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Spectrophotometric Methods for the Determination of Cefotaxime Sodium in Dosage forms

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Two simple and sensitive spectrophotometric methods (A and B) in the visible region have been developed for the determination of cefotaxime sodium (CFTS) in bulk and in dosage forms. Method A is based on the reaction of CFTS with nitrous acid under alkaline conditions to form a stable violet colored chromogen with absorption maximum of 560 nm and method B is based on the reaction of CFTS with 1,10-phenanthroline and ferric chloride to form a red colored Chromogen witht the absorption maximum of 520mm. The color obeyed Beer's law in the concentration range of 100-500 µg/ml for method A and 1.6-16 µg/ml for method B, respectively. When pharmaceutical preparations containing CFTS were analysed, the results obtained by the proposed methods are in good agreement with the labeled amounts and are comparable with the results obtained using a UV spectrophotometric method.

CFTS[1] chemically known as (6R, 7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-3-(hydroxy methyl)-8-oxo-5-thia-1-aza bicyclo [4,2,0] oct-2-ene-2-carboxylic acid α-(o-methyl oxime) acetate monosodium salt, is a third generation cephalosporin antibiotic used in the management of mild to moderate infections caused due to susceptible microorganisms. Many methods were reported for its determination such as HPLC[2-7], microbiological[8-10] and spectrophotometry[11-14]. A review of the literature reveals that considerable attention has not been paid to the colorimetric determination of this drug. In the present work, the reaction of CFTS with nitrous acid under alkaline conditions to form a stable violet colored chromogen (method A) and the reaction of CFTS with 1,10-phenanthroline and ferric chloride to form a stable red colored chromogen (method B) were used for the determination of CFTS.

A stock solution of CFTS (1 mg/ml) was prepared by dissolving 100 mg of the drug in 100 ml of distilled water. Working standard solutions were obtained by appropriate dilution of the stock solution. Solutions of sodium nitrite (1% w/v), hydrochloric acid (0.5 N), sodium hydroxide (5% w/v), 1,10-phenanthroline (0.01 M), ferric chloride (0.003 M) and ortho-phosphoric acid (0.2 M) were prepared in distilled water. All chemicals used were of

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AnalaR or pharmacopoeial grade. An ELICO UV-VIS spectrophotometer model SL-150 with 10 mm matched quartz cells was used for all absorbance measurements. CFTS was obtained as gift sample from M/s. Fytokem Formulations, Vijayawada.

In method A, aliquots of 1-5 ml of standard CFTS solution (1 mg/ml) were taken into a series of 10 ml volumetric flasks. To each flask 1 ml of sodium nitrite solution and 1 ml of hydrochloric acid were added and kept aside for 3 min. Then 1 ml of sodium hydroxide solution was added and the volume was made upto 10 ml with distilled water. The absorbance of the violet colored solution was measured at 560 nm against a reagent blank. The amount of CFTS was calculated from the calibration graph.

In method B, aliquots of drug solution (ranging from 0.4-4 ml) were transferred to a series of 25 ml volumetric flasks. To each flask 2.5 ml of ferric chloride solution and 2 ml of 1,10-phenanthroline reagent were added successively. The flasks were heated on a water bath at 90° for 30 min and then cooled to room temperature. Two milliliters of ortho-phosphoric acid was added to each flask. The solutions were diluted to the mark with distilled water and allowed to stand for 15 min. The absorbance of the blood red color developed was measured at 520 nm against a reagent blank.

The contents of 5 vials are pooled, accurately weighed and the average weight noted. An accurately weighed quantity of vial powder equivalent to 100 mg of CFTS was weighed and the solution was prepared and analysed as described above.

Recovery experiments in both the methods were performed by adding known amount of drug to previously analysed pharmaceutical preparations and also to various excipients like starch, lactose and magnesium stearate used in formulations. The results are given in Table 2.

Beer's law limits, molar absorptivity, sandell's sensitivity, slope and intercept of regression analysis using least square method, precision and accuracy of the analysis of six replicate samples containing 3/4 of the amount of upper Beer's law limits in each method are summarized in Table 1. When injections containing CFTS were analysed the results obtained by the proposed methods (Table 2) are in good agreement with the labelled amount and are comparable with the results of UV spectrophotometric method. Recovery in both the methods is 98-101%. Method B is more sensitive than method A. Diluents and excipients like starch, lactose and magnesium stearate present in dosage forms did not interfere in the proposed methods.

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameter	Method A	Method B
λ_{max} (nm)	560	520
Beer's law limits (µg/ml)	100-500	1.6-16
Molar absorptivity (lit.mole^{-1}.cm^{-1})	1.547×10^4	2.684×10^4
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.308	0.0178
Regression equation (Y = a+bX)		
Slope (b)	3.2×10^{-4}	2.25×10^{-3}
Intercept (a)	9.2×10^{-3}	1.0×10^{-2}
Correlation coefficient (r)	0.9939	0.9987
% RSD	3.46	0.4392
% Range of error		
0.05 level confidence limit	2.893	0.3672
0.01 level confidence limit	4.281	0.5433

The formation of colored species in method A is due to the fact that cephalosporins develop a stable, concentration dependent violet color after reaction with nitrous acid under alkaline conditions. Color formation is highly specific, as it requires certain structural features such as the simultaneous presence of an intact aminothiazole ring and an alkoxyimino group in syn configuration. The red colored complex (Ferroin) in method B is due to the reaction between 1,10-phenanthroline and ferrous ions (formed by the reduction of ferric chloride by CFTS).

The results indicate that the proposed methods are sensitive, accurate and precise and can be used for the routine determination of CFTS in bulk and in dosage forms.

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TABLE 2: ANALYSIS OF CEFOTAXIME SODIUM DOSAGE FORMS USING THE PROPOSED METHODS

Pharmaceutical Preparation	Labelled amount (mg/vial)	Amount found in mg by			% Recovery*	
		Method A	Method B	UV Method	Method A	Method B
Injection - I	250	249.50	249.80	249.20	99.75±0.5	98.75±0.4
Injection - II	250	249.30	249.75	249.70	99.50±0.7	100.95±0.2
Injection - III	500	501.50	499.30	500.50	100.25±0.4	101.50±0.1
Injection - IV	500	500.95	501.75	501.20	101.10±0.3	99.80±0.2
Injection - V	1000	1010.50	1005.50	1004.75	98.95±0.8	99.95±0.3

*Mean and standard deviation of 6 determinations.

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