CHAPTER 5

STABILITY STUDIES

INTRODUCTION

Stability of a drug has been defined as the ability of a particular formulation, in a special container, to remain within its physical, chemical, therapeutic and toxicological specifications. The period of stability of a pharmaceutical preparation may be defined as the time from the date of manufacture of the formulation until its chemical or biological activity is lost below 90 % of labeled potency and its physical characteristic have not changed appreciably or deleteriously.

Through the stability studies, the formulation pharmacist determines the means by which optimum stability of the final product may be achieved. The formulated products contain besides active ingredients, some fillers, matrices, preservatives and other adjuvants which may have unforeseen adverse effects on the stability of active ingredients. In addition, the stability of these adjuvants themselves may require considerations.

Accordingly the physical stability is of importance for four primary reasons;

- ***Appearance :*** a pharmaceutical product is expected to look fresh, elegant and professional no matter how long it stands on the shelf. Any slight change in physical appearance such as colour fading or haziness may cause a loss in competence in the formulations.
- ***Uniformity:*** since most products are sold in multiple doses in a single container, the manufacturer must ensure that the patient will receive proper amount of the ingredient in each dose. A cloudy solution or a broken emulsion can lead to a non-uniform dosage.
- ***Availability:*** the active ingredient must be available to the patient throughout the expected shelf-life of the preparation. A breakdown in the physical system can lead to non-availability of the medication.
- ***Therapeutic activity:*** changes in the physical characteristics may affect the therapeutic activity of the material.

MATERIALS AND METHODS

Based on the results of the in-vitro drug release studies, optimized formulations were found to provide the required oral controlled drug delivery. Accelerated stability studies were carried out on the optimized formulations. To access the accelerated stability, various optimized formulations were selected and stored at 40 ± 2°C and 75 ± 5% RH for three months and were observed for physical change (appearance) and drug content. The

method of stability studies was in conformity with the recommendations in WHO document pertaining to stability testing of products intended for global market (Mathews, 1999) and ICH guidelines. Various optimized formulations from each system design (SPOP, MOTS and CPOP) were selected for the studies. The samples were withdrawn periodically and evaluated for physical change (appearance) and drug content. At the end of the storage period, the formulations were subjected for drug release studies. (Mathews, 1999; Verma et al., 2003)

Determination of physical change (appearance) and drug content :

The selected formulations were packed in strips of 0.04 mm thick aluminium foil laminated with PVC. The packed formulations were stored for 3 months in ICH certified stability chambers (40 °C / 75 % RH). The samples were withdrawn every thirty days and evaluated for the appearance, hardness, burst strength and drug content. Drug content was determined by specific methods such as UV spectrophotometry and HPLC given in chapter 3 for the respective drug.

In-house Specifications and Limits

For determining the physical changes and drug content, in-house specifications were generated as mentioned below

	Parameter	Limits
1.	Appearance	Off-white, round, biconvex tablets with plain surface on both sides
2.	Hardness	± 0.5 mm of mean of initial observation
3.	Burst strength	± 30 g of mean of initial observation
4.	Drug content	Not less than 95.00 % w/w and not more than 105.00 % w/w, calculated on as is basis.

Determination of drug release during and after completion of storage period

After the completion of storage period (90 days) the samples were withdrawn and evaluated for the drug release. In vitro drug release from the formulations was studied as per method mentioned in chapter 3 for the respective drug.

Statistical analysis

The physical changes were compared with each other statistically to find the significant difference. Student's "t" test with Welch correction was employed for each data set by using Graphpad-Instat software (Graphpad Inc., CA, USA).

RESULTS AND DISCUSSION

Based on the results of in vitro drug release studies, optimized formulations were found to provide the required oral controlled drug delivery and hence stability studies were carried out by storing the formulations at 40 °C / 75 % RH for 3 months (Climate zone IV conditions for accelerated conditions-ICH guidelines) to assess the long term stability. During and at the end of the storage period, studies were conducted on different optimized formulations to assess their stability with respect to their physical appearance, hardness of core tablets, burst strength, drug content and drug release characteristics.

