Spectrophotometric Determination of Cefetamet Pivoxil Hydrochloride in Bulk and in Pharmaceutical Formulation

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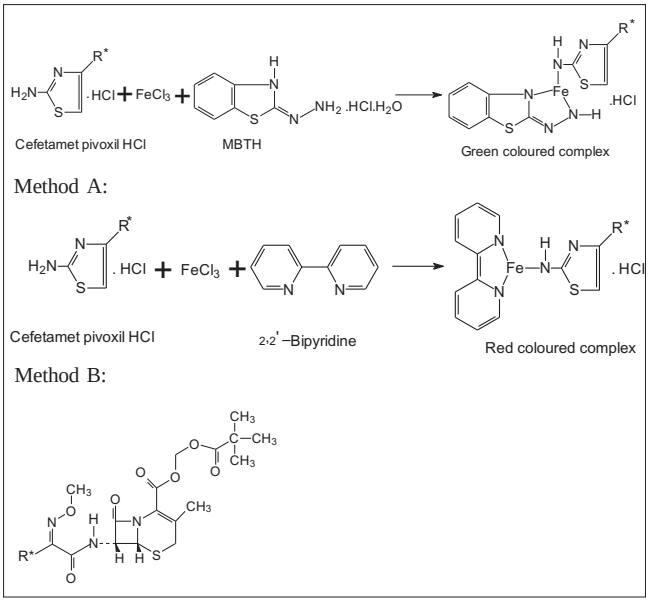
Two new simple and sensitive colorimetric methods were developed for the analysis of cefetamet pivoxil hydrochloride in bulk and in pharmaceutical formulations. In the first proposed method, colour of newly formed complex was measured at 645 nm (λ_{max}), and the calibration curve was linear in the range of 1-7 μg/ml; while in the second method, intensity of colour was measured at 524 nm (λ_{max}), and the calibration curve was linear in the range of 2-18 μg/ml. The developed methods were successfully applied to the pharmaceutical formulations.

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 (CPH), 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 infections[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 colorimetric methods for the determination of CPH in bulk and in its pharmaceutical formulation are described. In the first method (method A), 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) loses two electrons and one proton on oxidation, forming the electrophilic intermediate that couples with the drug molecule to form a green-coloured complex. The absorbance measurement was made at 645 nm; while in the second method (method B), each of the two nitrogen atoms in 2,2-bipyridyl has an unshared pair of electrons that can be shared with Fe (III) ion, and the product thus formed reacts with the drug molecule to form a red-coloured complex, which is measured photometrically at 524 nm. The methods are simple, rapid, sensitive and easy to apply in routine usage and do not require any costly instrumentation. The probable reactions involved in both methods have been shown in Scheme 1.

MATERIALS AND METHODS

All the absorbance measurements were made on Shimadzu UV-1601 UV/Vis spectrophotometer with 10 mm matched cells. Magnetic stirrer (Remi Equipment Pvt. Ltd.)



Scheme 1: Probable reactions involved in both methods

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was used in the initial steps of extraction. Whatman filter paper no. 42 was used to filter the solutions. The CPH standard was kindly gifted by Alembic Ltd., Vadodara. All chemicals were of analytical reagent grade, and solutions were prepared with purified water of IP[5] grade. Methanol AR was purchased from Suvidhinath Laboratories, Vadodara.

Optimization of parameters:

CPH was found to yield a green-coloured product with MBTH and ferric chloride ($FeCl_3$) and has absorbance maximum at 645 nm. It gives a red-coloured product with 2,2-bipyridyl and ferric chloride ($FeCl_3$) and has absorbance maximum at 524 nm. Therefore, studies were carried out to establish the most favourable conditions for the formation of these coloured products.

