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Development and Validation of Rp-Hplc Method for Simultaneous Estimation of Cefepime Hydrochloride and Amikacin Sulphate in Injection Dosage Form

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ABSTRACT:

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Cefepime and Amikacin in injection formulation. The separation was achieved by C18 (250 x 25mm) 25µm column and Acetonitrile: water (10:90 v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 212 nm. Retention time of Amikacin and Cefepime was found to be 2.51 min and 6.23 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity for Cefepime and Amikacin were in the range of 20-100 µg/ml. The percentage recoveries obtained for Cefepime and Amikacin were found to be in range of 98.22±0.56 and 99.63±0.57 respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Cefepime and Amikacin in injection. The proposed method was successfully applied for the simultaneous estimation of both drugs in commercial injection preparation.

KEYWORDS: Cefepime Hydrochloride, Amikacin Sulphate, RP-HPLC, Injection, Validation.

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INTRODUCTION:

Cefepime is chemically 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino) acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidin-1-ium[1], It is a fourth generation cephalosporin, and used as a broad spectrum antibiotic with improved activity against Gram-negative bacteria over other commercially available cephalosporin drugs, Amikacin (AK) is chemically (2S)-4-amino-N-[(1R,2S,3S,4R,5S)-5-amino-2-{[(2S,3R,4S,5S,6R)-4-amino-3,5-dihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}-4{[(2R,3R,4S,5S,6R)-6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy}-3-hydroxycyclohexyl]-2 hydroxybutanamide[11], It is a semi synthetic analogue of kanamycin, is an aminoglycosidic antibiotic active against most of gram-negative bacteria including gentamycin- and tobramycin-resistant strains. Combination of Cefepime and Amikacin are widely used in treatment of Pneumonia. Cefepime and Amikacin are official in USP [2, 10] and IP [3] describe Liquid chromatography method for its estimation. Literature Survey revealed that a number of UV-Spectrophotometric[4,5,9], Colourimetric[3], Flourimetry[7], RP-HPLC[6,12] and liquid chromatography[7,13,14] methods have been reported for estimation of Amikacin Sulphate, Cefepime Hydrochloride individually or in combination with other drug. Since no analytical method is reported for simultaneous estimation of these drugs in combined dose formulation. In present work a successful attempt has been made to estimate these drugs simultaneously by RP-HPLC method. 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.

MATERIALS AND METHODS

Apparatus

- Model: LC 100 HPLC Cyberlab
- Column: C18 (250 x 25mm, 25µm)
- Injector: Rhenodyne valve with 10µl fixed loop.
- Detector: LC-UV 100 UV Detector
- Software: WS-100 Workstation Software
- Analytical balance: Electronic analytical balance (Contech)
- Corning volumetric flasks and pipettes

Reagents and Materials

- Cefepime Hydrochloride API (Montage pharmaceutical Ltd. At Himmatanagar)
- Amikacin Sulphate (Montage pharmaceutical Ltd. At Himmatanagar)
- Water(HPLC grade)
- Acetonitrile (HPLC grade)
- Distilled water

METHODS

Preparation of solutions

Preparation of mobile phase / diluents: The mixture of Acetonitrile (HPLC) and Water was prepared in ratio of 10:90.

Stock solution for Cefepime and Amikacin (100µg/mL): An accurately weighed quantity of 100mg Cefepime and Amikacin was transferred into 100 ml volumetric flask, then made up to the mark with diluents. Take 10 mL from that and transfer to 100 ml volumetric flask to make up the volume up to the mark with diluents. From this stock solution different aliquots were prepared.

Method development and optimization

The standard solution of Cefepime and Amikacin were used for method development trials to optimize the method for determination of Cefepime and Amikacin.

Selection of detection wavelength

Standard solution of Cefepime (100 µg/ml) and Standard solution of Amikacin (100 µg/ml) were scanned between 200-400 nm using UV-visible spectrophotometer. Both solutions were scanned between 200-400 nm. Wavelength was selected from the overlay spectra of above solutions. Spectra Shown in the Figure 1.

