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FORMULATION AND EVALUATION OF DIMETHYL FUMARATE DELAYED RELEASE CAPSULES

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ABSTRACT

The main objective of the present study is to prepare robust and stable formulation and evaluvation of dimethyl fumarate delayed release enteric coated tablets in tablets in capsules. Since Dimethylfumarate degrades in the acidic environment, it is important to bypass the acidic pH of the stomach. protection of drug from acidic environment is done by coating the drug with enteric polymers by using suspension layering technique in fluidized bed process (FBP) with different enteric polymers like Methacrylic acid copolymer type A, Tri ethyl citrate, sodium lauryl sulphate. The Dimethl fumarate delayed release capsules used for treatment of multiple sclerosis patients with relapsing forms were prepared. The chemical structure of Dimethylfumarate was characterized by $_1H^1$ NMR and FTIR spectroscopy. Then Dimethyl fumarate (DMF), an anti-inflammatory drug choosen as a model drug &prepared as capsules in three steps: Formulation of Dimethylfumarate core tablets by direct compression method, Enteric coating of Dimethylfumarate core tablets, Filling of enteric coated tablets in to capsule shells. The delayed release Dimethyl fumarate delayed release capsules were characterized for particle size, solubility, in-vitro, in-vivo targeting studies The delayed release capsule formulation F3, F4, F5, was in size range of 524,534,535 µm respectively. The delayed release capsules were targeted for drug release was delayed from stomach to intestine so that the capsules when placed in the 0.1N HCl no drug release was observed, finally the release of drug

observed in phosphate buffer pH-6.8 in the 5 formulations F3, F4, F5 were showed good delayed release of drug. Because of that delaying of drug Dimethyl fumarate the capsules were used to treat sclerosis.

Keywords: Dimethyl fumarate, Delayed release, Multiple sclerosis

INTRODUCTION

Multiple sclerosis is also known as disseminated sclerosis or encephalomyelitis disseminate, is an inflammatory disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged **[1]**. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of signs and symptoms including physical, mental and sometimes psychiatric problems MS takes several forms, **[2, 3]** with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms) **[4-7]**. Between attacks, symptoms may disappear completely; however, permanent neurological problems often occur, especially as the disease advances. MS is the most common autoimmune disorder of the central nervous system. As of 2010, the number of people with MS was 2–2.5 million per globally, with rates varying widely in different regions formulated dimethyl fumarate delayed release capsules. In this formulation dimethylfumarate powder directly filled in the capsule shell **[8-12]**. So the drug release from the shell is immediately done. So the gastric irritation problems occurred and also the drug target ing not properly done here. so by avoiding this problem formulated dimethy fumarate capsule for treating multiple sclerosis half life is 1hr.The starting dose for dimethyl fumarate is 120mgtwice a day orally after 7 days the dose should be increased to maintenance dose of 240mg twice a day **[13-15]**.

MATERIALS

The materials selected for the preparation of capsules Dimethyl fumarate was a gift sample from Hetero labs limited, Micro crystalline cellulose, cross carmellose sodium, Talc, colloidal Silica, magnesium stearate, Triethylcitrate, Methacrylicacid Simeticone Polysorbate80 Gelatin Titanium dioxide was analytical purity and used as purchased from Lobachemie, Mumbai

Formulation of capsules:-

Preparation of Enteric coated tablets:-

Procedure:-

Dimethyl fumarate, crosscarmellose sodium, talc, silica colloidal anhydrous were mixed together to form a blend. The blend was then passed through sieve of 800 microns. Add microcrystalline cellulose and mix, add magnesium stearate and mix well.

Compression:-

The blend is then compressed on a suitable rotary tablet press equipped with multiple

tolling (16 multitiple tooling) having 2mm concave tips.

Coating 1:-

The resulting 2mm micro tablets are coated with a solution of methacrylic acid. Methyl methacrylate copolymer & Triethyl citrate in isopropanol.

Coating 2:-

The coated micro tablets are then coated with a second layer of coating consisting of methacrylicacid, Ethacrylic acid copolymer, polysorbate 80, SLS, Triethyl citrate, simethicone & talcum micronized suspended in water

Quality control of capsules:-

Whether capsules are produced on a small scale or large scale all of them are required to pass not only the disintegration test, weight variation test and percentage of medicament test but a visual inspection must be made as they roll off the capsule machine onto a conveyor belt regarding uniformity in shape, size, color and filling. As the capsules moves in front of the inspectors the visibly defective or suspected of being less than the perfect are picked out. The hard and soft gelatin capsules should be subjected to following tests for their standardization.

1. Shape and size
2. Color
3. Thickness of capsule shell
4. Leaking test for semi-solid and liquid ingredients from soft capsules
5. Disintegration tests
6. Weight variation test
7. Percentage of medicament test

Disintegration test:

For performing disintegration test on capsules the tablet disintegration test apparatus is used but the guiding disc may not be used except that the capsules float on top of the water **[9]**. One capsule is placed in each tube which are then suspended in the beakers to move up and down for 30 minutes, unless otherwise stated in the monograph. The capsules pass the test if no residue of drug or other than fragments of shell remains on No. 10 mesh screen of the tubes.

