SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF AMIKACIN AND CEFEPIME IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT
Simple and sensitive spectrophotometric methods have been developed for the quantitative estimation of Cefepime and Amikacin in pharmaceutical formulations. Method I and II are simultaneous analysis of Cefepime and Amikacin in UV region based on simultaneous equation method and first order derivative spectrophotometry method respectively. III and IV methods are visible spectrophotometric methods for the estimation of Cefepime and Amikacin individually. Simultaneous equation method under Ultraviolet condition was proposed with water as solvent and found to have absorbance maxima at 210 nm for Amikacin and 217 nm for Cefepime respectively. First order spectrophotometric method was applied for simultaneous estimation at 210 nm for Amikacin and 217 nm for Cefepime respectively. The linearity lies between 5-17.5 µg/ml for Amikacin and 20-70 µg/ml for Cefepime in both simultaneous UV and first derivative methods. Visible spectrophotometry method for Amikacin was proposed with p-Dimethyl Amino Benzaldehyde (PDAB) Method at 595 nm and for Cefepime it was proposed with Wool Fast Blue BL (WFBBL) method at 498 nm. The results of analysis have been validated statistically as per ICH guidelines. The accuracy and precision of the methods were determined and validated statistically. All methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Cefepime and Amikacin in pure and combined dosage form.

KEYWORDS: Cefepime, Amikacin, Spectrophotometry, Method development, Validation.

INTRODUCTION
Cefepime: Cefepime is a fourth-generation broad-spectrum cephalosporin antibiotic with greater activity against both gram-negative and gram-positive organisms than third-generation agents. Cefepime is developed in 1994 which is now available as a generic drug and sold under a variety of trade names worldwide.³,⁴ It is usually reserved to treat moderate to severe nosocomial pneumonia, infections caused by multi-resistant microorganisms (e.g. Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, Enterobacteriaceae etc.) and empirical treatment of febrile neutropenia. It has been used to treat bacteria responsible for causing pneumonia and infections of the skin and urinary tract. IUPAC name of Cefepime is (6R,7R,Z)-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 with molecular formula C₁₉H₂₁N₆O₅S₂ and molecular mass 480.56.

Figure 1: Chemical structure of Cefepime (a) and Amikacin (b).
Amikacin
Amikacin is an aminoglycoside antibiotic and is a semi-synthetic drug derived from kanamycin A. It is used for the treatment of different types of bacterial infections with multidrug-resistant gram-negative bacteria such as Pseudomonas aeruginosa, Acinetobacter, and Enterobacter. Serratia, arcrescens and Providencia stuartii are also included in the spectrum. Amikacin can also be used to treat non-tubercular mycobacterial infections and tuberculosis (if caused by sensitive strains) when first-line drugs fail to control the infection. Amikacin may be combined with a β-lactam antibiotic for empiric therapy for people with neutropenia and fever. It works by disrupting bacterial protein synthesis by binding to the 30S ribosome of susceptible organisms similar to other aminoglycosides. IUPAC name of Amikacin is (2S)-4-Amino-N-[2S,3S,4R,5S]-5-amino-2-[2S,3R,4S,5S,6R]-4-amino-3,5-dihydr oxy -6-(hydroxymethyl) oxan-2-y]oxy-4-[2R,3R,4S,5R,6R]-6-aminomethyl]-3,4,5-trihydroxy -oxan-2-y]oxy-3-hydroxy-cyclohexyl]-2-hydroxybutanamide with molecular formula C_{19}H_{27}N_{6}O_{9} and molecular mass 585.603. The structures of both Cefepime and Amikacin are given in Figure 1.

Combination therapy with an amino glycoside and an anti-pseudomonal β-lactam has been recommended commonly because this approach provides broad-spectrum coverage, bactericidal activity and potential synergic effects and minimizes the development of resistance during treatment. To minimize the toxicity caused by aminoglycosides, a fixed dose combination of cefepime and amikacin is preferred to be used as combined antibiotics for treatment. Combination of these two antibiotics has lesser nephrotoxicity as compared to other combinations. They are administered as a single composition parentally. There is plenty of literature for the analysis of cefepime (spectrophotometry, chromatography) as a single component and along with other combinations of drugs and few analytical methods have been reported with Amikacin drug (spectrophotometry, chromatography). But there is only one spectrophotometry method reported for the analysis of both Cefepime and Amikacin in combination. In the present work, it is aimed to develop Ultraviolet, visible and derivative spectrophotometric methods for estimation Cefepime and Amikacin in combined dosage forms.

Experimental

Chemicals and Materials
Analytically pure Amikacin and Cefepime were obtained as gifts from reputed Pharmaceutical companies. Methanol, water (Merck, Mumbai, India) were of HPLC grade, while Ethanol used for the preparation of mobile phase were of analytical grade (Merck Specialties Private Limited, Mumbai, India). Formulations of Potentox vials (Cefepime – 500 mg and Amikacin – 125 mg labeled amounts) contain a combination of Cefepime and Amikacin were procured from local market.

