CHAPTER 6

STABILITY TESTING OF AERODYNAMICALLY LIGHT AND LARGE PARTICLES

6.1 INTRODUCTION

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (Draft guidance, Stability Testing of Drug Substances and Drug Products, FDA, 1998). Physical, chemical, and microbiological data are generated as a function of time and storage conditions (e.g., temperature and relative humidity [RH]). Stability testing provides evidence that the quality of a drug substance or drug product under the influence of various environmental factors changes with time (Draft guidance, Stability Testing of New Drug Substances and Products, 2003). Attention has been focused on processes affecting the quality and therefore acceptability of novel inhalation formulations such as ALLP. Micron size particles of ALLP can aggregate and or fuse; forming larger particles would alter the disposition of the drug in vivo and thereby presumably affect the therapeutic index of the drug involved. Other physical parameters may also change during storage of these formulations include; hydrolysis of specialized excipients like phospholipids causes the formation of fatty acids and lysophopholipids (Grit et al, 1993). Though under low humidity conditions there is least possibility of the formulation to encounter hydrolytic degradation. Stability is generally considered as chemical stability of drug substance in a dosage form, however, the performance of a drug when given as a dry powder inhaler system is not only dependent upon the content of the drug substance, but also reproducible in vivo performance of the formulations. The design of a stability study for complex dosage forms such as DPIs should include special sampling plans. A special sampling plan (e.g., a predetermined number of DPI units may be randomly or otherwise sampled) may increase assurance that the resulting data for each batch are truly representative of the batch as a whole. In addition, the number of samples to be tested should be increased, if possible, near the end of the study, to better establish the various parameters and confidence levels at either side of the curve for determining the expiration dating period (Draft Guidance, MDI and DPI Drug Products, FDA, 1998). Drugs under study were considered chemically stable so the study can be focused on monitoring the drug deposition patterns in vitro. The stability protocol was designed as per ICH guidelines (Singh et al, 1999) for countries falling under zone III (hot, dry) and zone IV (very hot, humid).

6.2 METHODS

Comparative stability studies were carried out of the potential ALLP formulations at accelerated (40°C±2°C, 75±5% RH), intermediate storage (30°C ± 2°C, 60 ± 5 % RH and long term study at (25°C ± 2°C/60 ± 5 % RH) up to one year. Size '2'hard gelatin capsules were filled with 20 mg of powder. Set of 50 capsules from each batch were filled in the HDPE bottles containing 1 g silica bags as desiccant and were sealed with PVC coated aluminum foil. The study was done with three batches of same composition. Entire process was carried our at low humidity conditions (<30% RH).

During sampling, one bottle containing 50 capsules was withdrawn at predetermined time intervals, visually examined for the evidence of cacking and discoloration. The content of the capsule were tested for particle size, assay, degradation, water content, emitted dose (ED) and fine particle fraction (FPF). Drug content was analyzed as per the method explained in chapter 3 (section 3.4.3.3 for TB and section3.4.4.3 for AMK). ED and FPF were calculated by estimating level of fluorescent dye coumarin in the formulation as explained in chapter 3 (section 3.4.2.3). The stability results are summarized in Tables 6.1 and Table 6.2.

6.3 STATISTICAL ANALYSIS AND DATA INTERPRETATION

Three batches of each formulations was evaluated three times, data of nine experiments are expressed as Mean ± SD. The data were compared using ANOVA and student's t-test and difference larger than the value at $p<0.05$ were considered significant.

"Significant change" *was* considered under following conditions

- A 5 percent change in assay from its initial value
- Sum total of degradation product's exceeding its acceptance criterion (Total related substance not more then 2 %)
- Failure to meet the acceptance criteria for appearance, physical attributes, and functionality tests (e.g. ED, FPF).

Table 6.1 Stability data of TB-ALLP formulation (Mean± S.D, n=3).