Physical Stability and Drug Content

All the formulations during and after storing at 40 °C / 75 % RH for 3 months showed no change in physical appearance (Table 5.1). Slight variation in the hardness was observed with all the core tablet formulations however the difference was statistically insignificant ($P>0.05$). The burst strength values during and after storage were higher than the reported values of mechanical destructive forces in the GIT (Kamba et al., 2000) ensuring the formulations to be intact in the GIT without any incidence of dose dumping. The drug content was also found to be within the pharmacopoeial limits during and after the storage period.

Any variations observed were within the limits hence the formulations were found to be stable in terms of drug content and physical appearance.

Drug release characteristics

Drug release properties of various optimized formulations were determined during and after complete storage period for three months. The release profiles obtained before , during and after the storage period are depicted in Fig. 5.1-5.6. The release profiles were compared by calculating the similarity ($f1$) and difference ($f2$) factor (Table 5.2).

Fig 5.1. In-vitro release profile of Venlafaxine HCl (Batch No. VD) before and after storage for 3 Months at 40° C / 75 % RH

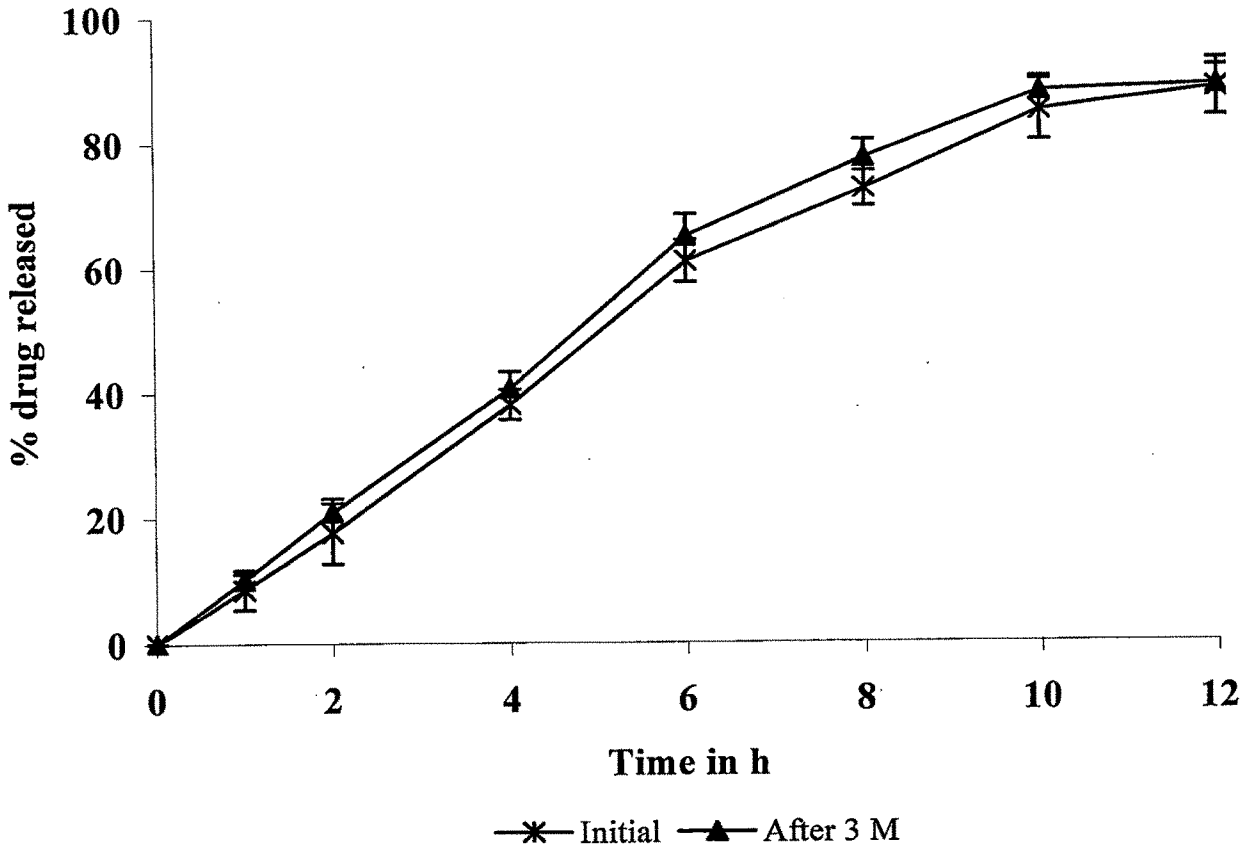


Fig 5.2 In-vitro release profile of Glipizide (Batch No. GD) before and after storage for 3 Months at 40° C / 75 % RH

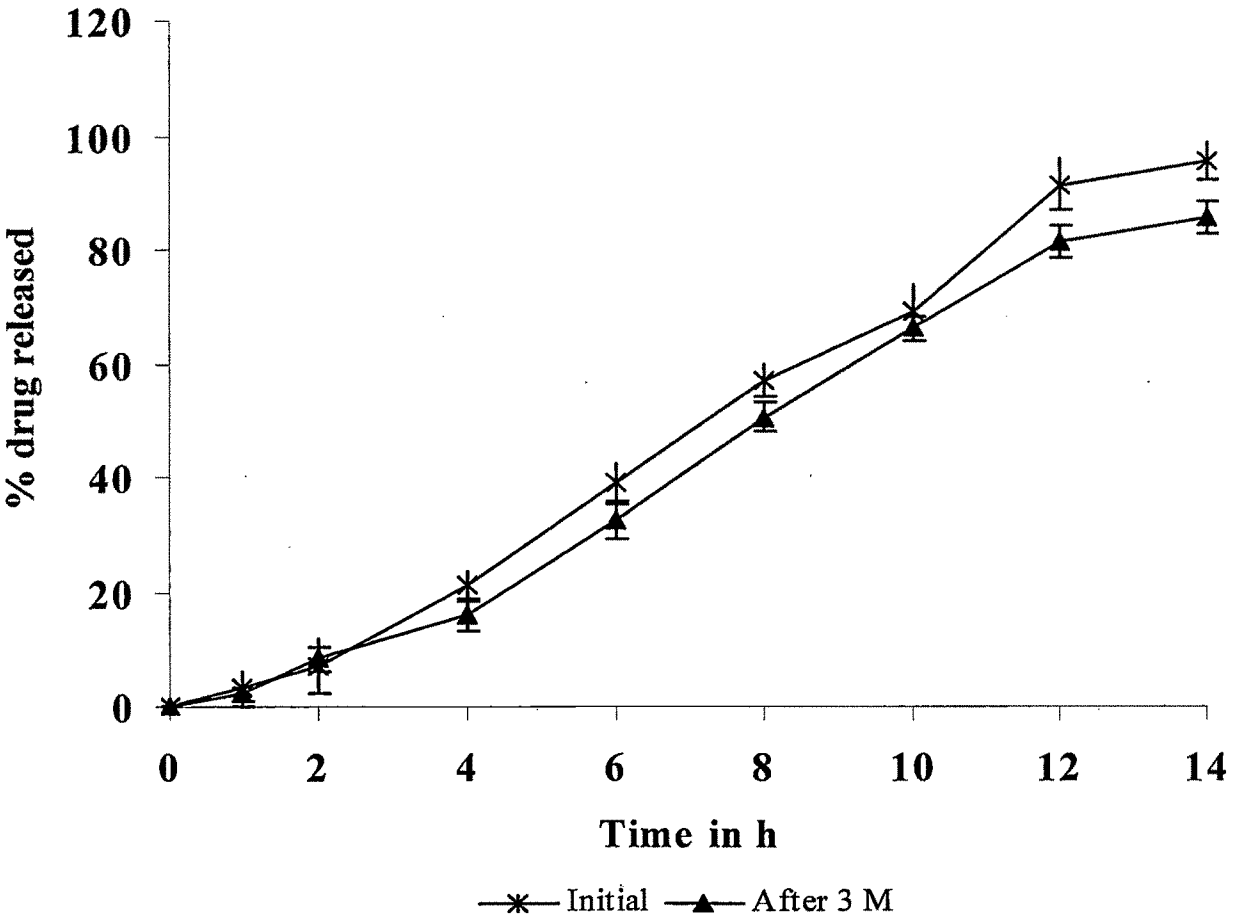


Fig 5.3 In-vitro release profile of Metoprolol tartrate (Batch No. ME) before and after storage for 3 Months at 40° C / 75 % RH

