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## Spectrofluorimetric Estimation of Cefdinir in Formulation

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Accepted 28 August 2004

Revised 27 April 2004

Received 19 October 2003

**A simple, accurate and sensitive spectrofluorimetric procedure was developed for the estimation of cefdinir containing heterocyclic fused ring structure, in 1 M sodium hydroxide at 95° for 1 h, which shows strong fluorescence having excitation and emission wavelength 262 and 530 nm, respectively. Linear relationship for the fluorescence intensity was obtained in the range of 0.2–1 µg/ml. The method was statistically validated and was applied successfully to determine the cefdinir in pharmaceutical dosage form.**

Cefdinir<sup>1,2</sup> is an extended-spectrum, semi-synthetic cephalosporin antibiotic for oral administration. Chemically it is [6R-[6 $\alpha$ ,7 $\beta$ (z)]-7-[(2-aminothiazolyl) (hydroxyimino) acetyl] amino] -3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (fig. 1). It is not official in any pharmacopoeia. Literature survey revealed that a microbiological assay<sup>3</sup> and HPLC<sup>4</sup> methods have been reported for the estimation of cefdinir from biological fluids. Therefore, it was thought necessary to develop a specific, simple, precise and accurate spectrofluorimetric method for the estimation of cefdinir in its pharmaceutical dosage form.

Generally fluorescence occurs because of transition from first excited singlet state to ground state by emission of light<sup>5,6</sup>. Cefdinir consists of a heterocyclic fused ring structure, which is responsible for fluorescent behaviour<sup>7</sup> in alkaline medium at 95° for 1 h<sup>8</sup>. This fluorophore showed an excitation wavelength at 262 nm with an emission wavelength at 530 nm.

Pure authentic sample of cefdinir was procured from Unichem Laboratories Ltd., Mumbai and 300 mg capsules Sefdin and Cefdiel were purchased from Thulasi Pharmacies India (Pvt.) Ltd., Coimbatore. All chemicals used in this

investigation were of AR grade. Triple distilled water was used in this study.

A Jasco model FP/750 fluorimeter with single quartz cell of 1 cm path length was used to measure fluorescence intensity of the resulting solutions. Buffer pH was measured and adjusted using a digital pH meter (Elico make) and a constant temperature water bath (Labtronics make) were also used in the study. Phosphate buffer of pH 7 was used for the preparation of solutions. The buffer was prepared by mixing 61.1 ml of dibasic sodium hydrogen phosphate solution (0.95 %w/v solution of Na<sub>2</sub>HPO<sub>4</sub> in water) with 38.9 ml of monobasic potassium dihydrogen phosphate solution (0.9 %w/v of KH<sub>2</sub>PO<sub>4</sub> in water).

As cefdinir was poorly soluble in other solvents like hydrochloric acid, acetic acid, ammonium hydroxide, acetate buffer, and phosphate buffer of pH 3, 4, 5 and 6, phosphate buffer of pH 7 was taken as the solvent of choice. Before developing the method the instrumental parameters like excitation bandwidth, emission bandwidth and response time were optimized at 5 nm, 5 nm and 0.02 s, respectively, as other bandwidth and response time such as 5 nm, 10 nm, 20 nm and 0.02, 0.05, 0.1, 0.25, 0.5, 1, 2 and 8 s have already been installed in the fluorimeter. Apart from these, different parameters like concentration and volume of sodium hydroxide, heating temperature, heating time

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TABLE 1: RESULTS OF ANALYSIS OF CEFDINIR BY SPECTROFLUORIMETRY

Drug	Amount ( mg/ cap)		Amount added for recovery (mg)	% recovery
	Labeled	Found		
Brand I	300	302.81	0.005	98.97 ± 0.07
Brand II	300	301.15	0.005	99.70 ± 0.12

All values are average of six determinations based on label claim. Brand I is cefdiel marketed by Ranbaxy Laboratories Ltd, New Delhi and Brand II is sefdin marketed by Unichem Laboratories Ltd, Mumbai.

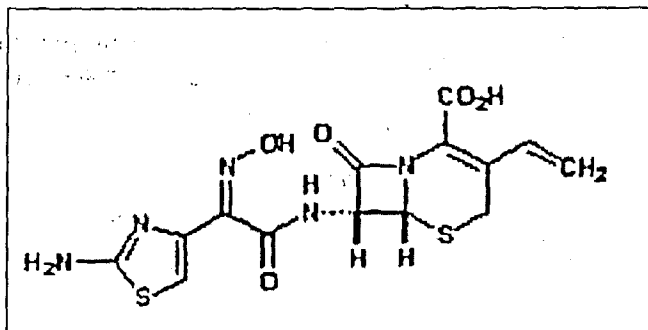


Fig.1: Structure of cefdinir.

required for development of maximum fluorescence intensity and stability of developed fluorophore were also optimized. Maximum fluorescence intensity was obtained with 9 ml of 1 M sodium hydroxide solution. On further increase in concentration and volume of sodium hydroxide, fluorescence intensity gets decreased. The minimum temperature and heating time required to get maximum fluorescence intensity was 95° for 1 h. On further heating fluorescence intensity gets decreased.

