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Visible spectrophotometric methods for the quantitative estimation of CPH in their formulations

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Abstract

Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of CPH (Cefetamet Pivoxil as Hydrochloride) based on the formation redox products. Method A, Method B involves the redox reactions of the CPH. The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (A-B) are given. Regression analysis using the method of least squares was made to evaluate the slope(b), intercept(a) and correlation coefficien(r) and standard error of estimation (Se) for each system. Determination of CPH in bulk form and in pharmaceutical formulations were also incorporated.

Key words: Determination, redox, CPH

Introduction:

Cefetamet,[6R-[(6a,7β(Z))]]-7,[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo-[4,2-O]oct-2-ene-2-carboxylic acid (CPH), is an oral third-generation cephalosporin which is hydrolyzed to form the active agent cefetame[1-5]t. Cefetamet, because of its broad coverage of most gram-negative and gram-positive community-acquired pathogens, is one of the drugs of choice in the empiric therapy of respiratory and urinary community-acquired-infections[6,7] Literature survey serves only HPLC method[8-15] for analytical estimation of CPH; however, no spectroscopic studies for its estimation have been reported till date. Hence it was thought worthwhile to develop spectrophotometric method for the same. As the analytically useful functional groups in CPH have not been fully exploited for designing suitable, visible spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing thirteen visible spectrophotometric methods. All these methods have been extended to bulk and in its pharmaceutical formulation as well are described.

Experimental

i) Instruments used:

An Elico, UV-Visible digital spectrophotometer (SL - 159) 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.

ii) Preparation of standard drug solutions:

For Methods:

The stock solution (1mg/ml) of CPH was prepared by dissolving 100mg of it in 100ml 0.1M HCl. This solution was further diluted step wise with distilled water to obtain working standard solution of corresponding concentrations 200 μgml^{-1} [M_1, M_2].

iii) Proposed procedures:

After systematic and detailed study of the various parameters involved, as described under results and discussions the following procedures; [Methods NBS/CB (M_1); NBS/PMAP-SA (M_2)] were recommended for the assay of CPH in bulk samples and pharmaceutical formulations.

a) For Bulk samples

Method – M_1

Aliquots of standard CPH solution (0.5-3.0mL, 200$\mu g.mL^{-1}$) were transferred into a series of 25mL calibrated tubes. Then 1.25mL (5.0M) of HCl and 2.5mL ($5.618x10^{-4}$ M) of NBS were added. The volume was brought to15mL with distilled water. After 10min, 10mL ($5.50x10^{-4}$M) of CB solution was added and mixed thoroughly. The absorbance was measured after 5min at 535nm against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances were measured against distilled water. The decrease in absorbance corresponding to consumed NBS 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 CPH was computed from its calibration graph (Fig. 3).

Method – M_2

Aliquots of standard CPH solution (1.0-5.0mL, 200$\mu g.mL^{-1}$) were transferred into a series of 25mL calibrated tubes. Then 0.5mL (8.75×10^{-1}M) of AcOH and 2mL (4.94×10^{-3}M) of NBS solutions were added and kept aside for 15min at room temperature. Then 1.5mL (8.71×10^{-3}M) of PMAP solution was added. After 2min 2.0mL (1.16×10^{-2}M) of SA solution was added. The volume was made upto the mark with distilled water. The absorbance was measured after 10 min. at 520nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in the absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of CPH was computed from its calibration graph (Fig. 4).

b) For pharmaceutical formulations:

An accurately weighed portion of tablet content equivalent to about 100 mg of CPH was transferred into a 100mL volumetric flask. Added about 80mL of warm isopropyl alcohol and shaken well for about 20min. The contents were diluted with isopropyl alcohol up to the mark and mixed thoroughly. The solution was filtered. The filtrate was evaporated to dryness. The residue was used for the preparation of formulation solutions for different methods as given under standard solutions preparations. These solutions were analyzed as under procedures described fro bulk solutions.

