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Stability Study of Optimized Formulation and Evaluation of Anti-Cancer Drug

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ABSTRACT

The present research work aim was to develop a ready-to-dilute simple liquid injectable composition of anti-cancer drug candidate called Decitabine. Since Decitabine is very sensitive to hydrolysis in the water and controlling the impurity levels by designing a suitable solvent was a challenging in the present research work was focused. During preformulation study, it was confirmed that the active substance is Decitabine. Based on the study outcome, It was concluded that the possibility of developing aqueous formulations is not possible due to severe degradation of hydrolytic impurity in the aqueous environment. Based on the un satisfactory results of aqueous formulation attempts, nonaqueous formulation trials were attempted. The attempt of non-aqueous was able to give better results compared to aqueous formulations of Decitabine. However, still there is a scope to work to fine tune non aqueous formulations of Decitabine with respect to further control of impurities.

INTRODUCTION:

Decitabine (trade name Dacogen), or 5-aza-2'-deoxycytidine, acts as a Nucleic Acid Synthesis Inhibitor[1]. Decitabine is a cytosine analog and an intravenously administered antineoplastic agent used in the therapy of myelodysplastic syndromes. Decitabine is associated with a low rate of transient serum enzyme elevations during therapy but has not been implicated in causing clinically apparent liver injury with jaundice. Therapeutic indication of this product for myelodysplastic syndromes[2]. It is also evaluated as a feasibility of therapy for treating for chronic kidney disease with some of the hypomethylating agents[3].It also acts as hypomethylating aqent[4&5]. It is known that the substance is a heat and hydrolysis sensitive[6] and hence it is approved as a freeze-dried product in the world. Decitabine has shown promising activity against acute myeloblastic leukemia and myelodysplastic syndrome. Also, it is significant in activity against chronic myeloid leukemia. It is rapidly metabolized in the liver by cytidine deaminase, which explains its short half-life of 8–30 minutes. Major pathways for the instability nature of Decitabine is heat, pH and water. The drug undergoes severe instability in the water under the influence of temperature and pH.

The Decitabine is indicated for the treatment of patients with 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. Myelodysplastic syndromes (MDS) are conditions that can occur when the blood-forming cells in the bone marrow become abnormal. This leads to low numbers of one or more types of blood cells. MDS is considered a type of cancer. MDS is a severe, chronic syndrome from which very few people successfully recover. It often progresses to AML, which is a form of leukemia. Depending on which scoring system a doctor uses, life expectancy can change, according to the progression of MDS. Decitabine (trade name Dacogen), or 5-aza-2'-deoxycytidine, acts as a Nucleic Acid Synthesis Inhibitor[7]. 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)[8]. Chemically, it is a cytidine analogy. 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[9]. It also has EU approval for acute myeloid leukemia (AML)[8]. Decitabine is a hypomethylating agent[10,11]. It hypomethylates DNA by inhibiting DNA methyltransferase. It functions in a similar manner to azacitidine, although decitabine can only be incorporated into DNA strands while azacitidine can be incorporated into both DNA and RNA chains.

A few azacytosine nucleosides[13], such as 5-aza-2'-deoxycytidine (also called decitabine) and 5-azacytidine (also called azacitidine), have been developed as antagonist of its related natural nucleoside, 2'-deoxycytidine and cytidine, respectively.

Stability Study of Optimized Formulation:

Based on the outcome of the total formulations made which were of aqueous and non-aqueous, it was understood that the non-aqueous formulations of trial NDF2 was found a optimized formulation based on the initial analytical characterization data. Hence, it was decided to evaluate the stability study for the optimized formulation to understand the overall behaviour of the product when subjected to accelerated and long-term stability condition for 6 Months.

Table No.: 1 Composition details of the Optimized Formulation.

Sl.No	Ingredients	NADF1	Qty for 500 mL	Quantity Taken
1	Decitabine	10 mg/mL	5.0 G	5.12G
2	PotassiumPhosphate Monobasic	6 mg/mL	3.0 G	3.002 G
3	Ethanol	100 mg/mL	50.0 G	50.01 G
4	DMSO	50 mg/mL	2.5 G	2.501 G
5	Propylene Glycol	400 mg/mL	200.0 G	200.002 G
6	Sodium Hydroxide	Qs to pH	--	--
7	Glycerine	QS to 1 mL	Qs to 500 mL	About 240 G

Note: Quantity of Decitabine is taken 5.12 G based on potency correction.

Brief manufacturing procedure:

The above composition was formulated as per the procedure described below.