Stability conditions	Description	Assay (%)	Degradation (%)	Water content(%w/w)	VMD (μm)	ED (%)	FPF (%)
Initial	Light, fluorescent yellow free flowing powder	101.32 ± 1.54	ND	3.06 ± 0.12	6.75 ± 0.23	86.72 ± 1.18	58.74 ± 2.01
40 ± 2°C / 75 ± 5% RH							
1M	Light, fluorescent yellow free flowing powder	100.47 ± 0.89	ND	3.88 ± 0.27	6.86 ± 0.12	87.08 ± 1.77	60.43 ± 3.21
2M	Light, fluorescent yellow free flowing powder	101.24 ± 0.94	ND	4.74 ± 0.14	6.44 ± 0.24	90.18 ± 1.97	57.33 ± 1.32
3M	Light, fluorescent yellow powder with small agglomerates.	99.86 ± 1.79	0.02	5.16 ± 0.41	9.19 ± 0.12	78.57 ± 2.07	45.22 ± 2.34
6M	Light, fluorescent yellow powder with small agglomerates	99.14 ± 2.02	0.024	5.57 ± 0.34	10.02 ± 0.27	74.68 ± 2.07	43.77 ± 1.75
30 ± 2°C / 60 ± 5% RH							
1M	Light, fluorescent yellow free flowing powder	101.06 ± 1.66	ND	3.54 ± 0.52	8.24 ± 0.21	89.12 ± 3.06	55.57 ± 1.14
2M	Light, fluorescent yellow free flowing powder	100.26 ± 3.51	ND	3.96 ± 0.44	7.85 ± 0.32	83.82 ± 2.43	54.65 ± 0.76

Stability conditions	Description	Assay (%)	Degradation (%)	Water content(%w/w)	VMD (μm)	ED (%)	FPF (%)
30 ± 2°C / 60 ± 5% RH							
3M	Light, fluorescent yellow free flowing powder	98.90 ± 3.51	ND	3.49 ± 0.47	9.18 ± 0.11	85.76 ± 1.76	55.37 ± 0.26
6M	Light, fluorescent yellow free flowing powder	99.86 ± 1.64	ND	3.63 ± 0.34	8.66 ± 0.24	89.67 ± 2.12	55.68 ± 0.74
9M	Light, fluorescent yellow free flowing powder	100.60 ± 0.87	ND	4.15 ± 0.47	7.67 ± 0.32	90.34 ± 3.72	54.56 ± 1.24
12M	Light, fluorescent yellow free flowing powder	98.74 ± 2.57	ND	4.24 ± 0.28	7.98 ± 0.11	85.26 ± 2.07	53.64 ± 1.21
25 ± 2°C/ 60 ± 5% RH							
3M	Light, fluorescent yellow free flowing powder	99.74 ± 3.23	ND	4.14 ± 0.18	7.92 ± 0.14	93.42 ± 1.65	58.14 ± 2.45
6M	Light, fluorescent yellow free flowing powder	97.67 ± 3.57	ND	4.34 ± 0.18	6.69 ± 0.21	86.32 ± 1.77	54.42 ± 3.07
9M	Light, fluorescent yellow free flowing powder	98.92 ± 0.65	ND	3.86 ± 0.87	9.87 ± 0.67	84.25 ± 3.25	50.84 ± 2.47
12M	Light, fluorescent yellow free flowing powder	97.20 ± 1.82	ND	4.44 ± 0.13	8.78 ± 0.17	86.06 ± 3.17	54.16 ± 1.19

Table 6.2 Stability data of AMK-ALLP formulation (Mean± S.D, n=3).

Stability conditions	Description	Assay (%)	Degradation (%)	Water content(%w/w)	VMD (μm)	ED (%)	FPF (%)
Initial	Light, fluorescent yellow free flowing powder	99.24 ± 1.24	ND	3.46 ± 0.19	6.82 ± 0.33	91.47 ± 2.26	57.76 ± 1.28
40 ± 2°C / 75 ± 5% RH							
1M	Light, fluorescent yellow free flowing powder	99.40 ± 1.46	ND	3.93 ± 0.15	9.72 ± 0.14	92.33 ± 1.78	57.56 ± 2.44
2M	Light, fluorescent yellow free flowing powder	98.72 ± 1.72	ND	4.42 ± 0.27	7.86 ± 0.23	88.42 ± 2.12	59.09 ± 0.87
3M	Light, fluorescent yellow powder with small agglomerates.	98.84 ± 0.90	ND	5.27 ± 0.34	10.13 ± 0.11	76.54 ± 1.28	44.46 ± 1.84
6M	Light, fluorescent yellow powder with small agglomerates	97.16 ± 1.76	0.06	6.14 ± 0.32	11.57 ± 0.25	74.54 ± 2.56	41.27 ± 1.65
30 ± 2°C / 60 ± 5% RH							
1M	Light, fluorescent yellow free flowing powder	100.7 ± 0.78	ND	3.67 ± 0.43	7.76 ± 0.11	90.32 ± 2.15	56.48 ± 1.64
2M	Light, fluorescent yellow free flowing powder	100.08 ± 1.14	ND	4.16 ± 0.55	7.85 ± 0.24	87.65 ± 1.72	56.25 ± 1.66

