KINETICS OF DEGRADATION OF CEFEPIME HYDROCHLORIDE IN VARIOUS AQUEOUS BASED SOLUTIONS

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Abstract:
The kinetics of degradation of cefepime hydrochloride has been investigated in water for injection, 0.5% metronidazole injection, 0.9% sodium chloride injection and 5% dextrose injection. The degradation studies were performed in three different types of containers i.e. glass, polyvinylchloride and polyethylene phthalate, at 5, 15 and 30 °C.

A validated HPLC method was employed in the study. The degradation of the compound showed first-order kinetics and the degradation rate constants ‘kobs’ were found in the range of 1.07-3.41 x 10⁻³ hr⁻¹ (r²=0.9999) at 5°C, 1.84-4.33 x 10⁻³ hr⁻¹ (r²=0.9999) at 15°C and 2.53-6.79 x 10⁻³ hr⁻¹ (r²=0.9997-0.9999) at 30 °C, respectively. The extent of degradation of the compound was also affected by the containers used during this study.

Key words: Cefepime hydrochloride, Degradation kinetics, Degradation rate constants.

Introduction:

Cefepime hydrochloride (7-[C-2-amino-1,3-Thiazol-4-yl-2-[(z)-methoxyimino] acetamido]-3-[1-methylpyridinidio-methyl]-3-cephem-4-carboxylic acid) is a fourth generation cephalosporin which has more gram positive spectrum while retaining the prolonged gram negative activity of third generation cephalosporins [USP,2008]. Like other cephalosporin injectable cefepime hydrochloride is also marketed in powder form because of its instability in the aqueous medium [Boylan and Fites, 1979]. These powders are reconstituted with suitable diluents
prior to use [Deluca and Boylan, 1992]. The stability of cefepime hydrochloride in powder and solution forms has remained an area of interest for a number of workers. The highest stability of cefepime hydrochloride in aqueous solutions has been found at a pH range of 4-5 [Fubara and Notari, 1998]. In a comparative study in aqueous media cefepime has shown a minor (~10%) difference in the apparent stability to that of ceftazidime [Viaence et al., 2002].

The stability of cefepime has also been evaluated in different infusion solutions [Robouan-Guyon et al., 1997]. They have reported the liberation of coloured degradation products in these reactions.

Temperature stability and antibacterial activity of cefepime during continuous I/V administration was also studied [Spauten et al., 2002]. They have shown that the degradation of cefepime includes breakage of the C₃-side chain and opening of the β-Lactam ring.

Bugnon et al., 2002 have studied the plasma and temperature related drug degradation of cefepime in vitro. The study shows that degradation of cefepime in deproteinized plasma at 37 °C is inversely related to its concentration, with half-lives ranging between 3.66 and 6.89 hours for concentrations ranging from 10 to 500mg/mL.

The stability of cefepime hydrochloride has also been evaluated in ethylene vinyl acetate plastic auto dose containers [Trissel and Xu, 2003]. They have shown that cefepime hydrochloride exhibits physical and chemical stability in these containers.

Stewart et al. [Stewart et al., 1999] investigated the stability of cefepime in polypropylene syringes at -20 °C, 4 °C and 22-24 °C. The influence of pH, temperature and buffers on the degradation kinetics of cefepime was also investigated [Fubara and Notari, 1998]. The stability and incompatibility of cefepime hydrochloride was made in comparison with ceftazidime in detail [Narine et al., 2003]. The loss of cefepime was found to reach to 10% within 24 hours at 25 °C. Solutions of cefepime also showed a substantial increase in pH. Solutions having less concentration were found more susceptible to pH change due to the small buffering capacity. The colour of the cefepime solutions were also found changed from light yellow to red-purple if stored at 30 °C for 12-16 hours.

The stability of cefepime has also been studied in polypropylene syringes and peritoneal dialysis solutions [Stewart et al., 1999]. Besides a number of studies on stability of the compound in various solvents and containers, no
systematic kinetic study is, however, reported in the literature. This fact has motivated the present study which focuses on the effect of various reconstitution solvents used commonly i.e. water for injection, 0.5% metronidazole, 0.9% sodium chloride and 5% dextrose solutions, on the kinetics of degradation of cefepime hydrochloride. Attempts have also been made to correlate the increase in temperature and reaction container with the rate of degradation of the compound. The effect of extended degradation on the order of the reaction has also been evaluated.