1. Dispensed the required quantity of ingredients as per the above composition table.
2. Dispensed quantity of Ethanol & DMSO were collected into the mixing vessel and maintain the temperature of the WFI at 5°C to 15°C and also purged with filtered nitrogen. (Observations: Temperature: 14.3°C.).
3. The drug Decitabine was added into above mixture and stirred for 15 minutes, a light-yellow color solution was obtained.
4 Weighed quantity of Propylene Glycol was taken and required quantity of Potassium Phosphate Monobasic followed by Sodium Hydroxide were added and stirred for about 10 minutes. A clear solution was obtained.
5.The solution was finally made to 100% using Glycerine and then stirred for 5 minutes. (Observations: Temperature: 15.8°C). pH of the solution (1:10 diluted with water) was recorded 6.36 (after 100% final volume make up).

Stability Design: In any rational design and evaluation of dosage forms for drug products, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability can be defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. Or Stability of a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a pre-determined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. The stability studies were carried as per ICH guidelines. The accelerated study was carried at temperatures of 25°C±2°C/60%±5% RH and real time condition was carried at temperatures of 5°C±3°C sample with drawn at respective intervals of I, II, III & VI month at accelerated condition and III & VI month at real time condition for evaluation parameters such as Description, pH, water content, Assay and Related Substances test parameters.

Stability Design of Decitabine Injection:

Table No.: 2. Stability Testing Schedule including Accelerated Condition: 25ºC ± 2ºC/60% RH ± 5% RH &Real time Condition: 5ºC ± 3ºC.

S. No.	Tests	25ºC ± 2ºC/60% RH				5ºC ± 3ºC	
		1M	2M	3M	6 M	3M	6M
1	Description	@	@	@	@	@	@
2	pH	1	1	1	1	1	1
3	Water Content						
4	Assay By HPLC	1	1	1	1	1	1
5	Related Substances	1	1	1	1	1	1

@: The stability exposed vials of respective month for pH study shall be used for measuring the description. Hence no separate vials are kept.

Evaluation of the formulations:

Description: The formulated trials of both aqueous and non-aqueous formulations were examined by the visual observation.

pH: pH test parameter in the case of aqueous formulations were measured directly by placing the formulation directly using pH meter, whereas, the case of non-aqueous formulations, pH was measured by diluting one part with 10 parts of water and then measured.

Water Content:

This test parameter was used to measure the non-aqueous formulations. Karl Fischer titration is a classic titration method in analytical chemistry that uses coulometric or volumetric titration to determine trace amounts of water in a sample. Karl Fischer titration was used as an analytical method for quantifying water content in the drug and methanol was used as a solvent.

The water content was checked by auto Karl Fischer Titration and the results were reported.

Assay by HPLC:

Assay was carried out using validated HPLC method. Results have been discussed in subsequent chapters.

Related substances by HPLC:

Related substances test parameter was carried using validated HPLC method. Results were discussed in subsequent chapters.

Table No.:3. Test parameters for evaluation.

S. No:	TEST	ACCEPTANCE CRITERIA
1.	Description	A Clear ,colourless to light yellow solution
2.	Assay of Decitabine by HPLC	Between 90.0 to 110.0%
3.	Related substances by HPLC	
	α-Decitabine	Not More Than 0.2%
	Ring Open	Not More Than 1.5%
	5-Azacytosine	Not More Than 0.2%
	Deformyl Impurity	Not More Than 2.0%
	Single Maximum unknown	Not More Than 0.2%
	Total Impurities	Not More Than 4.0%
4.	pH	Between 6.0 to 6.5
5.	Water Content*	Not more than 2.0% w/w

Note: Water Content for non-aqueous formulations.

RESULTS AND DISCUSSION

Table No.:4. Preformulation Study Results

Sl. No.	Test parameter	Results
1	Description	White fine powder
2	Solubility	**Buffer pH** / **Quantity Dissolved at 25°C (mg/mL)**: 1.2 — 0.1g/10 mL; 2.0 — 0.1g/10 mL; 4.0 — 0.1g/10 mL; 6.0 — 0.1g/10 mL; 8.0 — 0.1g/10 mL
3	Identification by IR	The sample spectrum exhibited maxima only at the same wave length as that of standard spectrum. The IR spectrum of Decitabine
4	Melting Point	Between 195°C and 205°C
5	Hygroscopic study	The material found hygroscopic.
6	XRD study	The result conformed material is amorphous
7	Water content	Not More Than 1.0%
8	pH	Between 6.0 and 7.0

Solubility: Found Soluble in DMSO at 90 mg/mL; soluble in ethanol at 2 mg/mL with warming; soluble in water at 25 mg/mL with warming; buffers, serum, or other additives may increase or decrease the aqueous.