Stability conditions	Description	Assay (%)	Degradation (%)	Water content(%w/w)	VMD (μm)	ED (%)	FPF (%)
30 ± 2°C / 60 ± 5% RH							
3M	Light, fluorescent yellow free flowing powder	98.96 ± 1.74	ND	3.87 ± 0.37	7.58 ± 0.27	89.19 ± 0.77	54.27 ± 1.54
6M	Light, fluorescent yellow free flowing powder	99.14 ± 1.47	ND	4.24 ± 0.27	8.52 ± 0.11	91.26 ± 1.24	56.42 ± 1.23
9M	Light, fluorescent yellow free flowing powder	97.75 ± 1.27	ND	4.35 ± 1.26	8.77 ± 0.11	84.34 ± 1.85	54.26 ± 1.08
12M	Light, fluorescent yellow free flowing powder	97.60 ± 1.07	ND	4.44 ± 0.21	7.94 ± 0.16	85.42 ± 1.64	54.73 ± 0.74
25 ± 2°C/ 60 ± 5% RH							
3M	Light, fluorescent yellow free flowing powder	101.30 ± 1.32	ND	3.67 ± 0.15	8.17 ± 0.14	87.55 ± 1.05	59.26 ± 1.27
6M	Light, fluorescent yellow free flowing powder	99.56 ± 0.54	ND	4.16 ± 0.37	7.74 ± 0.27	86.77 ± 1.56	54.65 ± 2.16
9M	Light, fluorescent yellow free flowing powder	100.27 ± 1.12	ND	4.43 ± 0.47	8.24 ± 0.27	85.75 ± 1.42	54.47 ± 1.43
12M	Light, fluorescent yellow free flowing powder	98.54 ± 1.24	ND	4.35 ± 0.22	7.76 ± 0.17	85.08 ± 1.54	52.34 ± 2.45

6.4 RESULTS AND DISCUSSION

During storage in DPIs, complex and subtle interactions may occur between the drug substance, carrier(s), and components of the container and closure system that significantly affect the safety and effectiveness of the drug product. For example, gravitational, fluid dynamic, and other interactive forces, such as electrostatic, van der Waals, and capillary forces, together are responsible for different fluidization behaviors exhibited by different powders in an inhaler. Electrostatic charge interactions influence the overall efficiency of a DPI, since such forces are considered to be significant for attraction and adhesion between the drug substance particles, excipient particles, and device surface. Additionally, alteration in particle size, size distribution, particle morphology, and moisture content during their storage period can greatly influence the bulk properties of the formulation and the product performance.

Due to the complexity of these products, accelerated stability studies (i.e., 40±2°C/75±5%RH) alone may not be predictive of the product performance throughout the extrapolated expiration dating period. Hence, intermediate and long term stability testing was carried out at 30 ± 2°C / 60 ± 5% RH and at 25 ± 2°C / 60 ± 5% RH (Draft guidance, Stability Testing of New Drug Substances and Products, 2003).

