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RESEARCH ARTICLE

# Spectrophotometric Determination of Ceftotaxime Following Azo Dye Formation with Resorcinol

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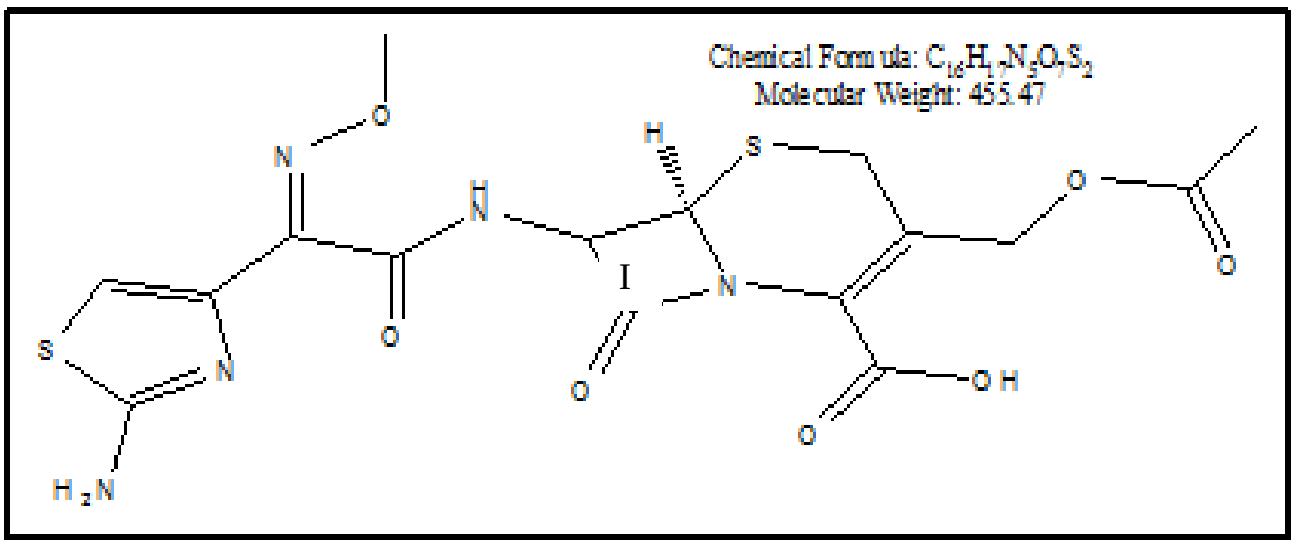
## Abstract

A sensitive, selective, rapid, simple and accurate of the spectrophotometric determination of ceftotaxime in bulk and in dosage forms. This method depend on diazotization of primary amine group of cefotaxime with sodium nitrate and hydrochloric acid followed by coupling with ceftotaxime in aqueous mildly acidic medium to form azo dye a stable orange. Showed obeyed beers law between 0.5- 15 ppm, with molar absorptive $4.4*10^4$L.mole$^{-1}$.cm$^{-1}$ at 456nm.Sandells sensitivity 0.008µg.cm$^{-2}$, limit of detection (LOD) 0.185ppm and limit of quantification (LOQ) 0.619 ppm. This method has been successfully applied to determination of ceftotaxime in bulk and in pharmaceutical preparation, tablet with a good recoveries 99.43-100.32 %.

**Keyword**: *Cefotaxime, Resorcinol, Diazotization-coupling, Spectrophotometry.*

## Introduction

The cefotaxime I are class of ß- lactam antibiotics[1], used to treat a number of bacterial infections[2], for example meningitis , pneumonia, urinary tract infection, sepsis and cellulitis[3].Cefotaxime discovered in 1976 but came in to commercial in 1980 [4], side effects came from taking cefotaxime include allergic reactions, nausea , and inflammation at the site of injection[5]. It is relatively safe for use breastfeeding and during pregnancy [6].Mechanism of action refer to the ß-lactams inhibit bacterial cell wall synthesis by binding to one or more of the penicillium-binding proteins [7]. According to this mechanism ß- lactams are considered to be bactericidal [8]. Cefotaxime is the only cephalosporin which has very low toxicity in plants [9].It is metabolized to both active and inactive metabolites by liver and large excreted in the urine [10].



Various method used of determination of Ceftotaxime such as chromatography (11), spectrophotometric [12], volume try [13], polarography [14] and flow injection analysis [15]. The aim of this research to develop simple, selective and sensitive spectrophotometric method based on diazotization-coupling reaction with resorcinol to determination of Ceftotaxime in pure and pharmaceutical preparation.

