Process Validation of Lyophilized Formulation of the Anticancer Drug Topotecan

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ABSTRACT

Sterile pharmaceutical products are produced under strict compliance with good manufacturing practices (GMP). The purpose of process validation is to provide documented evidence that the pharmaceutical system is capable of continuously supplying the clean and conditioned air with the specified quality attributes and establish its dependability of cytostere injection facility. Topotecan (Hycamtin) is an anticancer agent that is a topoisomerase inhibitor. It is a synthetic, water-soluble analog of the natural chemical compound camptothecin. This work is aimed to establish documentary evidence to demonstrate the anticancer drug topotecan hydrochloride process validation, under defined set of operating conditions are capable of producing the finished product as per the finished product specifications. The data generated were checked, compiled, and critically reviewed based on the final results. It has been observed that all the critical process parameters and analytical results for process validation batch are well within the limits and correlated with the expected compliance parameters.

KEYWORDS: Lyophilization, Topotecan Hydrochloride, Process Validation; GMP.

Introduction

Process validation is established documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics. The proof of validation is obtained through the collection and evaluation of data, preferably, beginning from the process development phase and continuing through the production phase. Validation necessarily includes process qualification, (the qualification of materials, equipment, systems, building, personnel), but it also includes the control on the entire process for repeated batches.

The element of validation is based on the qualification of equipment and system/utilities, which include risk assessment, design qualification, installation qualification, operational qualification, performance qualification. Other activities which are equally important and significant are sterilization validation, contaminant efficiency verification, and manufacturing process validation. The validation process mainly undergoes five different phases of documentation which are preparation and approval of the validation master plan (phase I), preparation and pre-approval of validation protocols (phase II), execution of the validation protocols and data collection (phase III), review of data collected during execution, preparation and authorization of validation report (phase IV) and ongoing review, change control and revalidation (phase V) (WHO, 2006; USP, 2007).

Topotecan hydrochloride is a specific inhibitor of Topoisomerase-I enzyme (Fig.1). It is a water soluble analogue of camptothecin, a natural substance found in several species of Asian tree. It is a cytotoxic alkaloid extracted from plants such as Camptotheca acuminata. It is used in the form of its hydrochloride salt to treat ovarian cancer, lung cancer and other cancer types. The cytotoxicity of topotecan results from the production of enzyme mediated DNA damage. It is an S-phase dependent cytotoxic drug and is a radiation sensitizing agent. Following intravenous administration of topotecan at doses of 0.5 to 1.5 mg/m² as a 30 min infusion daily for 5 days, it demonstrated a clearance of 1030 mm/min. with a plasma half life of 2 to 3 hrs. It has a volume of distribution of 130 L. Binding of topotecan to plasma proteins is about 35%. It is evenly distributed between blood cells and plasma. It is metabolized by pH dependent hydrolysis, with the equilibrium favoring the ring opened hydroxy acid form at physiological pH. The total measurement of topotecan in urine suggests that a variable fraction of the dose (20 to 60%) is excreted in urine. It is also measured in human bile samples indicating that topotecan is excreted by both biliary and urinary routes in humans (Louis and Joan, 2004; USP 2007).

Topotecan hydrochloride is used in the treatment of ovarian cancer, gliomas, leukemia, lung cancer and other cancer types. It is administered intravenously and the vial should be stored between 15 and 30 °C and protected from light in the original package. Topotecan hydrochloride injection is supplied as a sterile lyophilized, buffered, light yellow to greenish powder available in single dose vials. Each vial contains topotecan hydrochloride equivalent to 4 mg of topotecan as free base. Some generic versions are also available in Europe and other countries.

This study sought to establish documentary evidence to demonstrate the anticancer drug topotecan hydrochloride process validation, under defined set of operating conditions are capable of producing the finished product as per the finished product specifications.

Materials and Methods

Chemicals and Reagents

Various chemicals used for study are topotecan hydrochloride (TH), Mannitol USP, Tartaric acid USP, Hydrochloric acid USNF, Sodium hydroxide USNF, Water for injection USP and Soyabean casein digest medium.

Instruments

Digital hot wire anemometer, Aerosol photometer, Balance, CIP system I & II, Dynamic pass box, Dry heat sterilizer FTIR spectrophotometer etc.

Experiment Methodology

Process validation strategy: All master documentation necessary for the manufacturing, testing and validation of manufacturing process are prepared (Holly, 2007; Smith, 2004; Luis, 2004)

3 Master batch manufacturing record
  • Process flow chart
  • Specification and test methods
  • Certification of analysis
  • Validation protocols
  The process validation &/or exhibit batches are manufacturing as per the master batch manufacturing record

- The data generated during manufacturing and captured in the executed batch manufacturing records is compiled and analyzed against the acceptance criteria. Defined in the process validation protocol.
- If the analysis of the data related to process validation does not conclude to an acceptance process, appropriate corrective measures are implemented and revalidation is conducted after proper documentation of the corrective actions.
- If the analysis of the data related to process validation does conclude to an acceptable and reliable manufacturing process, the manufacturing process of anticancer formulation is considered validated.
- Final batch documentation based on the process validation report is prepared, if required.

Overview of aseptic manufacturing process

The entire aseptic manufacturing process can be divided into following steps

CIP and SIP of manufacturing and holding vessel: This was performed to sterile the validation process. Before sterilization, vacuum leak was carried out on the system to ensure leak tightness. The product and vent filters were also sterilized in place along with vessels.

Preparation of bulk solution and filtration: The formulation is prepared as per the manufacturing process in sterilized manufacturing tank. The formulation is filtered through a 0.22 micron sterilized grade filter into a sterilized holding vessel. The vessel is kept over pressurized with compressed air.