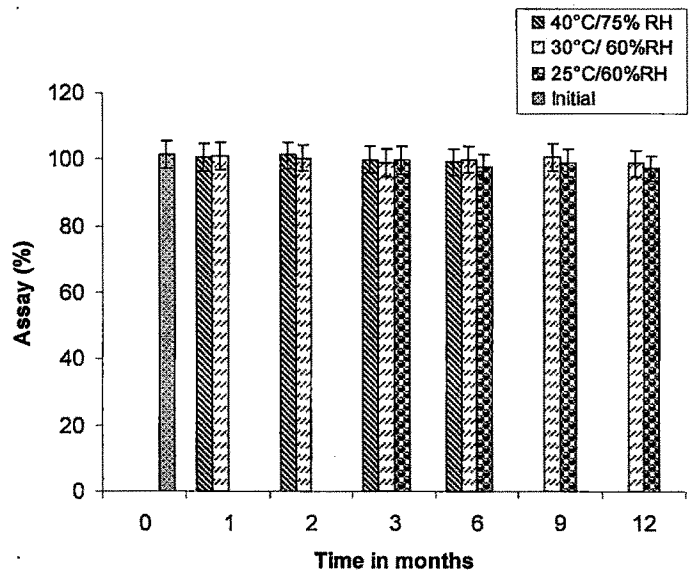
The results of stability data (Table 6.1 and 6.2) revealed formation of small agglomerates in the both TB and AMK-ALLP after storage period of 3 months under accelerated conditions. There was no *"significant change"* observed in the assay content of both formulations. No potential degradants also observed during their entire storage period at all conditions. Analysis of the Karl Fischer water contents suggested a significant (ANOVA $p<0.05$), positive increase in water content at 3M/40±2°C/75±5%RH. The moisture content in ALLP increased from 3.06 ± 0.12 to 5.16 ± 0.41 and 3.46 ± 0.19 to 5.27 ± 0.34 for TB and AMK-ALLP respectively. The results further supports the moisture sorption property of ALLP at high humidity conditions (>60%RH) as discussed in chapter 5 (section 5.3). The increased moisture level of ALLP may responsible for formation of small agglomerates in the formulation at 3M/40±2°C/75±5%RH. The high moisture sorption of ALLP at accelerated condition (40±2°C/75±5%RH) partly may be because of large proportion of hydrogenated soyaphospatidyl choline (>30%w/w) in the formulation. In addition to this, the crystalline nature of both TB and AMK would have further contributed to the high moisture sorption.

There was positive increase in particle size growth of ALLP at storage period of 3 months under accelerated conditions. The VMD of ALLP increased from initial value of 6.75 ± 0.23

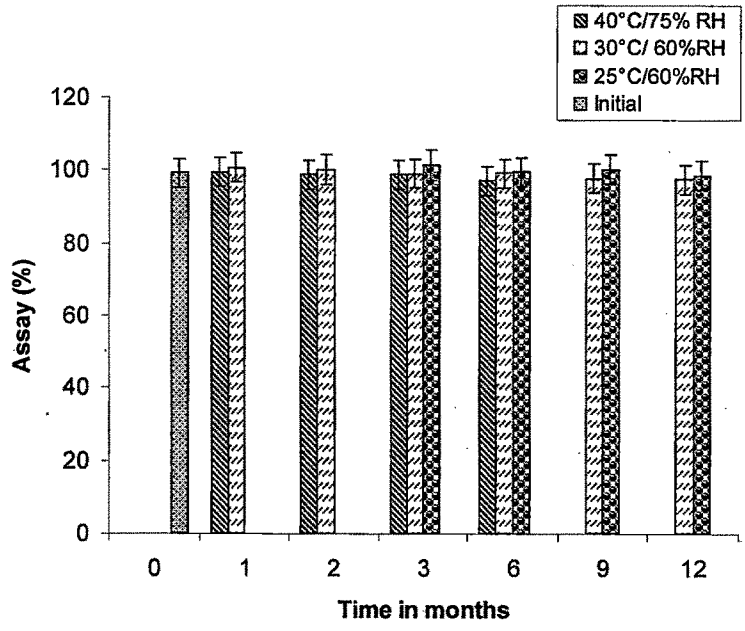
to 9.19 ± 0.12 for TB-ALLP and 6.82 ± 0.33 to 10.13 ± 0.11 for AMK-ALLP at 3M/40±2°C/75±5%RH, which are about 36% and 48% increase in particle size growth for TB and AMK ALLP respectively. However the altered particle size is still fits in the respirable size window of 5-30μm for aerodynamically light large particles (Vanbever et al, 1999).

