New spectroscopic estimation of cefotaxime in pure and pharmaceutical formulation using environmental-friendly method

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Abstract. Simple and cost low, accurate and fast spectrophotometric methods were developed for cefotaxime determination. First method including change cefotaxime to a colored Fe (II) complex in the alkaline medium. Colored product with absorbance at \( \lambda_{max}=505 \text{ nm} \) is reddish orange. Concentration ranged between (2-50 \( \mu \text{g.mL}^{-1} \)), the Beer’s law obeyed with correlation coefficient 0.9995, molar absorptivity was \( 0.405 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1} \), limit of detection 0.370 \( \mu \text{g.mL}^{-1} \) and limit of quantification as 1.123 \( \mu \text{g.mL}^{-1} \). The second cloud point extraction method was used to estimate the trace quantity of the colored product in the first method, followed by UV-Vis spectrophotometer calculation. The calibration curve was the range of (2.5-30 \( \mu \text{g.mL}^{-1} \)), the correlation coefficient and molar absorptivity were 0.9996 and \( 1.16 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1} \) respectively. The detection limit LOD and quantification limit LOQ were to be 0.026 and 0.079 \( \mu \text{g.mL}^{-1} \) respectively. This technique was effectively employed for cefotaxime detection within the pure and pharmaceutical preparations.

Keywords: cefotaxime, cloud point extraction, complex, UV-Vis spectrophotometer and Environmental-friendly

1. Introduction

Cefotaxime sodium is sodium 7-[2-(2-amino-4-thiazolyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate 72 (Z)-(o-methyloxime), acetate (ester). Fig1. (CTX), is a third generation cephalosporin antibiotic used intravenously for the treatment of disease caused by a wide variety of Gram negative and Gram positive species of bacteria [1,2]. The determination of cefotaxime is necessary not only for pharmacokinetic analysis in the field and human health, but also for quality control in the food and fermentation industry in for the illegal use of cefotaxime in food preservation, processing, introduction of antibiotics in refined sugar and as food products [3]. Several methods have been established for cefotaxime determination including HPLC [4–7], spectrofluorometry [8], voltammetry [9,10], chemiluminescence [11,12] and capillary zone electrophoresis [13]. These methods show good sensitivity and selectivity, the steps for analysis and sample preparation were time consuming and complicated. The UV-visible spectrophotometer can be used to sample the CTX in the visible spectrophotometric process. To determination the trace concentration of CTX. In aqueous solutions used the CPE and used surfactant such triton-X100, the temperature at which the
solution becomes turbid before separation into two phases (a surfactant-rich phase and an aqueous phase) known as the cloud point extraction CPE [14]. The CPE method has generated widespread interest as an alternative to conventional extraction [15]. As for the trace concentrations, cloud point extraction can be used for their determination by pre-concentration and using a non-ionic surfactant (Triton X-100) to increase the size of the phase rich in surfactant and increase the enrichment factor [16,17]. These methods have some compensations including low cost, sensitivity, accuracy, rapidity, low toxicity, less dangerous and simplicity of procedure. Therefore, researches have applied to pharmaceuticals determination and have proven to be successful in estimating the effective ingredient [18,19]. Here, in this paper the proposed approach was entirely based on the reaction CTX with Fe (II) metal in alkaline presence, which was then tested and pre-concentration cloud point extraction (CPE), which showed an absorption at $\lambda_{max}$ 505 nm. This procedure would then form a reddish-orange solution.

![Structure of Cefotaxime](image)

Figure1: Structure of Cefotaxime

2. Materials and methods

Spectrophotometric single-beam UV-visible 295 (Lasany- India), equipped with quartz cells of 1 cm. Chemicals and reagents were of analytical grade. However, cefotaxime was obtained from (SDI) and (FeCl$_2$. H$_2$O) from company Merck. A stock solution of iron (250 $\mu$g. mL$^{-1}$) was prepared by dissolving (0.056 g) of FeCl$_2$.H$_2$O in distilled water and dilution to (100 mL) volumetric flask was made. Stock cefotaxime solution (250 $\mu$g/ mL) was prepared by dissolving (0.025 g) in distilled water and completed to the (100 mL) volumetric flask. (0.01M) of hydrochloric acid, (0.01 M) sodium hydroxide, TritonX-100 (10%).

