fig. 6: FTIR spectrum for the pure CF

Fig. 7: FTIR spectrum for CF-Fe(III) complex
Consequently, we can propose the structure of the complex formed (Fig. 8).

![Chemical structure](image)

**Fig. 8: Probable chemical structure of CF-Fe(III) complex**

### 3.4 Analytical Data

Under the optimum experimental conditions described previously, linearity, detection limit, molar absorptivity, Sandell’s sensitivity, accuracy and precision. Beer's law was obeyed in the concentration range 5-120 µg ml⁻¹ of CF. The regression calibration equation obtained under optimum conditions was:

\[ Y = 0.001X + 0.001 \quad (n=11) \]
where $Y$ is the absorbance and $X$ the CF concentration as $\mu g \, l^{-1}$, with a correlation coefficient of $r = 0.9996$ and the coefficient of determination ($R^2$) of 99.92% which suggests statistically valid. this fitted linear calibration model was used to estimate the CF concentration in the drug samples which appears justified, on statistical basis. The confidence limits of slope and intercept of the regression line were computed using the formulas $b \pm t_s b$ and $a \pm t_s a$ at 95% confidence level and found to be $1 \times 10^{-3} \pm 0.6 \times 10^{-4}$ and $1.1 \times 10^{-3} \pm 0.5 \times 10^{-4}$ respectively, where $t$ is $t$-test and $s_b$, $s_a$ are the standard deviation of slope and intercept respectively. Limit of detection was calculated on the statistical basis from the calibration graph data and found to be $0.31 \, \mu g \, ml^{-1}$. The molar absorptivity for the complex CF-Fe(III) was $1.457 \times 10^3 \, L \, mol^{-1} \, cm^{-1}$ and Sandell’s sensitivity was $0.4541 \, \mu g.cm^{-2}$ . The accuracy in term of recovery percent and precision were achieved by spiking of 10, 60 and 100 $\mu g \, ml^{-1}$ using the recommended procedure previously mentioned under section (2.2.4). the results were shown in Table 1. These data indicate that the visible spectrometric determination of CF is not highly effected by the presence of other constituents in the drug sample.

Table 1: The accuracy and precision of the proposed method for the determination of CF in pharmaceutical preparation.

<table>
<thead>
<tr>
<th>Amount of Ceftriaxone taken ($\mu g.mL^{-1}$)</th>
<th>Amount of Ceftriaxone found ($\mu g.mL^{-1}$)</th>
<th>%Rec.</th>
<th>%E_rel.</th>
<th>%RSD n = 5</th>
<th>Conf. Limit. for %Rec.$\pm$S.D</th>
<th>Mean %E_rel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.10</td>
<td>101.00</td>
<td>1.00</td>
<td>1.51</td>
<td>101.18 $\pm$ 0.84</td>
<td>1.18</td>
</tr>
<tr>
<td>60</td>
<td>60.78</td>
<td>101.30</td>
<td>1.30</td>
<td>1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>101.23</td>
<td>101.23</td>
<td>1.23</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.5 Determination of CF in Triaxone,

The proposed method was applied for the detection of CF in Triaxone vials with stated value of 1000 mg per unite by using direct calibration and standard additions procedures (Fig.9) under optimum conditions. The CF was determined through the atomization of the complex extracted as a result of the reaction of CF present in the pharmaceutical preparation with Iron (III) ion and found to be 989 and 978.5 mg / unit with relative error of (-1.10%) and (-2.5%) respectively.
It can also be observed from (Fig. 8), that the ratio of the slopes of the direct calibration and standard additions is found to be one, which indicates that the interferences resulting from drug constituents are insignificant using the proposed procedure. Thus, it is possible to use direct calibration procedure for the determination of CF in drugs without need the standard additions method which requires more effort, more amount of sample and time-consuming. This is also support the specificity of the proposed method, indicating that the excipients did not interfere with the analysis of CF.

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في هذا البحث استخدمت طريقة جديده للمطيافيه المرئيه لتقدير المضاد الحيوي سلفرياكزون بشكل نقي وكذلك في المستحضرات الصيدلانيه حيث اعتمد الطريقة على تعين المضاد الحيوي بشكل مخالف مخلي ذي لون برتقالي مصفر من خلال تفاعل مع ايون الحيد الثلاثي. عند تطبيق الظروف الفضلي تراوحت الخطيه (5 - 120) مايكروغرام مل^{-1} وحد الكشف (0.31) مايكروغرام مل^{-1} ومدي الدقه (0.93 - 1.5%)، وتراوح معدل الاسترداد بالمئة 84 ± 101.18 %، طبقت الطريقة لتقدير المضاد الحيوي في المستحضر الصيدلاني التجاري ترياكزون بطريقة المعايرة المباشرة واداءات القياس ووجد انه يساوي 989 و 970.5 مغم على التوالي مقارنة بالقيمه المصرف بها وهي 1000 مغم.

الدكتور
أمين وليد قاسم