Selection of elution mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. Here, C18 250 × 4.6 mm column of 25µm particle packing was selected for separation of Cefepime and Amikacin. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

Selection of Mobile phase

The mobile phase should be sufficiently transparent at the wavelength of detection. i.e. minimum absorbance. It is best initial choice of organic solvent for the mobile phase. Acetonitrile-water mixture can be used with UV detection at low wavelength (200-240 nm) range. Acetonitrile-water mixture also has lower viscosity, resulting in somewhat higher number of plates and lower column back pressure than methanol-water mixture.

The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So, numbers of trial were taken. After number of trial Acetonitrile: Water (10:90v/v) was selected.

Selection of oven temperature: Oven temperature is keep ambient.

Standard curve:

Accurately weighed 5 mg Of Cefepime and 5 mg of Amikacin were transferred to 50 ml volumetric flask and diluted up to mark with diluent to give concentration of 100 µg/ml of Cefepime and 100 µg/ml of Amikacin. Similarly, different concentration (20, 40, 60, 80 and 100 µg/ml) for Cefepime and (20, 40, 60, 80 and 100 µg/ml) Amikacin were prepared and prepared the concentration V/S area plot. (Figure-2)(Table-1)

VALIDATION APPROACH

Validation of analytical method shall be done to establish by laboratory studies, that the performance of the method meet the requirement for the intended analytical application. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [15].

Specificity:

Specificity of an analytical method is ability to measure specifically the analyte of interest without interferences from blank and placebo.

Check for interference from blank: Diluent was used as blank. Standard and sample were prepared as per test procedure. Check for the interference of blank and peaks with the analyte peak and calculate % interference of blank peaks interferes with analyte peak against the standard peak area.

Linearity:

The linearity for Cefepime and Amikacin were assessed by analysis of combined standard solution in range of 20-100 µg/ml respectively, in term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Precision:

Method precision for assay was established by determining the assay of seven sample preparations under same conditions. Seven replicates of sample were prepared at

sample concentration by one analyst and analyzed on same day. Intraday precision was performed by standard Five times and measuring the area of drugs at same day with time interval. Inter day precision was performed by standard Five times and measuring the area of drugs at different day interval. (Table 2,3,4)

Accuracy:

Accuracy was determined over the range of 50%, 100% and 150% of the sample concentration. Calculated amount of Cefepime and Amikacin API was added in placebo to attain 50%, 100% and 150% of sample concentration. Amount as shown above was transferred into 50 ml volumetric flask and made up to the mark with diluent. Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated from regression equation as shown in (Table 5, 6).

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method for Cefepime and Amikacin were determined by injecting progressively known concentrations of the standard solutions using the developed HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response at signal to noise ratio of 3:1 and 10:1, respectively. (Table 7, 8)

System Suitability:

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (*N)*, resolution *(R),* and tailing factors *(T)* (Table 9).

Formulation Analysis:

The percentage assay of Cefepime and Amikacin was performed for commercially available innovator Venus Remedies Ltd. POTENTOX Injection.

RESULTS AND DISCUSSION

RP-HPLC method developed for simultaneously estimation of Cefepime and Amikacin in Injection Dose. Developed RP-HPLC method was validated according to ICH guideline. RP-HPLC method has shown adequate separation for Cefepime and Amikacin. Separation was achieved on Inertsil C18 (250 x 4.6mm) 5 µm column by using Acetonitrile: Water (10:90) as a mobile phase at a flow rate of 1.0 mL/min, and UV detection was carried out at 212 nm. (Figure 3)

In the present study the specificity of the method was determined by assessing interference from the placebo & diluents. There were no other co eluting, interfering peaks from excipients, impurities found and the method was specific for estimation of Cefepime and Amikacin.

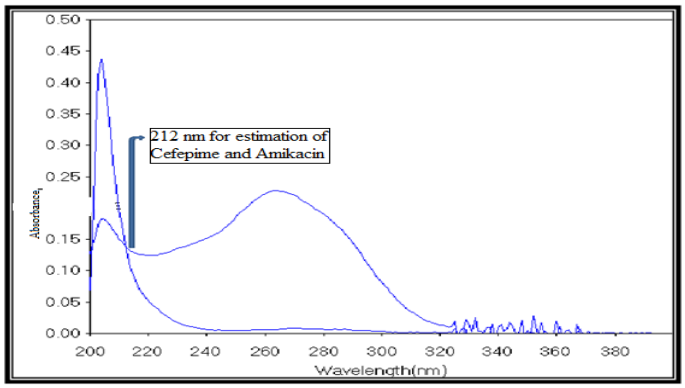

Figure 1: Overlay UV Spectrum of Cefepime and Amikacin showing selection of Wavelength detection