## Experimental

## Materials and Methods

### Instruments

All absorbance and spectral was carried by double-beam UV-Visible spectrophotometer (EMC 11 and T 80 Germany) with quartz cell of 1cm path length.

### Reagents

All chemicals were grade purity .Standard reference cefotaxime was obtained from (SAFA pharmceuteical Industries Co, Iraq). Pharmaceutical preparation containing ceftotaxime obtained from the commercial market.

### Solutions

All aquieous solution was prepared by distill water .100 ppm of cefotaxime was prepared by dissolving 0.01g of ceftotaxime then complete to the mark by distill water. Working standard solutions prepared freshly by diluting the stock solution by distill water to obtain the appropriate concentration. Sodium nitrate (BDH) 0.01M.Hydrochloric acid (BDH) 1M.cefotaxime 100 ppm prepared by dissolved 0.01g in 100 mL distills water.

### Recommended Procedure and Calibration Curve

Transfer volumes of cefotaxime solution. Covering the range 0.5-30 ppm, into series of 10 mL volumetric flask. Add 1 mL of 1M HCl and mixtures are shaken.

Then 2.0 mL ɔf 0.01 M NaNO2 solutions added and the mixture lives about 2 minutes. After that 1.0mL of 0.2 M sulphamic acid solution was added and the mixture allowed for 2min. After that 3 mL ɔf 0.01M resorcinol were added, and then completed to the mark with distill water. After 10 minutes measure the absorbance against reagent blank.

### Procedure for Dosage Forms

Take 0.01g from the ceftotaxime capsuol and dissolved in 20 mL distill water then transferred to the 100 mL volumetric flask, shaken and completed to the mark by distill water.

## Results and Discussion

### Choice of Coupling Reagent

Several coupling reagent used in this study such as procaine, resorcinol and thiozon, but useful analytical results obtained with resorcinol. This reagent gives a stable water azo dye with ceftotaxime. Therefore this reagent was selected and optimum condition of this reaction with ceftotaxime was further studied.

### Spectral Characteristics

Absorption spectrum of orange azo product with maximum absorption at 456 nm shown in Figure (1).

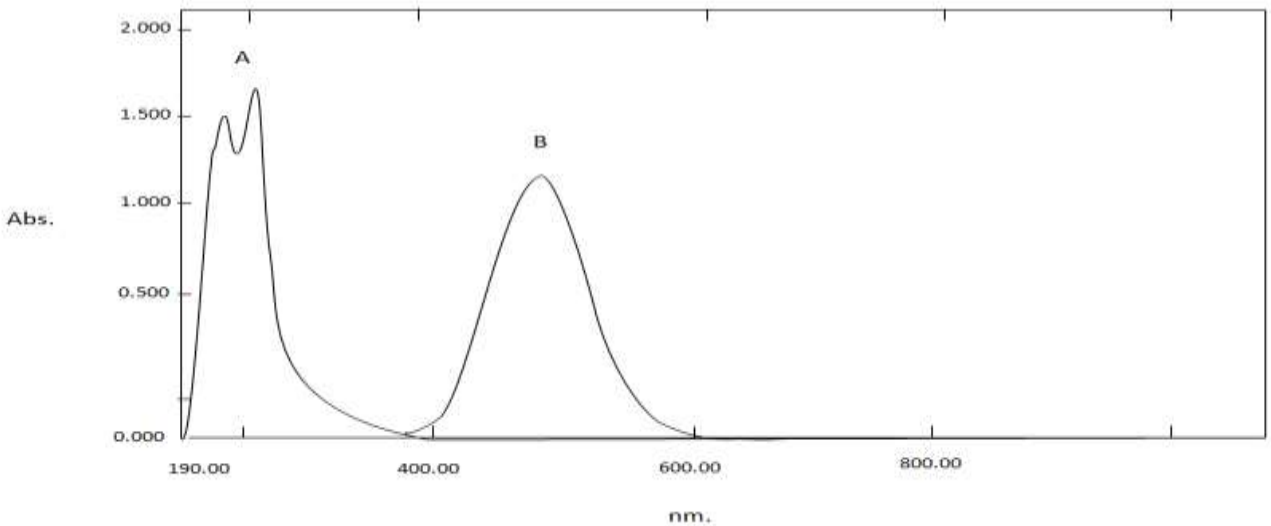



**Figure 1: A: Absorption spectra of ceftotaxime . B: Absorptions pectra of resorcinol with ceftotaxime**

## Optimization of Reaction Conditions

The effects of the various parameters on absorption intensity of the azo were studied and the conditions are optimized.

