Stability Studies of Cefepime Hydrochloride by Stability Indicating RP-HPLC Method

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ABSTRACT

Drug stability and compatibility are critical issues controlling accurate and appropriate delivery of drug therapy to patients. Stability is very important for antibacterial agents especially those given by intravenous route as they reach systemic circulation directly, and the clinical outcome and safety are directly correlated to drug levels in blood. The physical and chemical stability of Cefepime hydrochloride, a fourth generation cephalosporin, was determined at three different temperatures (5 °C/ 60 %RH, 25 °C/60 %RH & 45 °C/75% RH) and quantified by using a stability indicating RP-HPLC method. Decrease in drug concentration by more than 10% from initial concentration (0 time) was considered unstable (chemical instability). Change in pH by more than 1 was considered unstable (Physical instability). The drug solutions were clear and light yellow initially with intensity increasing over time, eventually becoming dark yellow for cefepime. HPLC analysis indicated that 40 mg/ml concentration of cefepime hydrochloride maintained adequate stability for 2 hours at 45°C and up to 24 hours at 25 °C and up to 7 days at 5 °C.

KEYWORDS: Cefepime; Stability studies; RP-HPLC; Physical instability; Chemical instability; Degradation.

Introduction

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (FDA guidelines, 1998a). Drug stability is aimed at ensuring that the drug product remains within specifications established to ensure its identity, strength, quality and purity. Drug stability and compatibility are critical issues controlling accurate and appropriate delivery of drug therapy to patients. Impurities and degradation can lead to change in the pharmacological, chemical and toxicological properties of the drug which will affect its safety and efficacy (Ahuja, 1998, Ahuja and Alsante, 2003, FDA guidelines, 1998b). Stability is very important for antibacterial agents especially those given by Intravenous (I.V route) as they reach systemic circulation directly, and the clinical outcome and safety are directly correlated to drug levels in blood.

Stability testing of an active substance or finished product gives evidence about the quality of the active substance or finished product which varies over time because of the influence of environmental factors like temperature, humidity and light. Stability testing also provides vital information about the interaction of drug with its ingredients, possible degradations and their mechanisms, and degraded products. The results of stability studies are widely used by the pharmaceutical industry in deciding the storage conditions, suitable packaging material, shelf life and expiration date of the product (ICH guidelines, 2000, Grimm and Carstensen, 2000a, Dean and Carstensen, 2000b).

The increased use of parenteral drugs is revealed in surveys that show in the average hospital, 40% of the total dosage forms dispensed to patients are in the form of injections (Turco, 1994). Newer generation of parenteral antibiotics have lead to increased role of parenteral therapy. Continuous infusion is an efficient means of administering beta-lactams to maintain drug concentrations higher than the Minimum Inhibitory concentration (MIC) throughout the dosing interval. Continuous infusion has a pharmaco-economic advantage over intermittent dosing by achieving the same effect with a lower daily dose of drug. Antibacterial agents, especially fourth generation cephalosporin like cefepime which has been selected for our study is commonly used by IV route, alone and in combination therapy for treating severe multidrug resistant infections (AHFS drug information, 2004, USP, 2004). Most monograph literature on cefepime indicates stability of the drug at...
room temperature (25°C) and refrigeration (5°C) (Data sheet, Maxipime). Hence we were interested in studying if cefepime, which are extensively used intravenously, maintain their stability when used clinically as CI, in a temperate country like India, where temperature reach up to 45°C in summer. The main objective of the present study was to develop a simple, rapid and reproducible HPLC method as per the ICH guidelines and apply to the stability studies of Cefepime hydrochloride formulations.

Materials and Methods

Drugs and Chemicals

Cefepime Hydrochloride was gifted by Orchid pharma, Chennai. Cefepime hydrochloride vials were procured from Alembic Limited, Vadodara, Gujarat. HPLC grade water was purchased from Qualigens fine chemicals, Mumbai. All other chemicals and reagents used were of laboratory or analytical grade.

Chromatographic Conditions

Chromatography was performed by the Reversed Phase (RP-HPLC) (Shimadzu) method. Compounds of interest were separated, employing Ammonium acetate: Acetonitrile (92:8) as mobile phase and Phenomenex ODS column (4.6 x 250 mm, 5 µ) as the stationary phase. During the investigation, the mobile phase composition remained unaltered. The flow rate of mobile phase was 1.5 ml/min. The internal standard was Cefotaxime sodium. The pH was adjusted to 4.9. The peak areas were measured at 256 nm using UV detector and scanned (Camag scanner). Cefepime calibration range used was between 4-20 mcg/ml.

Standard stock solution

1gm of cefepime hydrochloride was dissolved in 25 ml of HPLC grade water to produce 40mg/ml, and serially diluted to produce 400 µg/ml. From this solution further dilutions were made to the concentration range of 4 mcg – 20mcg/ml respectively.

