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Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Cefepime Hydrochloride and Amikacin Sulphate in Injection

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ABSTRACT:

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative Spectrophotometric method for the simultaneous determination of Cefepime Hydrochloride and Amikacin Sulphate in combined Parentral dosage form. The derivative Spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in 0.1N HCl and the determinations were made at 220.0 nm (ZCP of Cefepime Hydrochloride) for Amikacin Sulphate and 294.0 nm (ZCP of Amikacin Sulphate) for Cefepime Hydrochloride. The linearity was obtained in the concentration range of 20-100 µg/ml for both Cefepime Hydrochloride and Amikacin Sulphate. The mean recovery was 99.53 ± 0.37 and 98.94 ± 0.70 for Cefepime Hydrochloride and Amikacin Sulphate, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Cefepime Hydrochloride and Amikacin Sulphate in pharmaceutical Parentral dosage form. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Cefepime Hydrochloride, Amikacin Sulphate, First order derivative Spectrophotometric method, 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-methyl pyrrolidin-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-(hydroxymethyloxan-2-yl]oxy} 4{[(2R,3R, 4S,5S,6R)-6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy}-3-hydroxycyclohexyl]-2hydroxy butanamide[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 UV-spectrophotometric 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

Spectral and absorbance measurement were made on Helios alpha (Thermo Scientific) UVA 1002 E (Model) Spectrophotometer by using 1 cm quartz cell. Digital precision Balance (A series) Contech Model –CA34 was used for weighing the samples. Commercially available injection of Cefepime and Amikacin combination were procured from the local market and estimated.

Reagents and Materials

Cefepime Hydrochloride and Amikacin sulphate bulk powder was kindly provided by Montage Laboratory, Himmatanagar. The commercial fixed dose combination of Cefepime hydrochloride and Amikacin sulphate (Potentox Injection of Venus Remedies Limited) was procured from local market. Distilled water was used in present study. All the solution was protected for light and was analyzed on the day of preparation.

Preparation of standard stock solutions

An accurately weighed quantity of Cefepime (100 mg) and Amikacin (100 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with 0.1N HCl to obtain standard solution having concentration of Cefepime (1000 µg/ml) and Amikacin (1000 µg/ml).

Methodology

The standard solutions of Cefepime (1000 mg) and Amikacin (1000 mg) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two spectra were overlain and it appeared that Cefepime showed zero crossing at 220.0 nm, while Amikacin showed zero crossing at 294.0 nm. At the zero crossing point (ZCP) of Cefepime (220.0 nm), Amikacin showed a first-derivative absorbance, whereas at the ZCP of Amikacin (294.0 nm), Cefepime showed a first-derivative absorbance. Hence 220.0 and 294.0 nm was selected as analytical wavelengths for determination of Amikacin and Cefepime, respectively. These two wavelengths can be employed for the determination of Cefepime and Amikacin without any interference from the other drug in their combined dosage formulations.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [15].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 20-100 µg/ml for Cefepime and 20-100 µg/ml for Amikacin. Accurately measured standard solutions of Cefepime (20, 40, 60, 80, and 100 ml) and Amikacin (20, 40, 60, 80, and 100 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with 0.1N Hal. First-derivative absorbance (D1) was measured at 294.0 nm for Cefepime and 220.0 nm for Amikacin. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (n = 6) for Cefepime and Amikacin (20 µg/ml) without changing the parameter of the first-derivative spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Cefepime and Amikacin (20, 40 and 60 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of Cefepime and Amikacin by the standard addition method. Known amounts of standard solutions of Cefepime and Amikacin were added at 50, 100 and 150 % level to prequantified sample solutions of Cefepime and Amikacin (20 µg/ml for each drug). The amounts of Cefepime and Amikacin were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were 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 International Conference on Harmonization (ICH) guidelines [15].

$$LOD = 3.3 \times \sigma/S \ldots\ldots \quad (1)$$

$$LOQ = 10 \times \sigma/S \ldots\ldots \quad (2)$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of Cefepime and Amikacin in combined Injection dosage form

Marketed powdered injection formulation (Potentox)