## Effect of Acid

A different volume (0.5-5 mL of 1M) of different acid has been examined. 1 mL of 1M HCl gives a good result shown in Table (1).

**Table1: Effect of different acids on absorbance**

| Acids 1M | Absorbance | | | | | |
|---|---|---|---|---|---|---|
| Volume of acid(mL) | 0.5 | 1 | 2 | 3 | 4 | 5 |
| HCl | 1.123 | 1.291 | 1.283 | 1.219 | 1.114 | 1.021 |
| $HNO_3$ | 0.723 | 0.712 | 0.696 | 0.663 | 0.669 | 0.664 |
| $H_2SO_4$ | 0.592 | 0.681 | 0.571 | 0.592 | 0.611 | 0.621 |
| $CH_3COOH$ | 0.610 | 0.699 | 0.684 | 0.695 | 0.643 | 0.671 |
| $CH_2O$ | 0.318 | 0.322 | 0.325 | 0.358 | 0.319 | 0.301 |

## Effect of Sodium Nitrate Concentration and Time

The effect of concentration$NaNO_2$ was study by using different volumes (0.1 – 4.0 mL) of 0.01 M $NaNO_2$ solution. 2 mL of $NaNO_2$ and 2 minutes gives a maximum absorbance shown in Table (2).

**Table2: Effect of nitrate concentration**

| Volume of 0.01 M $NaNO_2$ mL | Absorbance |
|---|---|
| 0.5 | 0.486 |
| 1.0 | 0.835 |
| 1.5 | 1.082 |
| 2.0 | 1.292 |
| 2.5 | 1.291 |
| 3.0 | 1.290 |
| 3.5 | 1.291 |
| 4.0 | 1.291 |

## Effect of Sulfamic Acid Concentration and Time

In order to remove the excesses of nitrous acid, used different volumes (0.1 – 5.0 mL ) ɔf 0.2 M sulfamic acid solution , the maximum absorbance were with 1.0 mL of sulfamic acid and 2 minute shown in Table (3).

**Table 3: Effect of sulfamic acid**

| Volume of sulfamic acid in Ml | Absorbance |
|---|---|
| 0.5 | 0.896 |
| 1.0 | 1.281 |
| 2.0 | 1.281 |
| 3.0 | 1.281 |
| 4.0 | 1.281 |
| 5.0 | 1.281 |

## Effect of Reagent Concenrration

Table (4) shows different volumes (1.0-5.0 ml) of 0.01 M procaine used to testing on reagent concentration , in the results showed that 3.0 mL ɔf reagent is sufficient of the production of maximum colour intensity.

**Table 4: Effect 0f reagent concentration**

| Volume of reagent 0.01 M mL | Absorbance |
|---|---|
| 1.0 | 0.599 |
| 1.5 | 0.774 |
| 2.0 | 0.941 |
| 2.5 | 1.189 |
| 3.0 | 1.293 |
| 4.0 | 1.292 |

| | |
|---|---|
| 4.5 | 1.291 |
| 5.0 | 1.292 |

## Effect of Time

Figure (2) shows different times used to investigation of the formation of the azo product, the maximum absorbance obtained after 5 minute.