Equipment
Tec comp UV-2301 double beam UV/Visible spectrophotometer model was used to carry out the spectral analysis and the data was recorded by Hitachi software. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Preparation of standard drug solution
Standard stock solution of 1000 µg/ml was prepared by accurately weighing 10mg of standard drug Amikacin and Cefepime individually. From this 1 ml was again diluted to 10 ml to obtain a concentration of 100µg/ml solution of Amikacin and Cefepime separately. Required concentrations were prepared separately for calibration. Then 1 ml from each of the solution was mixed to obtain a combined solution for the simultaneous estimation Amikacin and Cefepime.

Preparation of formulation sample solution
10 vial formulations of Cefepime and Amikacin (Potentox; Cefepime- 500mg and Amikacin - 125mg) were soaked in 5ml diluents and were kept in it for 1 hr. Then it was filtered and made up to 10 ml with the same diluents to make 100 µg/ml stock solutions. From this by proper dilution a concentration of 40 µg/ml of Cefepime was prepared as per the label claim of the drugs; Amikacin concentration 10 µg/ml was obtained. The resultant solution was used for the simultaneous estimation of Cefepime and Amikacin in combined dosage forms.

Preparation of reagents
p-Dimethyl Amino Benzaldehyde (PDAB): 0.5 gm of p-Dimethyl Amino Benzaldehyde was dissolved in 100 ml of Methanol.

WFBBL solution: Weigh accurately 200 mg of Wool Fast Blue BL (WFBBL) and was dissolved in 100 ml of distill water.

HCl Solution: Dissolve 8.6 ml of analytical grade concentrated hydrochloric acid in 1000 ml of distilled water.

Simultaneous equation method
From the stock solution 100 µg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned for the entire UV range to determine the λ max. Amikacin has λ max of 210 nm while Cefepime has λ max at 217 nm (Figure. 2). Standard solutions were prepared having concentration 5-17.5 µg/ml for amikacin and 20-70 µg/ml for cefepime for linearity study. At the absorbance of these standard solutions calibration curves were plotted at these wavelengths. The simultaneous analysis of the drugs was carried using the following equation.

$$C_A = A_2a_{11} - A_1a_{21} + a_{12}a_{21} \cdot a_{11}$$

$$C_P = A_1a_{12} - A_2a_{12} + a_{21}a_{11} \cdot a_{12}$$
Where:

- $a_{x1}$ = Absorptivity of Amikacin at 210 nm
- $a_{x2}$ = Absorptivity of Amikacin at 217 nm
- $a_{y1}$ = Absorptivity of Cefepime at 217 nm
- $a_{y2}$ = Absorptivity of Cefepime at 210 nm

A1 and A2 are the absorbance of the diluted sample at 210 nm and 217 nm respectively.

Visible spectrophotometry method for Cefepine

In a series of separating funnels containing aliquots of standard drug solution was taken and 6 ml of HCl solution and 2 ml of WFBBL solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15 ml with distill water. To each separating funnel 10 ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured for scanning in the visible region to determine the maximum absorbance against a similar reagent blank.

RESULTS AND DISCUSSION

Simultaneous equation method

Study of overlain spectra has shown that Amikacin has max of 210 nm while Cefepime has max at 217 nm respectively. Linearity range was 5-17.5 μg/ml for Amikacin and 20-70 μg/ml for Cefepime at their respective maxima, which were validated by least square method. Linearity results were presented in Table 1, calibration graphs were presented in Figure 5. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 50, 100 and 150% level. The % of recovery ranges from 98.90 - 99.80 for Amikacin and 98.12 - 99.37 for Cefepime respectively. Precision was calculated as repeatability (% RSD is less than 2) and inter and intra-day variations (%RSD is less than 2) for both drugs.
Table 1: Results of linearity

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Amikacin Peak area</th>
<th>Cefepime Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.158</td>
<td>20</td>
</tr>
<tr>
<td>7.5</td>
<td>0.225</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>0.314</td>
<td>40</td>
</tr>
<tr>
<td>12.5</td>
<td>0.393</td>
<td>50</td>
</tr>
<tr>
<td>15</td>
<td>0.471</td>
<td>60</td>
</tr>
<tr>
<td>17.5</td>
<td>0.549</td>
<td>70</td>
</tr>
</tbody>
</table>

The validation limit that percentage of recovery are 100.04 - 100.27 for Amikacin and 99.72-100.64 for Cefepime respectively. Analysis of vial formulation Potentox was carried out and the amount recovered was expressed as percentage amount of vial claim (Table 4). The percentage recovery in formulation analysis was found to be 99.213 for Amikacin and 99.217 for Cefepime. The method can be successfully used for simultaneous estimation of Amikacin and Cefepime in combined dosage form.

