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Research Article

DEVELOPMENT AND EVALUATION OF ETHANOL FREE READY TO USE INJECTION OF GEMCITABINE HYDROCHLORIDE

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Abstract

Cancer known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. Cancer is one of the most life-threatening diseases. The mortality rate due to cancer is increasing even after presence of many chemotherapeutic agents in market. So, the ultimate objective of the present work was to developed Gemcitabine Hydrochloride aqueous injectable solution which is widely used as antineoplastic antimetabolite. Since, the Gemcitabine Hydrochloride is soluble in water as confirmed from the solubility studies, hence, formulated as an aqueous injectable solution for i.v. administration.

The present work is to formulate novel injectable formulation of Gemcitabine Hydrochloride using WFI as the only vehicle by ready to use technique. Marketed injectable formulation of Gemcitabine Hydrochloride has ethanol as its major excipient. Gemcitabine Hydrochloride is given in combination therapy either with platinum derivatives as Cisplatin or Carboplatin and Paclitaxel etc. for various cancer treatments. As both aqueous injectable formulation of Gemcitabine Hydrochloride and Paclitaxel have Ethanol as major excipient. However, when both are given in combination therapy the daily exposure of Ethanol to patients exceeds its limit and shows its side effects. The present work is envisaged to overcome the above limitations by formulating it into a stable ethanol free formulation.

Keywords: - : malignant, mortality, gemcitabine, injectable, ready to use technique etc.

Introduction

Gemcitabine Hydrochloride is an Anti-neoplastic agent under the category of Pyrimidine Analogues. It has been approved by the Food and Drug Administration (FDA) in 2004 for treatment of breast cancer and then in 2006 for treatment of ovarian cancer (FDA, 2008). It appears white to off-white in colour having molecular weight 299.66 and melting point 286-292 °C. The pK_a value of drug is 3.58 with half life 0.7-6 hours. It belongs to class II drug of BCS Scale. It is soluble in water; slightly soluble in methanol; practically insoluble in alcohol and in polar organic solvent. It belongs to the group of anti-metabolites. In this family, the structure of drug is very much similar to those of normal substances in living cells. The molecular formula of Gemcitabine hydrochloride is $C_9H_{11}F_2N_3O_4 \cdot HCl$. The IUPAC name of the drug is 4-amino-2-deoxy-1-(2, 2 difluoro-β-D erythropentofuranosyl) pyrimidine. The structural formula of the drug is given below.

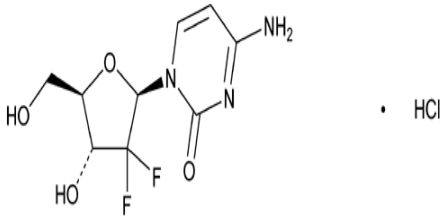

Figure 1: Structural representation of Gemcitabine Hydrochloride

Gemcitabine Hydrochloride is metabolized intracellularly by nucleoside kinases to the active 2', 2'- difluoro-2'-deoxycytidine diphosphate (dFdCDP) and 2', 2'- difluoro-2'-deoxycytidine triphosphate (dFdCTP) nucleosides. The cytotoxic effect of Gemcitabine Hydrochloride is attributed to a combination of two actions of the diphosphate and triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, Gemcitabine Hydrochloride diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA

synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including deoxycytidine triphosphate (dCTP). Second, Gemcitabine Hydrochloride triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of Gemcitabine Hydrochloride triphosphate into DNA (self-potentiation). After the Gemcitabine Hydrochloride nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the Gemcitabine Hydrochloride nucleotide and repair the growing DNA strands (masked chain termination). In T lymphoblastoid cells (CEM), Gemcitabine Hydrochloride induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death. Hence Gemcitabine Hydrochloride exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the Gl/S-phase boundary.

Materials and Methods

Gemcitabine Hydrochloride was obtained as a gift sample from Fresenius Kabi Oncology Ltd. Gurgaon. Water for Injection was obtained from Fresenius Kabi Oncology Ltd. Gurgaon.