"Significant change" in the functionality tests such as ED and FPF of ALLP were observed at 3M/40±2°C/75±5%RH. The capsule and device retention was significantly higher ($p < 0.05$) on 3rd month onwards under accelerated condition. The mean percentage delivered dose (ED) decreased from 86.72 ± 1.18 to 78.57 ± 2.07 and 91.47 ± 2.26 to 76.54 ± 1.28 for TB and AMK-ALLP respectively. The results indicate that about 8 and 15% of recoverable dose of TB and AMK-ALLP was retained in the capsules and/or in the inhaler device at 3M/40±2°C/75±5%RH. The reason for lower emission may be due to formation of agglomerates at high humid conditions. A plot of FPF at different time intervals (Figure 6.5A and 6.5B) suggests a decrease in mean FPF of both ALLP from 3rd month onwards at 40±2°C/75±5%RH, which may be attributed due to the increase (about 2% w/w) in moisture uptake. The relationship between increased humidity and decreased aerosolization performance of ALLP most likely is attributed to the hygroscopic nature of the powder, because water is rapidly absorbed into the crystal lattice at a specific humidity until it forms equilibrium with the surrounding environment (Cox et al, 1971). The presence of such a dynamic equilibrium can only promote the condensation of water between the capillaries of the powder particulates, thus increasing the interparticulate forces while decreasing the aerosolization efficiency.

The ED and FPF of both TB and AMK-ALLP at 40±2°C/75±5%RH were below the acceptable level as per the ICH guidelines. Hence, shelf life can not be assigned, until the formulation meets the criteria at intermediate conditions. Hence the formulations were tested on intermediate storage condition in order to assign a shelf-life at CRT. The product stability in terms description, chemical stability, particle size growth, moisture content and in-vitro deposition characteristics were conducted for one year at intermediate storage condition. No significant change was observed in the powder properties at all sampling points. Based on the results of intermediate condition and real time study data (Table 6.1,6.2 and Figure 6.1 to 6.5) both TB and AMK-ALLP can ascertain shelf life of 12 months as per the ICH and USFDA at CRT. The shelf life can be further re-ascertained base on the real time study data at 25±2°C/60±5%RH.

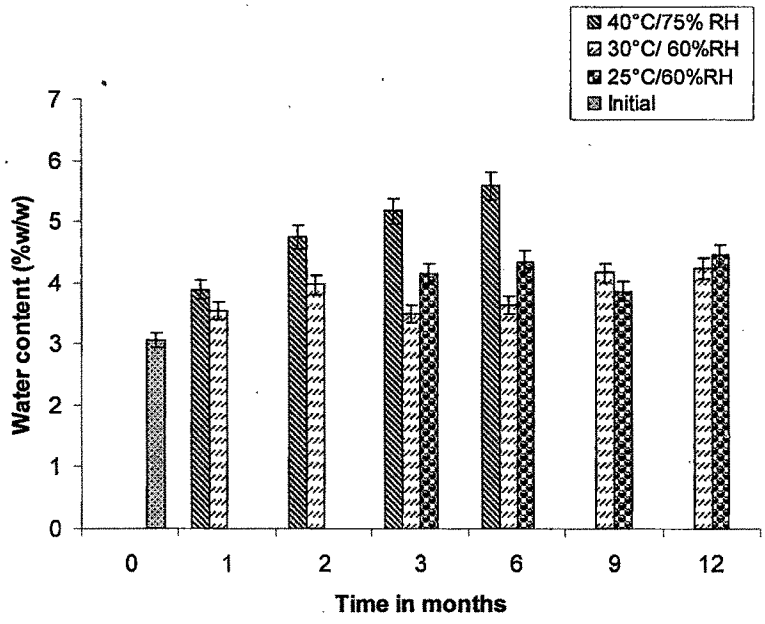


(A)

