Research article

Formulation and evaluation of Gemcitabine HCl anti-cancer lyophilized powder for injection

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Keywords: Gemcitabine, Lyophilization, Lyophilization Cycles

ABSTRACT

Gemcitabine belongs to BCS class III, it has high solubility and low permeability. Moreover the drug is highly unstable in the liquid formulation. These limitations in the formulation of gemcitabine were addressed in the present investigation. To solve the permeability issue, the drug can be administered through I.V route, because it has good aqueous solubility. The lyophilized form of the drug prevents its deterioration in liquid medium. So the lyophilized drug should be reconstituted with normal saline before administration through I.V route.

1. INTRODUCTION

Gemcitabine Hydrochloride is a white (or) almost white powder, it is soluble in water; slightly soluble in Methanol; practically insoluble in alcohol & polar organic solvents. It is used in the treatment of following types of cancer:

- **Non-small cell lung cancer**: Gemcitabine is indicated in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (stage III A or III B) or metastatic (Stage IV) non-small cell lung cancer.
- **Pancreatic cancer**: Gemcitabine is indicated as first line treatment for patients with locally advanced (Stage II or stage III) or metastatic (stage IV) adenocarcinoma of pancreas. Gemcitabine is indicated for patients previously treated with 5-Flourouracil.

![Figure 1. Molecular Structure of Gemcitabine HCl](image)

2. MATERIALS AND METHODS

**Procedure for the manufacturing of Gemcitabine for injection 1000mg in detail**: The preparation tank was cleaned and sterilized prior to the starting of the process. Water for injection was collected in the cleaned and sterilized tank and cooled to room temperature. Send a sample of water for Bacterial endotoxin test. After getting clearance form QA Mannitol was added to the tank contains water for injection. Sodium acetate anhydrous was added with stirring. Finally required quantity of Gemcitabine was added to the tank with stirring. Stirring was continued until all the ingredients were completely dissolved.

The solution was cooled to 25±2°C and pH of the solution was checked. If the pH was not within the limits then the pH was adjusted to 2.9±0.2 using 0.5 N NaOH solution or 0.1 NHCl solution the quantities consumed were recorded.

A sample was sent to QC for analysis as per IPQA. After getting the clearance from QA the solution was filtered through 0.2 micron PVTF filter. Finally the solution was filled to glass vials and sealed and labeled.

**Lyophilization cycle development of Gemcitabine for injection**: Lyophilization on freeze drying is a process in which water is removed from a product after it is frozen and placed under vacuum, allowing ice to change directly from solid state to vapor state without passing through liquid phase. This process consists of three different processes viz. freezing, primary drying and secondary drying.

**Process step: Freezing**

Process design criteria:
- Minimum freezing temperature of product 30°C.
- Chamber vacuum: The chamber shall be at atmospheric pressure. No vacuum will be applied.
- Process controls: Product temperature of less than -30°C shall be attained by gradually cooling shelf from room temperature -40°C.

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The freezing step shall comprise of 4 set points
-5°C : For beginning of ice formation
-25°C : For stabilization of frozen material
-40°C : For completion of freezing
-42°C : Extra freeze step to ensure that product reaches temperature at least -35°C.

**Process steps: Primary drying**

**Process design criteria:** Shelf temperature in primary drying shall be ramped up from -40°C to +40°C in 6 steps. The heating rates during early steps of this ramp up would be kept below 3°C per hour. This ensures a gradual heating of product via the vacuum would be maintained (<0.37 millibars) such that melt back during the process does not happen.

The chamber vacuum would be set at 0.37 millibar. This vacuum is derived from ice vapor pressure data at a temperature of -30°C, which is minimum temperature required for freezing the product. During ramp up of shelf temperature from -40°C to +40°C, the chamber vacuum would further be reduced from 0.37 milli bar to 0.035 milli bar (<1/10th of 0.37 milli bar).This is done gradually by reducing the vacuum in a programmed manner from 0.37 millibar, to 0.25mb, then to 0.035mb: This reduction vacuum <1/10th of 0.37mb is necessary because at end of primary drying when pressure increase test is performed, the chamber vacuum does not fall beyond 0.37mb. This ensures that product does not melt back during process.

**Process step: Secondary drying:**

**Process design criteria:** Secondary drying of product shall be done at +40°C. Vacuum of chamber shall be further reduced from 0.035milli bar to less than 1/18th of 0.035milli bar (ex: 0.0019mb). During secondary drying there will be rapid loss of free moisture from product. Hence, a low chamber vacuum would ensure that moisture is removed efficiently. Further, during pressure increase test in secondary drying stage the chamber vacuum would ensure to 0.035milli bar (vacuum at last step of primary drying). This ensures that no melt back of drug product would occur due to increase of chamber pressure during pressure increase testing in secondary drying.

Minimum duration of secondary drying shall be 360 minutes. Subsequently to which cycle has a 2 hours step at 40°C. This step is provided as a process control to ensure adequate drying when pressure in a case test fails, the secondary step shall be repeated at vacuum of 0.0019milli bars & shelf temperature of +40°C. The acceptance value of pressure increase shall be +10 steps from 0.0019milli bars (chamber press should be <0.02milli bars).