Identification by IR

The decitabine drug sample exhibited characteristic peaks such as C–H stretching (alkane) at 2918 cm^{-1} and stretching of NH group (3467 cm^{-1}).

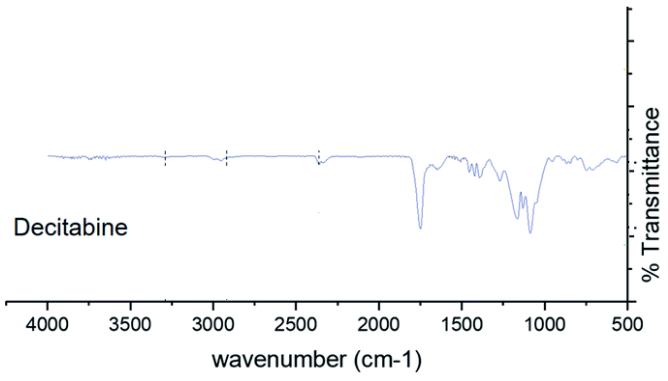

Figure No.: 1. IR Spectrum of Decitabine

Melting point by DSC chromatogram

By the onset of DSC graph of Decitabine where it was observed that the melting onset falls at about 200°C and a following exothermic decomposition of the sample.

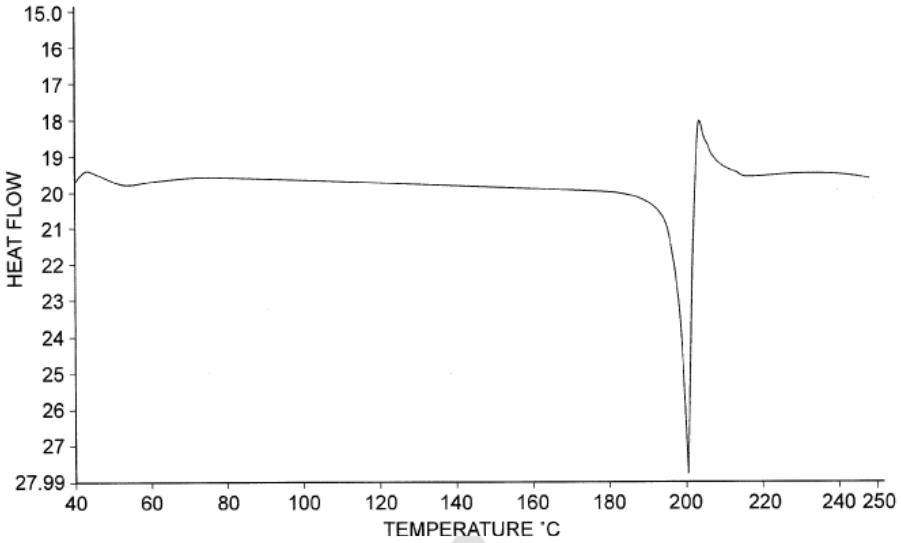

Figure No.:2. DSC Thermogram of Decitabine

Table No.:5. Hygroscopic Study Results**.**

API	**Weight (mg)**				**Weight Change (%)**			
	0 Day	7th Day	21st Day	28th Day	0 Day	7th Day	21st Day	28th Day
Decitabine	200.00	206.8	218.9	234.5	Nil	3.4%	9.45	17.25

The drug substance is hygroscopic nature in high humidity condition. The drug substance has absorbed the moisture around 17.00% of its weight after 28 Days. Hence the substance is found highly hygroscopic and unstable at high humidity condition.

XRD Chromatogram

The diffraction peak visible to about 37.62° is due to the sample container. crystalline decitabine having characteristic XRPD peaks located at approximately 7.0, 13.0, 14.3, 18.5, 21.5 and 24.5±0.2° 2θ, or having peaks located substantially as shown in the XRD diffractogram.

