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STABILITY STUDIES OF CONJUGATED NANOCONSTRUCTS OF DACARBAZINE IN VARIOUS CONDITIONS AS PER ICH GUIDELINES

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ABSTRACT

Background

The design of the stability studies for the finished pharmaceutical product (FPP) should be based on knowledge of the behaviour and properties of the API, information from stability studies on the active pharmaceutical ingredients (API) and on experience gained from preformulation studies, similar marketed formulations and investigational FPPs. The likely changes during storage and the rationale for the selection of attributes to be tested in the stability studies should be stated.

Objective

To determine stability studies of conjugated nanoconstructs of dacarbazine in various conditions as per ICH guidelines. Stability studies should include testing of stability-indicating attributes of the FPP, i.e. those that are susceptible to change during storage and are likely to influence quality, safety and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological and microbiological attributes, preservative content.

Methods:

Stability studies were carried out for DPGN-3. The prepared Dacarbazine loaded PLGA formulations were stored at The following conditions i.e., 5 ± 3 °C, 30±2 °C, 65% ± 5% RH (long term stability), 40±2 °C, 75% ± 5% (accelerated stability). Every three months the drug content, *in vitro* release studies were determined for the nano-formulation subjected for long term stability studies.

Results

The prepared Dacarbazine PLGA loaded formulations were stored at the various conditions as per ICH guideline, in every three months the drug content, *in vitro* release studies were carried out for the nanoformulation and the results were recorded.

Conclusion

The stability studies it was witnessed that the prepared the loaded PLGA nanoformulation DPGN-3 will be stable at 5±3 °C, 30 ± 2 °C, 65 ± 5% RH for a period of 12 months.

Key Words: Dacarbazine, PLGA, Nanoformulation, melanoma, ICH

INTRODUCTION

Stability studies should be performed on each individual strength, dosage form and container type and size of the FPP unless bracketing or matrixing is applied (refer to ICH Q1D).

Stability testing should be conducted on the dosage form packaged in the primary container-closure systems proposed for marketing. If the secondary container-closure system has protective properties, and labelling clearly indicates that the product is to be stored in the primary and secondary packaging (e.g. “store tablets in blisters in the provided cartons”), or if the product is packaged in a semi-permeable container where components from the secondary packaging can migrate into the product, the secondary packaging may also form part of the packaging system for stability samples.

Long-term, accelerated and, where appropriate, intermediate storage conditions for FPPs are detailed in the sections below. The general case applies if the FPP is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified.

Table 1. Long-term, accelerated and intermediate storage conditions

Study	Storage condition	Minimum time period covered by data at submission
Long-term[a]	25 °C ± 2 °C/40% RH ± 5% RH or 30 °C ± 2 °C/35% RH ± 5% RH	12 months or 6 months as referred to in section 2.2.7
Intermediate[b]	30 °C ± 2 °C/35% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C/not more than (NMT) 25% RH	6 months

[a]Whether long-term stability studies are performed at 25 °C ± 2 °C/40% RH ± 5% RH or 30 °C ± 2 °C/35% RH ± 5% RH is determined by the climatic condition under which the FPP is intended to be marketed. Testing at 30 °C/35% RH can be an alternative to the storage condition at 25 °C/40% RH.

[b]If 30 °C ± 2 °C/35% RH ± 5% RH is the long-term condition, there is no intermediate condition.

Products meeting the specifications when stored under the accelerated conditions and the long-term storage conditions appropriate to the intended market, as specified in the table above, have demonstrated the integrity of the packaging in semi-permeable containers. A significant change in water loss alone at the accelerated storage condition does not necessitate testing at the intermediate storage condition. However, data should be provided to demonstrate that the pharmaceutical product would not have significant water loss throughout the proposed shelf life if stored at 25 °C/40% RH or 30 °C/35% RH.

For long-term studies conducted at 25 °C ± 2 °C/40% RH ± 5% RH, that fail the accelerated testing with regard to water loss and show significant change with respect to any other parameters, additional testing at the "intermediate" storage condition should be performed as described under the general case to evaluate the temperature effect at 30 °C.

A 5% loss in water from its initial value is considered a significant change for a product packaged in a semi-permeable container after an equivalent of three months' storage at 40 °C and not more than (NMT) 25% RH. However, for small containers (1 mL or less) or unit-dose products, a water loss of 5% or more after an equivalent of three months' storage at 40 °C/NMT 25% RH may be appropriate, if justified.