2.1 The general method of cefotaxime -complexation.

1 mL of 250 $\mu$g .mL$^{-1}$ of cefotaxime was transferred into volumetric flask 10 mL, 1 mL FeCl$_2$.H$_2$O 250 $\mu$g .mL$^{-1}$ was added followed by pH 12 (1mL), then completed to the mark 10 mL with distilled water. The mixtures keeping in the water bath (35 $^\circ$C) for 40 min. The resulting solution were measured at $\lambda_{max}$ 505 nm against reagent blank treated similarly but without cefotaxime drug.

2.2 The general method of CPE for cefotaxime

Concentrations 2.5-30 $\mu$g. mL$^{-1}$ of cefotaxime complex volumes put within the 15 mL centrifuge tubes, 1 mL of Triton-X-100 and distilled water were added to complete the volume of solution to 12 mL, mixture of the solution keeping in the water bath (75 $^\circ$C) at 35 min. Two phases were separated via centrifugation for 5 min at 4000 rpm. Cooled mixture that increases the viscosity of the surfactant-
The rich phase and the aqueous phase was easily disposal by decantation. The rich-surfactant phase from this technique was diluted with 0.5 mL of EtOH and transferred into quartz cell to measure its absorption intensity at \( \lambda_{\text{max}} \) 505 nm.

2.3 Method for pharmaceutical preparations
Cefotaxime sodium Roth company of Germany \( 1g \) (1000 ppm) and Cefotaxime sodium Julphar company of Alemarat \( 1g \) (1000 ppm) have been carefully weight, extraction of average weight tablets. Weight of drug was dissolved in distilled water, then made up to volumetric flask (100 mL) and the solution was filtrated.

3. Results and Discussion
Absorption intensity of cefotaxime-Fe (II) complex against reagent blank in pH 12 at 35\(^\circ\)C have been produced a reddish-orange colored product with wavelength 505 nm, as shown in fig.2.

![Figure 2. UV-Visible spectrum of cefotaxime-Fe(II) complex](image-url)

3.1 Optimization of complexes reaction
The study of various parameters influence in order to found the optimized conditions is important for the quantitative and rapid formation reddish-orange colored product with the stability and sensitivity, including pH, pH metal concentration form and volume, temperature and response time. Different kinds of buffer solutions such as sodium hydrogen ortho-phosphate/sodium hydroxide and potassium chloride and sodium hydroxide were tested. The impact of pH on the absorption with a fixed compound concentration was reduced between 1-13. The best phosphate buffer and pH12 was the best. Then values absorbance decrease with increasing pH, may be attributed to the formation of metal hydroxides shown the result in fig.3. Different volume (0.2-2) mL of buffer solution studied, the best volume were 1.6 mL using in the fixed experiments the result shown in fig.4.
Concentration metal Fe (II) was studied using different concentrations 5-80 μg mL⁻¹ Fe (II) complexation procedure, found that 30 μg mL⁻¹ provided the optimum absorption intensity. Different volume of metal (0.2-1.4) mL was studied and the best volume that gave higher intensity was 1 mL shown the result in fig.5. Different temperatures ranged between (10-50) °C and incubation time (10-60) min were studied. It was found that the temperature 35 °C and incubation time 40 min were the optimum conditions to obtain the heights absorbance for cefotaxime shown in fig.6 and fig.7.

Figure 3. Effect buffer solutions.  
Figure 4. Volume of buffer solution CTX-Fe(II) mL.

Figure 5. Effect volume of metal ion Fe (II) mL  
Figure 6. Effect of Temperature °C.
Amino drug Cefotaxime (1mole) react with (1mole) Fe (II) to yield complex CTX-Fe (II) in present pH 12 [20] shown in fig.8.

3.2 Calibration Curve
After the optimum conditions were establish the calibration curve for CTX-Fe (II) of the linear calibration curve by plotting the different concentrations of CTX (2-50) μg / mL, against the absorbance, analytical data obtained from the calibration curve, the linear regression equation, the coefficient of correlation, slope and intercept, shown in fig.8 and the data of calibration in Table 1.
3.3 Optimization of cloud point extraction (CPE)

