ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CEFETAMET BY RP-HPLC METHOD

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Abstract:Theobjective of the present work is to develop an efficient, precise, accurate, linear, simple, rapid, reproducible and sensitive RP- HPLC method for the estimation of Cefetametin tablet dosage forms. The developed method was validated as per ICH guidelines. The HPLC method was developed using waters C_{18} column (250mm×4.6mm; 5_{μ} id) and flow rate 1.0ml /min. Detection was carried out at by absorption at 232nm and injection volume is 20µl. The mobile phase used was methanol, acetonitrile and 0.01Msodium perchlorate in the ratio of 60:40. The calibration curve was linear over the range of 1-6µg/ml. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Cefetametin tablet dosage forms.

Index terms: **Cefetamet, tablet dosage form, RP-HPLC method, validation**

I.INTRODUCTION:

Cefetamet is 2,2dimethylpropanoyloxymethane(6R,7R)-7-{{(2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl}-3-methyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-ene-2-carboxylateis one of the potential antibacterial agent and used to treat a number of bacterial infections. Cefetametpivoxil is a 3rd generation cephalosporin which exerts its bactericidal action by inhibiting bacterial cell wall synthesis. Cefetametpivoxil inhibits transpeptidase& thus prevents cross linking of bacterial cell wall. Transpeptidase& associated proteins constitute various types of specific binding proteins which have affinity for cephalosporins like Cefetametpivoxil. CefetametPivoxil Hydrochloride is the hydrochloride salt form of cefetametpivoxil, a pivalate ester prodrug form of a cefetamet. After oral administration of cefetametpivoxil hydrochloride, the ester bond is cleaved, releasing active cefetamet.

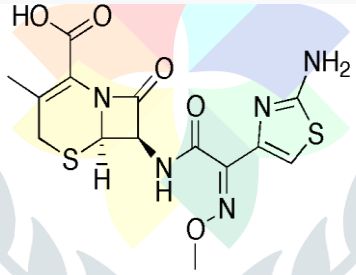

Figure 1: Structure of cefetamet

A literature survey revealed that a number of analytical methods have been developed for the determination of cefetamet alone and in combination in various dosage forms and biological samples using HPLC, liquid chromatography and spectrophotometry techniques.
We have developed a new accurate and precise RP-HPLC method for the determination of cefetamet in tablet dosage form. The developed method is validated as per ICH guidelines.

II. MATERIALS AND METHODS

2.1 Drugs, chemicals and solvents: HPLC grade water was purchased from Merck chemicals, Mumbai. HPLC grade methanol and all other laboratory grade chemicals were purchased from Merck chemicals, Mumbai.

2.2 Equipment and chromatographic conditions: Agilent 1100 series HPLC with Quaternary G1311 A pump, COLCOM G1316A thermostat column temperature control, Thermostatic auto sampler G 1329A with sample volume of 0. 1 – 1500 µL and variable programmable UV detector G 1314 A. The instrument was operated and integrated with Agilent chem. station LC software. The LC was coupled with Water mass detector model LAA 1369. Mobile phase used was methanol: 0.01M sodium perchlorate in the ratio of 60:40. All the chromatographic runs were carried out in isocratic elution mode with a flow rate of 1ml/min and the sample injection volume was 20 µL. The detector wavelength was set at 232nm.

2.3 Preparation of the mobile phase and diluent: Methanol 0.01M sodium perchlorate are mixed in the ratio of 60:40 after that, it is filtered by vaccum filtration using 0.45 micron filter paper and employed as the mobile Phase. The same solution was also used as the diluents for preparing drug dilutions.

2.4 Preparation of working standard solution of cefetamet: About 10mg of drug was weighed accurately and transferred into a 10ml volumetric flask. Methanol was added to it to dissolve the drug. The volume was made up to the quantity with the diluents and mixed well. This was used as a standard stock solution. 1.0 ml of the stock solution was transferred to 10ml volumetric flask and made upto the volume using diluents to get a 100µg/ml of cefetamet. This was used as working standard solution.