**First order derivative Spectrophotometry method**

Six points calibration curve were obtained in a concentration range from 5-17.5 μg/ml for Amikacin and 20-70 μg/ml for Cefepime respectively. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation \( y = 0.0316x - 0.0044 \) for Amikacin and \( y = -0.002x + 0.005 \) with correlation coefficient 0.999 for Cefepime (Table 2, Figure 6). The precision results were found to be within the limit where % RSD values for Amikacin found to be 0.11, 0.137 and 0.221 for intra-day, inter-day and ruggedness studies. And also % RSD values for Cefepime found to be 0.128 and 0.211 and 0.19 for intra-day, inter-day and ruggedness studies. Recovery results also found within the validation limit that percentage of recovery are 100.04 - 100.27 for Amikacin and 99.72-100.64 for Cefepime respectively. Analysis of vial formulation Potentox was carried out and the amount recovered was expressed as percentage amount of vial claim (Table 4). The percentage recovery in formulation analysis was found to be 99.213 for Amikacin and 99.217 for Cefepime. The method can be successfully used for simultaneous estimation of Amikacin and Cefepime in combined dosage form.

![Figure 5: Calibration graph of Amikacin and Cefepime](image)

![Figure 6: Linearity Graph of Cefepime and Amikacin](image)

Table 2: Results of linearity

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Amikacin Amplitude</th>
<th>Cefepime Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.00485</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>0.01085</td>
<td>7.5</td>
</tr>
<tr>
<td>40</td>
<td>0.01686</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>0.02301</td>
<td>12.5</td>
</tr>
<tr>
<td>60</td>
<td>0.02956</td>
<td>15</td>
</tr>
<tr>
<td>70</td>
<td>0.03539</td>
<td>17.5</td>
</tr>
</tbody>
</table>
Amikacin PDAB visible method
The absorbance maxima (λ max) of the colored solution were observed at 595 nm in visible region. Linear regression of absorbance on concentration gave the equation $y = 0.022x + 0.126$ with a regression coefficient ($R^2$) of 0.999 (Figure 7) and the linearity range was 5-30 µg/ml. Higher percentage recovery value (98.72-99.55%) indicates that there is no interference of the excipients present in the formulation. 99.5% of assay was observed when the method was applied for Alfakim (Amikacin) marketed formulation estimation. Thus the method is useful for the determination of Amikacin pharmaceutical formulations.

Cefepime WFBBL visible method
The absorbance maxima (λ max) of the colored solution were observed at 498 nm in visible region. Linear regression of absorbance on concentration gave the equation $y = 0.054x + 0.102$ with a regression coefficient ($R^2$) of 0.998 (Figure 7) and the linearity range was 2-12 µg/ml. Linearity results of both visible methods are presented in Table 3. The higher percentage recovery value (99.06-100.37%) indicates that there is no interference of the excipients present in the formulation. 99.5% of assay was observed when the method was applied for Forpar (Cefepime) marketed formulation estimation.

**Table 3: Linearity results of visible methods**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Amikacin - PDAB method</th>
<th>Cefepime - WFBBL method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration in µg/ml</td>
<td>Absorbance</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0.239</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.351</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.469</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.582</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0.691</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0.806</td>
</tr>
</tbody>
</table>

The proposed methods are found to be simple, accurate and rapid for the routine determination of Amikacin and Cefepime in formulation. Marketed brand of injection vials were analyzed and amount of drug determined by proposed methods ranges from 99.92 and 99.77 for Amikacin and Cefepime respectively (Table 4). For visible spectrophotometry analysis individual formulations of Amikacin and Cefepime were analyzed for assay and good recovery was achieved.

**Table 4: Formulation analysis Amikacin and cefepime by proposed methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Drug</th>
<th>Brand Name</th>
<th>Label Claim</th>
<th>Amount Prepared</th>
<th>Amount Found</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simultaneous</td>
<td>Amikacin</td>
<td>Potentox</td>
<td>125mg</td>
<td>10µg/ml</td>
<td>9.992µg/ml</td>
<td>99.920</td>
</tr>
<tr>
<td>Equation method</td>
<td>Cefepime</td>
<td></td>
<td>500mg</td>
<td>40µg/ml</td>
<td>39.906µg/ml</td>
<td>99.766</td>
</tr>
<tr>
<td>Derivative Method</td>
<td>Amikacin</td>
<td>Potentox</td>
<td>125mg</td>
<td>7.5µg/ml</td>
<td>7.441µg/ml</td>
<td>99.213</td>
</tr>
<tr>
<td></td>
<td>Cefepime</td>
<td></td>
<td>500mg</td>
<td>30µg/ml</td>
<td>29.765µg/ml</td>
<td>99.217</td>
</tr>
<tr>
<td>PDAB method</td>
<td>Amikacin</td>
<td>Alfakim</td>
<td>50mg</td>
<td>4µg/ml</td>
<td>3.98µg/ml</td>
<td>99.50</td>
</tr>
<tr>
<td>WFBBL method</td>
<td>Cefepime</td>
<td>Forpar</td>
<td>500mg</td>
<td>4µg/ml</td>
<td>3.98µg/ml</td>
<td>99.50</td>
</tr>
</tbody>
</table>
CONCLUSION
The proposed methods simultaneous equation method and derivative methods are found to be very simple, precise and linear. Visible methods are also simple methods in order to estimate the drugs in formulation. Both these methods have been shown good linearity values and sensitivity. These methods are successfully applied for the estimation of Amikacin and Cefepime in its pharmaceutical formulations. Thus the methods are applicable for simple and economic methods for estimation of Amikacin and Cefepime on their pharmaceutical dosage forms.

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