Certificate code:	GMP-QC-1106-02	**Internal Sample:**	GMP-110601A
Page No.:	Page 1 of 6	**External Sample code**:	N/A
Sampled on:	10/06/24	**Reported on:**	11/06/24
PO reference:	N/A	**Number of samples:**	1

S. NO.	PRODUCT	BATCH NO.
1	Vitamin C Liposomal (lipolife)	LVC1-0424

Purpose:

Quality testing of lipolife dry liposomal product.

Subject:

One bottle of the selected batch was forwarded to the Quality Control Department.

Methods

The following methods were employed as part of QC testing to verify the quality and authenticity of the product.

1. **Physical Appearance**:
   Description: Visual assessment of the powder to note colour, smell, and taste. Consistency in these attributes indicates uniform quality.

   Method: Powder is inspected visually and using organoleptic evaluation.

2. **Particle Size Analysis (Dynamic Light Scattering - DLS)**:
   Description: DLS measures the average size and size distribution of particles in suspension. It is critical for determining the uniformity and quality of liposomal formulations.

   Method: The powder content of one capsule is constituted in DI water. Samples are then further diluted in a suitable solvent and subjected to DLS to measure the average size and polydispersity index (PDI), indicating size uniformity.

3. **Moisture Content**:

   Description: Moisture content affects the stability and shelf life of the product. Low moisture content is generally preferred for dry formulations. However, the absence of moisture might indicate that the sample was not subject to freeze/spray drying.

   Method: An Infrared Moisture Analyser is used to determine the percentage of water content in the samples.

4. **Powder Re-dispersibility**:

   Description: Due to the amphiphilic properties of phospholipids forming the liposomes, dried liposomes are expected to fully disperse in water within a few minutes. No precipitation or clumping should be observed.

   Method: The dispersion time of the powder in water is measured. The capsule content is emptied in 30 ml water and shaken gently; the mixture is assessed after 5 minutes.

5. **Lipid Content (Sudan Test)**:

   Description: The Sudan Test is a qualitative test to confirm the presence of lipids, which are essential components of liposomal structures.

   Method: Samples are stained with Sudan dye, which binds to lipids/ fatty acids, confirming their presence. Briefly, a known weight of powder is dissolved in specified quantity of water to form a solution. Then, 1ml of Sudan reagent is added to the above solution and observed for the formation of a red ring.

6. **Lipid Extraction and Quantification**:

   Description: This test measures actual lipid content, providing a quantitative assessment of the lipids present in the product.

   Method: Lipids are extracted from the samples using an organic solvent, dried, and weighed to determine the lipid content per gram of sample.

7. **Electron Microscopy (TEM) Imaging**:

   Description: TEM provides high-resolution images of liposomal vesicles, allowing for the visualisation of their size and structure.

Method: A sample is prepared and imaged at high magnifications (20,000x) to observe the liposomal morphology. The process is performed by an independent third-party to avoid biased selection of the imaged area.

Results:

a) Physicochemical Properties

The lipolife vitamin C product consisted of creamy white powder with no smell and exhibited an acidic taste, which is characteristic of vitamin C. The powder exhibited excellent flow and compressibility properties. The product readily dispersed in water and the liposome size was measured in the nanometre range (205nm, 0.464) as determined via Zeta sizer (DLS). Nutrient assay was performed for drug content analysis, and drug content was found to be 102%. Moisture content was also found to be in the acceptable range, 3.99%. Results are displayed in Table 1, as given below;

Table 1: Physicochemical properties of lipolife Vitamin-C powder

SR. NO.	PARAMETER	METHOD	RESULT
1	Appearance, taste and smell	Organoleptic	Creamy colour fine powder, acidic taste
2	Flowability	Angle of Repose and Carr's index	Excellent
3	Size and PDI	DLS Zeta sizer	206nm, 0.464
4	Assay/ Drug content	HPLC	102%
5	Moisture content (%)	IR moisture analyser	3.99%
6	Powder re- dispersibility	Manual	re-dispersed within <15min

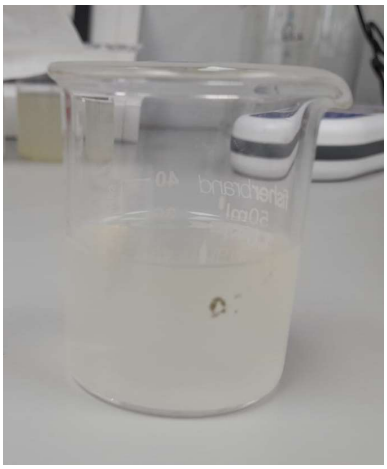

Figure 1: Dispersibility profile in water

lipolife Vitamin C was readily dispersed in water within <15min.

b) Sudan Test

Sudan III reacts with the lipids or triglycerides to stain red in colour. Lysochromes, such as Sudan III, bind to lipids but do not stick to any other substrate, resulting in confirmation of the presence of lipids. For this test, water was included as a negative control, 10% almond oil as a positive control, and lipolife Vitamin C as the test product. All products were treated with Sudan reagent using the standard protocol.