Table 1: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS	FIRST-DERIVATIVE-UV SPECTROPHOTOMETRY	
	Cefepime at 294nm	**Amikacin at 220nm**
Concentration range (µg/ml)	20-100	20-100
Regression equation (y = a + bc)	(Y=0.033x+0.078)	(Y=0.003x-0.026)
Slope (b)	0.033	0.003
Intercept (a)	0.078	-0.026
Correlation Coefficient (r^2)	0.9990	0.9990
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.0333	0.0254
Accuracy (% recovery) (n = 5)	98.80 ± 0.61 99.31 ± 0.31 98.78 ± 0.41	99.11 ± 0.38 98.89 ± 0.69 98.81 ± 1.0
Repeatability (%RSD[a], n = 6),	1.68	0.73
Intraday (n = 3) (%RSD[a])	0.15-1.05	0.48-1.85
Interday (n = 3) (%RSD[a])	0.36-1.02	0.16-1.42
LOD[b](µg/ml)	0.52	0.65
LOQ[c](µg/ml)	1.58	1.97

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

containing 500mg of Cefepime and 125mg of Amikacin were analyzed by this method. The response of sample solution was measured at 220nm and 294 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 and Amikacin in proposed method.

Results of analysis are shown in Table 1. The proposed methods were validated as per ICH guidelines [14].The accuracy of the proposed methods were determined by performing recovery studies at 50%, 100% and 150% of the test concentration. The statistical validation data of recovery study are given in Table 2. The results of the analysis and statistical validation data of the injection formulation are given in Table 3.

Table 2: Recovery data of proposed method

Drug	Level	Amount Taken (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	%Mean Recovery ± S.D (n=5)
Cefepime	I	20	5	24.96	99.41 ± 0.39
	II	20	10	29.94	99.39 ± 0.30
	III	20	15	34.82	98.79 ± 0.42
Amikacin	I	20	5	24.90	99.11 ± 0.39
	II	20	10	29.90	98.89 ± 0.71
	III	20	15	34.83	98.82 ± 1.00

S. D. is Standard deviation and n is number of determinations

RESULTS AND DISCUSSION

The standard solutions of Cefepime and Amikacin were scanned separately in the UV range, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two derivative spectra showed maximum absorbance at 220.0 nm (ZCP of Cefepime) for Amikacin and 294.0 nm (ZCP of Amikacin) for Cefepime. First-derivative absorbances (D1) were recorded 220.0 nm for Amikacin and 294.0 nm for Cefepime (Figure 2). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations. Second and third-ordered derivative spectra of the drugs were not tested because the first-order spectra give satisfactory ZCPs and good quantitative determination of both the drugs without any interference. Linear correlation was obtained between absorbances and concentrations of Cefepime and Amikacin in the concentration ranges of 20-100 µg/ml. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for Cefepime and Amikacin were found to be 1.68 and 0.73 %, respectively (Table 1). The low values of relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The low RSD values of interday (0.36-1.02 and 0.16-1.42 %) and intraday (0.15-1.05 and 0.48-1.85 %) for Cefepime and Amikacin, respectively, reveal that the proposed method is precise (Table 1). LOD values for Cefepime and Amikacin were found to be 0.52 and 0.65 µg/ml, respectively and LOQ values for Cefepime and

Table 3: Analysis of Cefepime and Amikacin by proposed method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	Cefepime	Amikacin	Cefepime	Amikacin	Cefepime	Amikacin
I	20	20	19.96	19.93	99.64±0.46	99.33 ± 0.59
II	20	20	19.97	19.96	99.73±0.72	99.65 ± 0.65

S. D. is Standard deviation and n is number of determinations

Amikacin were found to be 1.52 and 1.97 µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of Cefepime and Amikacin. The recovery experiment was performed by the standard addition method. The mean recoveries were 99.53±0.37 and 98.94±0.70% for Cefepime and Amikacin, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine Cefepime and Amikacin in their combined dosage form. The results obtained for Cefepime and Amikacin were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Cefepime and Amikacin in pharmaceutical dosage forms.

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 20-100 µg/ml and 20-100 µg/ml for Cefepime and Amikacin, respectively with co-efficient of correlation, (r^2)=0.9990 and (r^2)= 0.9990 for Cefepime and Amikacin, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of Cefepime and Amikacin. The method can be used for the routine analysis of the Cefepime and Amikacin in combined dosage form without any interference of excipients.