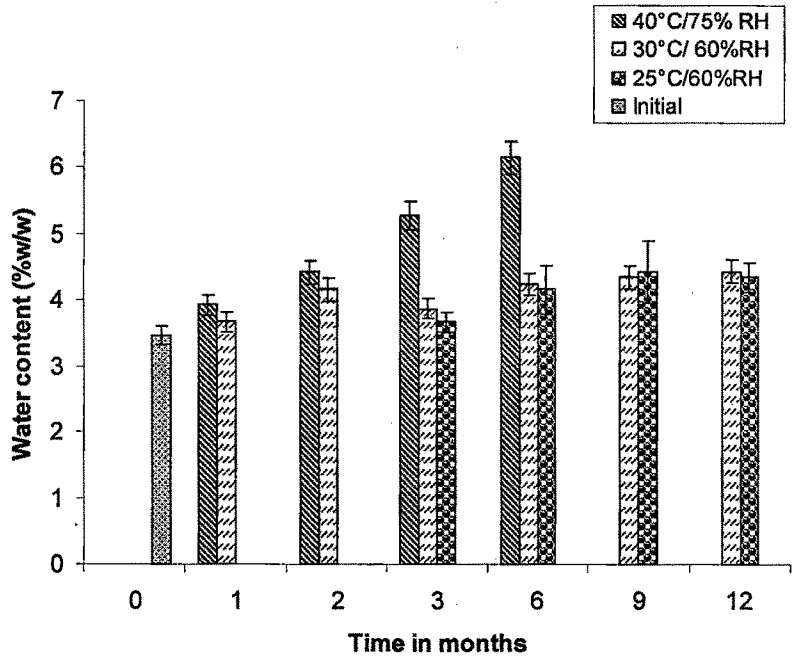


(B)

Figure 6.1 **Stability profile of Assay content of TB (A) and AMK (B) ALLP Vs. Time in months (Mean± S.D, n=3).**

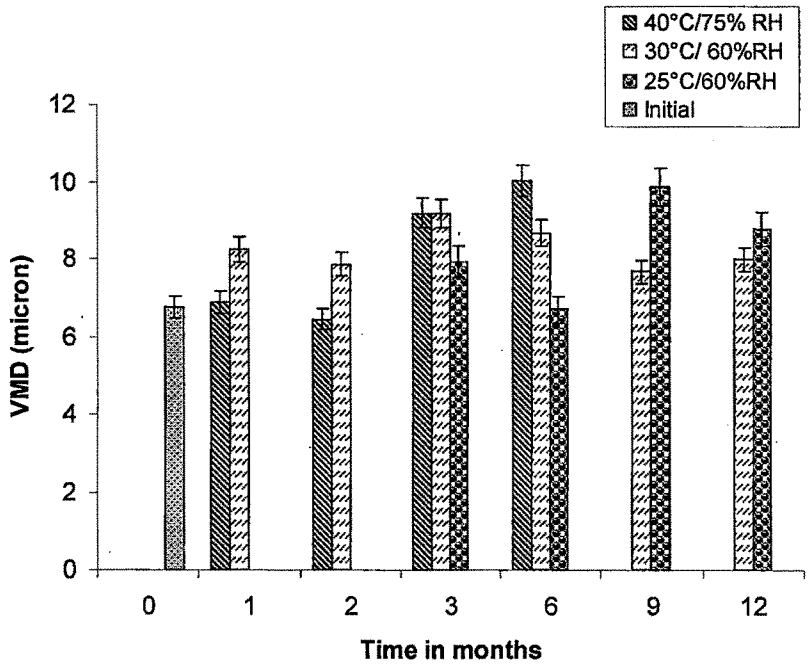


(A)

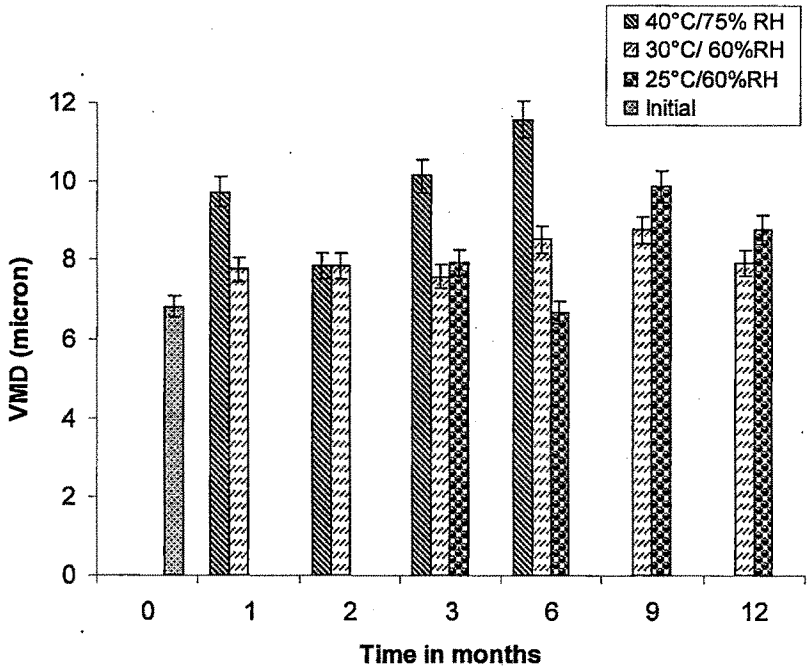


(B)

Figure 6.2 Stability profile of water content (% w/w) of TB (A) and AMK (B) ALLP Vs. Time in months (Mean± S.D, n=3).

