WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 1482-1491. Research Article ISSN 2277– 7105

# DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRY METHOD FOR SIMULTANEOUS ESTIMATION OF CEFEPIME HYDROCHLORIDE AND AMIKACIN SULPHATE

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Article Received on
21 Feb 2015,

Revised on 15 March 2015,
Accepted on 08 April 2015

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## ABSTRACT

A simple UV-Visible Spectrophotometric method was developed for the simultaneous equation estimation of cefepime hydrochloride and amikacin sulphate in combined dosage form. In this method the wavelength was selected at 257.40.40 nm For Cefepime Hydrochloride and 191 nm for amikacin sulphate. The method was validated for accuracy, precision, linearity. The linearity was found to be in the range of 20-44 µg/ml for cefepime hydrochloride and 5-11µg/ml for amikacin sulpate. The % recoveries were found in the range of 101.87% to 98.08% of the labeled value for cefepime hydrochloride and amikacin sulphate repectively. The proposed method was successfully applied for the routine quantitative analysis of combined dosage foam containing cefepime hydrochloride and amikacin sulphate.

**KEYWORDS:** cefepime hydrochloride, amikacin sulphate, UV-Visible Spectrophotometric method.

## INTRODUCTION

Cefepime hydrochloride is chemically 7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((1-methylpyrrolidinium-1-yl)methyl)-8-oxo-5-thia- 1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride. It is a fourth generation cephalosporin, and used as a broad spectrum antibiotic with improved activity against Gram-negative bacteria. Amikacin sulphate is chemically O-3-amino-3-Deoxy-alpha-D-glucopyranosyl-(1->4)-O-(6-amino-6-deoxy-alpha-D-glucopyranosyl-(1->6))-N(3)-(4-amino-L-2-hydroxybutyryl)-2-deoxy-L-streptamine sulphate. It is a semi synthetic analogue of

kanamycin, which is active against most of gram-negative bacteria including gentamycin- and tobramycin-resistant strains. The combination of cefepime hydrochloride and amikacin sulphate is widely used in treatment of Pneumonia.[1-4] Cefepime hydrochloride and amikacin sulphate are official in USP and IP.[5-6] Literature survey revealed that a number of UV-Spectrophotometric, colorimetric, flourimetry, liquid chromatography methods have been reported for estimation of amikacin sulphate and cefepime hydrochloride individually or in combination with other drug.[7-11] The first order derivative spectrophotometric and RP-HPLC methods have also been reported for simultaneous estimation of these drugs in combined dose formulation.[12-13]

The present manuscript describes simple, sensitive, accurate, precise, rapid and economic spectrophotometric method based on simultaneous equations, for simultaneous estimation of cefepime hydrochloride and amikacin sulphate in parentral dosage form. The method was validated according to the ICH Q2 (R1) guidelines.[14-15]

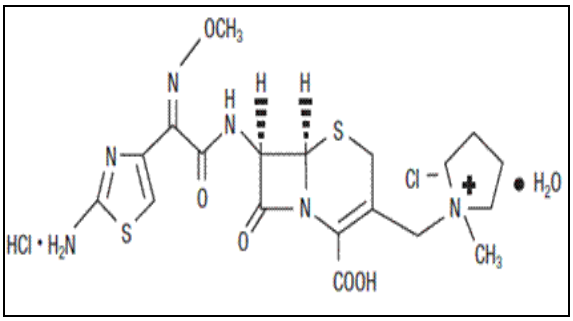


**Figure 1.Cefepime Hydrochloride**

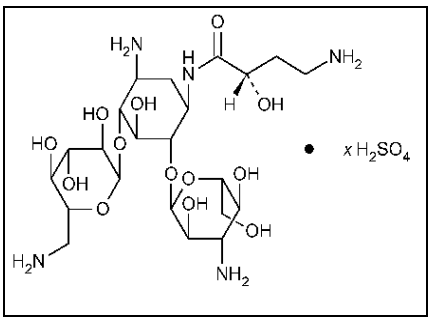


**Figure 2.Amikacin Sulphate**

## MATERIAL AND METHOD

### Materials

Cefepime hydrochloride and amikacin sulphate was provided by Montage Laboratory Pvt. Ltd., Himmatnagar. Distilled water used in present study was collected from college distilled Plant. POTENTOX Injection (Cefepime dydrochloride 500mg, Amikacin sulphate 125 mg) was purchased from local market.

