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Conventional and Advanced Spectrophotometric Methods for estimation of Cefetamet Pivoxil Hydrochloride in Bulk and in Pharmaceutical Formulation

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ABSTRACT: Purpose of the present study is to develop new, simple, cheap, fast, accurate, sensitive and precise colorimetric methods that can be used for the determination of cefetamet pivoxil hydrochloride. The first proposed method is colorimetric method using folin ciocalteu reagent and sodium hydroxide, color of newly formed complex was measured at room temperature within one hour; while the second method is derivative spectrophotometric method, first derivative spectra at sensitivity 2 were obtained for different standard drug solutions and the amplitude was calculated between maxima and minima. The developed methods were successfully applied to the determination of this drug in synthetic mixtures and commercially available tablets**.**

KEY WORDS: Cefetamet pivoxil hydrochloride (CPH), Colorimetric method, Derivative spectrophotometry, Method validation, Pharmaceutical formulation.

1. INTRODUCTION

Cefetamet [6R-[(6α, 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 (figure- 3) is an oral third generation cephalosporin which is hydrolyzed to form the active agent cefetamet [1,2]. 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 infection [3].

Literature survey serves only HPLC method [4] 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. In the present study, two methods for the determination of CPH in bulk and in its pharmaceutical formulation are described. First method is a colorimetric method in which phosphomolybdotugstic mixed acid of the folin ciocalteu reagent (FCR) is reduced by the cefetamet pivoxil in presence of sodium hydroxide (NaOH) to give a blue colour product having absorption maxima at 725 nm. The second method involves the principle of advanced spectrophotometric method in which a first derivative spectrum for CPH was obtained using derivative mode of the instrument. The maxima in the spectrum was at 221 nm and minima at 207 nm. The technique of derivative spectroscopy is convenient and precise as conventional spectroscopy, but offers advantage of increased specificity.

2. EXPERIMENTAL

2.1 Apparatus

Absorbance measurements were made on Shimadzu UV-1601 UV visible spectrophotometer with 10 mm quartz cells. Magnetic stirrer (Remi Equipment Pvt. Ltd., India) was used in the initial steps of extraction. Whatman filter paper no.42 was used to filter the solution.

2.2 MATERIALS AND METHODS

The CPH standard was kindly gifted by Alembic Ltd., Vadodara, India. All chemicals were of analytical reagent grade and solutions were prepared with purified water of IP [5] grade. Methanol-AR was purchased from Allied Chemical Corporation, Vadodara, India, S.D. Fine Chemicals, Mumbai, India and Qualigens Fine Chemicals, Mumbai, India. Commercial pharmaceutical preparation (Altamet[R], Alembic Ltd. Vdodara, India.) was procured from commercial source.

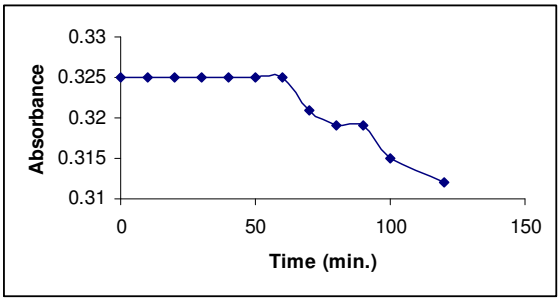
2.3 Optimization of parameters for colorimetric method

All the optimization parameters are estimated at room temperature. CPH was found to yield a blue-colored product with FCR and NaOH and has absorbance maxima at 725 nm. Therefore, investigations were carried out to establish the most favorable conditions for the formation of this colored product.

The influence of the concentration as well as volume of reagent on the reaction has been studied. Different concentrations and different volumes were tried for all the reagents, by varying one parameter at a time. The optimum concentration of FCR was 1 normal and of NaOH was 1 normal, similarly optimum volume of FCR and NaOH was found to be 0.8 ml and 1 ml respectively. The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance.

Also the color stability of newly formed complex was measured and the color was found to be stable for a period of one hour. Graphical presentations indicating stability of color of colored complex is given in **figure 1.**

Figure: 1 Stability of Colored Complex



2.4 Preparation of standard solutions

A stock solution of drug was prepared by dissolving 50 mg of CPH in required quantity of methanol and diluting up to 50 ml with methanol. From the above solution 2.5 ml was again diluted to 25 ml with purified water [5] to get 100 µg/ml solution of CPH. A standard solution of folin ciocalteu reagent (FCR) was prepared by diluting the reagent with purified water to get a concentration of 1 normal. A standard solution of sodium hydroxide was prepared by dissolving 4 gm of reagent in sufficient quantity of purified water and finally diluting to 100 ml with purified water.