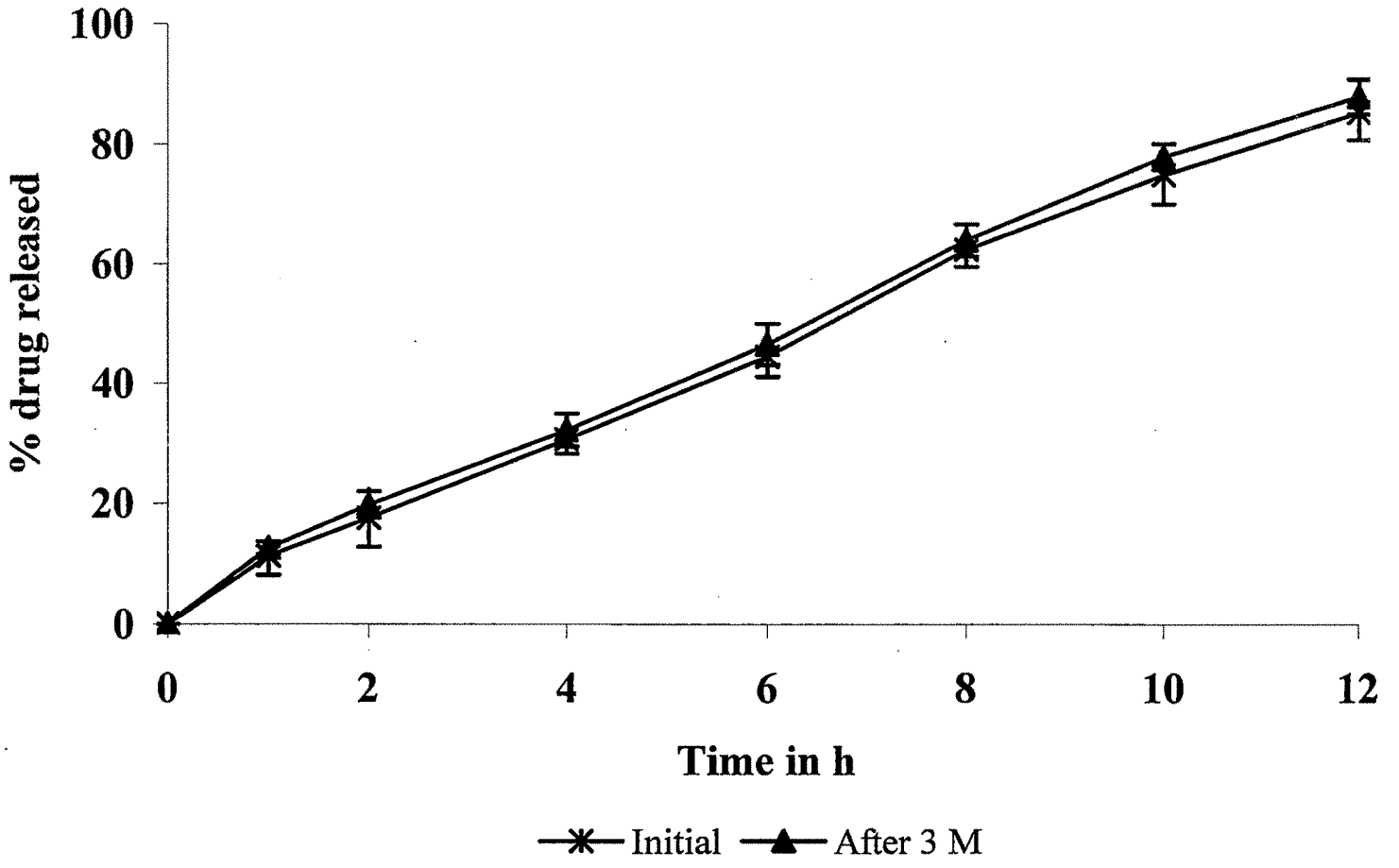


Fig 5.4 In-vitro release profile of Nifedipine (Batch No. ND) before and after storage for 3 Months at 40° C / 75 % RH