Results and Discussions:

i. Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of CPH were taken and colors were developed separately by following the above procedures. The absorption

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spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in Fig.1&2. The absorption curves of the colored species in each method show characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

Fig. 1: Absorption spectrum of CPH with NBS - CB (M_1)

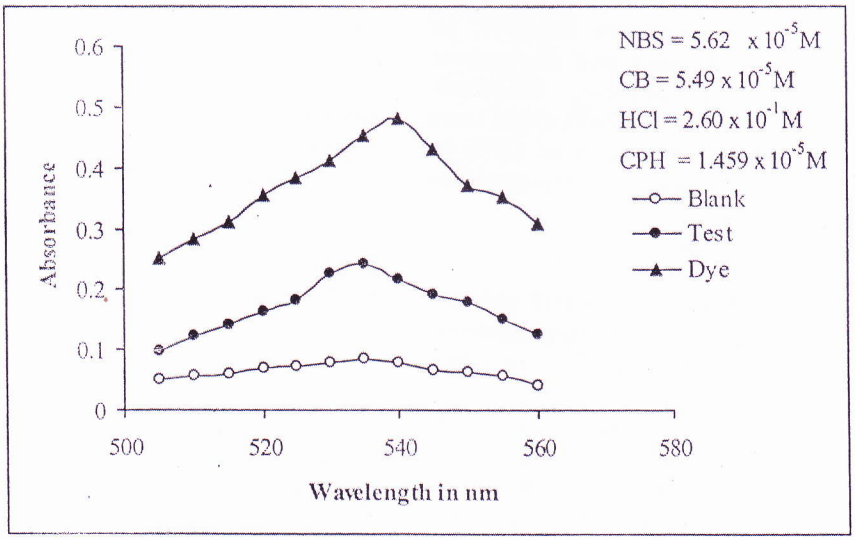
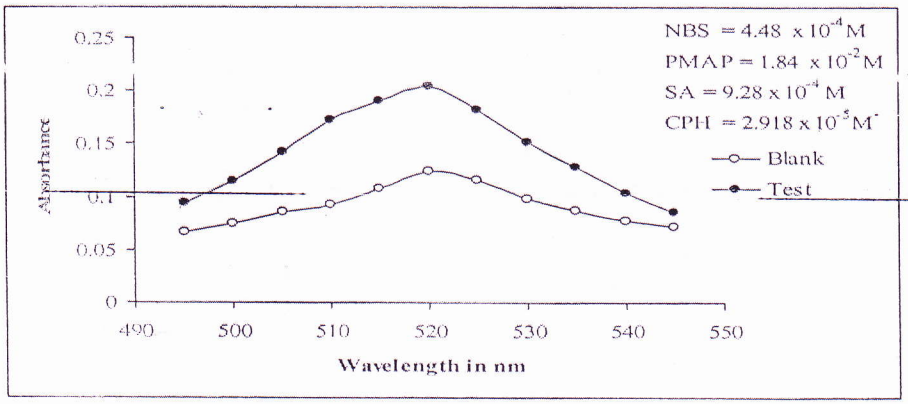


Fig. 2: Absorption spectrum of CPH with NBS – PMAP - SA (M_2)



appropriate wave lengths of a set of solutions containing varying amounts of CPH and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded graphically (Figs.3to4) against the corresponding reagent blanks. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range (Table.1) for CPH in each method developed. With mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values. (Table1).

iv. Precision:

The precision of each proposal methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of CPH in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table1).

v. Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of CPH within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in (Table1).

vi. Interference studies:

The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of CPH in methods (M_1 and M_2) under optimum conditions were investigated. The commonly used execipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

vii. Analysis of formulations:

Commercial formulations (tablets) containing CPH were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to different significantly. The results are summarized in (Table 2). Percent recoveries were determined by adding standard drug to preanalysed formulations. The results of the recovery experiments by the proposed methods are also listed in (Table 2).

Conclusions:

The proposed methods exploit the various functional groups in CPH molecule. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy with good sensitivity and higher λ_{max}. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations. The **order of sensitivity** among the proposed methods is: $M_1 > M_2$.

