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Spectrophotometric determination of Ceftiofur in pharmaceutical formulations by FGFCF, SFNO and MB

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Abstract:
Three simple spectrophotometric methods are proposed for the determination of Ceftiofur (CEFT) in bulk drug and in pharmaceutical preparations. The method is based on oxidation product with Fast green FCF (FGFCF)/$KMnO_4$-, and ion association complex with safranin O (SFNO) and Methylene blue (MB). These two methods yield good results, included evaluation of the range, linearity, precision, accuracy, recovery, and specificity. The spectrophotometric determinations were performed at 625 (FGFCF), 530 (SFNO) and 655 nm (MB). A prospective validation showed that the methods are linear (r = 0.9999) and precise. The methods were successfully applied to the assay of CEFT in tablet preparations and the results were statistically compared with those of the reference method by applying the Student's t-test and F-test. No interference was observed from the common tablet excipients. The accuracy of the method was further ascertained by performing recovery studies *via* standard addition method.

Key words: *Ceftiofur, spectrophotometric methods, oxidation, ion complex methods, statistical analysis, recovery studies*

Introduction:

Ceftiofur (Figure 1) is a (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.

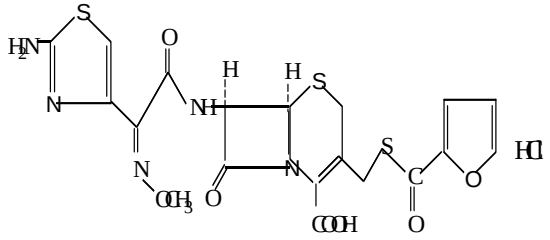


Figure 1: Molecular formula C_{19} H_{17} N_5 O_7 S_3. HCl

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 spectrophotometric 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.

Preparation of standard Drug solution: 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.

Method M_1: $KMnO_4$ solution (BDH; 0.0316%, 2.0 × 10^{-3} M): Prepared by dissolving 31.6 mg of $KMnO_4$ in 100 ml of 2.0M H_2SO_4 solution and this solution was kept aside for 2-3 hrs.

FGFCF solution (Chroma; 0.01%, 1.236 × 10^{-4}M): Prepared by dissolving 100 mg of Fast green FCF in 100 ml of 1.0M H_2SO_4 and 10.0ml of this solution was further diluted to 100ml with same strength of acid

Na_2SO_4 solution (BDH; 14.2 %, 1M): Prepared by dissolving 14.2g of sodium sulphate in 100ml of distilled water

Method M_2:SFNO solution (Fluka; 0.2 %, w/v 5.714 × 10^{-3}M): Prepared by dissolving 200 mg of safranin O in 100 ml of distilled water and subsequently washed with chloroform to remove chloroform impurities

Method M_3: MB solution(Fluka; 0.2%, w/v 6.25 × 10^{-3}M): Prepared by dissolving 200 mg of Methyleneblue in 100 ml of distilled water and subsequently washed with chloroform to remove chloroform soluble impurities.

Buffer solution pH 9.8 (NH_4OH – NH_4Cl): 7 g of NH_4Cl and 6.8ml of liquid Ammonia solutions were mixed and diluted to 100 ml with distilled water and pH was adjusted to 9.8.

Recommended procedures

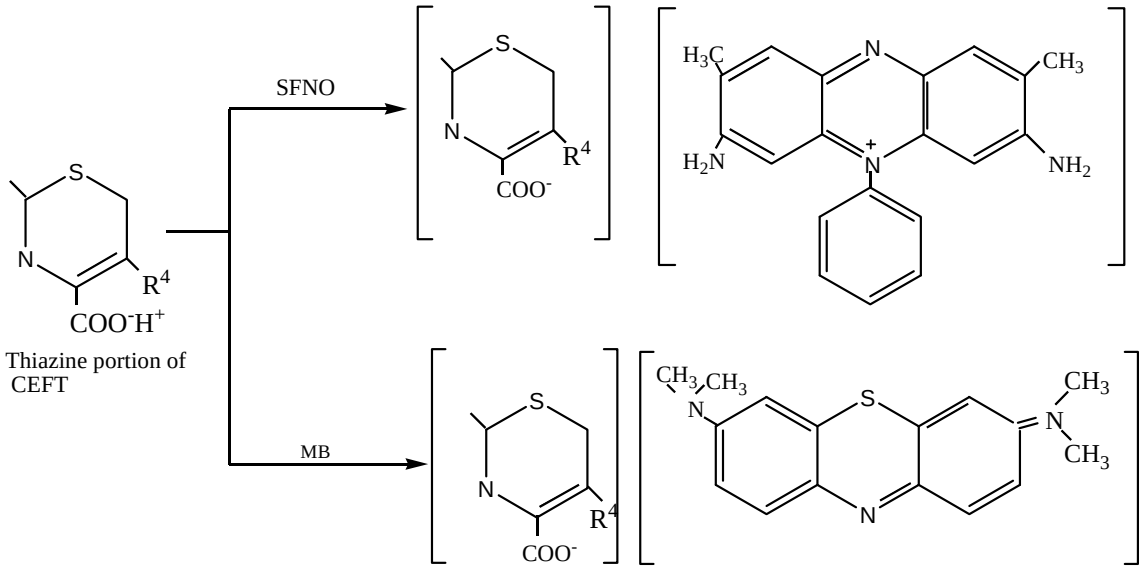
Method M_1: Aliquots (0.5-3.0ml, 20μg/ml) of standard CEFT solution were taken into a series of 25 ml calibrated tubes. To these tubes, 1.0 ml of $KMnO_4$ solution was added and the total volume in each tube was brought to 10 ml with distilled water and kept aside for 15 min at room temperature. Then 4.0 ml each of FGFCF solution and 1M sodium sulphate solution were added successively. After 10 min, the volume was made upto the mark with distilled water. The absorbance was measured at 625 nm against distilled water. A blank experiment was carried out in a similar manner omitting drug. The decrease in absorbance corresponding to consumed permanganate and in turn the drug concentration was obtained by subtracting the decrease in absorbance of the test solution (dye-test) from that of the blank solution (dye-blank). The amount of CEFT was deduced from its calibration graph.