2.5 Estimation of the drug from the tablet dosage form: Ten tablets of drug Cefetamet (Altamet-250mg) were grounded to finely powdered material. Powder equivalent to 10mg of drug was taken into a 10 ml of volumetric flask containing 10ml of mobile phase and was shaken to dissolve the drug and then filtered through Nylon membrane filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 1000µg/ml.

III. RESULTS AND DISCUSSION

During the method optimization studies trails were carried out for an ideal separation of the drug using different mobile phases and different chromatographic conditions. Finally the following conditions were found to be optimum after evaluating the column efficiency by parameters like theoretical plates and tailing factor.

Table 1: Optimized conditions for the proposed HPLC method

Stationary phase	kromasil C18 column, (250mm×4.6mm; 5µ)
Mobile phase	Methanol: 0.01M sodium perchlorate 60:40
Flow rate	1.0 ml/min
Column temperature	Ambient
Injection volume	20 µl
Detection wavelength	232nm
Run time	10 min
Retention time of the drug	2.369 min

Optimum wavelength was selected by injecting standard solution of drug into HPLC with UV detector G 1314 A and the wave length which gives higher response for the compound is selected. The wavelength was found to be 232nm. Under the optimized conditions the retention time of cefetamet was found to be2.369min.

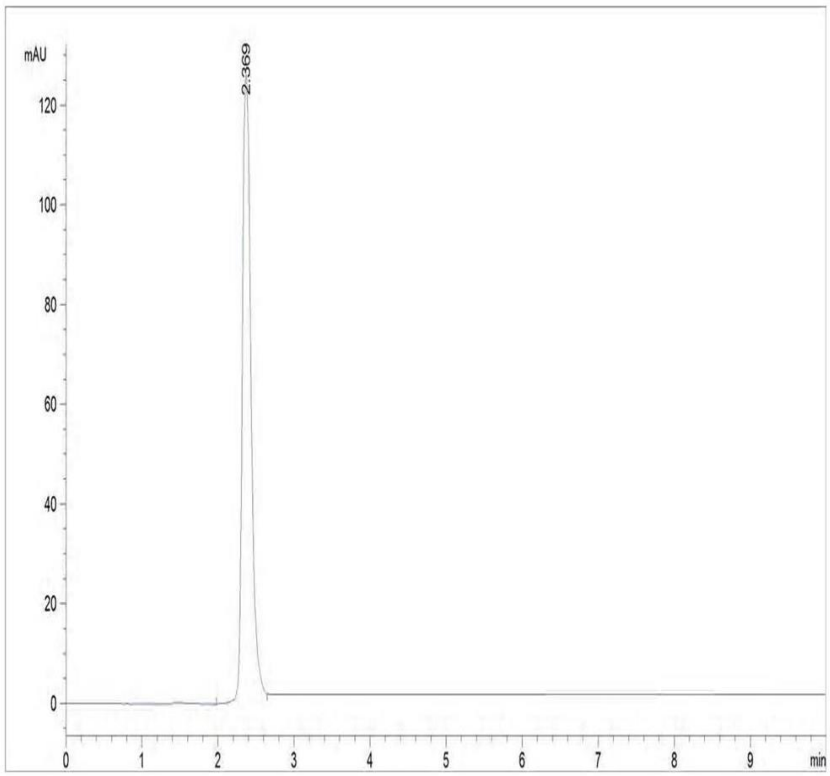

Figure 2: Chromatogram of standard cefetamet

3.1 Linearity: The regression of the plot was computed by least squares method and is shown in Figure 3. The calibration curve of the drug was linear over the concentration range of 1-6µg/ml with the correlation coefficient 0.999 and the % RSD for each component was less than 2.