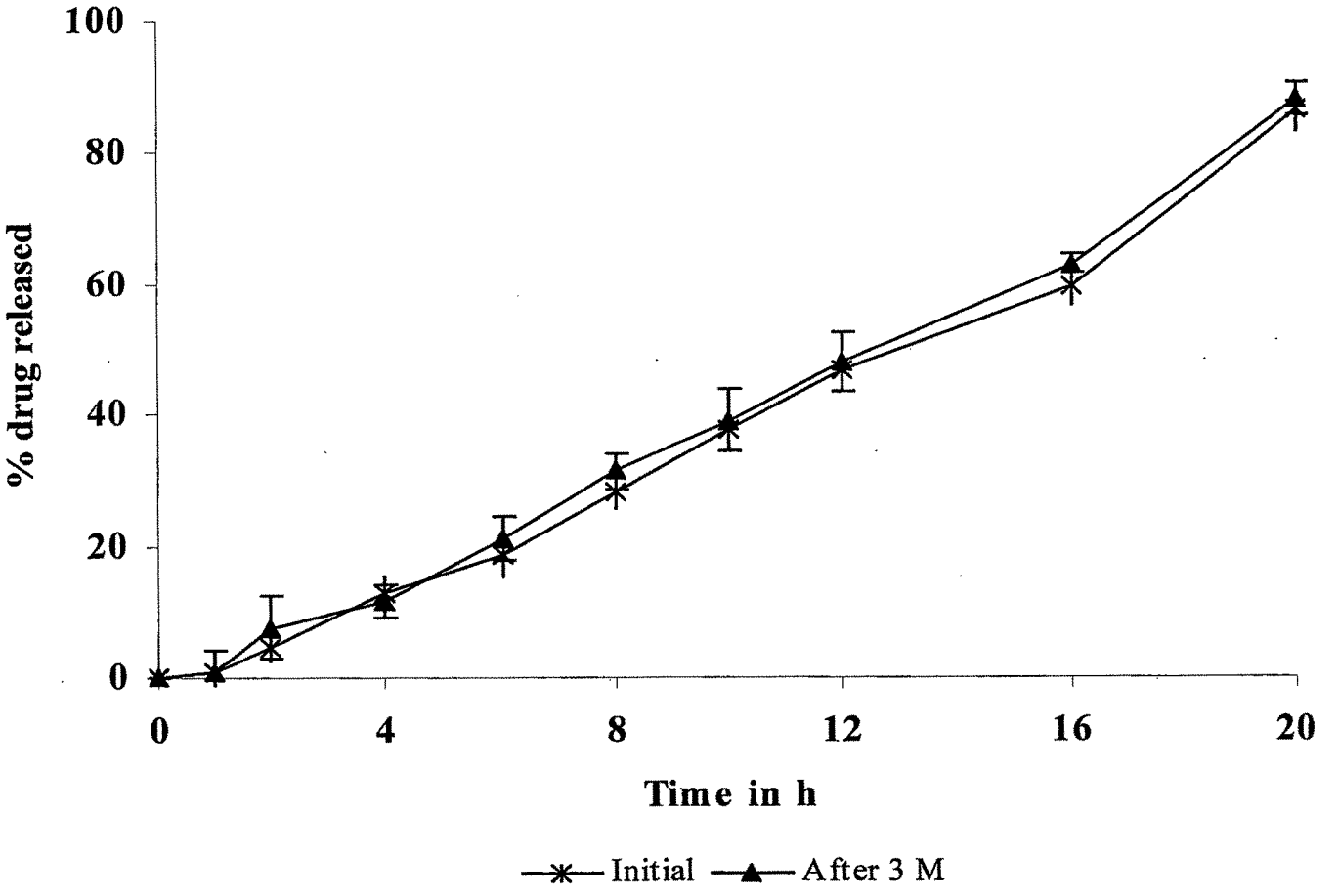


Fig 5.5 In-vitro release profile of Oxybutynin chloride (Batch No. OC) before and after storage for 3 Months at 40° C / 75 % RH

