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EXTRACTIVE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF CEFEPIME HYDROCHLORIDE IN TABLET FORMULATION

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ABSTRACT

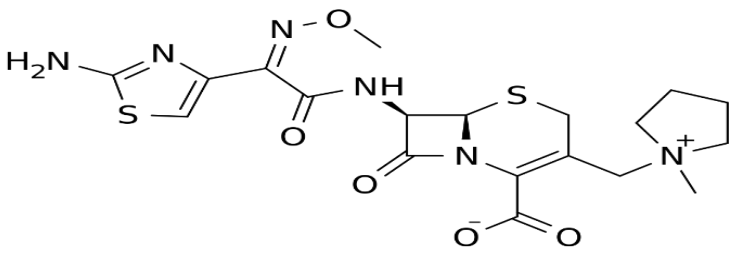
A Simple, accurate and convenient sensitive extractive spectrophotometric method based on the ion-association reaction between cefepime hydrochloride and a bromophenol blue, in phosphate buffer pH 3.0 was developed. The developed method involve formation of reddish orange chromogen extractable in chloroform layer. The method was validated according to international conference on harmonization (ICH) guideline, the extracted complex showed the λ_{max} at 417 nm, with good linearity in the range of 2 – 24 µg/mL with R^2 value of 0.9976.The % RSD was found to be < 2%. The accuracy, precision and sensitivity of extractive spectrophotometric method shows that the developed method is better for estimation of Cefepime Hydrochloride in tablet.

KEYWORDS: Cefepime hydrochloride, bromophenol blue, extractive spectrophotometric method, Validation, International conference on harmonization guidelines.

INTRODUCTION

Cefepime hydrochloride (CEF) belongs to fourth-generation cephalosporin antibiotics, which has an extended spectrum of activity against gram-negative and gram-positive bacteria. CEF is resistant to beta-lactamase, due to the presence of thiazolyl methoxyimino acetamido group Cefepime hydrochloride (Fig.1) is chemically 1-[[(6R,7R)-7-[2-(2-amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidiniumchloride,72-(Z)-(O-methyloxime), mono-hydrochloride,monohydrate.[1] Cefepime Hydrochloride is listed in the Indian Pharmacopoeia[2], British Pharmacopoeia[3]

and United State Pharmacopoeia.[4] CEF is used in the treatment of moderate-to-severe infections, such as pneumonia, urinary tract infections, skin and soft tissue infections, intra-abdominal infections and febrile neutropenia[5]



.HCl

Fig. 1: Chemical structure of Cefepime hydrochloride.

From the literature survey conducted, many methods have been reported for the estimation of CEF such as spectrophotometry[6-11], HPLC, stability indicating methods and degradation studies.[12-19] Hence, an extractive spectrophotometric method for CEF using bromophenol blue is proposed and carried out.

MATERIALS AND METHODS

a. Chemicals and reagents

HPLC grade Chloroform was obtained from (Rankem, Gujarat). HPLC grade Water was obtained from (Merck limited, Mumbai). LR grade 0.04% Bromophenol blue was obtained from Merck Pvt Ltd (Bangalore, India). CEF standard drug was obtained from Micro Labs limited (Bangalore, India). Marketed formulation **Ultipime O, 500 mg** (tablet dosage form), manufactured by Zydus Cadila Healthcare Ltd (Ahmedabad, India) was obtained from local pharmacy. The absorbance of extractable chromogen in chloroform layer was measured using Shimadzu 1601 model with a path of 1 cm.

b. Preparation of standard stock solution

Standard solution of CEF (1 mg/mL) was prepared in phosphate buffer pH 3.0. Series of dilutions were prepared in the concentration range of 2 – 24 µg/mL. 5 mL of each dilutions were taken, and mixed with 5 mL of chloroform and bromophenol blue into different separating funnels. Each of the separating funnel was shaken vigorously for 10 min. A reddish orange chromogen was formed. The chloroform layers were separated and checked for absorbance at 417 nm.

c. Preparation of sample solution

Twenty tablets of **Ultipime O** (500 mg) with label claim of 500 mg of CEF were accurately weighed and finely powdered. The powdered drug, equivalent to 100 mg of CEF, was dissolved in 60 mL of phosphate buffer pH 3.0. The solution was kept for sonication for about 10 min, followed by filtration (Whatman filter paper no.1). The filter paper was rinsed 3 times with 10 mL of phosphate buffer pH 3.0 and the volume of the filtrate and rinsing was made up to 100 mL. In a separating funnel, 5 mL of the sample solution was taken and mixed with 5 mL each of chloroform and bromophenol blue.[4] The separating funnel was shaken vigorously for 10 min. A reddish orange chromogen was formed. The chloroform layer was separated and checked for absorbance at 417 nm.