The influence of the concentration as well as volume of the reagents on the reaction has been studied. Different concentrations and different volumes were tried for all the reagents, by varying one parameter at a time. For method A, it was found that the optimum concentration of $FeCl_3$ was 0.7% and that of MBTH was 0.3%. The optimum volume of $FeCl_3$ was found to be 1 ml and that of MBTH was 0.8 ml. Also, for method B, the optimum concentration of $FeCl_3$ was 0.1% and that of 2,2-bipyridyl was 1.5%, whereas the optimum volume of $FeCl_3$ and 2,2-bipyridyl was found to be 1.5 ml and 1 ml respectively.

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. In case of method A, there was no effect of time on the stability of the colour up to 10 h; however, decrease in absorbance was noted after this period, while in case of method B, the colour was found to be stable for a period of 6 h. Graphical presentations indicating stability of colours of coloured complexes in methods A and B are given in figs. 1 and 2 respectively.

Preparation of standard solutions:

A standard solution of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) was prepared by dissolving 0.3 g of MBTH in required quantity of purified water and diluting to 100 ml with purified water. Standard solutions of ferric chloride ($FeCl_3$) were prepared by dissolving 0.7 g of anhydrous $FeCl_3$ for method A and 0.1 g of anhydrous $FeCl_3$ for method B in required quantity of purified water and diluting to 100 ml with purified water. A standard solution of 2,2-bipyridyl was prepared by dissolving 1.5 g of 2,2-bipyridyl in about 50 ml of purified water and diluting to 100 ml with purified water.

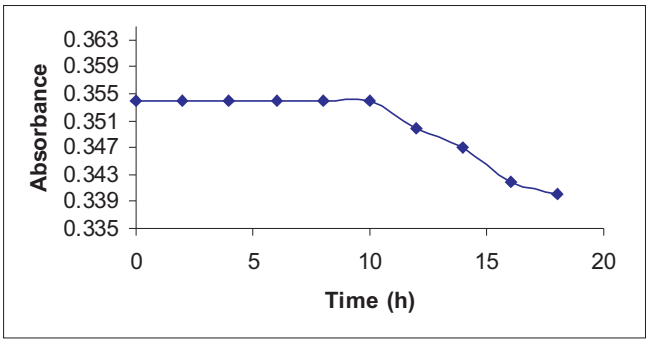

Fig. 1: Stability of colour for method A
The colour is stable for 10 h.

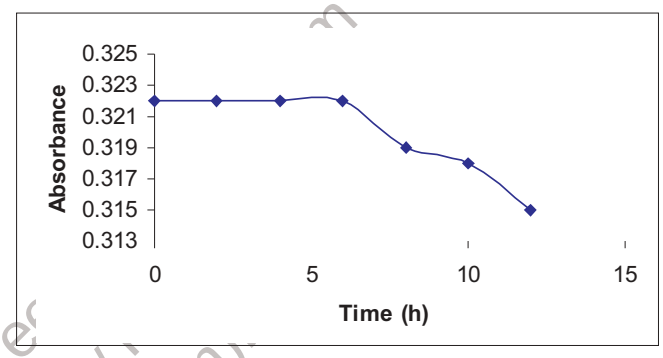

Fig. 2: Stability of colour for method B
The colour is stable for 6 h.

A standard stock solution of CPH was prepared by dissolving 0.1 g of CPH in methanol and diluting to 100 ml with methanol. From the above solution, 10 ml of solution was again diluted to 100 ml with methanol to get 100 µg/ml solution of CPH.

Method A:

Suitable aliquots of the standard stock solution of CPH (0.1 to 0.7 ml) were taken in 10 ml volumetric flasks. To each flask were added 1 ml of standard $FeCl_3$ solution and 0.8 ml of MBTH solution. The flasks were shaken well for about 2-3 min and allowed to stand for 5 min. The volume was then made up to the mark with purified water to prepare a series of standard solutions containing 1-7 µg/ml CPH. The absorbance of green chromogen formed was measured at 645 nm against the reagent blank within 10 h and the calibration curve was plotted.