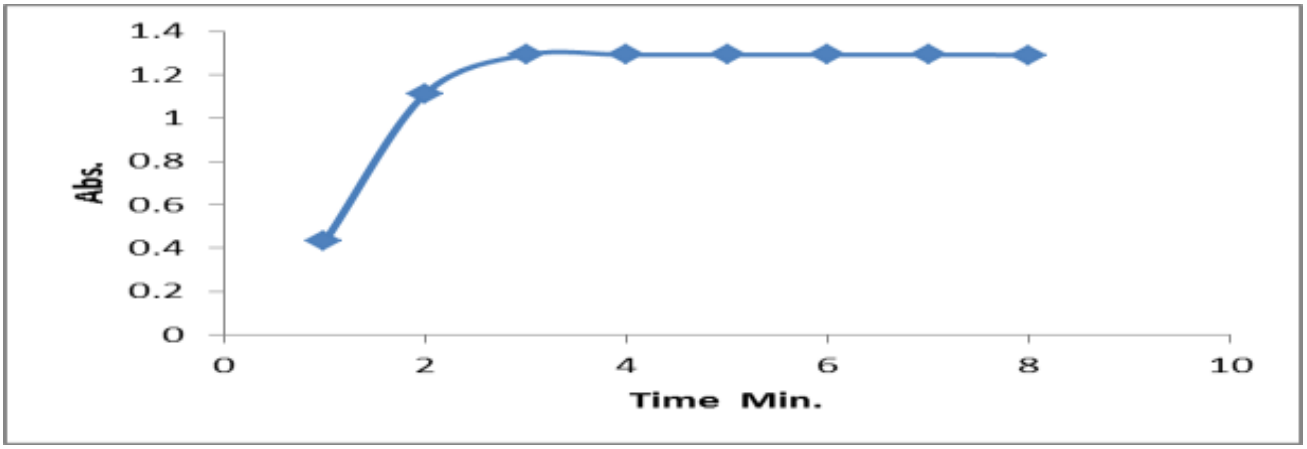



**Figure 2: Effect of time on absorbance**

## Effect of Temperature

Figure (3) shows different temperatures (15-80 $^0$C) using to investigation, which temperature used in study, from the Table (6) the range between (15 – 30 $^0$C) gives maximum absorbance. At higher temperature the absorbance value decreased, which was probably due to dissociation of product.

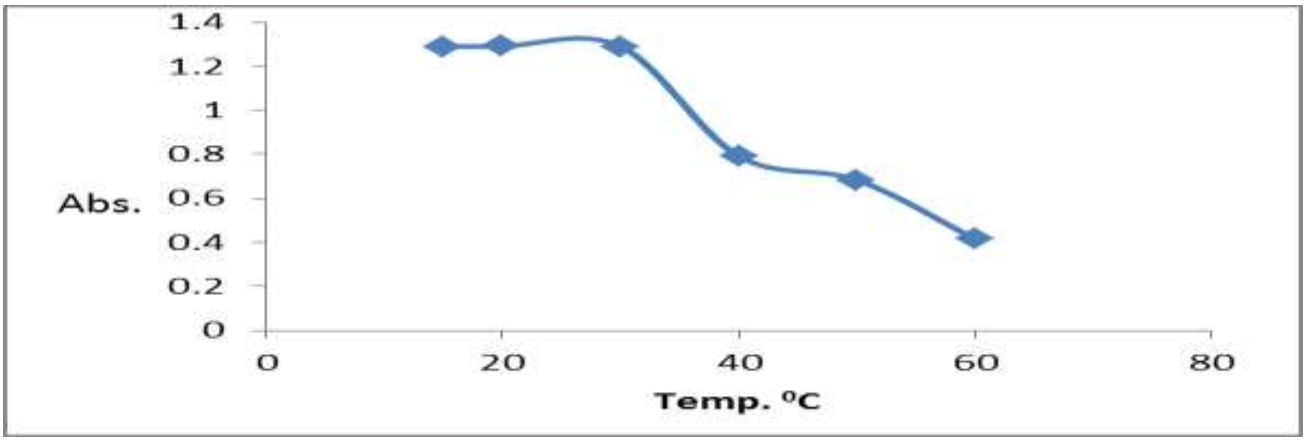



**Figure3: Effect of temperature on absorbance**

## Calibration Curve and Sensitivity

Under optimum conditions above, standard calibration curve of the ceftotaxime-cefotaxime azo product showed in Figure (4).