Method M_2 & M_3: Aliquots of standard solution 1.0 - 5.0 ml for method M_2 (0.5-3.0ml 25 μg/ml) and 1.0 ml of pH 9.8 buffer solution were placed separately in a series of 125 ml separating funnels. A volume of 1.0 ml of safranin O or 0.5 ml of MB was added respectively. The total volume of aqueous phase in each funnel was adjusted to 10 ml with distilled water. There 10 ml of chloroform was added in each separating funnel and the contents were shaken for 2 min and allowed to separate. The organic layer was collected through cotton plug and the absorbance was measured immediate at 530 nm (for method M_2) and at 655 nm (for method M_3) against reagent blank. Both the colored species were stable for 2 hours. The amount of drug in a sample was obtained from the Beer's Lambert plot

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:

The analytically important functional moieties such as 2-amino - 4-thiazolyl, hetero sulphur, unsaturated centers, β-lactam and six membered dihydro thiazine 4-carboxyl present in the ceftiofur were exploited in developing proposed methods. CEFT exhibits reducing property due to the presence of functional moieties vulnerable to oxidation selectively with oxidizing agents such as MnO_4^- (M_1) under controlled conditions. When treated with known excess of oxidant CEFT



Scheme 1

undergoes oxidation giving products of oxidation besides unreacted oxidant. The unreacted oxidant has been estimated colorimetrically either by decrease in the intensity of dye color due to disruption of chromophoric centers in the dye. The first step in the method mentioned above is the oxidation of CEFT with the oxidant.

CEFT + MnO_4^- → oxidation products + Mn (II) + unreacted MnO_4^-

The second step concerns with the estimation of the unreacted oxidant with appropriate dye or chromogenic reagent

MnO_4^- + FGFCF → FGFCF + Mn (II) + Mixture of compounds

As CEFT possesses carboxyl group (acidic), it forms an ion association complex with basic dye (saffranin O or methylene blue) which is extractable into chloroform from aqueous phase. The carboxylate anion (negative charge) of CEFT is expected to attract the oppositely charged part of the dye (positive charge, safranine O or methylene blue) and behave as single unit being held together by electrostatic attraction. It is supported by slope ratio method which was obtained as 1:1 in each method (M_2&M_3). Based on analogy the structure of ion association complexes are shown in scheme 1.

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 also given in Table 2.

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

Parameter	M_1	M_2	M_3
λ_{max} (nm)	625	530	655
Beer's law limits (μg/ml)	0.4-2.4	2.5-15	1.25-7.5
Detection limit (μg/ml)	0.04074	0.1532	0.1082
Molar absorptivity (1 mol/cm)	2.745×10^4	1.770×10^3	4.515×10^3
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	3.934×10^{-2}	6.171×10^{-2}	5.357×10^{-2}
Optimum photometric range (μg/ml)	1.2-2.4	7-14	1.8-7.1
Regression equation (Y=a+bc)			
slope (b)	0.127125	0.03672	0.08054
Standard deviation on slope (S_b)	1.286×10^{-3}	1.969×10^{-4}	6.964×10^{-4}
Intercept (a)	1.5×10^{-3}	4.749×10^{-3}	5.000×10^{-3}
Standard deviation on intercept (S_a)	1.7060×10^{-3}	1.632×10^{-3}	2.886×10^{-3}
Standard error on estimation (S_e)	1.627×10^{-3}	1.558×10^{-3}	2.752×10^{-3}
Correlation coefficient (r)	625	0.9999	0.9999
Relative standard deviation (%)*	0.4-2.4	0.7005	0.7007
% Range of error (confidence limits)	0.04074		
0.05 level	2.745×10^4	0.8052	0.8057
0.01 level	3.934×10^{-2}	1.263	1.263

* Average of six determinations considered

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_3		M_1	M_2	M_3
Tablet I	50	49.87±0.29 F = 2.09 t = 0.5366	49.73±0.33 F = 1.613 t = 1.1546	49.62±0.28 F = 2.18 t = 1.781	49.98± 0.42	99.83 ±0.42	99.93± 0.53	99.92 ±0.25
Tablet II	50	49.81±0.18 F = 1.92 t = 0.8861	49.78±0.13 F = 3.693 t = 1.276	49.80±0.21 F = ±1.417 t = 0.9036	49.92± 0.25	99.92 ±0.33	99.92± 0.63	99.83 ±0.18
Tablet III	50	49.92±0.22 F=2.983 t=0.8082	49.88±0.31 F = 1.502 t = 0.9169	49.81±0.29 F = 1.717 t = 1.312	50.06± 0.38	99.84 ±0.31	99.91± 0.61	99.96 ±0.24
Tablet IV	50	49.54±0.28 F= 4 t = 1.732	49.78±0.41 F = 1.865 t = 0.6495	49.59±0.38 F = 2.171 t = 1.363	49.96± 0.56	99.93 ±0.39	99.85± 0.23	99.93 ±0.35

* 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 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

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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