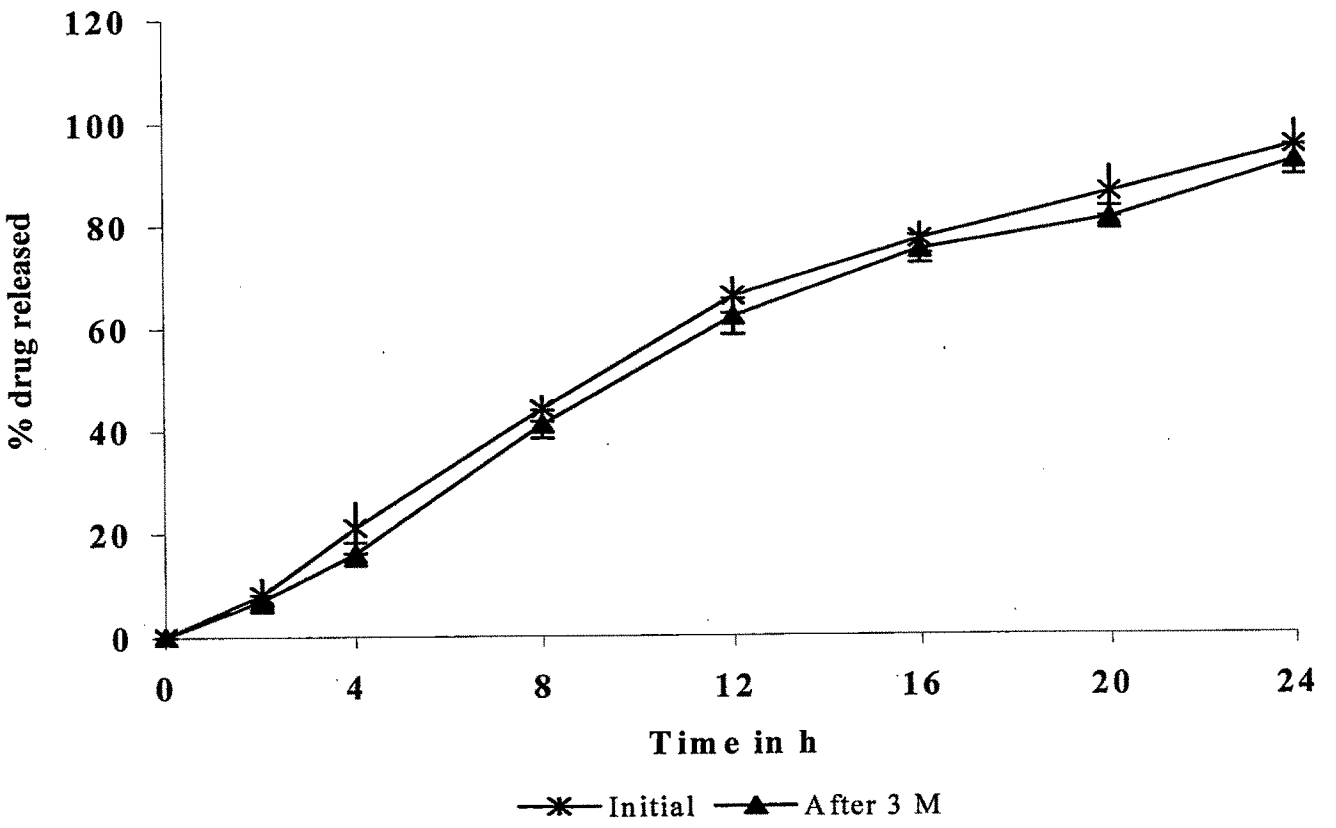