Preformulation Studies

Solubility

The solubility of Gemcitabine Hydrochloride was determined in various aqueous and non-aqueous solvents and data is presented in table below:

Table 1: Solubility profile of Gemcitabine Hydrochloride in various solvents

S. No.	Solvent	Specification	Observation
1.	Water	Soluble	Soluble in water
2.	Methanol	Slightly soluble	Slightly soluble in methanol
3.	Alcohol & Organic solvents	Insoluble	Practically insoluble in alcohol & in polar organic solvents

Solution State Stability Studies

The Gemcitabine Hydrochloride was dissolved in WFI and stored in vials for 48 hours at 2-8°C and room temperature, samples were withdrawn and analyzed at regular intervals and assay was carried out.

pH Determination

The pH of the Gemcitabine Hydrochloride solution was checked with pH instrument and it was found to be 2.4.

Degradation Pathway

The main mechanism of Gemcitabine Hydrochloride degradation is hydrolysis at higher temperature. The Gemcitabine Hydrochloride is remarkably stable in the solid state. In 0.1N Hydrochloric acid (HCl) solution at 40°C, deamination of Gemcitabine Hydrochloride occurs, yielding its uridine analogue. Approximately 86% of the initial Gemcitabine Hydrochloride remains after 4 weeks under these conditions used. Cleavage of the N-glycosidic bond of Gemcitabine Hydrochloride or conversion to its alpha-anomer in 0.1 N HCl solutions is not observed during 4 weeks period. However, this work has shown that Gemcitabine Hydrochloride anomerizes in 0.1N Sodium hydroxide (NaOH) at 40°C. Approximately 72% of the initial Gemcitabine Hydrochloride remains after 4 weeks under the basic conditions used. Uridine hydrolysis products are also formed under these conditions. A mechanism involving an acyclic intermediate is proposed.

Effect of temperature

It is reported in the literature that Gemcitabine Hydrochloride is sensitive to heat.

Formulation of Injection

Gemcitabine Hydrochloride injection by using ready to use technique was formulated as follows: WFI (90% quantities required for batch) was taken in a compounding beaker. Weighed amount of Gemcitabine Hydrochloride was then added to the WFI and stirred to dissolve Gemcitabine Hydrochloride. After Gemcitabine Hydrochloride dissolution, pH was adjusted if necessary using 1N NaOH/1N HCl. After pH adjustment final volume was then made up to batch quantity using WFI. Finally the solution was filtered through 0.22µ PVDF membrane filter and filtrate collected was filled in 20 mm neck, 6 mL USP Type-I, tubular vials with fill volume of 5.26 mL, stopper and sealed .

Formulation Optimization Studies

WFI was selected as a vehicle to dissolve the Gemcitabine Hydrochloride (38 mg/ml). The concentration i.e. (38 mg/ml) was selected with reference to its lyophilized product (40 mg/ml) available in market. Lyophilized product was available with concentration of 200 mg/vial which is diluted with 5 ml of WFI which on dilution gives 5.26 ml because of volume displacement by Gemcitabine Hydrochloride which further gives 38 mg/ml conc. NaOH/HCl had been selected for pH adjustment. Proposed manufacturing formula for Gemcitabine injection is given in the table below:

Table: Optimized formula for Gemcitabine injection

S. No.	Ingredients	Quantity per mL
1.	Gemcitabine Hydrochloride	38.0 mg
2.	HCl	q.s to adjust pH
3.	NaOH	q.s to adjust pH
4.	Water for Injection	q.s to 1 mL

Evaluation of Injection

Stability testing was carried out for evaluation of injectable formulation. The purpose of stability testing was to provide evidence on how the quality of a Gemcitabine Hydrochloride substance or Gemcitabine Hydrochloride product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for the Gemcitabine Hydrochloride substance or a shelf life for the Gemcitabine Hydrochloride product and recommended storage conditions.

Since the product is intended to be stored in the refrigerator i.e. at 2-8ºC stability conditions for the product will be:

- For accelerated conditions: 25±2ºC/60±5%RH
- For long term / real time conditions: 2-8ºC.

Final 3 product batches were subjected to stability study in following conditions:-

i.Accelerated : 25±2ºC/60±5% RH for 1 & 3 months.

ii.Long term : 2-8ºC for 1 & 3 months.