Standard stock solution cefdinir was prepared by dissolving 25 mg of the drug in 25 ml of pH 7 phosphate buffer to get 1mg/ml solution and this solution was suitably diluted with distilled water to get stock solution of concentration 25 µg/ml. Stock solution (0.2-1 ml ) was pipetted in to 25 ml volumetric flask. Then 9 ml of 1 M sodium hydroxide was added to each flask and mixed. The mixture was heated at 95° for 1 h in boiling water bath, cooled to room temperature and volume was adjusted up to the mark with distilled water. The fluorescence intensity of resulting solution was measured at emission wavelength 530 nm keeping excitation wavelength 262 nm. The calibration curve was prepared by plotting concentration of cefdinir Vs relative fluorescence intensity of the respective solution. The method was found to follow the regression equation  $y=0.0228X+0.012$  with a correlation coefficient of 0.9989.

Twenty capsules of cefdinir were weighed and average weight was calculated. The quantity equivalent to 25 mg was weighed accurately and taken in to 25 ml volumetric flask. Cefdinir was extracted in pH 7 phosphate buffer, filtered and the volume was adjusted to 25 ml with distilled water. This solution was suitably diluted again with distilled water to get stock solution of concentration 25 µg/ml. From this, 0.4 ml was pipetted out in to a 25 ml volumetric flask, 9 ml of 1 M sodium hydroxide was added and was heated at 95° for 1 h. The solution was cooled to room temperature and volume was made upto 25 ml with distilled water. The fluorescence intensity of resulting solution was measured at emission wavelength 530 nm keeping excitation wavelength 262 nm and the concentration of cefdinir in formulations were calculated from the standard graph. The results are given in Table 1.

The developed method was validated for accuracy and reproducibility by carrying out the recovery studies after confirming non interference from diluents like lactose, mannitol, magnesium carbonate and lubricant, magnesium stearate in capsule formulation. To the preanalyzed sample

TABLE 2: VALIDATION PARAMETERS FOR SPECTROFLUORIMETRIC METHOD

Parameters	Range
Linearity range (µg/ml)	0.2-1
Detection limit (µg/ml)	0.04
Quantification limit (µg/ml)	0.2
Correlation Coefficient ( r )	0.998931
Quantum efficiency ( Ø )	
(Ref: quinine sulphate)	0.9767
Accuracy ( % w/v) ( n = 6)	98 -102
Precision ( n = 6)	1.32-2.58
Specificity	Specific

formulation of 5 µg, 0.4 ml (10 µg) of stock solution, 9 ml of 1M sodium hydroxide were added and heated for 1h in water bath at 95°. The volume was made upto 25 ml with distilled water to get a concentration of 0.6 µg/ml and was reanalyzed as mentioned earlier. The recovery was close to 100 % indicating the reproducibility and accuracy of the method.

The fluorescence intensity has linear relationship in the concentration range of 0.2-1 µg/ml. The quantum efficiency ( $\phi$ ) was calculated for the present method using reported method and was found to be 0.9767 using quinine sulphate as reference<sup>7</sup>. Stability study proved that the developed fluorophore was stable upto 2 h at room temperature ( $28 \pm 1^\circ$ ). After that fluorescence intensity diminished gradually. The LOD and LOQ were found to be 0.04 and 0.2 µg/ml, respectively (Table 2). Thus the developed method was specific, accurate, simple, precise and reproducible, implying its use in the routine analysis of cefdinir in formulation.

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## Involvement of Potassium Channels in the Release of Various Hormones

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Accepted 1 September 2004

Revised 5 May 2004

Received 5 March 2003

The present investigation was taken up to study the effect of long term treatment (30 days) of various potassium channel openers and blockers on serum insulin, glucose,  $T_3$ , TSH and plasma cortisol in rats. Treatment with cromakalim for 30 days produced a significant decrease in serum insulin levels in rats. However, treatment with pinacidil and glibenclamide produced a significant increase in serum insulin levels. KRN 2391 did not produce any effect on serum insulin levels. Glucose levels were decreased significantly only with glibenclamide and no significant alteration in serum glucose levels was observed with any of the potassium channel openers. Serum  $T_3$  levels were significantly increased with cromakalim and glibenclamide. However no significant alteration in serum  $T_3$  levels was observed with pinacidil and KRN 2391. Serum TSH levels were significantly decreased with pinacidil. No significant alteration was observed in serum TSH levels by other potassium channel modulators. Serum cortisol levels were significantly decreased with all the three potassium channel openers while glibenclamide did not produce any significant change in serum cortisol levels. Our data suggest that potassium channels may be involved in the release of

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