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# Spectrophotometric determination of Ceftiofur in pharmaceutical formulations by folin cio calteu & ammonium molybdate

**M.Kishore[1]* Y.Hanumantharao[2]**

[1]Department of chemistry, SVRM College of post-graduate studies and research center, Nagaram, Gunturu (District), Andhra Pradesh, (INDIA)

[2]Department of chemistry, Andhra Loyola College (Autonomous), Vijayawada, Krishna (District), Andhra Pradesh, (INDIA)

E-mail : medikissi@gmail.com

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## ABSTRACT

Simple, accurate and reproducible spectrophotometric methods were established for the assay of Ceftiofur (CEFT) based on the formation of reduction, polymerization and condensation products. These two methods yield good results, included evaluation of the range, linearity, precision, accuracy, recovery, and specificity. The spectrophotometric determinations were performed at 750 and 710 nm. A prospective validation showed that the methods are linear (r = 0.9999) and precise. The results demonstrated the validity of the proposed method as a simple and useful alternative for the determination of Ceftiofur in routine QC analyses.



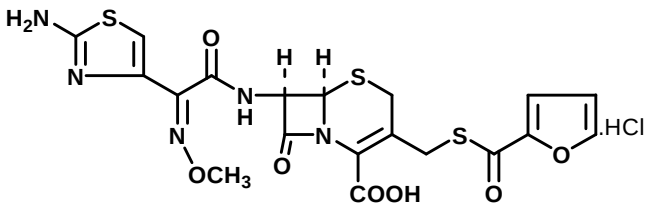
## KEYWORDS

Ceftiofur;
Spectrophotometric methods;
Reduction;
Polymerization and condensation products;
Statistical analysis;
Recovery studies.

## INTRODUCTION

Ceftiofur (Scheme 1) (CAS Number: [80370-57-6]; IUPAC Name: (6R,7R)-7- [[(2z)-(2-Amino-4-thiozolyl (methoxyimino) acetyl] amino]-3-[[2-furanyl carbonyl} thio], methyl]-8-oxo-5-thia-1-azabicyclo [4-2.0] oct-2-ene-2-carboxylic acid) is a part of a family of powerful antibiotics[1]. They are known as the third generation cephalosporins. The non-steroidal anti-inflammatory drugs (NSAIDS), such as flunixin, ketoprofen and carprofen were used in conjuction with ceftiofur, in the treatment of naturally occurring bovine respiratory diseases. Ceftiofur (CEFT) has worldwide approvals for respiratory disease in swine, ruminants and horses and has also been approved for foot rot and metritis infections in cattle.

A very few physicochemical methods have been reported in the literature for the assay of CEFT in biological fluids and pharmaceutical formulations. Most of them are based on spectrophotometric methods[2,3,20-22], HPLC[4-8], GC[9,10], fluorimetry[11-13], LC-MS[14], GC-MS[15-17], TLC[18] and Mass[19]. The analytically useful functional groups in CEFT includes 2-amino-4-thiazoyl, β-lactam, carboxyl and double bond in dihydrothiazine have not been fully exploited for designing suitable spectrophotometric methods and so still offer a scope to develop more visible spectrophoto-




**Scheme 1 : Molecular formula $C_{19}H_{17}N_5O_7S_3$. HCl**

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**TABLE 1 : Optical and regression characteristics, precision and accuracy of the proposed methods for CEFT**

| Parameter | $M_1$ | $M_2$ |
|---|---|---|
| $\lambda_{max}$ (nm) | 750 | 710 |
| Beer's law limits (µg/ml) | 4-24 | 5-30 |
| Detection limit (µg/ml) | 0.0382 | 0.1416 |
| Molar absorptivity (1 $mol^{-1}.cm^{-1}$) | $9.502\times10^{3}$ | $8.768\times10^{3}$ |
| Sandell's sensitivity (µg.$cm^{-2}$/0.001 absorbance unit) | $7.969\times10^{-2}$ | 0.1578 |
| Optimum photometric range (µg/ml) | 7.08-21 | 12.59-25.12 |
| Regression equation (Y=a + bc) slope (b) | 0.1743 | 0.01644 |
| Standard deviation on slope ($S_b$) | $1.6900\times10^{-4}$ | $4.536\times10^{-5}$ |
| Intercept (a) | $2.500\times10^{-3}$ | $5.000\times10^{-4}$ |
| Standard deviation on intercept ($S_a$) | $2.2420\times10^{-3}$ | $7.522\times10^{-4}$ |
| Standard error on estimation ($S_e$) | $2.138\times10^{-3}$ | $7.172\times10^{-4}$ |
| Correlation coefficient (r) | 0.9999 | 0.9999 |
| Relative standard deviation (%) * | 0.4165 | 1.0021 |
| % Range of error (confidence limits) | | |
| 0.05 level | 0.4812 | 1.1522 |
| 0.01 level | 0.7510 | 1.8069 |

***Average of six determinations considered**

metric methods with better sensitivity, selectivity, precision and accuracy, method to compare the results obtained by the proposed methods.

## EXPERIMENTAL

An Elico UV-Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

### Preparation of the reagents

All the chemicals and reagents used were of analytical grade and the aqueous solutions were freshly prepared with triple distilled water.