Results of Stability study carried out on three different batches are discussed in tables below::

Table 2: Stability study results (B.NO: API/11/A)

Stability Condition	Description	Assay (%)	pH	Total Impurities
Initial	Clear and colourless solution	100.00	2.28	0.12
1month/25±2ºC/60±5%RH	Clear and colourless solution	97.80	2.22	0.09
3months/25±2ºC/60±5%RH	Clear and colourless solution	96.10	2.16	0.10
1month/2-8ºC	Clear and colourless solution	99.50	2.23	0.10
3months/2-8ºC	Clear and colourless solution	99.60	2.17	0.11

Table 3: Stability study results (B.NO: API/11/B)

Stability Condition	Description	Assay (%)	pH	Total Impurities
Initial	Clear and colourless solution	100.00	2.28	0.12
1month/25±2ºC/60±5%RH	Clear and colourless solution	95.90	2.19	0.10
3months/25±2ºC/60±5%RH	Clear and colourless solution	99.40	2.23	0.10
1month/2-8ºC	Clear and colourless solution	96.80	2.30	0.12
3months/2-8ºC	Clear and colourless solution	100.30	2.18	0.11

Table 4: Stability study results (B.NO: API/11/C)

Stability Condition	Description	Assay (%)	pH	Total Impurities
Initial	Clear and colourless solution	100.00	2.28	0.12
1month/25±2°C/60±5%RH	Clear and colourless solution	97.50	2.25	0.09
3months/25±2°C/60±5%RH	Clear and colourless solution	96.40	2.22	0.10
1month/2-8°C	Clear and colourless solution	99.40	2.24	0.10
3months/2-8°C	Clear and colourless solution	100.10	2.20	0.11

During the study the product was found to better comply with the specified limits of assay and pH at 2-8°C. All the individual and total impurities were found to comply the specified limits. Hence 2-8°C was selected as the storage temperature for the product compliance.

Summary

The objective of the study was to perform preformulation studies of the drug, selecting WFI as a suitable vehicle, perform various compatibility studies such as Stainless steel vessel compatibility studies, filter compatibility studies and tubing compatibility studies to select the suitable material for fabrication of batches and carry out photostability study to study the effect of light on formulation.

Formulation and evaluation of aqueous injectable dosage forms have been carried out and finally short term stability studies were performed.

All the formulated aqueous injectable formulations were analyzed initially and subjected to short term accelerated stability studies and further evaluation of formulation were studied at time interval of 1st month & 3rd month.

The stability results till 3rd month concluded that all batches have showed satisfactory results. Passing all the evaluation criteria like pH, assay and related substances impurities were within the limits.

Conclusion

The present work was designed to develop a ready to use ethanol free aqueous injectable of Gemcitabine Hydrochloride a broad spectrum antitumour antimetabolite. The Gemcitabine Hydrochloride is found to be soluble in WFI (as evidenced during solubility studies). Hence, the present goal of research work is to overcome the drawbacks associated with ethanol when used as major excipient in injectable formulation and to formulate a stable ethanol free formulation.

Based on the physicochemical properties and solubility profile of the drug, ready to use technique was adopted and WFI is selected as vehicle inspite of ethanol, to reduce the side effects of exceeding limit of ethanol in patients.Various studies were carried out on different batches to select the manufacturing procedure and to optimize the formulation.

The results of all batches were found to be satisfactory with respect to various parameters such as assay, pH and related substances impurities. Results of tubing and filter compatibility studies showed that Peroxide cured tubing and PVDF membrane filter give more satisfactory results as compared to Platinum cured tubing and PTFE membrane filters. Photostability study concluded that there was no adverse effect of light on formulation. Therefore, it was concluded that the aqueous injectable formulation was found to be satisfactory with respect to stability results and overcome the adverse effects of ethanol in patients.

From the above results it was concluded that ethanol free ready to use injectable formulation proved to be an advantageous tool for the development of stable injectable dosage form of Gemcitabine Hydrochloride. Hence, our objective to develop an ethanol free ready to use injectable formulation of drug candidate was achieved.

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