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Nonaqueous Formulation and Evaluation of Decitabine

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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 simple nonaqueous Decitabine formulations was attempted and it is learned that the levels of impurities in the nonaqueous were better when compared to aqueous Decitabine Formulations.

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 hypomethylates 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 aqueous solution, it was justified to use a lyophilized powder for reconstitution at the time of use.

Decitabine (5-aza-2'-deoxycytidine), an analogue of the 4 natural nucleoside 2'-deoxycytidine. Decitabine is a fine, white to almost white powder with the 5 molecular formula of $C_8H_{12}N_4O_4$ 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:

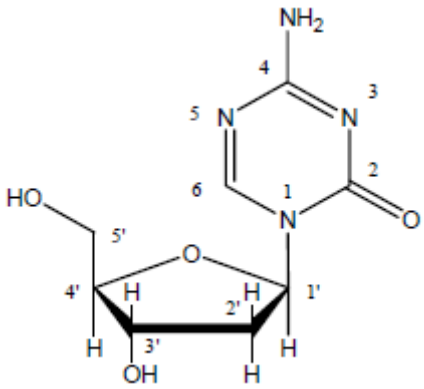

Figure No. 1: Structure of Decitabine

In India, the drug Decitabine was approved as the Lyophilized powder Injection of 50 mg/ 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. Ethanol, Propylene Glycol, DMSO, PEG 400 were purchased from commercial sources. All required chemicals used were of standard grade.

Preparation of Decitabine Formulations

A total of 3 formulations were prepared. The concentration chosen of Decitabine was chosen 10 mg/mL based on the solubility. Initially, DMSO was taken, the drug was dissolved and

added one by one excipient as per the below formulation table. The pH of the formulation was adjusted using Sodium Hydroxide. Glycerine was used to make 100 % volume.

Table No. 1: Formulation of Decitabine Injection.

Sr. No.	Ingredients	NDF1	NDF2	NDF3
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	100 mg/mL	100 mg/mL	300 mg/mL
4	DMSO	50 mg/mL	50 mg/mL	--
5	Propylene Glycol	--	400 mg/mL	--
6	PEG 400	--	--	400 mg/mL
7	Sodium Hydroxide	QS to pH	QS to pH	QS to pH
8	Glycerine	QS to 1 mL	QS to 1 mL	QS to 1 mL

NDF stands for Nonaqueous Decitabine Formulations

Evaluation of Decitabine Formulations:

Physical evaluation

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

pH: pH was measured using a pH meter at about 25°C temperature by diluting the one part of formulations with 10 mL of water.

Water Content: water content of all the formulations was measured using Karl-Fischer USP 921 <Method 1a>.

Light Transmission: All formulations were tested for light transmission at 650 nm using a UV spectrophotometer. The formulations were diluted in a 1:10 ratio with water and then measured.

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 the 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 the HPLC method.

RESULTS AND DISCUSSION:

The results are compiled in Table No. 2. A clear colourless to a light yellow solution was observed in all the studied formulations. The 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 a UV spectrophotometer at 650 nm. Concerning the chemical analysis of all the three formulations, it was observed that all the three formulations have shown assay value of about 97.0 % 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 substances, it is observed that higher levels of Open ring impurity and deformyl impurity contents were observed in all three formulations. However, the % level of unknown impurity in all three formulations is satisfactory.

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

Sr. No.	Formulation Codes	Description	pH	LT (in %)	Water Content	Assay (in %)	Related Substances
1	DF1	@	6.14	99.5	0.58%	96.8%	α-Decitabine : 0.04 % Ring Open Imp:1.84% 5-Azacytosine Imp: 0.03% Deformyl Imp: 1.92% Single Highest UNK Imp: 0.16% Total Imp: 4.06%
2	DF2	@	6.34	99.92	0.61%	98.4%	α-Decitabine : 0.01 % Ring Open Imp:1.24% 5-Azacytosine Imp: 0.06% Deformyl Imp: 1.43% Single Highest UNK Imp: 0.13% Total Imp: 3.02%
3	DF3	@	6.26	99.3	0.66%	98.1%	α-Decitabine : 0.02% Ring Open Imp:1.44% 5-Azacytosine Imp: 0.03% Deformyl Imp: 1.79% Single Highest UNK Imp: 0.09% Total Imp: 3.58%

@: 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, light transmission, and water content test parameters were found satisfactory. The pH of the formulations was adjusted towards the neutral side by considering the stability nature of the drug substance. Chemical evaluation such as assay test parameter result was observed satisfactory. However, concerning impurity formation, overall control on the alpha-decitabine and 5-azacytosine impurities were noticed in all three formulations. However, better control on the Ring open and deformyl impurities was noticed in all three formulations.

However, the % content of unknown impurities in all the formulations was satisfactory. From the above experiment, it can be concluded that non-aqueous Decitabine formulation can give better levels of impurities when compared to aqueous formulations.

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