**Materials and Methods**

**Materials**

Cefepime hydrochloride ( ) samples and reference standard were kindly donated by M/S. GSK Pakistan (Pvt) Ltd Karachi. Samples of metronidazole (flagyl) injection, sodium chloride injection (plasaline) dextrose (plades-5) injection and tabros pharma water for injection were purchased from the market. All the reagents and solvents, used in this study were of analytical and spectroscopic grades, respectively. Freshly prepared double distilled water was used throughout this work.

**HPLC Apparatus and conditions**

An HPLC system (Class 20A, Kyoto, Japan) provided with of an LC-20 AT pump with gradient mixer, an SPD-20A UV visible detector, a stainless steel column (C-18, 5µ, 4.6x150mm id, Hypersil, Thermo Quest, USA) and an inbuilt CBM-20A lite communication bus module. The data collection and integration were obtained by using Schimadzu LC Solution Computer software version 1.2 (Kyoto, Japan). All separations were achieved isocratically at room temperature (20±1°C). The mobile phase was a degassed and filtered mixture of acetonitrile: water (6:94 v/v) containing sodium-1-pantane sulfonate (2.88mg/mL) with a pH adjusted to 3.4with glacial acetic acid. The flow rate was maintained at 1.5mL / min with a detection at 254nm.

**pH Measurement**

The pH measurements were performed with a pH meter (Wertheim, Germany). Electrode of the pH meter was standardized with buffer solutions (pH 2.0, 4.0 and 7.0, Merck) at 25°C.
Degradation studies of cefepime hydrochloride in admixture with IV solutions

An accurately weighed quantity of 10g of cefepime hydrochloride was taken in 1000ml volumetric flask. A volume of about 500ml of 0.9% sodium chloride solution or 5% Dextrose solution or 0.5% Metronidazole solution or water for injection was added to the flask. The flask was kept in ultrasonic bath to promote dissolution of the drug in the solvent. After complete dissolution of the drug powder in the solvent, the volume was made up to the mark with additional volume of the respective solvent. Zero time samples were withdrawn for analysis while nine aliquots, each of 50ml, of the remainder samples solution were withdrawn into three groups, each of PVC, Glass and PET containers. One sample from each group was placed at 5°C, the other at 15°C while the third at 30°C in refrigerator or oven for 24 hours. Samples were withdrawn at regular interval of 6 hours, diluted with the mobile phase (final concentration 100µg/mL) and analyzed by HPLC. Quantification was made by comparing peak area or height of the sample to the peak area or height of the standard solution.

To determine the effect (if any) of extended degradation on the order of degradation reaction of cefepime hydrochloride, the drug powder was dissolved in water for injection in glass container and kept at 50°C for two hours to produce sufficient amount of the degradation products. The degraded solution was analyzed by HPLC to estimate the extent of degradation. The degraded solution was kept further at 30°C in an oven and samples were withdrawn at regular time intervals. Any change in the kinetic behavior of cefepime hydrochloride was determined by comparing the kinetic data at 30°C of the pre-heated sample and the sample which was not initially heated at 50°C for two hours.

Statistical Analysis

The orders of the degradation reactions were determined graphically using the half-life methods. The observed degradation rate constants ($k_{obs}$) were estimated from the slope of the log-linear phase of declining cefepime hydrochloride concentration versus time plots. All first-order plots reported in this study were linear with the square of correlation coefficient ($r^2$) greater than 0.999. The half-lives were calculated using the half life equation. Data are
expressed as the mean of replicate determinations (n = 3). Statistical analyses were achieved using Microsoft Excel (version 2003).

**Results and Discussion**

**Validation of analytical method**

The USP,2008 method for analysis of cefepime hydrochloride injection was partially validated by including parameters like specificity, linearity, accuracy and precision. A linear response ($r^2=0.9995-0.9998$) was shown by the compound in all solvents when measured by both peak area and height within the concentration range of 5-125µg/mL (Table 1).

**Table-1: Linearity data of cefepime hydrochloride in I.V solutions.**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Slope</th>
<th>Y-Intercept</th>
<th>Correlation Coefficient($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water for injection</td>
<td>0.6732</td>
<td>-0.0681</td>
<td>0.9996</td>
</tr>
<tr>
<td>5% dextrose</td>
<td>0.5983</td>
<td>-0.0553</td>
<td>0.9996</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>0.6811</td>
<td>-0.0654</td>
<td>0.9998</td>
</tr>
<tr>
<td>0.5% Metronidazole solution</td>
<td>0.6183</td>
<td>-0.0483</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

The reconstitution solvents did not interfere with the peak of the compound (Figure 1-4). The method was also found accurate as overall mean of the recoveries of the method was found within 99-101% of the 50-150% range of the nominal content (100µg/mL). The inter-day and intra-day precision of the method were also found within limits i.e. % RSD below 2% (Data not shown).
Figure-1: HPLC chromatogram of cefepime hydrochloride dissolved in water for injection.

