10. STABILITY STUDIES

10.1 INTRODUCTION

Stability study of any formulation on storage is necessary as it reflects whether the desirable properties of the formulation are retained on storage. The testing of such product should be carried out to check whether any changes take place in the product whether physical or chemical. So, after storage period, the formulation, on rehydration, should retain the same characteristics it possessed before lyophilization.

10.2 MATERIALS AND METHODS

Dry powder formulations of HNCs were evaluated for stability as per ICH guidelines. Briefly, 20 mg of formulations were filled in hard gelatin capsules kept in tightly closed HDPE container. At different time points, formulations were reconstituted with nuclease free water and evaluated for assay of cisplatin, complexation efficiency, zeta potential and water content.

As per the ICH stability study guideline Q1A (R2), stability studies should be performed on a drug product intended for storage in refrigerator at following storage conditions. (Table 10.1) The stability protocol was designed as per ICH guidelines (1)for countries falling under zone III (hot, dry) and zone IV (very hot, humid); however, only short-term studies for 3 months storage period were performed for having the idea of the stability of the product.

Table 10-1 Stability Testing Conditions for Drug Product Intended for Storage in Refrigerator as per ICH Guideline Q1A(R2).

Study	Storage condition	Time period for which study should be carried out
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months

Any "significant change" for a drug product as per ICHand its extrapolated parameters to HNCs is defined as:

1. A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures; →Assay and complexation efficiency of siRNA

2. Any degradation product's exceeding its acceptance criterion; → zeta potential
3. Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions; → Description of dry powder formulation and water content.

Statistical analysis

Experiments were performed in triplicate. Unless stated, data are expressed as the mean ± standard deviation (SD). The statistical significance of the results was determined using a Student's t-test where $p<0.05$ denotes significant difference.

10.3 RESULT AND DISCUSSION

The stability studies were carried out, for optimized formulations, in accordance with the ICH guidelines to evaluate the suitability of the storage condition for the developed formulations. The critical parameters of the formulations have to be preserved during stability studies (2,3). The assay, zeta potential and complexation efficiency was determined after reconstitution at each time point(Table 10.2). There was no significant difference in assay and complexation efficiency of formulations at different storage time points in real time condition. However, at accelerated condition there was a drop of around 10% for assay, though it was within specification (80-120%). The water content showed a gradual increase with time and this cloud have been responsible for a minor drop in complexation efficiency at accelerated storage conditions. The increase in water content may influence complexation efficiency as siRNA are prone to hydrolytic degradation. However, water content up to3% has been reported to be non-detrimental from aerosolization point of view.Therefore, it is recommended that water content of such formulations be strictly controlled < 3%.Stability studies at accelerated and refrigerated conditions demonstrate that the product was stable at both conditions for a period of 3 months and suggest that the product will be stable for longer periods at refrigerated conditions.

Table 10-2 Results of stability study (mean± SD)

Storage conditions	Time	Description of powder filled in capsules	Assay of Cisplatin caprylate	siRNA Complexation Efficiency (%)	Water content (%w/w)	Zeta potential
Target spec	**-**	**White free flowing powder**	**80%-120%**	**80%-120%**	**NMT 3.0%**	**>10mV**
Before lyophilization		HNCs	98.5±0.8	97.01±1.5	1.79	26.31±0.183
5 ± 3°C	Initial	White free flowing powder	98.2±0.5	94.59±2.44	1.82	25.21±0.205
	1M	White free flowing powder	97.1±0.4	92.87±2.12	2.01	22.81±0.253
	3M	White free flowing powder	95.5±0.9	91.28±2.86	2.30	24.39±0.313
25 ± 2°C /60 ± 5% RH	1M	White free flowing powder	94.2±1.1	90.10±2.74	2.17	24.57±0.190
	3M	White free flowing powder	90.7±1.5	86.13±2.52	2.59	29.83±0.248

10.4 REFERENCES

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