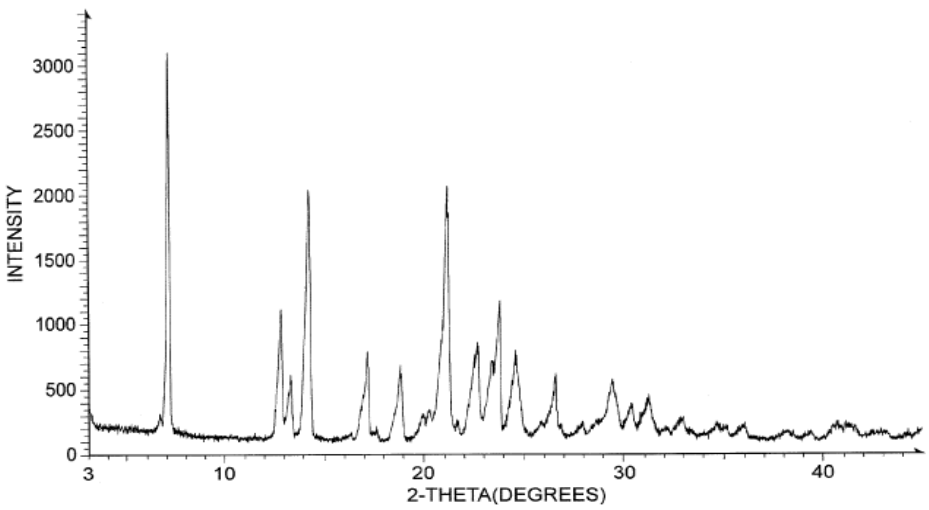

Fig No.: 3. XRD diffractogram of Decitabine

Water Content: The water content of the drug substance Decitabine was measured by using KF titrator and result was 0.51%.

pH: pH of the drug substance was measured using 0.5% of the concertation in carbon dioxide free water and the result is 6.42.

Table No.:6. Physical and Chemical Evaluation of Non-Aqueous Decitabine Formulations.

Sl. No.	Formulation Codes	Description	pH	LT (in%)	Water Content	Assay (in %)	Related Substances
1	NDF1	@	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	NDF2	@	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	NDF3	@	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.

NDF stands for Non Aqueous Decitabine Formulations.

Stability Study of Optimized Formulation: Decitabine Injection

Table No.:7. Stability Study of Optimized Decitabine Formulation. [Composition Reference.: NDF2]

Sl. No.	Test Parameters	Results							
		$25^0C/60\%$ RH					5 ± 3^0C	
		Initial		1M	2M	3M	6M	3M	6M
1	Description	$		$	$	$	$	$	$
2	pH	6.19		6.08	5.95	6.02	5.88	6.12	6.25
3	Water content by KF [% w/w]	0.51		0.58	0.47	0.53	0.54	0.49	0.56
4	Assay by HPLC [in %]	97.2		96.1	94.2	93.3	90.4	96.8	95.9
5	Related substances by HPLC [in %]	α-Decitabine	0.02	0.03	0.02	0.03	0.02	0.03	0.02
		Ring Open	1.32	1.66	2.09	3.46	4.89	1.82	2.36
		5-Azacytosine	0.04	0.03	0.04	0.04	0.03	0.04	0.03
		Deformyl	1.38	1.88	2.67	3.28	4.26	1.78	2.01
		Single Highest UK	0.15	0.19	0.18	0.21	0.19	0.18	0.18
		Total Impurities	3.16	3.94	5.13	7.28	9.89	4.01	4.72

@: A Clear yellow colour solution, HUNK: Highest Unknown impurity.

Table No.:8. Photostability Study of Optimized Formulation

[Optimized Batch of Composition Reference: NDF2]

Sl. No.	Condition	Description	pH	Water Content	Assay (in %)	Related Substances
1	Primary Pack	@	6.21	0.49	96.9	α-Decitabine : 0.05 % Ring Open Imp :1.69% 5-Azacytosine Imp: 0.05% Deformyl Imp: 1.91% Single Highest UNK Imp: 0.19% Total Imp: 3.92%
2	Dark Control	@	6.51	0.38	98.6	α-Decitabine : 0.02 % Ring Open Imp :1.29% 5-Azacytosine Imp: 0.04% Deformyl Imp: 1.71% Single Highest UNK Imp: 0.09% Total Imp: 3.12%
3	With Secondary Pack	@	6.41	0.44	98.9	α-Decitabine : 0.03 % Ring Open Imp :1.31% 5-Azacytosine Imp: 0.05% Deformyl Imp: 1.65% Single Highest UNK Imp: 0.11% Total Imp: 3.17%

@: A Clear yellow colour solution.

Non-Aqueous Formulations:

Based on the physicochemical outcome of aqueous formulations of Decitabine, it was decided to formulate non-aqueous formulations to evaluate the overall nature of the drug substance when formulated in non-aqueous formulations.

A clear colourless to light yellow solution was observed in all the studied formulations. pH of all 3 formulations were 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 the each of the formulations were transmitted through UV spectrophotometer at 650 nm. With respect to the chemical analysis of all the three formulations, it was observed that all the three formulations have shown satisfactory assay results around 97.0 %. From the related substances analysis, it is observed that higher levels of Open ring impurity & deformyl impurity contents were observed in all the three formulations. However, the % level of unknown impurities in all the three formulations are satisfactory.