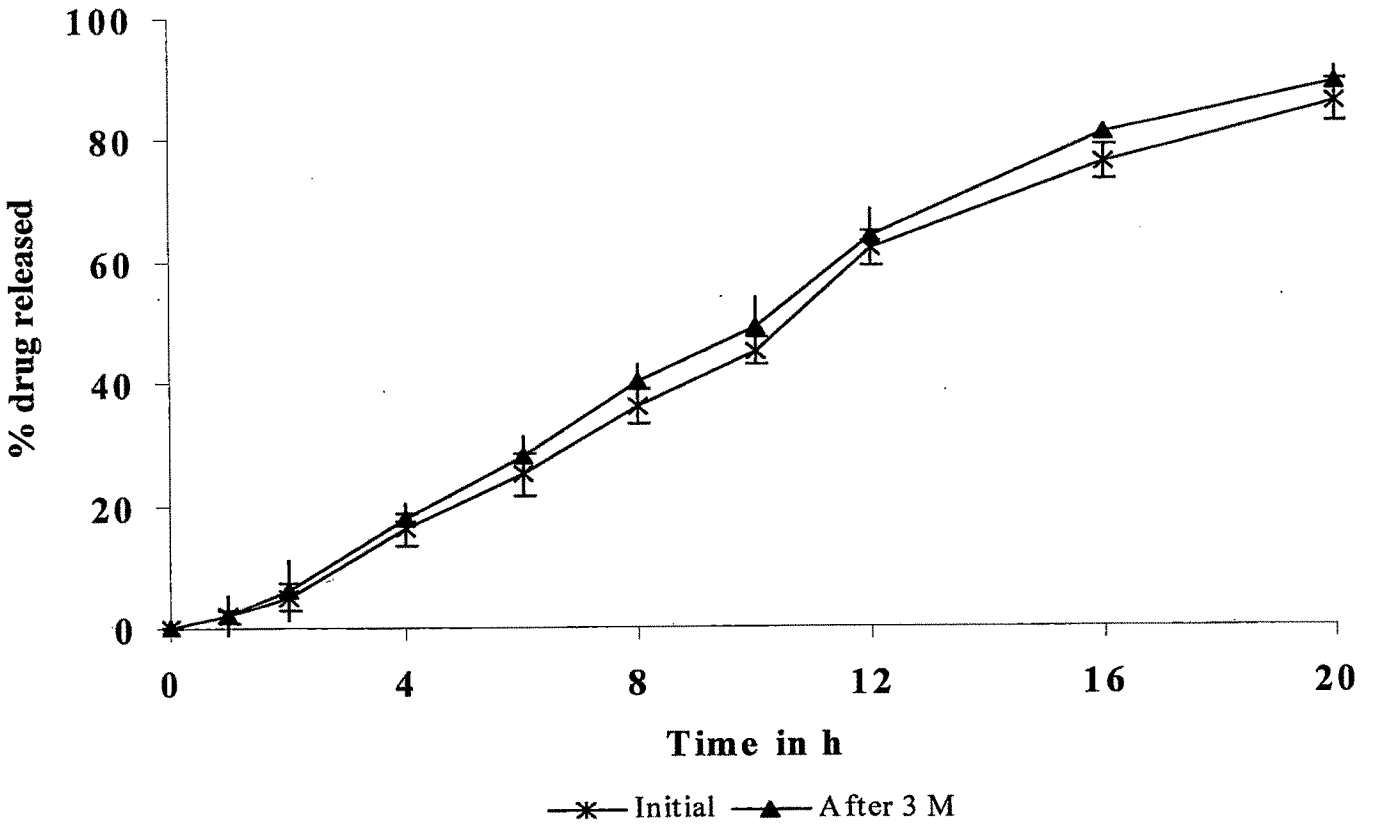