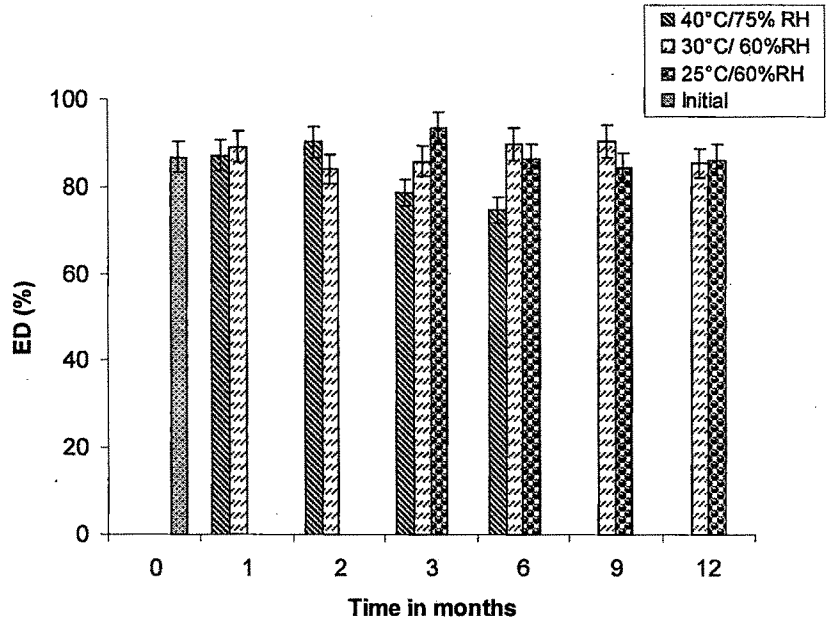


(A)

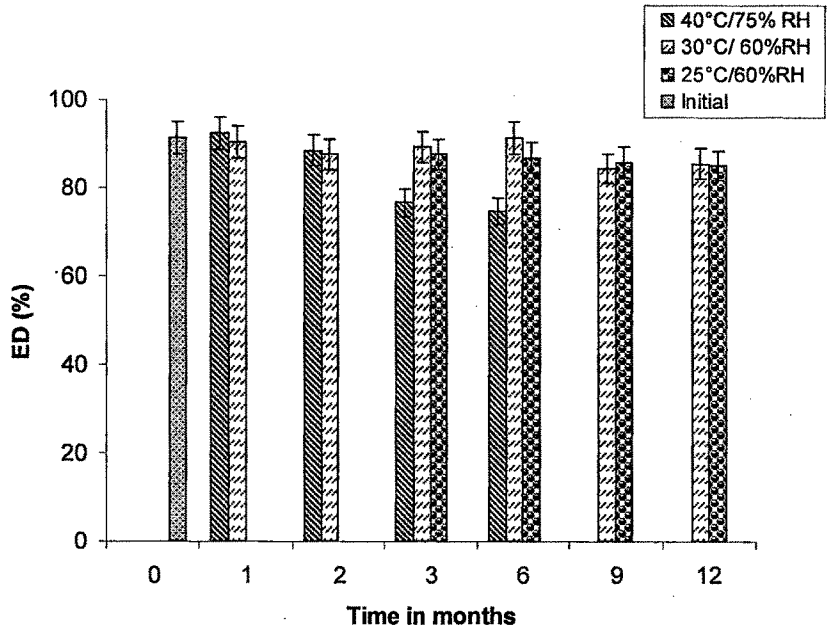


(B)

Figure 6.3 Stability profile of volume mean diameter (µm) of TB (A) and AMK (B) ALLP Vs. Time in months (Mean± S.D, n=3).