Depyrogenation of vials and sterilization of equipments and accessories: Approved lot of glass vials are received from the warehouse and transferred into the class C preparation area. After washing the vials with vial washing machine, vials are depyrogenated in the depyrogenated tunnel. Prior to manufacturing of drug products, all manufacturing, filling and other small equipments to be used are subjected to standard cleaning and sterilization process.

Aseptic filling, half stoppering, and tray loading: Vials are transferred into feed turn table of filling machine from the depyrogenation tunnel. Sterilized flurotec lyotec slotted rubber stopper are transferred into the stopper hopper are aseptically within CRABs. The filling CRABs is under air classification of class 100 conditions with a positive pressure while filling the product solution into the vials. The filling process followed by partial stoppering of vials using slotted sterilized rubber stopper under CRABs. After stoppering the vials are transferred in to the loading tray for loading into the lyophilizer without sealing under the CRABs.

CRAB technology: A well designed positive pressure CRAB, supported by adequate procedures for its maintenance, monitoring and control offers tangible advantages over traditional aseptic processing including fewer opportunities for microbial contamination during processing. The CRAB was used in the following operations.
Transferring of vials from the depyrogenation tunnel

Vial filling, stoppering and sealing

**Lyophilization process:** Partially stoppered vials are transported and loaded into the lyophilizer. A partial vacuum is drawn on the chamber at ambient temperature and maintained for shortened hold time. The chamber is then vented and stoppered vial are sealed within the chamber.

**External decontamination:** Lyophilized vials are sealed with aluminum seal and transported for external decontamination purified water and dried with compressed air.

**Vial coding:** External denominated vial are neck coded with respective batch number.

**Manufacturing procedure**

Validation batch of 12 liters for topotecan hydrochloride injection 4 mg (Base)/vial was taken as per following process flow diagram (WHO, 2006; 2007).

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**Fig. 2.** Flowchart of manufacturing procedure.
Results and Discussion

TABLE 1
Summary of results for finished product.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Specification</th>
<th>Batch result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Description</td>
<td>Light yellow color powder or cake</td>
<td>Light yellow color cake</td>
</tr>
<tr>
<td>2</td>
<td>Identification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>By U.V.</td>
<td>The absorption maxima of the test solution should correspond to the absorption maxima of test solution</td>
<td>Complies</td>
</tr>
<tr>
<td>2.2</td>
<td>By H.P.L.C.</td>
<td>The retention time of drug peak in test solution should be concordant with retention time of drug peak in standard solution as obtained in test for assay</td>
<td>Complies</td>
</tr>
<tr>
<td>3</td>
<td>Water (By KF)</td>
<td>NMT 4.0%/w/w</td>
<td>1.4%</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>Between 2.7 &amp; 3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>Uniformity of dosage units (by weight variation)</td>
<td>Must comply with USP</td>
<td>6.4</td>
</tr>
<tr>
<td>6</td>
<td>Sterility</td>
<td>Must comply with test for sterility</td>
<td>No growth observed</td>
</tr>
<tr>
<td>7</td>
<td>Bacterial endotoxin</td>
<td>NMT 64.8 EU/mg of drug</td>
<td>Sample passes at 16.21 EU/mg of drug</td>
</tr>
<tr>
<td>8</td>
<td>Reconstituted Solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.1</td>
<td>Reconstitution time</td>
<td>NMT 3mins</td>
<td>Less than 1 min</td>
</tr>
<tr>
<td>8.2</td>
<td>Description of solution</td>
<td>Pale yellow to yellow green color solution free from visible particles</td>
<td>A clear pale yellow colored solution free from visible particles</td>
</tr>
<tr>
<td>8.3</td>
<td>Completeness and clarity of solution after reconstitution</td>
<td>The solid that dissolves completely, leaving no visible residue as undissolved matter</td>
<td>The solid is completely dissolved</td>
</tr>
<tr>
<td>8.4</td>
<td>Particulate matter (visible foreign particles)</td>
<td>Should be free from visible foreign matter</td>
<td>It is free from visible foreign matter</td>
</tr>
<tr>
<td>9</td>
<td>Particulate matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.1</td>
<td>10µm</td>
<td>NMT 6000 particles per vial</td>
<td>06 particles per container</td>
</tr>
<tr>
<td>9.2</td>
<td>25µm</td>
<td>NMT 600 particles per vial</td>
<td>02 particles per container</td>
</tr>
<tr>
<td>10</td>
<td>Assay (by HPLC)</td>
<td>95-105.05% of label claim</td>
<td>99.5%</td>
</tr>
<tr>
<td>11</td>
<td>Degradation products (by HPLC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.1</td>
<td>10-hydroxy camptothecin</td>
<td>NMT 0.2%</td>
<td>0.00%</td>
</tr>
<tr>
<td>11.2</td>
<td>Any other unknown impurity</td>
<td>NMT 0.2%</td>
<td>0.06%</td>
</tr>
<tr>
<td>11.3</td>
<td>Total impurity</td>
<td>NMT 1.5%</td>
<td>0.27%</td>
</tr>
<tr>
<td>12</td>
<td>Seal integrity test</td>
<td>There should be no leakage in any vial</td>
<td>Complies</td>
</tr>
</tbody>
</table>

The data generated are checked, compiled and critically reviewed based on the final results. It has been observed that all the critical process parameters and analytical results for process validation batch are well within the limits.

Conclusions

The data generated are checked, compiled and critically reviewed based on the final results and discussion. It has been observed that all the critical process parameters and analytical results for the process validation batch are within the limit. It can be finally conclude that the drug product when manufacturing process consistently meets critical process control parameters as defined in the validation protocol and approved for batch manufacturing record. No deviation was observed during the manufacturing of the validation batch of anticancer lyophilized formulation.

References


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