### Instrumentation

Digital analytical balance (Shimadzu ATX 224), UV-Visible, Spectrophotometer (Shimadzu 1601), IR Spectrophotometer (FTIR 8400s), Ultrasonicator (Ultrasonicator cleanser $FS_4$).

**Method Development**

- **Selection of solvent**

Both the drugs are soluble distilled water. The overlain spectra of cefepime hydrochloride and amikacin sulphate were taken to check feasibility of this solvent for spectrophotometric analysis for simultaneous estimation of these drugs.

**Preparation of standard solution**

➢ **Prepare main stock solution of Cefepime hydrochloride (100 µg/ml)**

Standard Cefepime hydrochloride (10 mg) was accurately weighed and transferred to 100ml volumetric flask. It was dissolved properly and diluted up to mark with distilled water to obtain final concentration of 100 µg/ml. This solution was used as working standard solution.

➢ **Prepare main stock solution of Amikacin Sulphate (100 µg/ml)**

Standard amikacin sulphate (10 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was dissolved properly and diluted up to mark with distilled water to obtain final concentration of 100 µg/ml. This solution was used as working standard solution.

- **Calibration curve for Cefepime hydrochloride and amikacin sulphate**

Appropriate aliquots of the working standard solutions of cefepime hydrochloride (100 µg/ml) were transferred to series of 10 ml volumetric flask and diluted up to 10 ml with methanol to give final concentration in the range 20-440 µg/ml. Appropriate aliquots of the working standard solutions of amikacin sulphate (100 µg/ml) were transferred to series of 10 ml volumetric flask and diluted up to 10 ml with methanol to give final concentration in the range 5-11 µg/ml µg/ml. The UV spectrum of each standard solution was recorded against methanol as a blank solution and the absorbance at two selected wavelengths 257.40 nm and 191 nm was measured.

- **Simultaneous equation method**

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the $\lambda_{max}$ of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).

$$Cx = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2}$$

$$Cy = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Where,

$a_{x1}$ and $a_{x2}$ = absorptivity of cefepime hydrochloride at $\lambda_1$(257.40) and $\lambda_2$(191), respectively.

$a_{y1}$ and $a_{y2}$ = absorptivity of amikacin sulphate at $\lambda_1$ (257.40) and $\lambda_2$, (191) respectively.

$A_1$ and $A_2$ = absorbance of the diluted sample at $\lambda_1$ (257.40) and $\lambda_2$ (191), respectively.

**Validation of method**

**Linearity and Range**

Accurately measured standard solutions of cefepime (2, 2.4, 2.8, 3.2, 3.6, 4.0 and 4.4 ml) and amikacin sulphate (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 1.1 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with distilled water. The absorbance was measured at 257.40.40 nm for cefepime hydrochloride and 191 nm for amikacin sulphate. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

**Precision**

**Repeatability**

Aliquots of 2.8ml of working standard solution of Cefepime Hydrochloride (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.7 ml of working standard solution of Amikacin Sulphate (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with Distilled Water to get 28 µg/ml solution of Cefepime Hydrochloride and 7 µg/ml solution of amikacin sulphate. The absorbance of solutions was measured spectrophotometrically six times and relative standard deviation (%R.S.D) was calculated.

**Intraday precision**

Aliquots of 2.0, 2.4 and 2.8 ml of working standard solution of cefepime hydrochloride (100µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.5, 0.6 and 0.7 ml of working standard solution of amikacin sulphate (100 µg/ml) were transferred to series of 10 ml volumetric flask. The volume was adjusted up to mark with distilled water to get 20-28 µg/ml solution of Cefepime hydrochloride and 5-7µg/ml solution of amikacin sulphate. The absorbance of solutions was measured spectrophotometric three times on same day and relative standard deviation (%R.S.D) was calculated.