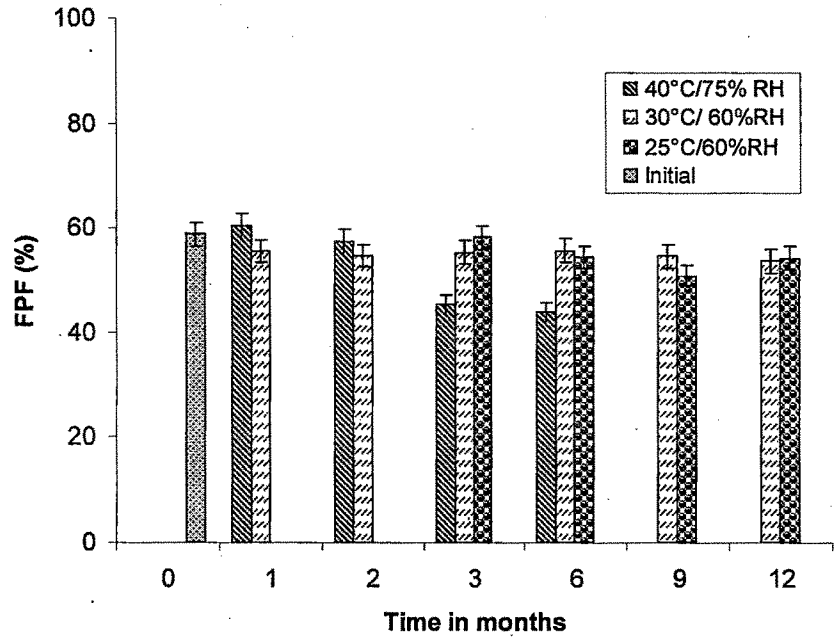


(A)

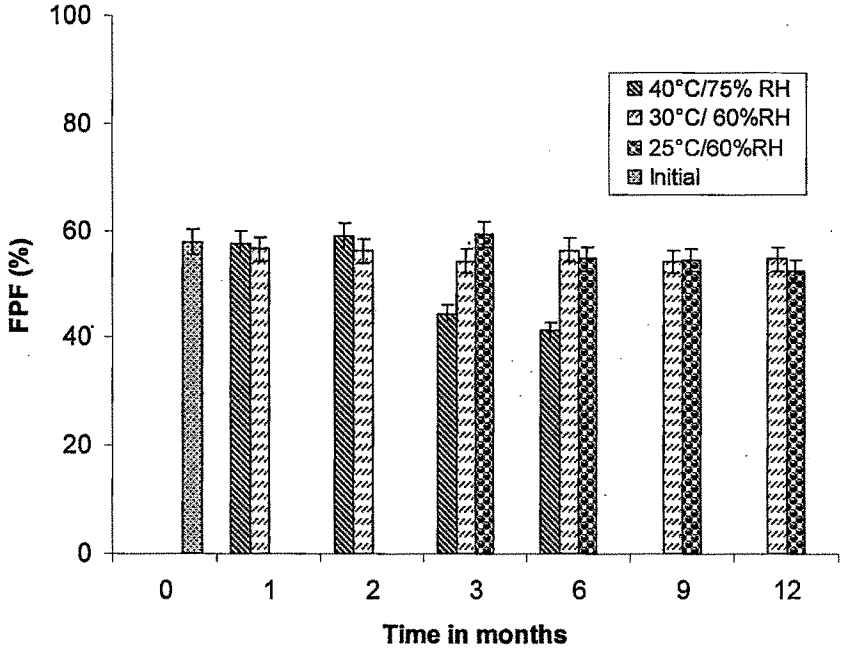


(B)

Figure 6.4 Stability profile of emitted dose (%) of TB (A) and AMK (B) ALLP Vs. Time in months (Mean± S.D, n=3).



(A)



(B)

Figure 6.5 Stability profile of fine particle fraction (%) of TB (A) and AMK (B) ALLP Vs. Time in months (Mean± S.D, n=3).

6.5 REFERENCES

Draft Guidance for Industry, Stability Testing of Drug Substances and Drug Products. FDA, Rockville,MD, June 1998.

Guidance for Industry, Q1A (R2): Stability Testing of New Drug Substances and Products, International Conference on Harmonization (ICH),.November 2003, http://www.fda.gov/cber/gdlns/ichstab.pdf.

Guidance for Industry, Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products, Chemistry, Manufacturing, and Controls Documentation, FDA, Rockville,MD, November 1998.

Vanbever R., Mintzes J., Wan J., Nice J., Chen D., Batycky R., Langer R., and Edwards D. A. Formulation and physical characterization of large porous particles for inhalation. Pharm. Res. 1999; 16: 1735–1742.