Table.1. Formulation table of Gemcitabine HCl injection

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ingredients</th>
<th>Specification</th>
<th>Formulation Qty per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gemcitabine HCl equivalent to Gemcitabine</td>
<td>USP Active</td>
<td>45.5408 mg</td>
</tr>
<tr>
<td>2</td>
<td>Mannitol (Pyrogen free)</td>
<td>USP Bulking agent</td>
<td>40 mg</td>
</tr>
<tr>
<td>3</td>
<td>Sodium acetate anhydrous</td>
<td>USP Buffer</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>4</td>
<td>WFI</td>
<td>IP/BP/USP/PH.Eur. Solvent</td>
<td>Q.s to 1ml</td>
</tr>
<tr>
<td>5</td>
<td>NaOH</td>
<td>NF pH adjustment</td>
<td>Q.s TO adjust the pH 2.7 to 3.3</td>
</tr>
<tr>
<td>6</td>
<td>Hydrochloric acid</td>
<td>NF pH adjustment</td>
<td>Q.s TO adjust the pH 2.7 to 3.3</td>
</tr>
</tbody>
</table>

Table.2. Lyophilization cycle used in exhibit batch of Gemcitabine 1000 mg.

<table>
<thead>
<tr>
<th>Step no.</th>
<th>Step</th>
<th>Shelf temperature (°C)</th>
<th>Chamber pressure</th>
<th>Time (mins)</th>
<th>Cumulative time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hold</td>
<td>20</td>
<td>NIL</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Rate</td>
<td>10</td>
<td>0.20</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Rate</td>
<td>-5</td>
<td>0.20</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>Rate</td>
<td>-25</td>
<td>0.20</td>
<td>50</td>
<td>140</td>
</tr>
<tr>
<td>5</td>
<td>Rate</td>
<td>-40</td>
<td>0.035</td>
<td>75</td>
<td>215</td>
</tr>
<tr>
<td>6</td>
<td>Rate</td>
<td>-42</td>
<td>0.035</td>
<td>180</td>
<td>395</td>
</tr>
<tr>
<td>7</td>
<td>Hold</td>
<td>-40</td>
<td>0.035</td>
<td>30</td>
<td>425</td>
</tr>
</tbody>
</table>

**Primary Drying:**

| 8        | Hold | -40                     | 0.37             | 60          | 485                    |
| 9        | Rate | 20                      | 0.37             | 1560        | 2045                   |
| 10       | Rate | 30                      | 0.25             | 60          | 2105                   |
| 11       | Hold | 30                      | 0.20             | 120         | 2225                   |
| 12       | Rate | 35                      | 0.20             | 300         | 2525                   |
| 13       | Hold | 35                      | 0.035            | 300         | 2825                   |
| 14       | Rate | 40                      | 0.035            | 120         | 2945                   |

**Secondary Drying:**

Total cycle time: 56 hours 35 minutes
Pressure raise : 0.0133 mbar

Storage program:

<table>
<thead>
<tr>
<th></th>
<th>Start value</th>
<th>Rate</th>
<th>Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>25</td>
<td>0.0019</td>
</tr>
<tr>
<td>2</td>
<td>0.0019</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3. Flow chart of steps involved in the formulation of Gemcitabine lyophilized product

### 3. RESULTS AND DISCUSSION

Physical observation of the finished product was done and the parameters like water content, clarity of the reconstituted solution, weight variation and identification were done according to USP specifications. It was observed that the product complies all the parameters.

Thermal stability studies were conducted at various temperatures ranging from 2-8°C, at 40°C and at 60°C. The bulk solution of Gemcitabine was stable up to 72 hours at 40°C, it is stable up to 8 hours at 60°C and it is stable up to 48 hours at 2-8°C.

In-process tests like appearance, stopping, sealing quality and leak test were conducted after lyophilization before going for sealing; and the results obtained were satisfactory.

Chemical analysis was conducted on final product for finding out water content, uniformity of dosage, reconstitution time, pH and assay: It was observed that the water content was from 0.5% to 0.6%. Uniformity of dosage was from 1.2 to 1.5 units. Reconstitution time was 0.4 minutes. pH was 2.9. Assay was 101 to 102. Assay was conducted by HPLC.

Microbial analysis was conducted to find out the sterility of the product by conducting bacterial endotoxin test. It was observed that the product passed the test.

Stability studies were conducted at various conditions, it was found that the product was stable till the end of three months. All the samples were analyzed by HPLC method.

### Table 3. Stability data at various conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>pH</th>
<th>Color of solution</th>
<th>H2O content (w/w)</th>
<th>Assay by HPLC</th>
<th>% of impurities</th>
<th>Sum of all impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specification</td>
<td>White to off white Lyophilized cake or discontinuous powder aggregates or free flowing powder</td>
<td>Between 2.7 &amp; 3.3</td>
<td>To be monitored</td>
<td>NMT 50%</td>
<td>NLT 95% &amp; NMT 105%</td>
<td>NMT 0.1%</td>
<td>NMT 0.1%</td>
</tr>
<tr>
<td>Stage initial</td>
<td>Complies</td>
<td>3.01</td>
<td>0.004</td>
<td>0.820</td>
<td>99.7</td>
<td>ND</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Stability condition: 40°C ± 2°C, 75% ± 5% RH
4. CONCLUSION
It can be concluded that the procedure is suitable to manufacture Gemcitabine HCl lyophilized injection. The results are satisfactory and reproducible.

REFERENCES