**Inter day precision**

Aliquots of 2.0, 2.4 and 2.8 ml of cefepime hydrochloride and 0.5, 0.6 and 0.7 ml of of Amikacin sulphate (100 µg/ml) working standard solution were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with water to get 20-28 µg/ml cefepime hydrochloride and 5-7 µg/ml solution of amikacin sulphate. The absorbance of solutions were measured spectrophotometrically three times in three different days and relative standard deviation (% R.S.D) was calculated

**Accuracy**

Accuracy of the measurement of cefepime hydrochloride in combination with amikacin sulphate was determined by standard addition and method. The known amounts of standard solutions of cefepime hydrochloride and amikacin sulphate were added at 50, 100 and 150 % level to prequantified sample solutions of cefepime hydrochloride and amikacin sulphate.

**Limit of detection**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated derived by calculating the signal-to- noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ), using the following equations designated by the International Conference on Harmonization (ICH).

**LOD = 3.3 X σ/S**

**LOQ = 10 X σ/S**

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

**Analysis of Cefepime and Amikacin in combined Injection dosage form**

Marketed powdered injection formulation (Potentox) containing 500mg of Cefepime and 125 mg of Amikacin were analyzed by this method. The response of sample solution was measured at 191 nm and 257.40 nm for quantification of Amikacin and Cefepime respectively. The amount of Cefepime and Amikacin present in sample solution were calculated by fitting the responses in to the regression equation for Cefepime hydrochloride and Amikacin sulphate in proposed method.

## RESULT AND DISCUSSION

### Linearity and Range

Linear relationship was found in the concentration range of 20-44 µg/ml and 5-11 µg/ml for cefepime hydrochloride and amikacin sulphate, respectively with co-efficient of correlation, (r2)=0.999 and (r2)= 0.999 for cefepime hydrochloride and amikacin sulphate respectively (Fugure 1-6, Table1).