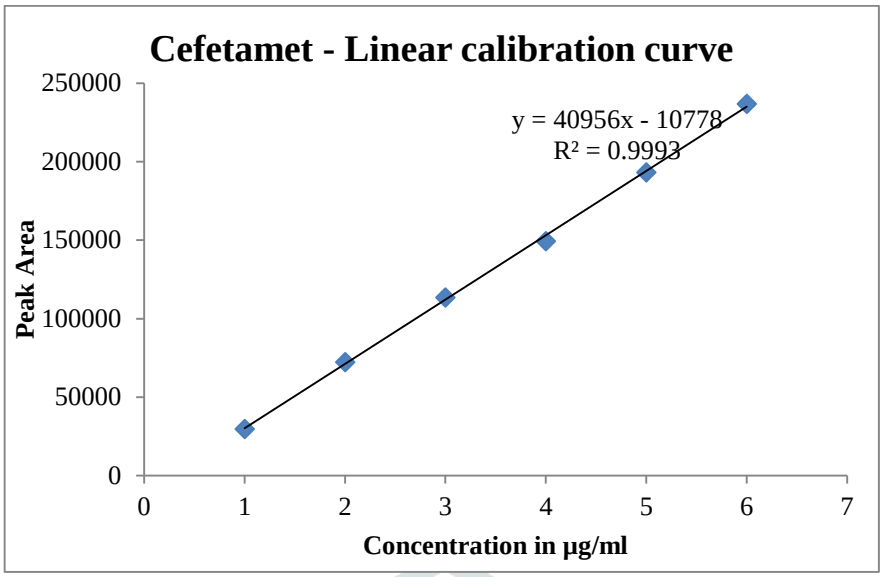

Figure 3: Linearity of detector response graphs for cefetamet.

3.2 Accuracy and precision: The accuracy of the method was determined by recovery experiments. Individual percentage recovery, mean percentage recovery, percentage RSD and squares correlation coefficient for linearity of the test method were calculated and the results were presented in table 2. The high percentage recovery indicates that the developed method is highly accurate. The precision of the method was demonstrated by intraday variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and presented in Table 3. From the data obtained the developed RP-HPLC method was found to be precise.

Table 2: Accuracy data of developed method

Level	Concentration in µg/ml			Peak Area observed	Amount of Recovered (µg/ml)	% Recovery
	Target	spiked	Total			
	2	1	3	112257	2.964	98.79
	2	1	3	112013	2.957	98.58
50 %	2	1	3	112049	2.958	98.61
	2	2	4	148056	3.962	99.06
	2	2	4	147839	3.957	98.91
100%	2	2	4	147705	3.953	98.82
	2	3	5	190137	4.919	98.38
	2	3	5	189979	4.915	98.29
150%	2	3	5	191473	4.953	99.07

Table 3: Precision data of developed method

S.No	Retention time	Area
1	2.367	149920
2	2.379	150255
3	2.374	150049
4	2.379	148987
5	2.375	149827
6	2.672	149946
Avg area		148987
%RSD		0.59

3.3 System suitability: System suitability parameters were studied with six replicates of standard sample solution and the corresponding values are presented in Table 4.

Table 4: System suitability parameters of developed method

Parameter	Value
Retention time (min)	2.369
Tailing factor	1.42
Theoretical plates	3620

3.4 Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ in the sample were determined with acceptable precision and accuracy. The results were presented in Table 5.

Table 5: Limit of detection and limit of quantification data

S.No	Sample name	LOD		LOQ	
		Conc (µg/ml)	Retention time	Conc (µg/ml)	Retention time
1.	Cefetamet	0.06	2.370	0.20	2.381

3.5 Robustness: Robustness of the proposed analytical method was determined by varying flow rate and mobile phase composition. Percentage RSD was given in Table 6.

Table 6: Robustness of proposed method

Variability		%RSD
pH	5.2	0.83
	5.0	0.22
Mobile phase ratio	55:45	0.36
	65:35	0.39
Wave length	242nm	0.29
	222nm	0.30

3.6 Method Suitability: The commercial tablet formulation, altamet-250mg, was analyzed by the proposed method and the average percent recovery was found to be 99.68. The value is in good agreement with the labeled amount, which confirms the suitability of the method for the analysis of cefetamet in pharmaceutical dosage forms.

IV. CONCLUSION:

The developed RP-HPLC method is simple, sensitive, precise and accurate and can be used for the estimation of cefetamet in the tablet dosage form for quality control analysis and the method is validated by ICH guidelines.

V. ABBREVIATIONS

HPLC- High performance liquid chromatography, µg- microgram, ml- millilitre, %- Percent.

VI. ACKNOWLEDGEMENT

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