Thus, the proposed methods are simple, sensitive or selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of CPH in bulk form and pharmaceutical formulations

Fig. 3: Beer's Law plot of CPH with NBS - CB (M_1)

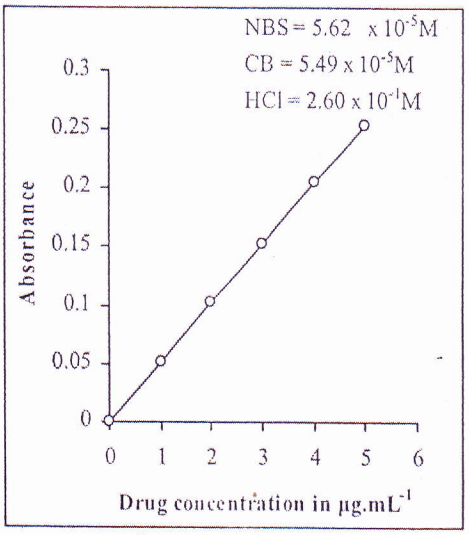
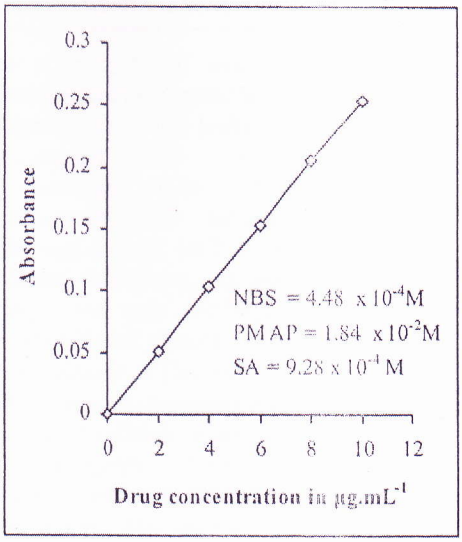


Fig. 4: Beer's Law plot of CPH with NBS – PMAP - SA (M_2)



ii. Optimum conditions fixation in procedures:

The optimum conditions for the color development of methods (M_1 and M_2) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Method – M_1 [NBS/CB]

The procedure involves two steps. The first step in the procedure is the reaction of CPH with an excess of NBS giving products involving oxidation, substitution or addition and the estimation of unreacted NBS using a known excess of CB (second step). The excess dye remaining was then measured with a spectrophotometer. The effect of reagent concentration (acidity, NBS and CB), waiting period in each step with respect to maximum sensitivity, minimum blank, adherence to Beer's law, reproducibility and stability of final color were studied by means of control experiments varying one parameter at a time .

Method – M_2 [NBS/PMAP/SA]

This is an indirect spectrophotometric method which involves two steps. In the first step, the volume of NBS required for oxidation of drug, the time and temperature for oxidation of the drug and volume of acetic acid were established through control experiments. In the second step, the volume of PMAP and the intermittent time between additions, volume of SA and the solvent for final dilution were found by varying one parameter at a time .

iii. Optical Characteristics:

In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at

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

Parameter	M_1	M_2
λ_{max} (nm)	535	520
Beer's law limits (µg/mL)	1.0-5.0	2.0-10.0
Detection limit (µg/mL)	1.316	5.994
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	2.808×10^4	2.212×10^4
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.13968	0.11240
Optimum photometric range (µg/mL)	0.3-0.7	0.6-1.0
Regression equation (Y=a+bc) slope (b)	4.86×10^{-2}	3.995×10^{-2}
Standard deviation on slope (S_b)	2.37×10^{-3}	6.994×10^{-4}
Intercept (a)	7.0×10^{-3}	4.50×10^{-3}
Standard deviation on intercept (S_a)	7.876×10^{-3}	4.639×10^{-3}
Standard error on estimation (S_e)	7.509×10^{-3}	4.423×10^{-3}
Correlation coefficient (r)	0.9964	0.9995
Relative standard deviation (%)*	1.6096	1.0288
% Range of error (confidence limits)		
0.05 level	1.6895	1.0799
0.01 level	1.6492	1.6935
% error in Bulk samples **		

* Average of six determinations considered
** Average of three determinations

Table 2: Assay of CPH 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	250	249.51±0.46 F=4.355 t=1.146	249.62±0.56 F=2.938 t=0.8204	249.98±0.96	99.81±0.98	99.85±0.99
Tablet II	500	499.36±0.46 F=3.680 t=1.356	499.20±0.96 F=1.855 t=1.530	499.52±0.79	99.88±0.98	99.87±0.55

* 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 = 2.262

*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

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