Fig 5.6 In-vitro release profile of Atenolol (Batch No. AD) before and after storage for 3 Months at 40° C / 75 % RH



While calculating the f_1 and f_2, initial analysis was taken as a reference and the 1M, 2M and 3M data was taken as test. While comparing the 1M and 3M data, it was observed that the *f1* value increased and *f2* value decreased for all formulations. However the values were within the limit of < 15 and > 50 for f_1 and f_2, respectively.

Table 5.1 Evaluation of optimized formulations during three months of storage at accelerated stability conditions (40 °C / 75 % RH)

Parameters	Time (days)	Venlafaxine (VD/VIII)	Glipizide (GD/VIII)	Metoprolol tartrate (ME/IV)	Nifedipine (ND/IV)	Oxybutynin chloride (OD/III)	Atenolol (AD/III)
Physical appearance	Initial	Off-white, round, biconvex tablets with plain surface on both sides	Off-white, round, biconvex tablets with plain surface on both sides	Off-white, round, biconvex tablets with plain surface on both sides	Off-white, round, biconvex tablets with plain surface on both sides	Off-white, round, biconvex tablets with plain surface on both sides	Off-white, round, biconvex tablets with plain surface on both sides
	30	No change	No change	No change	No change	No change	No change
	60	No change	No change	No change	No change	No change	No change
	90	No change	No change	No change	No change	No change	No change
Hardness of core tablets (kg/cm^2)	Initial	7.5 ± 0.4	6.1 ± 0.4	5.7 ± 0.2	8.8 ± 0.2	5.2 ± 0.8	4.6 ± 0.6
	30	7.4 ± 0.3	5.9 ± 0.2	5.6 ± 0.4	8.7 ± 0.2	4.8 ± 0.6	5.1 ± 0.3
	60	7.5 ± 0.2	6.2 ± 0.3	5.8 ± 0.4	8.8 ± 0.4	5.2 ± 0.4	4.8 ± 0.4
	90	7.3 ± 0.5	6.3 ± 0.4	5.5 ± 0.3	8.5 ± 0.5	5.4 ± 0.6	5.6 ± 0.7
Burst strength (g)	Initial	308 ± 5	357 ± 5	351 ± 9	354 ± 8	336 ± 6	312 ± 2
	30	296 ± 6	344 ± 2	355 ± 7	347 ± 6	351 ± 4	328 ± 5
	60	312 ± 8	358 ± 7	360 ± 10	356 ± 9	366 ± 9	336 ± 5
	90	318 ± 4	349 ± 3	348 ± 8	359 ± 7	337 ± 10	324 ± 4
Drug content (%)	Initial	99.87 ± 3.45	96.04 ± 3.52	100.57 ± 2.45	99.94 ± 2.23	101.28 ± 1.88	101.76 ± 4.45
	30	95.27 ± 2.28	94.73 ± 2.11	97.63 ± 1.81	100.34 ± 3.33	98.78 ± 2.61	99.49 ± 2.48
	60	96.26 ± 3.17	98.17 ± 2.98	99.61 ± 2.84	96.49 ± 3.78	96.18 ± 3.48	97.99 ± 1.78
	90	98.85 ± 3.76	97.76 ± 3.76	96.27 ± 1.89	98.67 ± 3.47	97.65 ± 2.74	97.67 ± 2.79

Table 5.2 Comparison of release profile *

Product	1 M		2 M		3 M	
	f_1	f_2	f_1	f_2	f_1	f_2
Venlafaxine	8.49	63.79	9.48	61.48	10.76	60.79
Glipizide	6.78	71.16	7.86	68.47	7.97	67.49
Metoprolol tartrate	9.74	66.68	9.88	64.28	10.64	61.85
Nifedipine	9.49	69.13	10.16	64.89	11.48	62.23
Oxybutynin chloride	12.45	62.49	12.14	63.48	13.66	61.13
Atenolol	6.49	71.49	6.99	69.37	7.19	67.46

* Reference: initial analysis

Conclusion:

It can be concluded from the results of accelerated stability studies that the optimized formulations were found to be stable and consistent in terms of Physical Parameters as well as Release profile. The optimized formulations could provide a minimum shelf life of one year.