Under negative control, the Sudan reagent dispersed in water instantly.
Under positive control, a very distinct, sharp red ring was formed on the top layer, as shown in figure 2.
The lipolife product displayed a distinctly formed, sharp red ring on the top layer.

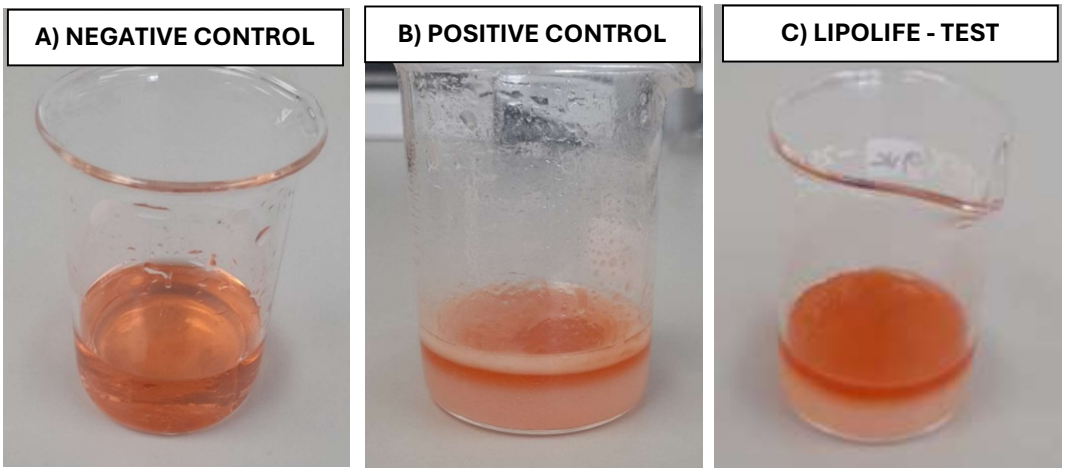

Figure 2: Sudan test for lipid content;
a) Water as a negative control with no red ring formation,
b) Oil as a positive control, where sharp red ring can be seen on top layer
c) lipolife as test product, where a sharp and distinct red layer can be seen.

c) Lipid Extraction

The Sudan test provides initial indication of high lipid content. The next step was to extract lipid from the product and quantify it. A turbid yellow solution was obtained, showing a high content of lipid. Following air-drying of the extracted solution, the amount of lipid quantified was 67.58mg/g of the sample. The lipolife product contained a considerable amount of lipid and can be therefore classified as a liposomal product. (Note, this method is not expected to achieve an extraction yield of 100%. Our validation methods indicated that the yield of extraction is 65-75% of the lipid content.)

Table 2: Weight of Extracted Lipid

SAMPLE	WEIGHT OF EXTRACTED LIPID
lipolife	0.06758g or 67.58mg/g sample

d) TEM imaging

The TEM micrograph revealed a uniform distribution of spherical liposomes in the nanometre range (100-200nm).

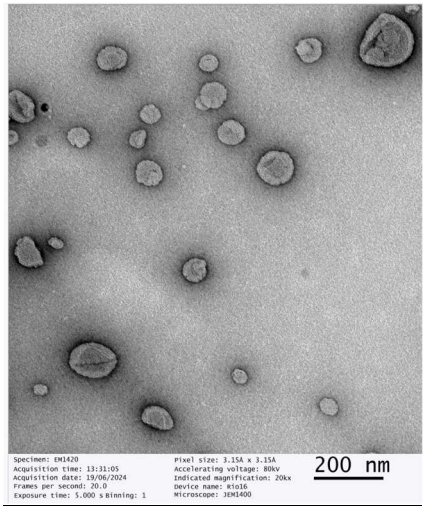

Figure 3: Transmission electron microscopic images at 20kx magnification. lipolife liposomal Vitamin C powder displaying presence of uni-lamellar, nanosized liposomes, uniformly distributed throughout the frame.

Findings and Conclusion

The lipolife product meets all standards for physical properties, particle size, active content, re-dispersibility and presence of lipid.

Based on the results obtained from these tests, it can be confirmed that the lipolife product meets the required quality attributes for liposomal product and can therefore be labelled as liposomal.

	PRINT NAME	POSITION	SIGNATURE	DATE
Operated by	Dr. Sahrish Rehmani	Principal Scientist and Regulatory Lead	Sahrish Rehmani	11/06/2024
Reviewed by	Dr. Hanan Abdalmaula	Lead Scientific Researcher	Dr. Hanan Abdalmaula	11/06/2024