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Development of RP-HPLC methods for the simultaneously Estimation of cefetamet pivoxil HCL in formulations

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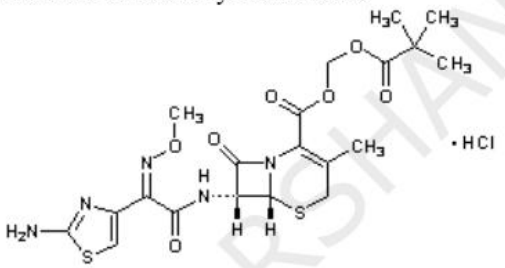
ABSTRACT

A simple and precise reversed phase HPLC method was developed for estimation of cefetamet pivoxil HCL cefetamet pivoxil HCL formulation. The method was carried out on a Hypersil ODS ®$C_{18,}$ (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Acetonitrile: Water (70: 30 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 232 nm. The aceclofenac was used as an internal standard. The retention time of aceclofenac and cefetamet pivoxil HCL were found to be 1.750, 3.708 minutes respectively. The validation of proposed method is specific, accurate, precise and linear. The linearity of 0.5 - 50 µg/ml of cefetamet pivoxil HCL. The proposed method can be used for estimation of cefetamet in formulation by HPLC method.

Keywords: cefetamet pivoxil HCL, RP-HPLC Method

1. INTRODUCTION

Cefetamet Pivoxil Hydrochloride



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 cefetamet.

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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.

A literature survey revealed that there are number of methods have been reported for the estimation of domperidone and omeprazole, individually or in combination with other drugs. However there is no method reported for the simultaneously estimation of these drugs. Hence the present work describes a reversed phase HPLC method for simultaneous estimation of these drugs in capsule. The validation of the proposed method was also carried out.

2. EXPERIMENT

REAGENT AND CHEMICALS

Acetonitrile HPLC grade were supplied by Merck. Water HPLC grade was obtained from Milli-Q RO system. Reference drugs were obtained as gift samples from manufacturers.

CHROMATOGRAPHIC CONDITIONS

A Shimadzu® LC10AT VP series HPLC system was used for analysis. The method was carried out on a Hypersil ODS ®$C_{18,}$ (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Acetonitrile: Water (70: 30 v/v) at a flow rate of 1.0 ml min^{-1} a Rheodyne ® 7725*i* injector with a 20 µl loop was used for the injection of samples. The SPD-10 Avp UV-Visible spectrometer detector was used to detect the drugs at 232 nm and CLASS-VP data station was used to process the chromatogram the mobile phase was filtered through a 0.45 µ membrane filter and degassed. The separation was carried about at room temperature of about 20±2° C. And the OPTIMIZED CHROMATOGRAPHIC CONDITION is presented in Table: 1.

Table: 1 OPTIMIZED CHROMATOGRAPHIC CONDITION

Parameters	Status
Stationary Phase (column)	Hypersil ODS ®$C_{18,}$ (25 cm x 4.6 mm i.d., 5 µ)
Mobile Phase	Acetonitrile: Water (70: 30 v/v)
Flow rate (ml/min)	1 ml/min
Run Time (minutes)	10
Column Temperature(°C)	Ambient
Volume Of Injection Loop (µl)	20
Detection Wavelength (nm)	232
Internal Standard	Aceclofenac
Drug RT (min.)	3.708
Internal Standard RT (min.)	1.750

PREPARATION OF SOLUTIONS

Standard Stock solution is prepared by adding of 10 mg cefetamet and 10 mg aceclofenac, were taken into 10 ml standard volumetric flask separately using a mixture of water and Acetonitrile (30: 70 v/v). From the standard stock solutions, mixed standard solution was prepared to contain 10 µg /ml, 100 µg /ml and 1 mg /ml of cefetamet, and spiked with 5ml of 5 µg/ml of aceclofenac as internal standard respectively.

Twenty tablets weighed and collect the powder, weight a quantity of powder equivalent to 10 mg of cefetamet, transferred to a sintered glass crucible and to this add 5ml of 5 µg/ml of aceclofenac as internal standard, extract with mixture of Acetonitrile and water (70: 30 v/v) and makeup volume to 10 ml and filter. Resulting solution was further diluted to get a concentration and this was used for the estimation (sample solution).

PROCEDURE

With the above chromatographic conditions, the standard solution and sample solution containing internal standard were injected and the chromatograms were recorded. The retention time of aceclofenac and cefetamet were found to be 1.750, 3.708 minutes respectively. The response factor (peak area ratio of standard peak area and the internal standard peak area) of the standard solution and the sample solution were calculated.

The concentration of the drugs were calculated using the following formula-

$$\text{Concentration Of Drug} = \frac{\text{Response Factor of the Sample}}{\text{Response Factor of the Standard}} \times \text{Concentration of Standard}$$

ANALYSIS OF THE FORMULATION:

The amount of drug present in each formulation was calculated through peak area ratio of drug to that internal standard by using the standard calibration curve and was plotted by taking concentration of drug on X- axis and peak area ratio on Y-axis and the result were shown in Table: - 2, A typical chromatogram of cefetamet in formulation as well as in pure form were shown in the chromatogram.