d. Analytical method validation[20]

a. Linearity

Standard solutions of CEF over a concentration range of 2 – 24 µg/mL were prepared from a 1 mg/mL stock solution and absorbance was measured. Five replicates were carried out. Calibration curve was constructed by plotting the concentration level of drug versus absorbance.

b. Precision

Three replicates each of intra and inter day studies in five cycles were carried out to determine the repeatability of the method.

c. Accuracy

The sample solutions were spiked with 80, 100, and 120 % of the standard CEF to carry out accuracy studies. Five replicates were conducted. This method was carried out to evaluate the recovery of the drug at different levels.

d. Sandell's sensitivity

Sensitivity of the proposed method is determined by calculating the Sandell's sensitivity, which is defined as the smallest weight of substance that can be detected in column of unit cross section.

Sandell's sensitivity is calculated by

$$\text{Sandell's sensitivity} = \frac{\text{Concentration of the drug (µg/mL) x 0.001}}{\text{Absorbance}}$$

e. Limit of detection and limit of quantification

Limit of detection and limit of quantification were calculated on the basis of the Standard Deviation of the Response and the Slope

The limit of detection (LOD) may be expressed as:

$$LOD = 3.3 \times \sigma/S$$

The limit of quantitation (LOQ) may be expressed as:

$$LOQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The estimation of slope (S) can be done from the data obtained from calibration curve of the analyte. The estimate of σ may be carried out using standard deviation of the response.

f. Analysis of the marketed formulation

Sample solution of CEF containing concentration of 12 µg/mL was prepared and absorbance was taken at 417 nm. Five replicates were carried out. The risk and possibility of interference of the excipient in the analysis was studied.

RESULTS AND DISCUSSION

a. Absorption spectra for CEF

Spectrum of CEF by spectrophotometric method is shown in (Fig. 2) given below. The λ_{max} of CEF was found to be 417 nm.

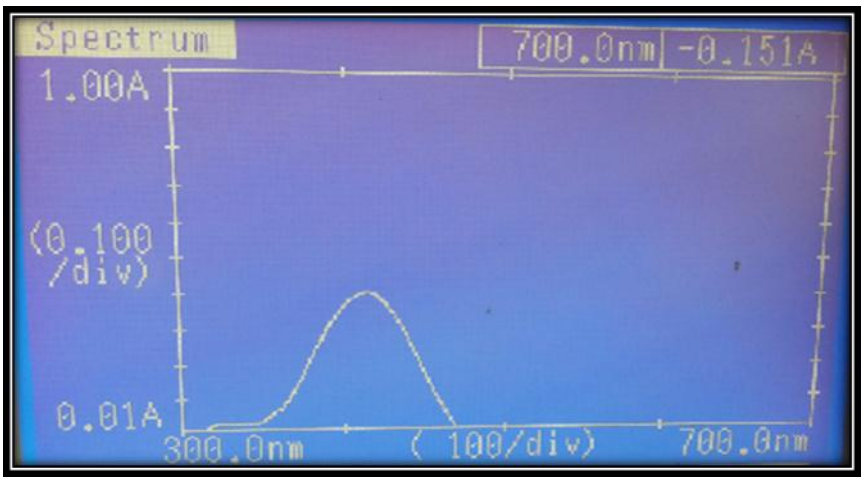


Fig. 2: UV Spectrum of CEF.

b. Method Validation

a. Linearity

Good linear relationships were obtained over a concentration range of 2 - 24 µg/mL for CEF with respect to absorbance. The linear regression data is tabulated in the (Table 1 and 2) given below. From the graphical data of CEF shown in (Fig. 3), it is evident that the absorbance values of CEF are linear over the concentration range of 2 - 24 µg/mL.

Table 1: Linearity data for CEF.

SL. NO.	Concentration (µg/mL)	Mean Absorbance*
1	2	0.147
2	4	0.2133
3	8	0.3873
4	12	0.5643
5	16	0.7147
6	20	0.875
7	24	0.9953

***Mean of five replicates**

Table 2: Linear regression data CEF.

