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Formulation and Evaluation of Aqueous Decitabine Formulations

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ABSTRACT

Cancer is a disease of uncontrolled cell division, invasion, and metastasis. Decitabine is a cytidine analog and is a drug for the treatment of myelodysplastic syndromes, a class of conditions where certain blood cells are dysfunctional, and for acute myeloid leukemia (AML). Decitabine is commercially available in the market as a lyophilized dosage form across the globe. By considering the product criticality and nature, there is a need to evaluate this product for a better stability profile in liquid form. Available literature stated that Decitabine is very unstable and undergoes hydrolytic degradation in the presence of water. Hence an attempt for developing a simple liquid Decitabineformulations is attempted and it is learned that the huge levels of impurities and a significant drop in the assay of Dectabine were formed when attempted to develop liquid formulations. Hence, there is a need to attempt the development of a non-aqueous injectable dosage form.

INTRODUCTION:

Decitabine (trade name Dacogen), or 5-aza-2'-deoxycytidine, acts as a Nucleic Acid Synthesis Inhibitor.[1] It is a drug for the treatment of myelodysplastic syndromes, a class of conditions where certain blood cells are dysfunctional, and for acute myeloid leukemia (AML).[2] Chemically, it is a cytidine analog.

Decitabine is used to treat myelodysplastic syndromes (MDS) including previously treated and untreated, de novo and secondary MDS of all French-American-British subtypes (refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) and Intermediate-1, Intermediate-2, and High-Risk International Prognostic Scoring System groups. In patients with chronic kidney disease, Batty and colleagues reported the first case series on the feasibility of therapy with hypomethylating agents in patients with chronic kidney disease[3]. It also has EU approval for acute myeloid leukemia (AML)[2]. Decitabine is a hypomethylating agent[4,5]. It hypomethylated DNA by inhibiting DNA methyltransferase. It functions similarly to azacitidine, although decitabine can only be incorporated into DNA strands while azacitidine can be incorporated into both DNA and RNA chains.

According to European scientific discussion[6], since decitabine is heat sensitive and prone to hydrolysis in an aqueous solution, it was justified to use a lyophilized powder for reconstitution at the time of use.

Decitabine (5-aza-2'-deoxycytydine), an analog of the 4 natural nucleoside 2'-deoxycytidine. Decitabine is a fine, white to almost white powder with the 5 molecular formula of C8H12N4O4 and a molecular weight of 228.21. Its chemical name is 4-amino-1-(2- 6 deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one and it has the following structural formula:

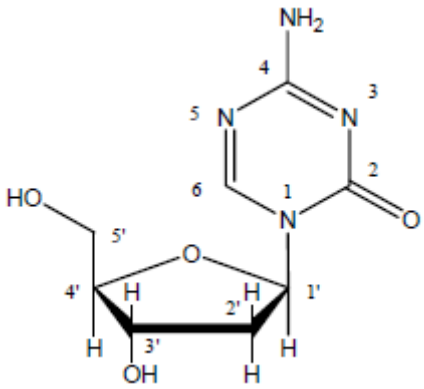

Figure No. 1: Chemical Structure of Decitabine

In India, the drug Decitabine was approved as the Lyophilized powder Injection 5omg/ vial. As per the literature available, the lengthy exposure of Decitabine in water increases the potential loss of potency and impurity formation due to the hydrolysis of the product by water. Also, pH and temperature are the two critical factors that cause Decitabine to degrade in the aqueous environment. Lyophilization is time-consuming, tedious, and involves cumbersome procedures. Further, it involves expensive technology to develop a lyophilized product. One of the main disadvantages of lyophilization is the expensive and the lyophilization cycle development and criticality of the freeze-drying process, which requires very low temperatures, can be quite costly. Further, the product needs to be handled with the precautions while dispensing, manufacturing and lyophilization. The lyophilization cycle recipe needs to be set carefully based on the load of the vials that go into the lyophilizer. Hence, an attempt to develop a non-lyophilized drug product such as liquid formulation would offer convenience for practitioners by avoiding the reconstitution step when preparing the drug for administration.

MATERIALS AND METHODS:

Decitabine was gifted from the SML, Raichur, Karnataka. Sucrose, Ethanol, and L-Arginine were purchased from commercial sources. All required chemicals used were of standard grade.

Preparation of Decitabine Formulations

Total 3 formulations were prepared. The concentration chosen of Decitabine was chosen 10 mg/mL based on the solubility. Initially, purified water was taken and the drug was added.

Ethanol was then added and the drug was dissolved. Later on, added one by one excipient per composition table. pH of the formulation was adjusted using Sodium Hydroxide.