TABLE: -2 ANALYSIS OF THE FORMULATION

Formulation	Labeled amount (mg)	Observed amount (mg)	% amount found	% RSD
ALTAMET (Alembic)	500 mg	496.33 ±2.886	99.26	0.5816
Cefime-O (Alembic)	500 mg	498.66± 1.1547	99.73	0.2315

VALIDATION OF THE METHOD

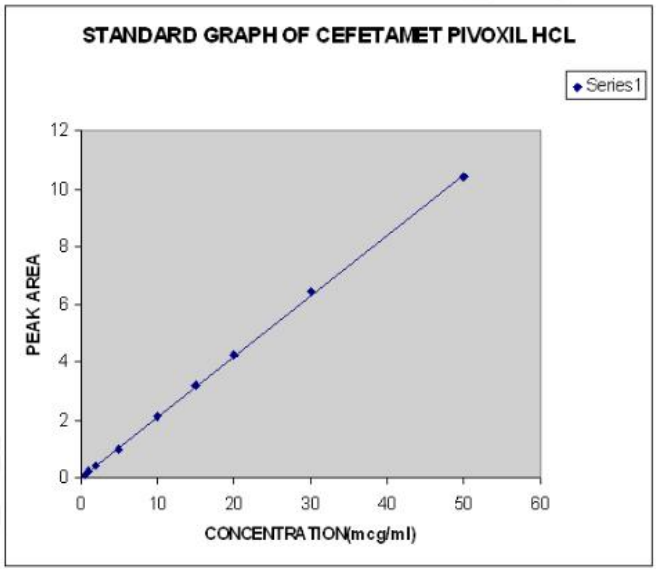
3. LINEARITY: -

Linearity of the method was done by analyzing mixed standard solution of cefetamet containing 0.5 - 50 µg/ml of the targeted level and also containing 5ml of 5 µg/ml of aceclofenac as internal standard respectively. These were analyzed and the response factor was calculated. The calibration curve was plotted using response factor Vs concentration of the standard solutions. The limit of Detection (LOD) and limit of Quantification (LOQ) of the method was determined by injecting progressively low concentrations of the standard solutions. The system suitability studies were also carried out and the parameters like column efficiency, resolution and peak asymmetry were calculated and presented in Table-3 & Fig: 1

TABLE: 3 LINEARITY:-

CONCENTRATION (mcg/ml)	Peak area Ratio of Drug / I.S	Statistical analysis
0.5	0.107	Slope(a) : 0.2096 Intercept(b) : 0.123 Correlation Coefficient ; 0.9997
1.0	0.218	
2.0	0.424	
5.0	0.998	
10	2.11	
15	3.19	
20	4.22	
30	6.44	
50	10.40	

Fig: 1



2. Precision

The precision of the method was by repeatability studies. The percentage recovery was demonstrated by the standard solution for 8 times and passing them through the assay procedure. From these results mean, SD and %RSD were calculated and presented in Table-4.

TABLE: 4 PRECISION READINGS:

S.No.	Concentration (µg/ml)	Peak area Ratio	Statistical analysis
1	10	2.10	Mean : 2.10 SD : 0.00517 %RSD ; 0.2457
2	10	2.10	
3	10	2.10	
4	10	2.11	
5	10	2.11	
6	10	2.10	
7	10	2.10	
8	10	2.11	

3. Accuracy

Accuracy of method was studied by the recovery experiments. To the powdered tablet formulations (each containing 150 mg cefetamet reference standard at the level of ± 20% of the label claim and 5ml of 5 µg/ml of aceclofenac as internal standard drugs were added. The extraction of drugs was followed using sample preparation procedure and these were analyzed. The percentage recovery was calculated and presented (Table-5).

TABLE: 5 Accuracy

Sample ID	Concentration (µg/ml)		%recovery of pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1: 80%	8	10	100.6	Mean : 100.4 SD : 0.3464 %RSD ; 0.3450
S_2: 80%	8	10	100.0	
S_3: 80%	8	10	100.6	
S_4: 100%	10	10	99.0	Mean : 99.2 SD : 0.3464 %RSD ;0.3491
S_5: 100%	10	10	99.0	
S_6: 100%	10	10	99.6	
S_7: 120%	12	10	99.66	Mean : 99.8 SD : 0.2436 %RSD ;0.2440
S_8: 120%	12	10	99.66	
S_9: 120%	12	10	100.08	

3. system suitability parameters:

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability were usually developed after method development and validation have been completed (or) the USP 2000 defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical Plates(N), Resolution(R), LOD and LOQ were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of cefetamet in the pharmaceutical formulation was validated or not. The results were shown in Table: - 6

TABLE: 6 SYSTEM SUITABILITY PARAMETERS:

S.No.	Parameters	Values
1.	Theoritical Plates (N)	NLT 2000
2.	LOD µg/ml	0.1975
3.	LOQ µg/ml	0.5984

3. RESULTS AND DISCUSSIONS

The results of proposed HPLC method showed that the results are consistent with the label claim of the formulation the accuracy of the method was determined by recovery studies. The recovery studies were carried out and the percentage recovery was calculated from the data obtained, recoveries of the standard drugs were accurate. The reproducibility data shows that the assay procedure was precise.

The linearity and range of the assay method was done and the calibration curves were plotted. The calibration curve showed linear response over the range of concentration used in the assay procedure, which justifies the use of single point calibration. From the linearity Table: - 1 it was found that the drug cefetamet obey linearity within the concentration range of 0.5 – 50 µg/ml. from the result shown in the precision Table : - 2 it was found that % RSD is less than 2 %, which indicates that he method has good Reproducibility. The limit of Detection (LOD) of 0.1974 µg/ml for the HPLC method. The limit of Quantification (LOQ) was 0.5984 µg/ml. The 3D chromatogram of the sample solution showed that there is no interference's due to formulations excipients. The developed method is simple, accurate, precise, linear and rapid. Hence this method is suitable for the quality control of raw materials, formulations and can be applied in dissolution studies.

4. CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination of cefetamet from pure and its dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation.

Hence, this method can be easily and conveniently adopted for routine analysis of cefetamet in pure form and its dosage form

and can be used for dissolution or similar studies.

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