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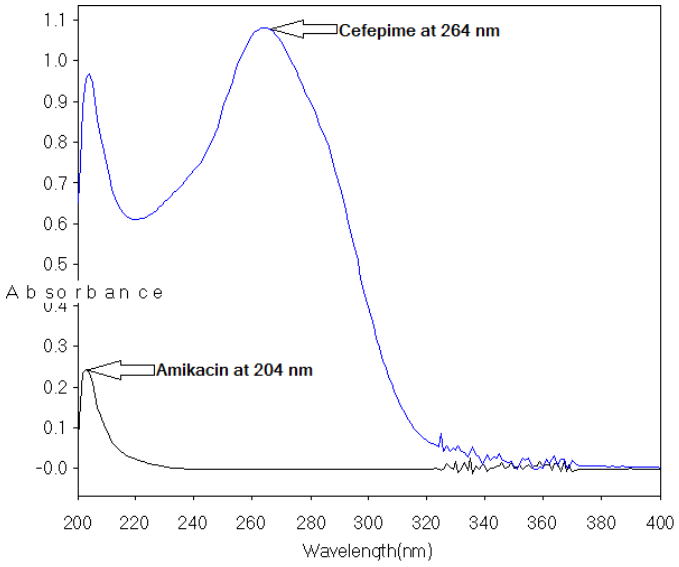

Figure 1: UV Spectrum for Cefepime Hydrochloride (100µg/ml) at 264 nm and Amikacin Sulphate (100µg/ml) at 204nm

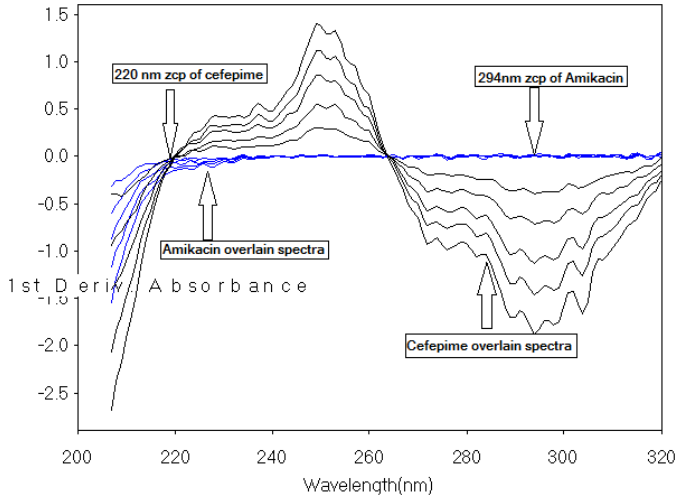

Figure 2: Overlain first-order derivative spectra of Cefepime and Amikacin in 0.1N HCl

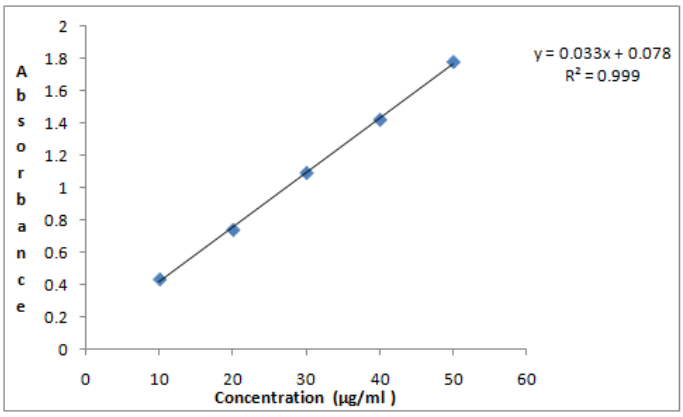

Figure 3: Calibration curve of Cefepime Hydrochloride at 294nm in 0.1N HCl

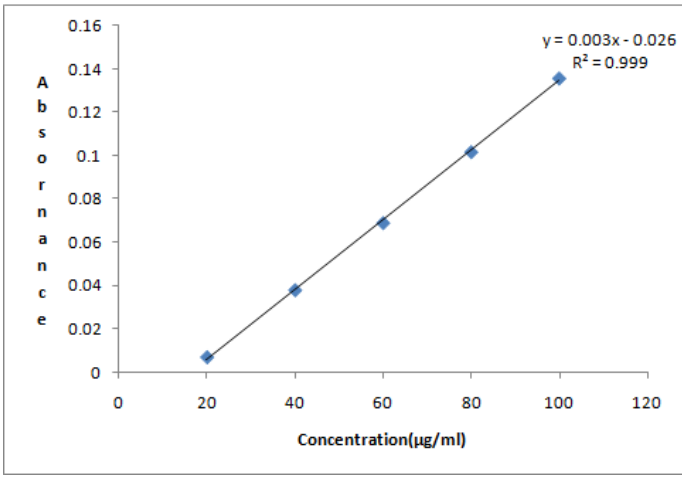

Figure 4: Calibration curve of Amikacin Sulphate at 220nm in 0.1N HCl

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