An alternative approach to studies at the low RH as recommended in the table above (for either long-term or accelerated testing) is to perform the stability studies under higher RH and to derive the water loss at the low RH through calculation. This can be achieved by experimentally determining the permeation coefficient for the container-closure system or, as shown in the example below, using the calculated ratio of water loss rates between the two humidity conditions at

the same temperature. The permeation coefficient for a container-closure system can be experimentally determined by using the worst-case scenario (e.g. the most diluted of a series of concentrations) for the proposed FPP.

Example of an approach for determining water loss

For a product in a given container-closure system, container size and fill, an appropriate approach for deriving the rate of water loss at the low RH is to multiply the rate of water loss measured at an alternative RH at the same temperature, by a water loss rate ratio shown in the table below. A linear water loss rate at the alternative RH over the storage period should be demonstrated.

The purpose of the stability study is to establish, based on testing a minimum number of batches of the FPP as specified in section 2.2.3, a shelf life and label storage instructions applicable to all future batches of the FPP manufactured and packaged under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout its shelf life.

METHODS

The comparison of The results of optimized Dacarbazine PLGA nanoparticles, created on The particle size, zeta potential, drug content, entrapment efficiency and *in vitro* release studies, The formulation DPGN-3 was indicated The least particle size, higher zeta potential, drug content, entrapment efficiency and sustained drug release. Hence among The different trials of Dacarbazine loaded PLGA nanoparticles. DPGN-3 has been identified to carry out further studies.

Table 2. Characterization studies report of optimized nanoformulation

Code	Particle size (nm)	Zeta potential (mV)	Drug content (µg/ml)	Entrapment Efficiency (%)	*In vitro* drug Release (%) (24 hrs)
DPGN-3	246±2.4	-27±1.4	0.955±1.3	83.15±1.4	86.67±2.4

Stability studies

As per the procedure, stability studies were carried out for DPGN-3. The prepared Dacarbazine loaded PLGA formulations were stored at The following conditions i.e., 5 ± 3 °C, 30±2 °C, 65% ± 5% RH (long term stability), 40±2 °C, 75% ± 5% (accelerated stability). Every three months the drug content, *in vitro* release studies were determined for the nano-formulation subjected for long term stability studies the results were shown in The following Table.

The drug content of DPGN-3 stored at 5±3 °C for a period of 12 months exhibited a slight fall in the drug content when compared to the initial drug content of the nano-formulation after storing the sample for 12 months.

Table 3. Stability studies-*In vitro* drug release of DPGN-3 stored at 5±3 °C (after 0,3,6,9 & 12 months as per QA1 (R))

Time in hrs	0 Months	3 Months	6 Months	9 Months	12 Months
0	0	0.00	0.00	0.00	0.00
1	29.32±1.8	29.12±1.8	29.10±1.8	28.92±1.8	28.05±1.6
2	34.15±1.4	34.00±2.5	33.72±2.8	33.12±1.7	32.24±1.3
4	38.25±1.1	37.74±1.9	37.52±1.5	37.12±2.4	36.22±1.4
6	44.88±1.7	44.45±3.2	44.13±1.8	43.93±2.8	43.72±1.6
8	49.21±3.1	49.80±3.2	49.31±1.8	49.12±2.5	48.68±2.1
10	56.36±1.9	54.72±1.6	54.28±1.7	54.02±1.2	53.32±2.6
12	64.87±2.7	61.75±2.6	61.13±1.7	60.76±1.8	60.26±1.5
14	68.69±3.2	66.74±3.2	65.58±2.8	64.39±1.2	63.92±2.4
16	74.25±3.1	71.48±1.3	71.28±1.2	70.62±1.9	70.23±2.5
18	78.27±1.4	76.28±2.1	75.73±1.5	74.33±1.6	73.39±1.7
20	83.35±1.3	80.34±2.1	80.00±2.7	79.22±1.5	79.62±1.5
22	85.73±1.8	83.84±1.8	83.24±2.2	83.02±1.6	82.70±1.1
24	85.25±2.5	84.27±2.6	83.72±1.4	82.41±3.2	82.16±1.4

Table 4. Stability studies-*In vitro* drug release of DPGN-3 stored at 30 ± 2 °C, 65 ± 5%

RH (after 0,3,6,9 & 12 months as per QA1 (R))