2.5 Colorimetric method

Suitable aliquots of the drug solution (0.2 to 2.5 ml) were taken in a series of 10 ml volumetric flasks. To each flask was added 0.8 ml of standard FCR solution and 6 ml of purified water. All the flasks were shaken well for at least 3 to 5 min., followed by addition of 1 ml of standard NaOH solution. Finally volume was made up to the mark with purified water to prepare a series of standard solutions containing 2 to 25 µg/ml CPH. The absorbance of blue color chromogen was measured at 725 nm against reagent blank within one hour and the absorbance were plotted against the respective concentrations to obtain the calibration curve.

2.6 Derivative spectroscopic method

Suitable aliquots of the drug solution (0.5 to 4.0 ml) were taken in 10 ml volumetric flasks. In each flask volume was made up to the mark with purified water, to prepare a series of standard solutions containing 5 to 40 µg/ml CPH.

First derivative spectra at sensitivity 2 were obtained (figure 2) for different standard drug solutions and the amplitude was calculated between maxima (221 nm) and minima (207 nm). The amplitudes were plotted against the corresponding concentration to obtain the calibration curve.

2.7 Estimation of CPH in pharmaceutical formulation

Twenty tablets (Altamet[R], Alembic Ltd. Vdodara, India.) were weighed and ground to fine powder. An accurately weighed quantity of powdered sample, equivalent to 50 mg of CPH, was transferred to a conical flask and extracted with 15 ml of methanol by stirring on a magnetic stirrer for about 30 min. Then it was filtered through Whatman filter paper no. 42 into a calibrated 50 ml volumetric flask. Filter paper was rinsed twice with 2 ml of methanol and the volume was made up to 50 ml with methanol. Appropriate aliquots were then taken in such a way that the final concentrations in 10 ml volumetric flasks were within the range used for testing the drug by the two methods.

2.8 Method validation[6]

The developed methods were validated for its accuracy, precision and reproducibility. Accuracy of the methods was determined by performing recovery studies for the tablet formulation and for synthetic mixture of CPH. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The percent coefficient of variance (% CV) was calculated at each concentration level. The reproducibility was confirmed by repeating the methods, taking methanol from three different

manufacturers and by three different analysts, and the percent relative standard deviation (% RSD) was calculated. The values of method validation are given in **table 1.** Limit of detection (LOD) and limit of quantification (LOQ) were calculated by repeating the blank measurements twelve times for both the methods and the results are shown in **table 2.**

3. RESULTS AND DISCUSSION

The proposed methods are simple, rapid and precise. They do not suffer from any interference due to common excipients of tablet. In case of colorimetric method, in the presence of alkaline medium, cefetamet pivoxil hydrochloride reacts instantaneously with the FCR resulting in blue colored product. Formation of blue color product is due to a common reaction mechanism i.e., the oxidation of the CPH and the reduction of FCR. Folin ciocalteu reagent is a mixture of acids and involves the following chemical species:

3 $H_2O.P_2O_5$ 13 $WO_3.5MoO_3$ .10 H_2O and **3$H_2O.P_2O_5$ .14WO_3 .4MoO_3 .10 H_2O**

The blue color formation by FCR with CPH seems analogous to Folin phenol protein reaction. CPH probably reduce tungstate and/or molybdate in FCR producing reduced species which have a characteristic intense blue color with λ max at 725 nm.

The stability of colored complex was checked with respect to time and it is clearly seen from the figure-1 that color was stable for 1.0 hour, so it is recommended that the readings should be taken within the specified time range. While second method is derivative spectroscopic method and first derivative spectra was recorded, as shown in figure- 2. The maxima and minima were found to be 221 nm and 207 nm respectively. The calibration curve was plotted as difference in amplitude against concentration in µg/ml.

Figure: 2. First Derivative Spectra of Cefetamet pivoxil hydrochloride

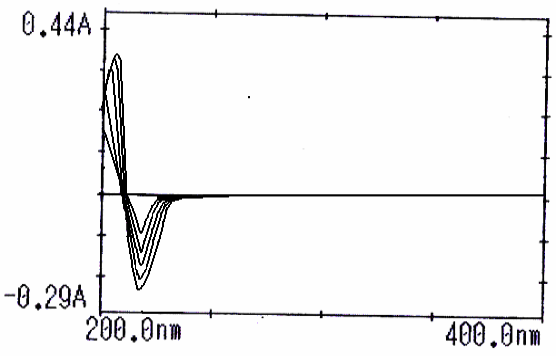
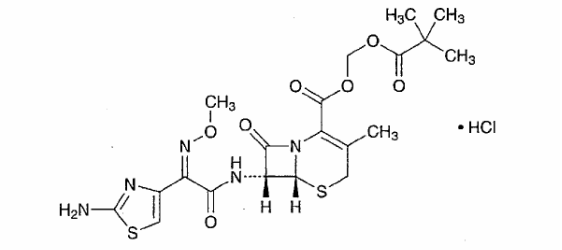


Figure: 3. Structure formula of Cefetamet pivoxil hydrochloride



The proposed colorimetric method was linear in the range of 2 to 25 µg/ml. In this method, the correlation coefficient (R^2) was found to be 0.9997, the slope was 0.0309 and the intercept was 0.0165. Derivative method obeys Beer's law in the concentration range of 5-40 µg/ml and value of correlation coefficient (R^2) was found to be 0.9990, the slope was 0.001 and the intercept was 0.0022.