Figure-2: HPLC chromatogram of cefepime hydrochloride dissolved in 5% dextrose solution.

Figure-3: HPLC chromatogram of cefepime hydrochloride dissolved in 0.9% sodium chloride.
The kinetic treatment of the data on degradation of cefepime hydrochloride in the solvents studied revealed that degradation of the drug follows first-order kinetics. This observation is an agreement with the previous studies [Narine et al., 2003]. The observed rate constants; $k_{obs}$, for the degradation of the drug in the solvents stored in glass, polyethylene phthalate and polyvinylchloride containers at 5, 15 and 30 °C, are in the range of $1.07-6.79 \times 10^{-3}$ hr$^{-1}$ with square of a correlation ranging from 0.9997-0.9999 (Table 2-4). The half-lives of the reactions were found to be in the range of 4.25-26.98 days. The highest rate of degradation of the drug was found in metronidazole injection followed by 5% dextrose, water for injection and 0.9% sodium chloride injection, respectively. The rate of degradation was also found to accelerate with increase in temperature by 0.3 and 2.0 folds, respectively, at 15 °C and 30 °C as compared to at 5 °C.
Table-2: Apparent first-order rate consents ($k_{obs}$) of cefepime hydrochloride in various solvents in various containers at 5 °C.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Glass Containers</th>
<th>PET Containers</th>
<th>PVC Containers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs} \times 10^3$, (hr$^{-1}$)</td>
<td>$r^2$</td>
<td>Half-life ($t_{1/2}$), (day)</td>
</tr>
<tr>
<td>0.5% Metronidazole</td>
<td>2.76</td>
<td>0.9999</td>
<td>10.46</td>
</tr>
<tr>
<td>5% dextrose solution</td>
<td>2.53</td>
<td>0.9999</td>
<td>11.41</td>
</tr>
<tr>
<td>WFI</td>
<td>1.68</td>
<td>0.9999</td>
<td>17.18</td>
</tr>
<tr>
<td>Saline</td>
<td>1.15</td>
<td>0.9999</td>
<td>25.10</td>
</tr>
</tbody>
</table>

Table-3: Apparent first-order rate consents ($k_{obs}$) of cefepime hydrochloride in various solvents in various containers at 15 °C.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Glass Containers</th>
<th>PET Containers</th>
<th>PVC Containers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs} \times 10^3$, (hr$^{-1}$)</td>
<td>$r^2$</td>
<td>Half-life ($t_{1/2}$), (day)</td>
</tr>
<tr>
<td>0.5% Metronidazole</td>
<td>3.53</td>
<td>0.9999</td>
<td>8.17</td>
</tr>
<tr>
<td>5% dextrose solution</td>
<td>2.64</td>
<td>0.9999</td>
<td>10.93</td>
</tr>
<tr>
<td>WFI</td>
<td>2.53</td>
<td>0.9999</td>
<td>11.41</td>
</tr>
<tr>
<td>Saline</td>
<td>1.91</td>
<td>0.9999</td>
<td>15.11</td>
</tr>
</tbody>
</table>
The increase in degradation rate of the drug with increase in temperature has also been evidenced by earlier investigations [Bugnon et al., 2002]. The reaction container also influenced the rate of degradation. In metronidazole injection the highest rate was noted in PVC containers and the lowest in glass containers. In dextrose and sodium chloride injection the highest degradation rates were seen in glass and PVC, respectively while the lowest in PET containers. The variable degradation rate in different containers clearly indicates the role of containers of the degradation. A slight increase in pH of the admixtures was also observed, along with a change in colour which is persistent to the earlier observations [Viaence et al., 2002]. The change in colour of the admixture may be due to the liberation of coloured products as reported previously [Spauten et al., 2002]. Extended degradation in many cases results in change in the order of the degradation reaction but in the present case the drug at 50 °C while dissolved in water for injection did not show any change in the order of the reaction.

**Conclusion**

The thermal degradation of cefepime hydrochloride in admixture with 5% dextrose injection, 0.9% sodium chloride injection or 0.5% metronidazole injection follows first-order kinetics. The kinetics of degradation of the compound are influenced by temperature, solvent and container used; therefore, it is paramount to use appropriate storage conditions and containers while storing solutions of the product. Cares must also be made while admixing intravenous solutions with the product during clinical use.
Acknowledgment

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References


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