Internal standard solution

10 mg of cefotaxime sodium was dissolved in 10 ml of HPLC grade water to produce 1 mg/ml, and serially diluted with HPLC water to the concentration of 10 µg/ml.

Standardization of cefepime hydrochloride

Different concentrations of cefepime hydrochloride (0.1, 0.2, 0.3, 0.4, 0.5 ml) were pipetted from the stock solution corresponding to the concentrations of 4-20 µg/ml respectively and 3 ml of internal standard (cefotaxime sodium) solution was added and made up to 10ml with HPLC water. 1 ml of this solution was injected into C18 column using ammonium acetate: acetonitrile (92:8) as mobile phase (pH 4.9 and flow rate 1.5 ml/min) and the peak areas were measured at 256 nm.

Stability Studies (Quantification by RP- HPLC method)

Chemical stability of cefepime hydrochloride was determined using a stability indicating RP-HPLC method for quantification (Sprauten et al., 2003). Three 1 gm vials of cefepime hydrochloride I.V were reconstituted with 25 ml of HPLC grade water. These vials were marked as refrigeration (5°C/60%RH), room temperature (25°C/60%RH) and 45°C/75%RH for identification and were kept at different storage conditions.

From the above reconstituted solution 0.4 ml (equivalent to 16 mg) solution was withdrawn and diluted to 10 ml, from this solution 0.1 ml (equivalent to 160 mcg) was withdrawn and 3ml of cefotaxime sodium (internal standard) was added and made upto 10 ml with HPLC water to get a concentration of 16 µg/ml of cefepime. 1ml of drug solution was injected into C18 column, with ammonium acetate: acetonitrile (92:8) as the mobile phase, with pH 4.9 and flow rate 1.5 ml/min and peaks were detected at 256 nm. The above procedure was repeated for 7 days with samples withdrawn from vials kept at 5°C, 25°C, 45°C at various time intervals of 0 min, 1 hr, 2 hrs, 4 hrs, 7hrs, 24 hrs, 120 hrs & 168 hrs. Color change, pH and clarity were noted. The results obtained were observed and recorded.

Results and Discussion

Physical Stability Study

There was a drastic change in pH in the 45°C samples, along with the change in color. At the end of 24 hours study the samples were completely dark yellow in color clearly indicating the degradation of the product. There was a minimum change in pH and color in the 5 °C samples even at the end of 7 days, indicating the stability of the product. The 25°C samples had major color change after 5 days, indicating stability up to 5 days. In the entire period of study the samples remained clear.

Chemical Stability Study

The 45°C samples had a major change in the concentration from the third hour with further loss of drug by 24 hours (Figure 1B). In 25°C samples the change in concentration started at the fourth hour and major changes occurred by 22 hours (Figure 1D). The 5°C samples retained their concentration with little loss of drug at the end of 7 days study (Figure 1C). The change in concentration or loss of drug indicates the degradation of drug.

Degradation Profile

Nearly 92% drug remained at the end of 7 days when stored at 5 ºC, indicating it as the best temperature to store the drug. Whereas in 25 ºC only 83% drug remained at the end of 7 days, hence the drug can be stored in room temperature for a week. At 45 ºC only 79% of drug remained at the end of 24 hours, indicating faster degradation or loss of drug at higher or accelerated temperature (Figure 2).
Fig. 1. HPLC Chromatograms of cefepime hydrochloride (24 hour samples) stored at three different temperatures. HPLC chromatograms: (A) initial concentration (0 time); (B) 24 hour (45°C); (C) 24 hour (5°C); and (D) 24 hour (25°C).
Stability study was conducted on pure drug of the investigated compound (Cefepime hydrochloride). The mobile phase was selected with reference to Sprauten et al., 2003 and preliminary experiments showed the elution of cefepime hydrochloride in the solvent system. Cefepime hydrochloride I.V. infusion (100 mg/ml) was stored at different temperatures such as room temperature (25°C), refrigeration (5°C) and 45°C. Samples were withdrawn at different time intervals for 7 days and concentration was found out by RP-HPLC method. Observations at the end of 7 days were noted. The stability study of cefepime hydrochloride I.V. infusion stored at refrigeration temperature proved to be more stable even at 7 days (% deviation = 7.92) than the other solutions stored at room temperature (% deviation = 16.46) and 45°C (% deviation > 20.74) (Table II).

At 45°C, the % deviation was > 10% (considered unstable) after nearly 4 hours of storage (value indicates between 4-24 hours). The degradation was confirmed by the change in pH of the solution at 4 hours and color change started at 1 hour and the color intensified over 3 hours and further darkened at 24 hours (Table 1). At 25°C > 10% degradation was seen between 4 hours and 24 hours. The physical changes also prove the degradation by change in pH of the solution at 24 hours and color started to appear at the end of 1 hour (Table 1). At 5°C drug solution was stable even after 5 days. The results obtained for pH, color and clarity test showed changes mainly in samples stored at 45°C and 25°C, while 5°C remained stable with minimum changes (Table 1).