Both the methods were validated in terms of accuracy, precision, reproducibility and the results are recorded in table-1. The accuracy of the methods was proved by performing recovery studies for the commercially available formulations and for synthetic mixture, which is made of common tablet excipients like starch and lactose, 0.2 g of each was separately mixed with 0.1 g of cefetamet pivoxil hydrochloride. Each such synthetic mixture was analyzed by proposed methods. Values greater than 99.0% indicate that the proposed methods are accurate for the analysis of drug. The precision of the proposed methods was checked in terms of inter-day and intra-day, where methods were repeated on three different days and also repeated for three different time periods in the same day. The results given in table-1 showing % CV of less than 1% at each level which clearly indicate that the proposed methods are precise enough for the analysis of drug. The reproducibility of the methods was checked by getting the proposed methods performed by three different analysts and by taking solvent methanol from three different manufacturers. The values of % RSD less than 1% indicate that the proposed methods are reproducible for the analysis of CPH. The values of LOD and LOQ were found to be 0.0920 µg/ml and 0.3067 µg/ml respectively for colorimetric method and 1.440 µg/ml and 4.830 µg/ml respectively for derivative method (table-2).

The optical characteristics, such as Beer's law limit, Molar absorptivity, Sandell's sensitivity[7], are recorded in table-2. The regression analysis using the method of last sequence was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations. The results are summarized in

table-2. Rigorous analysis of the results shows that the presence of excipients in tablet formulation did not interfere with the final determination of the active component. This reveals the potential utility of these developed methods for the routine analysis of CPH in pharmaceutical preparations.

CONCLUSION

Two new, simple and sensitive spectrophotometric methods were developed for the analysis of CPH in bulk and in pharmaceutical formulations. One of the methods was colorimetric method in which the color of newly formed complex was measured at λ max 725 nm. The stability of color was also checked with respect to time and it was found that the color was stable for one hour. The second method was the derivative spectrophotometric method in which the maxima and minima were found to be 221 nm and 207 nm respectively. As the instrument can do all the calculations, the method becomes quite simple and rapid, and is associated with high sensitivity and selectivity. Both the developed methods were also validated and from the statistical data, it was found that methods were accurate, precise, reproducible and can be successfully applied to the pharmaceutical formulation without interference of excipients

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Table: 1 Validation Parameters and Recovery data.

Data	Accuracy			Precision		Reproducibility
Developed methods	**Dosage form**	**Label claim (mg)**	**% Recovery[a]**	**Intraday (% CV)[b]**	**Interday (% CV)[c]**	**(% RSD)[d]**
Colorimetric method	S.M[e].	100	99.52 ± 0.31	0.882 ± 0.09	0.894 ± 0.12	0.955 ± 0.22
	Tab.-1	250	100.02 ± 0.67			
	Tab.-2	500	99.09 ± 0.51			
Derivative method	S.M[f].	100	99.66 ± 0.42	0.865 ± 0.41	0.835 ± 0.68	0.990 ± 0.82
	Tab.-1	250	100.37 ± 0.19			
	Tab.-2	500	99.14 ± 0.69			

a, b, c, d : Average value ± standard deviation of six determinations
e, f : Synthetic mixture

Table: 2 Optical Characteristics and other Parameters.

Parameters	Colorimetric method	Derivative method
λ max (nm)	725	221
λ min (nm)	-	207
Beer's law limit (µg/ml)	2 to 25	5 to 40
Molar extinction (l/mol.cm)	$1.326 X10^4$	$4.050 X 10^2$
Sandell's sensitivity (µg/cm^2 per 0.001 absorbance unit)	0.02996	0.9811
Regression equation (Y=mX+c)	0.0309 x + 0.0165	0.001 x + 0.0022
Slope	0.0309	0.001
Intercept	0.0165	0.0022
Limit of detection (µg/ml)	0.0920	1.440
Limit of quantification (µg/ml)	0.3067	4.830
Coefficient of determination	0.9993	0.9982
Correlation coefficient	0.9997	0.9990
% RSD	< 1%	< 1%
Accuracy	> 99%	> 99%

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