9. STABILITY STUDIES

9.1 Introduction

Stability testing is generally recommended during the product development of new drugs to establish a shelf-life for the product and to recommend a suitable storage condition (1). Stability testing should include the testing of all parameters that are susceptible to change during transportation and storage and are likely to influence the safety, efficacy, and quality of these products (2).

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 (3).

9.2 Methods

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 9.1). The stability protocol was designed as per ICH guidelines for countries falling under zone III (hot, dry) and zone IV (very hot, humid); however, only short-term studies for 6 months storage period were performed for having the idea of the stability of the product (4).

Polymer lipid hybrid nanocarriers (PLHNCs) were evaluated for stability as per ICH guidelines. Briefly, 50 mg of lyophilized formulations were kept in tightly closed HDPE container. The samples were subjected to two different conditions: i) Real time conditions (5°C ± 3°C) and ii) Accelerated conditions (25°C ± 2°C/ 60% RH ± 5% RH). The formulations were reconstituted with water and evaluated for assay, particle size and zeta potential.

Table 9.1 Stability Testing conditions for drug product intended for storage in refrigerator as per ICH guidelines Q1A (R2).

Study	Storage conditions	Time of study
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 ICH and its extrapolated parameters to nanoparticles is defined as:

1. A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency.→ Assay
2. Any degradation products exceeding its acceptance criterion.
3. Failure to meet the acceptance criteria for appearance, physical attributes, and hardness, however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be excepted under accelerated condition. → Particle size

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.

9.3 Results and Discussion

9.3.1 Stability study for Fulvestrant loaded PLHNCs

There was no significant difference in assay and particle size of formulations at different storage time in real time conditions (5). However, at accelerated condition, there was a drop of in assay, though it was within the limits of drug content as per USP (95 – 105%).

Table 9.2 Stability of FA FLV PLHNCs

Storage conditions	Time	Description of sample	Assay (%)	Particle Size (d. nm)	Zeta Potential (mV)
5°C ± 3°C	0M	White fluffy powder	100 ± 1.84	133.7 ± 4.68	+28.1 ± 1.15
	1M	White fluffy powder	99.52 ± 2.36	131.4 ± 5.12	+27.8 ± 1.24
	3M	White fluffy powder	99.16 ± 2.85	134.5 ± 6.21	+28.3 ± 1.08
	6M	White fluffy powder	98.84 ± 2.16	135.8 ± 4.17	+29.2 ± 1.23
25°C ± 2°C/	0M	White fluffy powder	100 ± 2.13	132.5 ± 3.54	+29.6 ± 1.62

60% ± 5% RH	1M	White fluffy powder	99.24 ± 3.24	134.2 ± 4.58	+28.5 ± 1.28
	3M	White fluffy powder	98.78 ± 2.86	135.1 ± 5.42	+28.7 ± 1.37
	6M	White fluffy powder	98.14 ± 3.12	135.4 ± 3.68	+27.9 ± 1.24

The nanoparticle were stored after lyophilization as they have phospholipids into its outer layer and lipids are unstable below the refrigerated condition if not lyophilized (6). Lyophilization increases the shelf-life of lipid based formulations and preserves it in dried form as a lyophilized cake to be reconstituted with water prior to administration. To maintain the same particle size distribution after lyophilization- rehydration cycle, a cytoprotectant needs to be added (7). After lyophilization the particles were subjected to both the conditions, wherein the assay, particle size and zeta potential were not found to be variating too much so both the condition found to be suitable for storage of nanoparticles after lyophilization. As a cryoprotectant trehalose was added which had high Tg and stable up to 30°C, the nanoparticles were stable at both storage conditions.

9.3.2 Stability study for Exemestane loaded PLHNCs

Table 9.3 Stability of FA EXE PLHNCs

Storage conditions	Time	Description of sample	Assay (%)	Particle Size (d. nm)	Zeta Potential (mV)
5°C ± 3°C	0M	White fluffy powder	100 ± 1.84	131.7 ± 3.78	+13.1 ± 1.15
	1M	White fluffy powder	99.22 ± 2.36	132.4 ± 4.12	+12.8 ± 1.24
	3M	White fluffy powder	98.76 ± 2.85	135.1 ± 5.21	+11.3 ± 1.08
	6M	White fluffy powder	98.84 ± 2.16	133.8 ± 3.87	+13.2 ± 1.23

25°C ± 2°C/ 60% ± 5% RH	0M	White fluffy powder	100 ± 2.13	129.5 ± 3.54	+11.6 ± 1.62
	1M	White fluffy powder	99.34 ± 3.24	132.2 ± 4.58	+12.5 ± 1.28
	3M	White fluffy powder	98.78 ± 2.86	134.1 ± 5.42	+13.7 ± 1.37
	6M	White fluffy powder	97.86 ± 3.12	136.4 ± 3.68	+12.9 ± 1.24

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