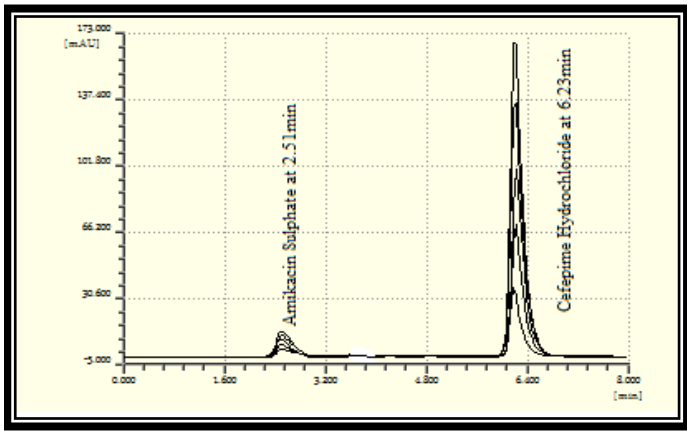

Figure 2: Chromatogram of binary mixture of Cefepime Hydrochloride and Amikacin Sulphate (20- 100 µg/ml)

The method was validated in terms of linearity, precision, accuracy, specificity, System Suitability. The linearity of the proposed method was investigated in the range of 20-100 µg/ml of test concentration for Cefepime and Amikacin. Accuracy was determined by recovery study & it was found to be 98.97 for Cefepime and 98.91% for Amikacin injection. The mean assay (*n=5*) were 98.22 and 99.63 respectively. The percentage RSD value for the five assay values was 0.56 for Cefepime and 0.57 for Amikacin.

Table 1: SERIES A Linearity data for Cefepime Hydrochloride and Amikacin Sulphate

Sr. No	CEF		AMK	
	Conc. (µg/ml)	Peak area(n=5)	Conc. (µg/ml)	Peak area(n=5)
1	20	71348.4 ± 117.0173	20	7556.6 ± 621.12
2	40	114328.6 ± 107.1286	40	11968.2 ± 216.46
3	60	160560.2 ± 116.9424	60	15953.8 ± 563.11
4	80	210415.2 ± 209.6710	80	20783.9 ± 108.58
5	100	256961.9 ± 314.1043	100	25341.7 ± 310.22

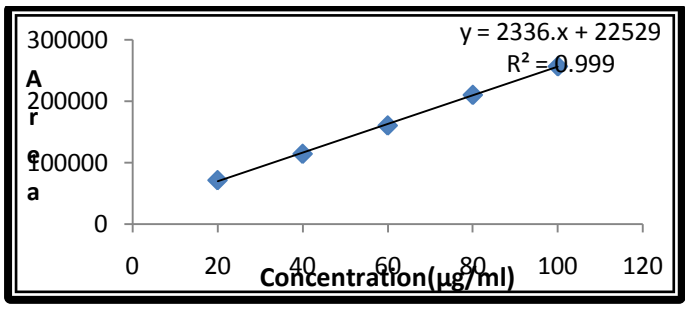

Figure 3: Calibration Curve of Cefepime (20-100 µg/ml)

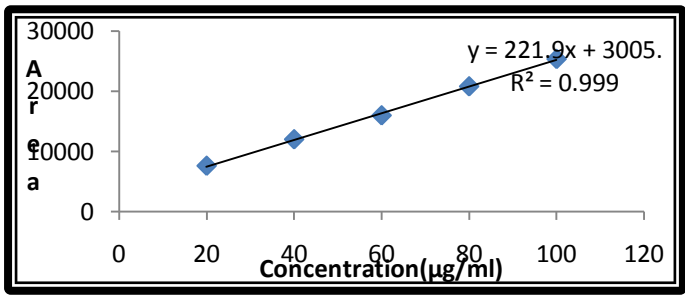

Figure 4: Calibration curve of Amikacin (20-100 µg/ml)