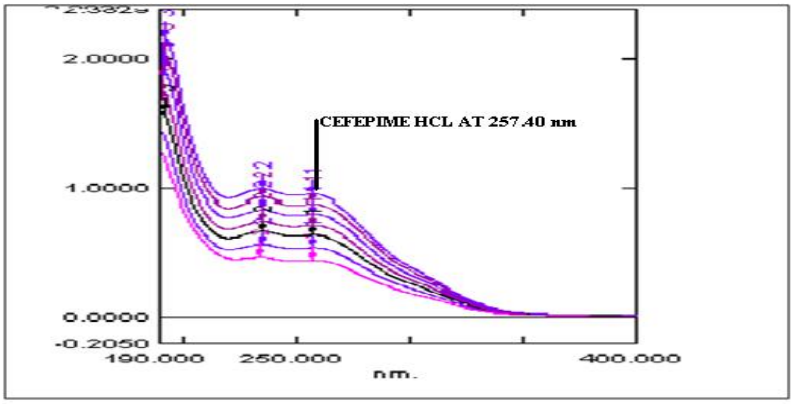


**Figure 1: Cefepime hydrochloride linearity (20-44 µg/ml).**

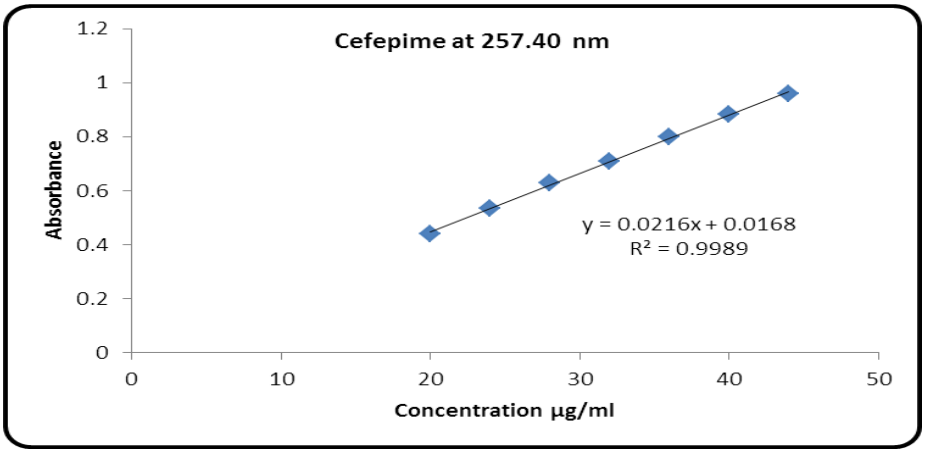


**Figure 2: Calibration curve of cefepime hydrochloride at 257.40**

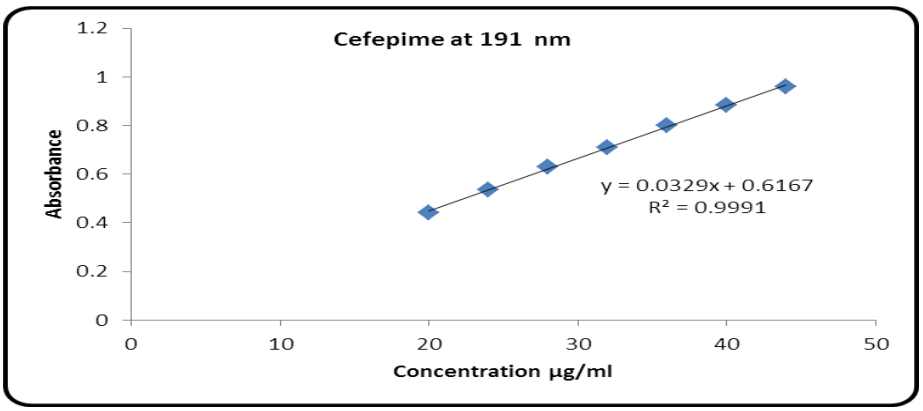


**Figure 3: Calibration curve of cefepime hydrochloride at 191 nm.**

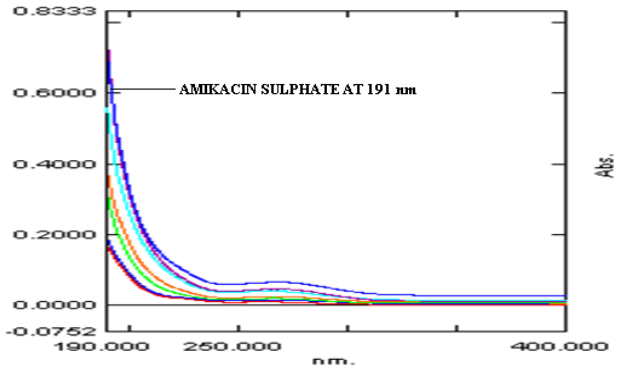


**Figure 4: Amikacin sulphate linearity (5-11 µg/ml)**

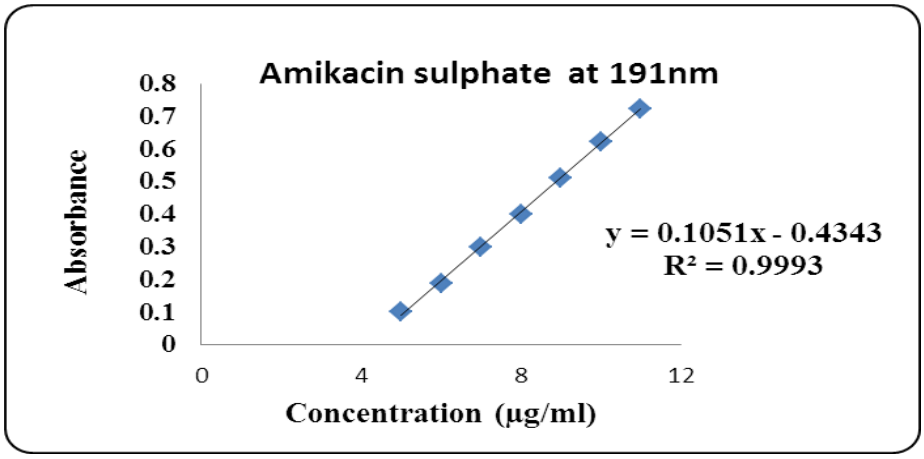


**Figure 5: Calibration Curve of amikacin sulphate at 191 nm.**

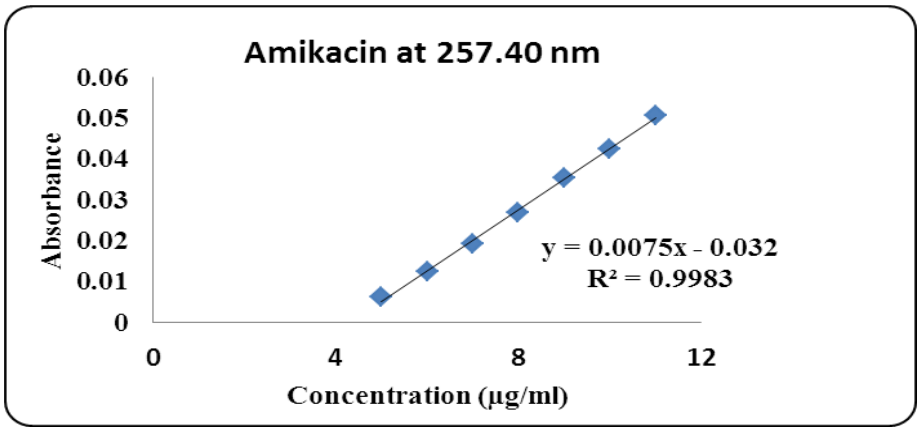


**Figure 6: Calibration curve of amikacin sulphate at 257.40.**

**Precision**

The results of intra-day and inter-day precision were expressed as % RSD and it was found to be NMT 2. The results of intra and inter day precision are shown in (Table 1).

**Accuracy**

The recovery studies were carried out at three levels and three determinations were made at each levels and percentage recovery was calculated. From the data obtained, it was observed that the recovery of standard drugs cefepime hydrochloride and amikacin sulphate was accurate and within the limits employing both methods. The results are shown in Table 1.

**LOD and LOQ**

The values for limit of detection and limit of quantitation by both methods are mentioned in (Table 1).

**Table 1: Summary of validation Parameters of UV Spectrophotometer**

| Parameters | Cefepime hydrochloride At 257.40 nm | Amikacin sulphate At 191 nm |
|---|---|---|
| Linear Range (µg/ml) | 20-44 | 5-11 |