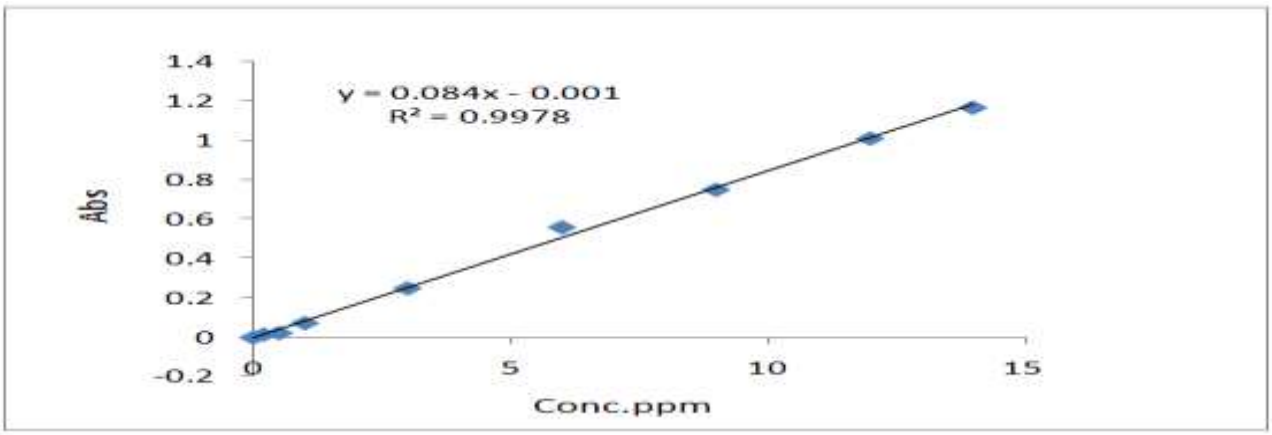



**Figure4: Calibration curve for ceftotaxime determine by using procaine as coupling reagent**

Various parameters of analytical performance of the proposed method in Table (5).

**Table 5: Analytical feature of the procedure developed to the determine of the ceftotaxime**

| Parameter | Proposed Method |
|---|---|
| Regression equation | Y = 0.125X – 0.007 |
| Slope | 0.125 |
| Correlation coefficient | $R^2$ = 0.9996 |
| Linear range (ppm) | 0.5 – 15 |
| Molar absorpitivity(L.mol$^{-1}$.cm$^{-1}$)[16] | 4.4 * $10^4$ |
| Limit of detection(LOD)(ppm)[17] | 0.185 |
| Limit of quantitative (LQD)(ppm)[17] | 0.619 |
| Sandells sensitivity, S (µg.cm$^{-2}$)[18] | 0.008 |
| Recovery(%)[16] | 99.32 |

## Nature and Stability Constant of the Product

Stoichiometric ratios were determined by using Jobs method [17] Figure (5). From the results showed a 1:1 ceftotaxime and resorcinol. The formation of product occurs as given in Scheme 1.

Stability constants of the product was 3.24* $10^8$ $M^{-1}$ according to the equation cited [19] Table (6). This result refer to stable products are formed between ceftotaxime and resorcinol.

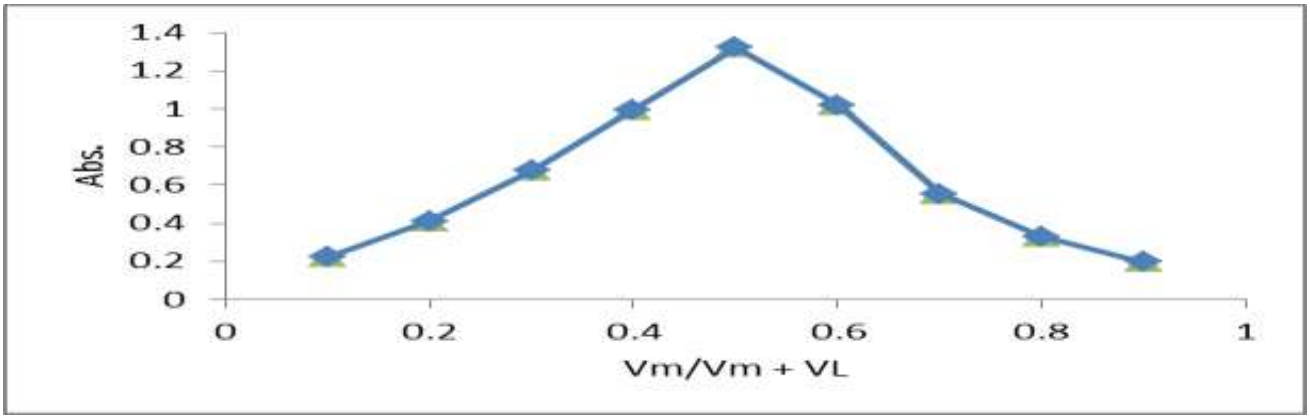
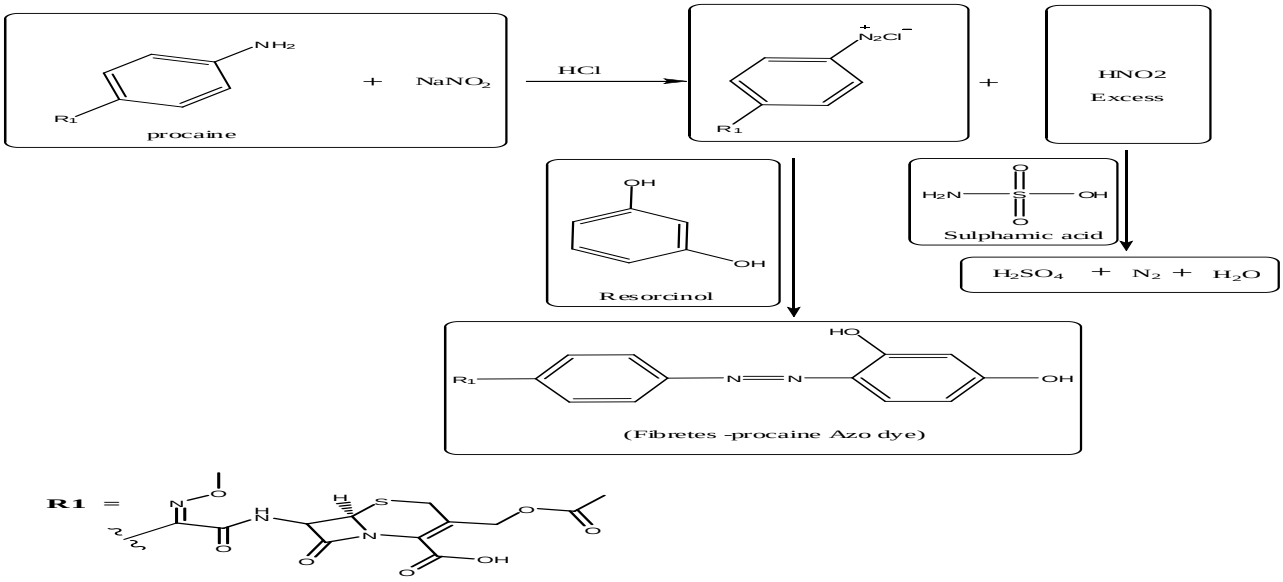