Time in hrs	0 Months	3 Months	6 Months	9 Months	12 Months
0	0.00	0.00	0.00	0.00	0.00
1	32.94±1.4	29.42±2.5	28.21±2.1	27.41±1.5	26.16±1.3
2	36.86±1.4	33.34±1.8	33.12±1.5	32.46±1.8	32.08±2.4
4	40.49±1.1	36.62±2.3	36.30±3.1	36.08±2.3	35.26±1.5
6	46.93±2.1	45.93±2.1	44.81±2.5	43.51±1.5	43.31±2.2
8	51.79±3.5	47.36±2.6	46.62±2.1	45.73±1.5	45.36±1.1
10	56.37±2.1	53.68±1.4	53.16±1.2	52.65±2.4	52.10±1.7
12	62.35±3.5	61.21±3.1	60.13±2.3	59.23±1.3	59.25±1.4
14	67.32±1.8	64.52±2.4	63.17±1.4	62.82±1.8	62.66±2.3
16	74.55±2.1	71.63±1.3	71.12±2.5	70.59±2.5	70.22±3.5
18	78.27±1.4	73.79±2.6	72.19±1.8	71.92±2.5	71.33±2.1
20	83.33±1.3	79.65±3.2	79.86±3.1	79.26±1.5	78.65±1.3
22	84.73±2.1	81.62±1.6	80.32±1.2	79.21±1.8	79.67±1.6
24	84.94±2.2	83.72±2.1	81.22±2.5	78.38±2.6	78.18±2.1

Table 5. Stability studies-*In vitro* drug release of DPGN-3 stored at 40±2 °C, 75±5% RH (after 0, 6, & 12 months as per QA1 (R)

Time in hrs	0 Months	6 Months	12 Months
0	0.00	0.00	0.00
1	31.96±1.4	21.45±2.0	12.35±2.5
2	35.86±1.8	27.63±3.2	18.33±2.2
4	39.49±1.1	31.65±3.3	23.51±2.3
6	45.93±1.5	35.31±2.6	27.19±3.0
8	50.79±3.0	38.83±4.3	34.67±2.1
10	56.37±2.2	43.72±2.6	38.42±2.5
12	63.35±3.2	49.62±2.7	36.32±2.8
14	67.32±1.8	54.58±2.0	47.34±1.3
16	73.55±2.2	58.73±2.5	52.28±3.0
18	77.27±1.6	64.64±2.6	54.41±1.6
20	82.33±1.8	70.54±2.6	58.38±2.2
22	84.73±2.2	75.64±3.2	61.72±1.4
24	85.25±2.3	77.24±2.6	62.45±1.7

The optimized nanoformulation DPGN-3 at 40±2 °C, 75±5% RH after 0, 6, & 12 months indicated substantial decrease in cumulative drug release when related to The initial cumulative drug release of The same nanoformulation.

On comparing the drug content after storing the optimized nanoformulation DPGN-3 at 5±3 °C, 30 ± 2 °C, 65 ± 5% RH, when compared to previous data of the same formulation there was a minor decrease in the drug content after 12 months of the storage.

Every three months the *in vitro* drug release almost remains the same for the optimized nano-formulation DPGN-3 stored at 5±3 °C, 30 ± 2 °C, 65 ± 5% RH up to 12 months.

However, The Dacarbazine Nano formulation, which was subjected for accelerated stability studies on 40 ±2 ° C, 75±5% RH, shows a major decrease in drug content and *in vitro* drug release. It may be due to storage at high temperature which leads the degradation of Dacarbazine loaded PLGA nanoparticles. Hence, there is a major decrease in the drug content and cumulative percentage of *in vitro* drug release. Therefore from The stability studies it was observed that the prepared Dacarbazine loaded PLGA nano-formulation DPGN-3 will be stable at 5±3 °C, 30 ± 2 °C, 65 ± 5% RH for a period of 12 months.

CONCLUSION

Dacarbazine loaded polymeric nanoparticles were designed optimized and prepared by emulsion polymerization method. The physiochemical characterization and other performances of the formulations were evaluated and found suitable for treatment of Melanoma disease. In this study, Dacarbazine hydrochloride is selected as a model drug. The prepared formulations were found to be stable at room temperature for period of 1 year. Hence the nanoparticular system of Dacarbazine indicates the possibilities to bring back the drug to the market. However more patient studies are suggested.

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