Overall characterization of all the three non-aqueous formulations concluded that satisfactory results of physical description was noticed. Analytical results of pH, light transmission and water content test parameters were found meeting to the acceptance criteria. pH of the formulations were adjusted towards 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 was noticed in all the three formulations. However, better control on the Ring open and deformyl impurities were noticed in all the three formulations.

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

Stability Study

The analytical results of optimized formulation of Decitabine Injection was found satisfactory. Though the impurities levels are high in the optimized formulation, however it is to be noted that N-formyl and ring open impurities are expected to be part of Decitabine drug product and the

non-aqueous optimized formulation has better levels when compared to aqueous formulations and also comparable level of impurities against local market available lyophilized formulation. However it is always preferred to have lesser impurities in the injection formulations. To understand suitable storage condition for the product, different temperature conditions were opted to charge the product and the stability study of optimized batches was found satisfactory at 2^0C to 8^0C condition indicating that the product is suitable to store at refrigerated.

Market Available Formulation Evaluation:

Before assessing the stability evaluation, the available three Indian market samples were subjected to evaluation for critical analytical test parameters. From the analytical evaluation of the three local market samples, it is understood that % content of known and unknown impurities is found on higher levels despite the product is lyophilized product and also comparable to the proposed nonaqueous product of second composition [NDF2].

Analytical Method Development & Validation:

An in-house analytical method development for Assay and Related substances was developed and it is learnt that methods were found stability indicating nature.

As a part of analytical method validation, Assay, and Related substances test parameter of the finished product, forced degradation study was carried. It is found that the drug product is sensitive to alkali, neutral, heat and peroxide conditions. The analytical method validation was carried out satisfactorily with the parameters like precision, accuracy, robustness, and linearity. The validated method was applied while analysing the stability exposed and photostability exposed samples.

Photostability: In order to understand the light/photo stability of the drug product, the photostability evaluation was carried as per ICH Q1B conditions. Optimized formulation of non aqueous formulation of Decitabine [NDF2] was chosen for conducting the photostability study. The chosen formulation was exposed to 200 watt-hours/m^2 of near UV light and 1.2 million Lux hours of cool fluorescent light in a photostability chamber maintained at 25°C.

Conclusion and Summary

In conclusion, the overall results of this study revealed clearly that Decitabine can easily be formulated in liquid form but with the aid of non-aqueous solvents. Huge rise of impurities and also a significant amount of assay test parameter drop in the aqueous based formulations was noticed based during the initial time point analysis of all the three formulations. Hence, an attempt to evaluate the nature in non-aqueous was attempted. It is learnt that non aqueous formulations is able to give better and stable composition against aqueous formulations of Decitabine. The present research work aim was to develop a ready to dilute simple liquid injectable composition of anti-cancer drug candidate called Decitabine. Since, Decitabine is very sensitive to hydrolysis in the water and controlling the impurity levels by designing suitable solvents was a challenging in the present research work was focused.

During preformulation study, it was confirmed that the active substance is Decitabine. Based on the study outcome, it was concluded that the possibility of developing aqueous formulations is not possible due to severe degradation of hydrolytic impurity in the aqueous environment. Based on the un satisfactory results of aqueous formulation attempts, non aqueous formulations trials were attempted. The attempt of non-aqueous was able to give better results compared to aqueous formulations of Decitabine. However, still there is a scope to work to fine tune non aqueous formulations of Decitabine with respect to further control of impurities.

As a part of the objective, pre-formulation studies of the drug, selecting a suitable composition system which includes solvents, vehicle etc evaluation of the formulated batches and finally to perform short-term stability studies. The analytical method developed and validated was found stability indicating. All the formulated aqueous and non aqueous formulations were analyzed initially to understand the drug product behavior. The optimized formulation [NDF2] was subjected to the time interval of 6^{th} month of accelerated and real time studies to understand the stability nature.

The stability results at the end of 6^{th} month of both condition concluded that the optimized formulation is able to perform better in the stability window period. All the studied critical test parameters like Description, Water Content, pH, Assay and Related substances data is found satisfactory. The real-time stability condition data dictated that the impurities levels would be

stabilizing over a period of time. Further, the assay test parameters results are found promising during the real time stability window. Photostability study data also indicated the optimized composition stability behaviour is satisfactory and able to support a clear glass vial. Finally, it is concluded that the optimized formulation [NDF2] has the capability to overcome presently market available product limitations.

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