**Figure 5: Jobs method**

**Table 6: Stability constant for product**

| Drug | Conc. of drug M | Absorbance with quantitative conc. ($A_s$) | Absorbance with increasing in conc. of reagent( $A_m$) | α | Kst. L.mole$^{-1}$ |
|---|---|---|---|---|---|
| Ceftotaxime | 1.0×10$^{-4}$ | 0.466 | 0.478 | 0.007 | 1.55×10$^9$ |




**Scheme 1: Proposed mechanism of the reaction between ceftotaxime and resorcinol**

## Interference Study

In the beginning study we must determine which excipients found in the ceftotaxime drugs, the study done by taking 8.0 ppm of ceftotaxime with excess amount of excipients then measuring the absorbance. An error of the 5% in the absorbance readings was considered tolerable [20], none of these excipients interfered seriously Table (7).

**Table 7: Interference effect on ceftotaxime**

| Interference | (8 ppm) Ceftotaxime | | |
|---|---|---|---|
| | Conc. | E% | Rec% |
| **Tween 80** | 7.98 | - 0.25 | 99.75 |
| **PVP** | 8.18 | + 2.25 | 102.25 |
| **Acacia** | 7.96 | - 0.50 | 99.50 |
| **NaCl** | 7.97 | -0.37 | 99.62 |
| **Mennitol** | 8.14 | +1.75 | 101.75 |
| **Talc** | 8.12 | +1.50 | 101.50 |
| **Benzoic acid** | 8.03 | +0.37 | 100.37 |
| **Lactose** | 8.04 | +0.50 | 100.50 |
| **Sucrose** | 8.19 | +2.37 | 102.37 |

## Pharmaceutical Applications

The proposed methods were applied to analysis for three different dosage forms contain ceftotaxime in order to evaluation the analytical usefulness of the spectrophtometric method.

Good results with good recoveries and reproducibility were obtained when determination of three different concentration of each pharmaceutical preparation tablet, therefore the proposed method successfully to the analysis.

**Table 8: Application on ceftotaxime capsuol**

| ceftotaxime capsuol | Concentration | | E% | Rec% |
|---|---|---|---|---|
| | Prepared ppm | Measured ppm | | |
| | 5 | 5.15 | +0.03 | 100.03 |
| | 10 | 10.09 | +0.9 | 100.9 |
| | 15 | 14.93 | -0.4 | 99.6 |

## Conclusion

The proposed method is found to be rapid, simple, selective and highly sensitive than must of spectrophotometric methods available in the literature; the recovery study data indicate the reproducibility and accuracy of method. This method can be adopted as excellent spectrophotometric method.

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