11.1. Introduction

Pharmaceutical stability studies are an essential component of the development and lifecycle of pharmaceutical products, in particular, supporting the development process and IND / NDA submission activities. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a shelf life for the drug product and recommended storage conditions. Data from these studies enable recommended storage conditions, retest intervals and shelf lives to be established. International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use provides guidelines on Stability Testing of New Drug Substances and Products (Q1A(R2)) [1].

Physical instability of a formulation in terms of aggregation-flocculation and fusion-coalescence influence the shelf life of a vesicular system. This can bring about loss of encapsulated drug and increment in size of the vesicles [2]. Aggregation implies formation of bigger units where particles comes together and generated larger particles. Aggregation could result into coalescence where new colloidal structures are formed and entrapped drug is leached out, which is an irreversible process. These two instabilities affects encapsulation efficiency and drug release which are critical to gain desired therapeutic effect. Moreover, increase in size critically affects the behaviour of the formulation in in vivo conditions [3].

11.2. Materials and Equipments

Materials

HPLC grade methanol (MeOH) and acetonitrile (ACN) were procured from Fisher Scientific (Vadodara, Gujarat) to carry out chromatographic analysis. Double distilled water used in the study was filtered using 0.22 micron nylon filter, Nylon N66 membrane filters 47 mm, Rankem, India. All other reagents were purchased from S.D. finechem Ltd, India and were of analytical grade.

Equipment

- Electronic weighing balance (ELB300, Shimadzu, Japan)
- Vortex Mixer (Spinix-Vortex Shaker, Tarsons, India)
- Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)
- Cooling Centrifuge (Remi Equipment, Mumbai, India)
- HPLC (Shimadzu LC-20AT, Japan)
- Zeta sizer (Nano ZS Malvern Instruments, UK)
- Magnetic Stirrer (Remi Instruments, Mumbai, India)

11.3. Methods

Short term stability of developed UDNVs formulations and IVRs of both the drugs was performed over three months at two different stability conditions i.e. refrigerated (2-8 °C) and at Room Temperatures (25 -30 °C). The UDNVs formulations were monitored for % Entrapment efficiency (%EE), Deformability Index and mean vesicle size over stability tenure. The IVRs were evaluated for in vitro drug release during stability. The UDNVs formulations and IVRs were hermetically packed and stored in glass vial and kept at refrigerated (2-8 °C) and at Room Temperatures (25 -30 °C) conditions. Samples were withdrawn and analysed using the methods described in earlier chapters for the parameters decided to evaluate its stability at specific time intervals i.e. Initial, 1, 2 and 3 months.

11.4. Results and Discussion

The results of % Entrapment efficiency (%EE), Deformability Index and mean vesicle size for PTX-UDNVs and CBP-UDNVs when stored at refrigerated (2-8 °C) and at Room Temperatures (25 -30 °C) conditions were summarized in **Table 11-1** .

Table 11-1: Three month stability results of PTX and CBP UDNVs

Storage Condition	Stability Time Interval (Months)	% Entrapment efficiency	Deformability Index	Vesicle size (nm)
PTX-UDNVs				
RT (25 - 30 °C)	Initial	90.41 ± 1.93	25.37 ± 1.84	255.2 ± 3.76
	1M	85.56 ± 2.42	25.45 ± 2.43	267.4 ± 3.08
	2M	84.97 ± 1.87	25.05 ± 2.05	273.1 ± 2.51
	3M	84.09 ± 1.95	25.19 ± 2.34	279.5 ± 2.84
Refrigerated (2 - 8 °C)	Initial	90.41 ± 1.93	25.37 ± 1.84	255.2 ± 3.76
	1M	89.92 ± 2.12	25.33 ± 1.98	259.8 ± 3.68
	2M	89.58 ± 2.23	25.49 ± 2.49	261.8 ± 2.79
	3M	89.27 ± 2.43	25.51 ± 2.51	262.2 ± 3.47
CBP-UDNVs				
RT (25 - 30 °C)	Initial	69.86 ± 2.66	88.97 ± 3.59	316.6 ± 2.83
	1M	62.77 ± 2.72	87.73 ± 3.76	331.3 ± 3.07
	2M	61.24 ± 3.10	87.85 ± 3.50	337.5 ± 3.46
	3M	60.39 ± 3.18	88.51 ± 3.86	339.2 ± 2.71
Refrigerated (2 - 8 °C)	Initial	69.86 ± 2.66	88.97 ± 3.59	316.6 ± 2.83
	1M	67.66 ± 2.87	88.39 ± 3.47	322.1 ± 3.27
	2M	67.34 ± 3.54	88.03 ± 3.71	324.9 ± 3.56
	3M	66.95 ± 2.92	87.79 ± 3.78	325.4 ± 3.44

It was observed that the % EE was slightly reduced over three month stability study for both the formulations when stored at Room Temperatures (25 -30 °C). However, %EE was not significantly affected when stored at refrigerated (2-8 °C) conditions for both the formulations. Deformability Index and vesicle size of both the formulation remained insignificantly affected by storage conditions during stability period.

The results of in vitro drug release from IVRs of PTX and CBP when stored at refrigerated (2-8 °C) and at Room Temperatures (25 -30 °C) conditions were tabulated in **Table 11-2**.

Table 11-2: Three month stability results of PTX and CBP IVRs

Storage Condition	Stability Time Interval (Months)	In vitro Drug Release after 48 h (%)	In vitro Drug Release after 120 h (%)
PTX-IVR			
RT (25 - 30 °C)	Initial	49.78 ± 1.86	96.45 ± 1.78
	1M	48.34 ± 1.93	96.04 ± 1.86
	2M	51.76 ± 1.75	96.55 ± 1.89
	3M	48.53 ± 1.58	97.14 ± 1.80
Refrigerated (2 - 8 °C)	Initial	49.78 ± 1.86	96.45 ± 1.78
	1M	45.18 ± 1.79	96.19 ± 1.75
	2M	46.69 ± 1.58	95.32 ± 2.37
	3M	48.12 ± 1.66	96.93 ± 2.34
CBP-IVR			
RT (25 - 30 °C)	Initial	53.27 ± 1.82	97.16 ± 1.95
	1M	55.04 ± 2.30	97.04 ± 1.84
	2M	54.73 ± 2.43	97.93 ± 2.06
	3M	57.89 ± 2.86	97.27 ± 1.94
Refrigerated (2 - 8 °C)	Initial	53.27 ± 1.82	97.16 ± 1.95
	1M	53.11 ± 1.91	97.89 ± 1.91
	2M	53.23 ± 2.26	96.81 ± 1.78
	3M	54.07 ± 2.18	97.37 ± 1.66

It was observed that the drug release after 48 h and 120 h time points for both the formulation loaded IVRs not affected during storage at refrigerated (2-8 °C) as well as at Room Temperatures (25 - 30 °C) conditions. This might be due to freeze dried form of the formulation inside rods.

The results of the short term stability studies indicate that both the formulations are acceptably stable at refrigerated (2-8 °C) as well as at Room Temperatures (25 - 30 °C). However, storage of the formulations at refrigerated (2-8 °C) conditions would be preferred to sustain stability of the formulation over long term.

References

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