Table 2: Repeatability data for Cefepime and Amikacin

Cefepime			
Conc. (µg/ml)	**Area**	**Area Mean ± S.D. (n=7)**	**% RSD**
60	160861.1 160505.6 160491.4 160746.5 161564.6 160486.2 160522.5	160739.7 ± 392.2452	0.97
Amikacin			
Conc. (µg/ml)	**Area**	**Area Mean ± S.D. (n=7)**	
60	15958.3 15933.7 15899.2 15546.8 15962.6 15911.2 15966.3	15882.59 ± 150.3344	0.95

Table 3: Intraday precision data for estimation of Cefepime and Amikacin

Cefepime			Amikacin		
Conc.	**Area Mean ± S.D. (n=5)**	**%RSD**	**Conc.**	**Area Mean ± S.D. (n=5)**	**%RSD**
20	71290.53 ± 877.685	1.24	20	7581.833 ± 94.61418	1.24
40	116729.2 ± 1564.944	1.35	40	11844.67 ± 247.3035	2.08
60	161659.9 ± 1342.759	0.83	60	15412.77 ± 378.4537	2.46
80	219554.6 ± 929.3279	0.43	80	21390.8 ± 392.3522	1.83
100	251555.2 ± 607.6647	0.25	100	25403.8 ± 387.6611	1.52

Table 4: Interday precision data for estimation of Cefepime and Amikacin

Cefepime			Amikacin		
Conc.	**Area Mean ± S.D. (n=5)**	**%RSD**	**Conc.**	**Area Mean ± S.D. (n=5)**	**%RSD**
20	71324.27 ± 486.7133	0.68	20	7546.133 ± 113.0512	1.49
40	123835.1 ± 2085.837	1.68	40	12849.53 ± 137.2226	1.06
60	171011.6 ± 963.6319	0.56	60	16177.57 ± 216.6444	1.34
80	221885.4 ± 2364.483	1.06	80	22999.9 ± 109.7719	0.47
100	263465.7 ± 4111.967	1.56	100	26521.4 ± 456.2084	1.72

CONCLUSION

The proposed method is accurate, simple, economical, rapid and selective for the simultaneous estimation of Cefepime and Amikacin in Injection dosage form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. Hence, the developed HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulation.

Table 5: Accuracy data for Cefepime

Conc. Level	Amount spiked	Area	Amount recovered	% recovery	% mean recovery ± SD
50	10	45923.1	10.01	100.10	100.96 ± 2.23
	10	45719.8	9.92	97.27	
	10	46723.5	10.35	103.50	
100	20	70238.4	20.42	102.10	101.43 ± 1.51
	20	70432.1	20.50	102.50	
	20	69128.4	19.94	99.70	
150	30	91692.5	29.60	98.67	98.97 ± 1.59
	30	90913.8	29.27	97.56	
	30	93113.2	30.21	100.7	

Table 6: Accuracy data for AMK

Conc. Level	Amount spiked	Area	Amount recovered	% recovery	% mean recovery ± SD
50	10	5256.3	10.14	101.4	101.2 ± 2.36
	10	5196.3	9.87	98.70	
	10	5311.2	10.34	103.4	
100	20	7413.2	19.80	99.60	100.6 ± 1.71
	20	7567.1	20.50	102.50	
	20	7431.9	19.90	99.50	
150	30	9563.9	29.50	98.33	98.91 ± 1.30
	30	9512.8	29.40	98.00	
	30	9688.7	30.12	100.4	

Table: 7 Limit of detection

CEF	AMK
LOD=3.3 x (SD/Slope)	LOD=3.3 x (SD/Slope)
LOD=3.3x	LOD=3.3x
(172.98/2336)	(363.9/221.9)
LOD=0.24 µg/ml	LOD= 5.5µg/ml

Table: 8 Limit of quantitation

CEF	AMK
LOQ=10 x (SD/Slope)	LOQ=10 x (SD/Slope)
LOQ=10 x (172.98 /2336)	LOQ=10 x (363.9/221.9)
LOQ=0.74 µg/ml	LOQ=16.40 µg/ml

Table 9: Results for system suitability test

Parameters	Data obtained	
	Cefepime	Amikacin
Theoretical plates	3291	1021
Symmetry factor/Tailing factor	0.02	1.70
Resolution	7.56	

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