A 1 mg/ml stock solution was prepared by dissolving 100 mg of pure CEFT in 100 ml of distilled water and working standards of required concentration were prepared. Solution of Folin Cio Calteu (FC) (Loba; 2N) reagent used as it is, $Na_2CO_3$ solution (Loba, 10%, $9.43\times10^{-1}$M) were prepared for method1; for method 2, By dissolving 2 g of Ammonium Molybdate (AM) (Loba; 2%, $1.618\times10^{-2}$ M) in 100 ml of distilled water, 105 ml Conc. $H_2SO_4$ to 100 ml of distilled water (1M) initially followed by diluting to 1000 ml with distilled water.

**TABLE 2 : Assay of CEFT in pharmaceutical formulations**

| Formulations * | Amount taken (mg) | Amount found by proposed Methods** | | Reference method | Percentage recovery by proposed methods*** | |
|---|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | | $M_1$ | $M_2$ |
| Tablet I | 50 | 49.91±0.52<br>F = 2.735<br>t = 0.7781 | 49.88±0.72<br>F = 1.42<br>t = 0.8178 | 50.22±0.86 | 99.93±0.41 | 99.85±0.36 |
| Tablet II | 50 | 49.53±0.51<br>F = 1.477<br>t = 0.866 | 49.75±0.42<br>F = 2.17<br>t = 0.4453 | 49.93±0.62 | 99.73±0.61 | 99.89±0.39 |
| Tablet III | 50 | 49.82±0.38<br>F = 2.09<br>t = 0.3276 | 49.72±0.44<br>F = 1.56<br>t = 0.55 | 49.96±0.55 | 99.83±0.35 | 99.91±0.43 |
| Tablet IV | 50 | 49.98±0.39<br>F = 3.503<br>t = 0.3576 | 49.82±0.43<br>F = 2.882<br>t = 0.6448 | 50.17±0.73 | 99.85±0.31 | 99.91±0.62 |

*** Tablets from four different pharmaceutical companies**
**** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57**
*****Recovery of 10mg added to the pre-analyzed pharmaceutical formulations (average of three determinations)**

### Recommended procedures

### Method $M_1$ (FC)

Delivered aliquots of standard drug solution (0.5-3.0 ml 100µg/ml) in to a series of 25 ml calibrated tubes and the volume were adjusted to 3.0 ml with distilled water. To each of test tubes 5.0 ml of $Na_2CO_3$ and 1.5 ml of FC reagent were added and kept aside for 5 min. The volume was brought to the mark with distilled water. The absorbance was measured after 15 min at 740 nm against reagent blank prepared under identical conditions. The amount of CEFT present in the sample was computed from the calibration graph.

### Method $M_2$ (AM)

Aliquots of standard drug solution (0.5 - 2.5ml, 100 µg/ml) were delivered in to a series of 10 ml calibrated tube. To each tube 1.0 ml of AM ($1.618\times10^{-2}$ M) reagent and 0.5 ml of 1 M $H_2SO_4$ were added to each tube and the contents were heated for 20 min in boiling water bath. After cooling the volume was made up to 10 ml with distilled water. The resulting absorbance of the green color was measured at 710 nm against a reagent blank. The amount of drug was computed from to appropriate calibration graph.

**Reference method**

An accurately weighed amount of formulation (Tablets powder) equivalent to 100 mg was dissolved in a few ml of ethyl alcohol evaporated to dryness and dissolved made upto 100 ml. 50 ml of this filtrate was further diluted to 100 ml with distilled water to obtain to a concentration of 500 μg/ml. It was further diluted step wise with distilled water to get the concentration of 25 μg/ml. Aliquots of CEFT solution 1.0-5.0 ml, 25 μg/ml were taken into a series of 5 ml calibrated tubes and made upto the mark with distilled water. The absorbance of each solution was measured at 250 nm against distilled water. The concentration of the drug was computed from its calibration graph.

## RESULTS AND DISCUSSION

CEFT probably affects a reduction 1,2 or 3 oxygen atoms from tungstate and / or molybdate in FC reagent (phosphomolybdo tungstate), thereby producing one or more of the possible reduced species which have a characteristic intense blue colour. In the tetrahedral anion $MoO_4^{-2}$ in aqueous medium, which is strongly oxidized form, on acidification with conc. $H_2SO_4$ exist as isopolyanionic species as a result of polymerization and condensation reaction having an arrangement $Mo_6$ octahedra as exemplified by $[Mo_7O_{24}]^{-6}$ and $[Mo_6O_{26}]^{-4}$.

The optical characteristics such as Beer's law limits, absorption maxima, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation and percent range of error (0.05 level and 0.01 confidence limits) were calculated for the proposed methods and the results are summarized in TABLE 1. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation (R) obtained from different concentrations and the results are summarized in Table 1.The optimum conditions for the colour development were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The values obtained for the determination of CEFT in tablets by the proposed and Spectroscopic methods is compared in TABLE 2. To evaluate the validity and reproducibility of the proposed methods, known amounts of pure drug was added to previously analyze pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are given in TABLE 2.

## CONCLUSIONS

The developed Spectrophotometric methods for the estimation of CEFT were found to be simple and useful with high accuracy, precision, and reproducible. Sample recovery in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, this suggesting the validity of the methods and non interference of formulation excipients in the estimation.

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