Method B:

Suitable aliquots of the standard stock solution of CPH (0.2 to 1.8 ml) were taken in separate conical flasks. To each flask was added 1.5 ml of standard $FeCl_3$ solution and 1 ml of 2,2-bipyridyl solution, and all the flasks were shaken well for at least 10 to 15 min. They were then heated on a boiling water bath for about 5 min, allowed to cool at room temperature and transferred to separate 10 ml volumetric flasks. The conical flasks were rinsed

with purified water and the rinsings were added to the respective volumetric flasks. Finally, volumes were made up to the mark with purified water to prepare a series of standard solutions containing 2-18 µg/ml CPH. The absorbance of the red-coloured chromogen was measured at 524 nm against the reagent blank within 6 h and the calibration curve was plotted.

Estimation of CPH in tablets:

Twenty tablets 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.

Method validation[6]:

Accuracy of the methods was determined by recovery studies in the tablet formulation of CPH. Recovery studies were carried out by addition of known quantities of standard drug solution to pre-analyzed sample at two different concentrations. 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. The proposed method A obeys Beer's law in the concentration range of 1-7 µg/ml. In this method, the correlation coefficient (R^2) was found to be 0.9957, the slope was 0.0822 and the intercept was 0.0396. Method B obeys Beer's law in the concentration range of 2-18 µg/ml. In this method, the correlation coefficient (R^2) was found to be 0.9975, the slope was 0.032 and the intercept was 0.0083. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by repeating the blank measurements twelve times at 645 nm for method A and 524 nm for method B. The values were found to be 0.01876 µg/ml and 0.06253 µg/ml respectively for method A, and 0.0820 µg/ml and 0.2734 µg/ml respectively for method B (Table 2).

RESULTS AND DISCUSSION

The proposed methods are simple, rapid and precise. They do not suffer from any interference due to common excipients of tablet. Beer's law is obeyed in the concentration range of 1-7 µg/ml and 2-18 µg/ml for Method A and Method B respectively. The stability of coloured complex was checked with respect to time, and it is clearly seen from the figs. 1 and 2 that colour was stable for 10 h in case of method A and 6 h in case of method B; so it is recommended that the reading should be taken within the specified time range, i.e., 10 h for method A and 6 h for method B.

Both the methods were validated in terms of accuracy, precision and reproducibility, and the results are recorded in Table 1. The accuracy of the methods was proved by performing recovery studies in the commercially available formulations. 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 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.

TABLE 1: RECOVERY STUDY AND VALIDATION DATA

DATA	ACCURACY			PRECISION		REPRODUCIBILITY
	Dosageform	Label claim (mg)	% recovery*	Intraday (% CV)*	Interday (% CV)*	(% RSD*)
METHOD A	S.M.	100	99.54			
	TAB-1	250	99.78	0.654	0.626	0.587
	TAB-2	500	100.09			
METHOD B	S.M.	100	100.85			
	TAB-1	250	101.21	0.762	0.801	0.834
	TAB-2	500	99.43			

*Average of six determinations, S.M. = synthetic mixture.

TABLE 2: OPTICAL CHARACTERISTICS AND OTHER PARAMETERS

Parameters	Method A	Method B
λ_{max} (nm)	645	524
Beer's law limit (μg/ml)	1 to 7	2 to 18
Molar extinction (l/mol.cm)	8.9 X10^4	1.2 X10^4
Sandell's sensitivity(μg/cm^2 per 0.001 absorbance unit)	0.004450	0.03226
Regression equation (Y=mX+c)	0.0822x + 0.0396	0.032x -0.0083
Slope (b)	0.0822	0.032
Intercept (a)	0.0396	0.0083
Limit of detection (μg/ml)	0.01876	0.0820
Limit of quantification (μg/ml)	0.06253	0.2734
Coefficient of determination	0.9957	0.9975
Correlation coefficient	0.9978	0.9987
% RSD	< 1%	< 1%
Accuracy	> 99%	> 99%

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, CPH. This reveals the potential utility of these developed methods for the routine analysis of CPH in pharmaceutical preparations.

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