Regression parameters	Data obtained*
Correlation coefficient	0.9976
Slope	0.0396
Intercept	0.0706

***Mean of five replicates**

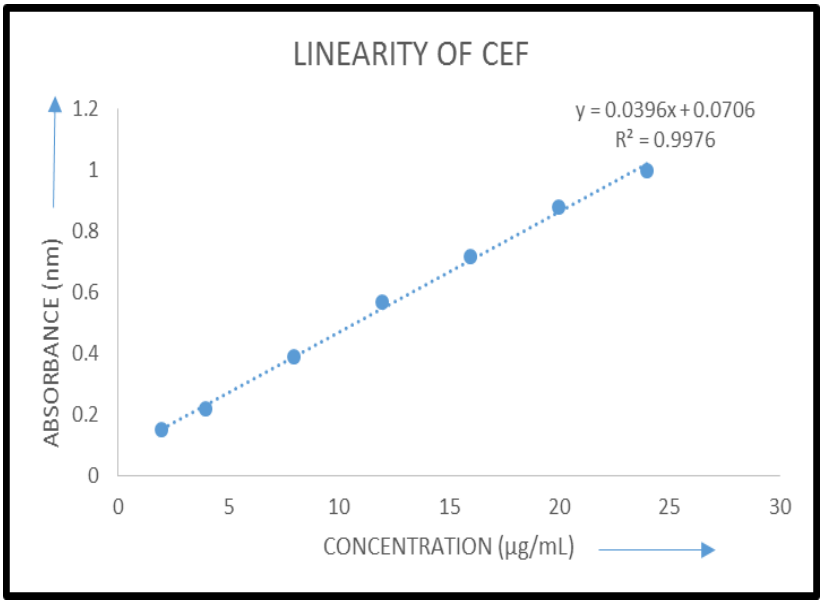


Fig. 3: Graphical representation of CEF for linearity studies.

b. Optical Characteristics

Optical characteristics of CEF are tabulated in the (Table 3). The data obtained for the optical characteristics, LOQ and LOD, clearly indicated that the proposed method for CEF is sensitive and that very small quantities of the compounds can be estimated accurately.

Table 3: Optical Characteristics of CEF.

Parameters	Cefepime
λmax	417 nm
Linearity (µg/mL)	2-24
Molar absorption Coefficient (L /cm/mol)	3.5336×10^4
Sandell's Sensitivity (µg/ cm^2)	0.0014
Limit of Detection	0.4134 µg/mL
Limit of Quantification	1.2538 µg/mL

c. Accuracy

The proposed method was carried out by spiking the tablet formulation with 80, 100 and 120% of the standard. The results are tabulated in (Table 4). The excellent mean recoveries with % RSD suggested good accuracy of the propose methods and no interference from formulations recipients.

Table 4: Recovery studies of CEF.

Drug	Amount of drug present (µg/mL)	Amount of drug added (µg/mL)	Total amount of drug added (µg/mL)	Mean* % Recovery ± RSD
Cefipime	6	4.8	10.8	99.9031 ± 0.4124
	6	6	12	100.1290 ± 0.3792
	6	7.2	13.2	101.0113 ± 0.5323

± Standard deviation

* Mean of five replicates

d. Precision

Three replicates of each concentration for intra and inter day studies of five cycles were carried out. The results are tabulated in the (Table 5 and 6). Low values of the % RSD for inter and intra-day variation suggested an excellent precision of the proposed method.

Table 5: Intra-day precision results of CEF.

Sl No	Concentration (µG/ML)	Mean* Absorbance	Mean Standard Deviation	Mean % Rsd
1	2	0.147	0.0018	1.2245
2	4	0.213	0.0042	1.9718
3	8	0.387	0.0019	0.4910
4	12	0.564	0.0017	0.3014
5	16	0.714	0.0040	0.5602
6	20	0.875	0.0017	0.1943
7	24	0.995	0.0027	0.2714

***Mean of five replicates**

Table 6: Inter-day precision results of CEF.

Sl No	Concentration µG/ML)	Mean Absorbance	Mean Standard Deviation	Mean % Rsd
1	2	0.143	0.0015	1.0490
2	4	0.202	0.0030	1.4851
3	8	0.394	0.0015	0.3807
4	12	0.561	0.0019	0.3387
5	16	0.719	0.0035	0.4868
6	20	0.869	0.0012	0.1381
7	24	0.998	0.0034	0.3407

***Mean of five replicates**

e. Analysis of marketed formulation

There was no interference from any of the formulation excipients and any other impurities in the sample. The mean percentage of the drug present was found to be 99.12 % for CEF.

Table 7: Assay of CEF tablets.

Sl No	Concentration (mg/mL)	% Assay
1	2	99.12

***Mean of five replicates**

CONCLUSION

The proposed extractive spectrophotometric method for CEF in bulk and formulation dosage form was said to be accurate, precise, sensitive and linear. The method is specific to CEF. Good linear relationships were obtained over a concentration range of 2 - 24 µg/mL for CEF with respect to absorbance. LOQ and LOD, clearly indicated that the proposed method for CEF is sensitive and that very small quantities of the compounds can be estimated accurately. Low values of the % RSD for inter and intra-day variation suggested an excellent precision of the proposed method. The excellent mean recoveries with % RSD suggested good accuracy of the proposed method and no interference from formulations recipients.

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