Table No. 1: Formulation of Decitabine Injection

Sr. No.	Ingredients	DF1	DF2	DF3
1	Decitabine	10 mg/mL	10 mg/mL	10 mg/mL
2	Potassium Phosphate Monobasic	6 mg/mL	6 mg/mL	6 mg/mL
3	Ethanol	300 mg/mL	300 mg/mL	300 mg/mL
4	L-Arginine	--	400 mg/mL	--
5	Sucrose	--	--	400 mg/mL
6	Sodium Hydroxide	Qs to pH	Qs to pH	Qs to pH
7	Purified Water	QS to 1 mL	QS to 1 mL	QS to 1 mL

DF stands for Aqueous Decitabine Formulations

Evaluation of Decitabine Formulations:

Physical evaluation

***Description*:** This is a physical observation made by the individual.

pH: pH was measured using a pH meter at about 25°Ctemperature.

Light Transmission: All the formulations were tested for light transmission at650 nm using a UV spectrophotometer.

Chemical Evaluation:

Assay: HPLC method was adopted to measure the active drug content from the 3 formulations. The active obtained is expressed as a percent of labeled amount of Decitabine content. The obtained value of drug content is expected to be within limits of 90.0% to 110.0% (General compendia like USP & BP requirement).

Related Substances: % content of known and unknown impurities were determined by HPLC method.

RESULTS AND DISCUSSION:

The results are compiled in table 2. A clear colorless solution was observed in all the studied formulations. pH of all 3 formulations was observed in the range of 6.2 to 6.6. Light transmission measured for the three formulations found between 95 to 100% indicating the clear transmission of the liquid formulation when each of the formulations was transmitted through UV spectrophotometer at 650 nm. For the chemical analysis of all the three formulations, it was observed that all the three formulations have shown assay value of about 95.0 % indicating the correct input of % content of Decitabine vs label claim. It also indicates that the analytical method employed for estimating the % content of Decitabine is correct. From the analysis of the related substance, it is observed that open ring impurity &deformyl impurity contents were observed in all three formulations in a significant amount. However, the % level of unknown impurity in all three formulations are satisfactory.

Table No. 2: Physical and Chemical Evaluation of Aqueous Decitabine Formulations

Sl. No.	Formulation Codes	Description	pH	LT (in%)	Assay (in %)	Related Substances
1	DF1	@	6.34	98.5	95.1%	α-Decitabine :0.05% Ring Open Imp :7.84% 5-Azacytosine Imp: 0.05% Deformyl Imp: 6.42% Single Highest UNK Imp: 0.12% Total Imp: 15.13%
2	DF2	@	6.28	98.9	96.2%	α-Decitabine : 0.03 % Ring Open Imp :6.14% 5-Azacytosine Imp: 0.02% Deformyl Imp: 6.13% Single Highest UNK Imp: 0.09% Total Imp: 12.84%
3	DF3	@	6.56	99.6	97.1%	α-Decitabine : 0.04 % Ring Open Imp :6.84% 5-Azacytosine Imp: 0.06% Deformyl Imp: 5.89% Single Highest UNK Imp: 0.13% Total Imp: 12.98%

@: Description: A clear colorless solution. LT is Light Transmission.

CONCLUSION:

The overall characterization of all three formulations concluded that no physical description complications were observed. Analytical results of pH and light transmission test parameters were found satisfactory. pH of the formulations was adjusted towards the neutral side by considering the stable nature of the drug substance. Chemical evaluation such as assay test parameter result was observed satisfactory. However, for impurity formation, overall control on the alpha-decitabine and 5-azacytosine impurities were noticed in all three formulations. However, a significant amount of Ring open and deformyl impurities was noticed in all three formulations. The probable mechanism of the formation of 2 major impurities is given below. The drug substance undergoes opening of its ring structure in aqueous solution followed by irreversible deformylation and formation of guanylurea derivatives. In an aqueous solution, Decitabine (I) is in equilibrium with its ring-open-formylated derivative (II), followed by irreversible deformylation and formation of the guanylurea derivative (III).

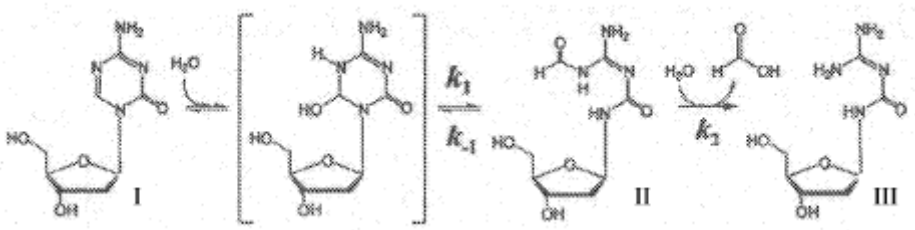

Figure No. 2: Derivatives of Decitabine

However, % content of unknown impurities in all the formulations was satisfactory. From the above experiment, it can be concluded that Decitabine formulation needs fine-tuning with respect to the presence of water to arrest the degradation of drug substance in the aqueous environment. Impurities levels in all the formulations are also not in line with the requirements of ICH Q3 B R(2). As an alternate, the scope of developing non-aqueous-decitabine Injection shall be attempted.

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