To estimate the trace concentration of the complex cefotaxime – Fe (II), used the cloud point extraction. Study effect of surfactant (Triton-100, TritonX-114, Tween 80, CTAB and SDS). The best absorbance for the surfactant was TritonX-100 on the extracted and determination of cefotaxime. Different volume of Triton X-100 (0.5-3) mL was studied. When increasing the amount of TritonX-100 up to 2 mL, the absorption of the process increased and the absorption decreased at higher concentrations. In this work, 2 mL TritonX-100 were therefore selected. Fig.10 displays the results. Similar (30-80) °C temperature and incubator time (10-50) min. As shown in fig.11 and fig.12, the temperature of 70°C and 40 min was chosen. After 40 min in heating at 70°C and 5 min in centrifuge at 4000 rpm and ice bath cooling at 20 min, the cefotaxime is recovered very rapidly. After the CPE extraction process was completed, the aqueous phase was extracted by decantation and ethanol was added to the surfactant-rich phase to decrease the viscosity of the surfactant-rich phase and ease its transition to the spectrophotometric.

Figure 9. Calibration curve of complex cefotaxime –Fe (II).

![Figure 9](image9)

Figure 10. Effect volume of Triton X-100 mL.

![Figure 10](image10)

Figure 11. Effect of Temperature °C.

![Figure 11](image11)
3.4 Calibration Curve

After optimizing all conditions, the calibration curve for CTX of the linear calibration curve began by plotting the different concentrations of CTX (2.5-30) μg / mL, against the absorbance, analytical data obtained from the calibration curve, the linear regression equation, the coefficient of correlation, slope and intercept shown in fig.13 and the data of calibration in Table 1.

![Figure 13](image)

**Figure 13.** Calibration curve of cloud point complex cefotaxime Fe (II).

| Characteristic parameter for the regression equation of the proposed CPE of CTX |
|---------------------------------------------------------------------------------|
| Accuracy and precision.                                                          |

**Table 1.** Characteristic parameter for the regression equation of the proposed CPE of CTX.
3.5 Accuracy and precision

Determination the precision and accuracy of the proposed procedure was done by using different concentrations under optimum conditions and calculating absorbance average at a minimum of five readings per concentration. Determination of precision and accuracy by RE %, R % and RSD %, as shown in Table 2.

Table 2. Application of the proposed CPE for the evaluation of cefotaxime.
3.6 Effect of Interference

The effect of the assumed interference presented in the cefotaxime drug was tested in terms of knowing the deductive research selectivity by adding 1 mL (100 μgml⁻¹) of each interference [Lactose, Starch, Arabic Gum, Glucose, Talc, Ca₃(PO₄)₂, CaCO₃] with 1 mL of 10 μg/mL of each complex and the of the addition are optimal conditions then diluted in volumetric flask 10 mL with distilled water then measured the absorbance. The results in Table 3 suggest that there has been no intervention from any of the excipients.

| Interference compounds | Recovery % of complex CTX-Fe II |
|------------------------|---------------------------------|
| Lactose                | 98.76                           |
| Starch                 | 99.87                           |
| Fructose               | 98.87                           |
| Glucose                | 99.21                           |
| Ca₃(PO₄)₂              | 98.98                           |
| CaCO₃                  | 99.15                           |

Table 3. Compassion for LOD and LOQ values of the CPE method with different literature recorded methods.

| Method                          | LOD μg/mL | LOQ μg/mL | Ref.   |
|---------------------------------|-----------|-----------|--------|
| RP-HPLC                         | 0.3       | 0.6       | [21]   |
| Spectrophotometric              | 0.301     | 0.294     | [22]   |
| Indirect Spectrophotometric     | 0.019     | 0.063     | [23]   |
| Extractive of Spectrophotometric| 0.42      | 1.26      | [24]   |
| Flow injection analysis         | 0.106     | -         | [25]   |
| Cloud point extraction          | 0.026     | 0.079     | Present work |

4. Conclusions

The proposed CTX estimation approach has the advantages in pharmaceutical preparedness of high sensitivity, low cost, streamlined, recurrent and reproducible CTX drug assessment techniques which can be applied to real samples. The surfactant has been used in pharmaceutical preparations for separation and pre-concentration of the CTX compound. For this procedure, a comparison between the
methods already recorded using different instrumental techniques appears to be more responsive and stable, simple, fast, quick and cheap.

Acknowledgment
The author would like to thank the Mustansiriyah University, Baghdad, Iraq, for helping to complete the work and the general company for the manufacture of medicines and medical supplies (Samarra) for supplying pure cefotaxime drug.

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