Results of our study involving cefepime hydrochloride (by RP-HPLC method) were found similar to those reported by Sprauten et al., 2003 and Baririan et al., 2003. The degraded products may or may not exhibit antibacterial activity. Further studies are necessary to elucidate the structure of the degraded products and to find out their activity.

Fig. 2. Degradation of cefepime hydrochloride at different temperatures (5°C, 25°C and 45°C). Samples at 25°C and 25°C showing degradation compared to 5°C at shorter period of time.
TABLE 1

Physical stability of cefepime Hydrochloride I.V. at three different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>pH</th>
<th>clarity</th>
<th>color</th>
<th>pH</th>
<th>clarity</th>
<th>color</th>
<th>pH</th>
<th>clarity</th>
<th>color</th>
<th>pH</th>
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<th>color</th>
<th>pH</th>
<th>clarity</th>
<th>color</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ºC</td>
<td>6.8</td>
<td>clear</td>
<td>colorless</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>25ºC</td>
<td>6.8</td>
<td>clear</td>
<td>colorless</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>45ºC</td>
<td>6.8</td>
<td>clear</td>
<td>colorless</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
<td>6.8</td>
<td>clear</td>
<td>Pale yellow</td>
</tr>
</tbody>
</table>

TABLE 2

Chemical stability of cefepime hydrochloride I.V. at three different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Expected concentration</th>
<th>0 hour</th>
<th>0.5 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>4 hour</th>
<th>8 hour</th>
<th>22 hour</th>
<th>24 hour</th>
<th>120 hour</th>
<th>168 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ºC</td>
<td>16 µg/ml</td>
<td>440994</td>
<td>440990</td>
<td>440875</td>
<td>435625</td>
<td>430728</td>
<td>427257</td>
<td>424775</td>
<td>425890</td>
<td>415106</td>
<td>413226</td>
<td>412267</td>
</tr>
<tr>
<td>25ºC</td>
<td>16 µg/ml</td>
<td>440994</td>
<td>440893</td>
<td>440557</td>
<td>432655</td>
<td>429387</td>
<td>395239</td>
<td>390143</td>
<td>388941</td>
<td>369855</td>
<td>366832</td>
<td>359745</td>
</tr>
<tr>
<td>45ºC</td>
<td>16 µg/ml</td>
<td>440994</td>
<td>440797</td>
<td>421628</td>
<td>415336</td>
<td>405826</td>
<td>390942</td>
<td>389657</td>
<td>383728</td>
<td>354863</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Values recorded are the peak area values and the values given within brackets are the concentration of cefepime hydrochloride I.V. in mcg/ml.

Percentage deviation = \( \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial Concentration}} \times 100 \)

TABLE 3

Degradation profile of cefepime hydrochloride at three different temperatures.

<table>
<thead>
<tr>
<th>Sampling Time</th>
<th>Amount of Drug Remaining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5ºC</td>
</tr>
<tr>
<td>0 time</td>
<td>100</td>
</tr>
<tr>
<td>0.5 hour</td>
<td>99.39 ± 2.3</td>
</tr>
<tr>
<td>1 hour</td>
<td>99.39 ± 1.8</td>
</tr>
<tr>
<td>2 hour</td>
<td>98.78 ± 1.1</td>
</tr>
<tr>
<td>3 hour</td>
<td>97.56 ± 1.9</td>
</tr>
<tr>
<td>4 hour</td>
<td>96.95 ± 1.1</td>
</tr>
<tr>
<td>8 hour</td>
<td>96.95 ± 1.7</td>
</tr>
<tr>
<td>22 hour</td>
<td>96.34 ± 1.0</td>
</tr>
<tr>
<td>24 hour</td>
<td>93.90 ± 1.6</td>
</tr>
<tr>
<td>120 hour</td>
<td>93.3 ± 0.7</td>
</tr>
<tr>
<td>168 hour</td>
<td>92.08 ± 1.3</td>
</tr>
</tbody>
</table>

Conclusions

From the above study it can be suggested that cefepime hydrochloride was stable up to 7 days at refrigeration (5ºC) and 24 hours at room temperature (25ºC) and only for 4 hours at 45ºC. Even though extensive studies have been conducted on the dosage form, additional studies on the product in the container of choice are necessary to ensure the total stability characterization of the product. The use of motorized portable infusion pumps and the bag system may be more suitable for CI regimens with drugs that exhibit temperature dependent stability. A HPLC-MS or GC-MS combined analytical data of the drug studied in our work could be of greater help in elucidation of the structure of degraded products of cefepime. The toxicity of the degraded compounds, if found, also needs to be confirmed for safe use of cefepime as CI especially in a country like India, where very low priority is given to storage and safe use of I.V administered drugs.

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References

Data Sheet. RxMed: pharmaceutical Information – MAXIPIME.

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