| Limit of Detection (µg/ml) | 0.4609 | 0.248 |
| Limit of Quantitation (µg/ml) | 0.139 | 0.751 |
| Repeatability | 1.30 | 0.99 |
| Intra day | 0.49-1.52 | 0..31-1.23 |
| Inter day | 0.49-0.142 | 0.36-.0.49 |
| Accuracy(% Recovery) | 98.98-100.69 | 98.9-102.57 |
| % Assay | 101.87% | 98.08% |

**Assay of market formulation**

The marketed brand of powder injection was analyzed and amount of cefepime hydrochloride and amikacin sulphate determined by the proposed method was found to be 101.87.5% for cefepime hydrochloride and 98.08% for amikacin sulphate, respectively by simultaneous equation method (Table 1).

**Table 2: Assay Results of Marketed formulations**

| Injection Formulation | Labeled Claim | | Amount found | | % Recovery | |
|---|---|---|---|---|---|---|
| | Cefepime HCl | Amikacin Sulphate | Cefepime HCl | Amikacin Sulphate | Cefepime HCl | Amikacin Sulphate |
| POTENTOX | 500mg | 125mg | 509.4mg | 122.50 mg | 101.87% | 98.08% |

**CONCLUSION**

These entire factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, and rapid and chip and can be applied successfully and routine analysis for estimation cefepime hydrochloride and amikacin sulphate in pharmaceutical formulations without interference from commonly used excipient and additives.

**ACKNOWLEDGEMENT**

The authors are thankful to Montage Laboratory, Himmatanagar, India for providing gift sample of cefepime hydrochloride and amikacin hulphate for research.

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