Weight variation test:

20 capsules are taken at random and weighed. Their average weight is calculated, then each capsule is weighed individually and their weight noted. The capsule passes the test if the weight of individual capsule falls with in 90-110% of the average weight. If this requirement is not met, then the weight of the contents for each individual capsule is determined and compared with the average weight of the contents.

The contents from the shells can be removed just by emptying or with the help of small brush. From soft gelatin capsules the contents are removed by squeezing the shells which has been carefully cut. The requirements are met if (1) not more than 2 of the differences are greater than 10% of

the average net content and (2) in no case the difference is greater than 25%.

Content uniformity test: This test is applicable to all capsules which are meant for oral administration. For this test a sample of the contents is assayed as described in individual monographs and the values calculated which must comply with the prescribed standards **[9]**.

Invitro release of drug from Dimethylfumarate delayed release capsules:-

Invitro release of drug from Dimethyl fumarate delayed release capsules was determined as follows Transfer 500 ml of 0.1 N HCl into each of 6/12 dissolution vessels. place one capsule into each jars set parameters as mentioned in acid stage & start apparatus run for 2 hr, after 2 hrs in 0.1 N HCl, discord 0.1N HCl & careful transfer 500 ml of phosphate buffer pH 6.8& run apparatus at 100 Rpm for specified time, withdraw 10 ml of sample from each vessel. Filter through 0.45 μm filtrate. The amount of Dimethylfumarate was evaluated by UV-spectroscopy at 210 nm.

***In Vivo* Pharmacokinetic Study:**

The pharmacokinetic study was approved by the Chalapathi Institute of Pharmaceutical Sciences (approval number:CIPS/06/1987/2021). Twelve rabbits weighing 2.25 ± 0.22 kg (divided into two groups) were fasted over night. The optimized formulation and marketed formulation (tablet) were administered orally via gastric intubation. The first group received marketed formulation and second group received delayed release capsules (F). Rabbits were kept in rabbit restainers during blood sampling. Blood samples were collected from ear vein at pre determined intervals of 1, 2, 4, 8, 12, 16, 20, and 24hr into heparinized tubes. The collected blood samples were centrifuged at 3500rpm for 5mins. The collected plasma was stored at 4ºC and frozen at -71ºC until further analysis. A sensitive HPLC method was used for analysis of plasma. Pharmacokinetic analysis was done by using software PK solutions 2.0 TM.

Stability studies:

The optimized formulation were kept under accelerated storage conditions 40 ± 2 °C and 75 ± 5% relative humidity according to ICH guidelines using a stability chamber (Thermolab, Mumbai) for a period of three months. The samples were withdrawn at predetermined time intervals and evaluated for drug content and physical parameters.

RESULTS & DISCUSSION

Characterization of dimethyl fumarate delayed release capsules:-

1) Solubility:

The % solubility of dimethyl fumarate in methanol and Water is 32.5%, Ethanol 45.7%, Methanol 59.4%

2) FTIR:-

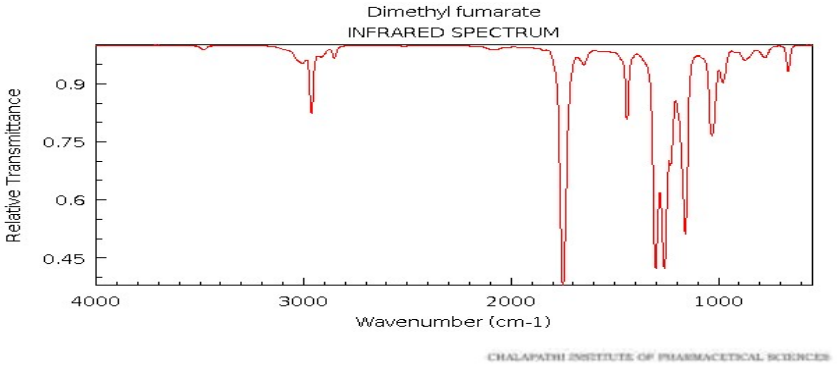

Figure 1: IR spectrum of Dimethylfumarate

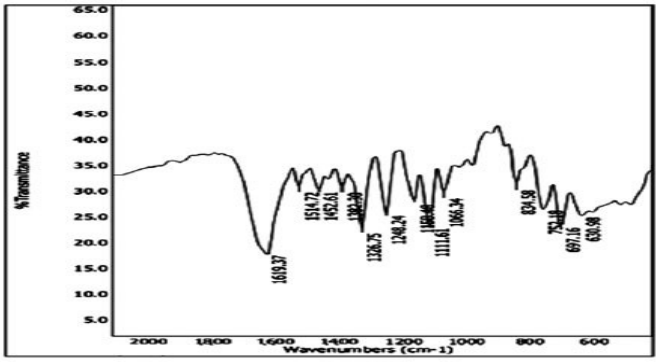

Figure 2: FTIR studies of dimethyl fumarate

3) MASS SPECTRUM:

Mass spectrum of dimethyl fumarate Molecular weight:114

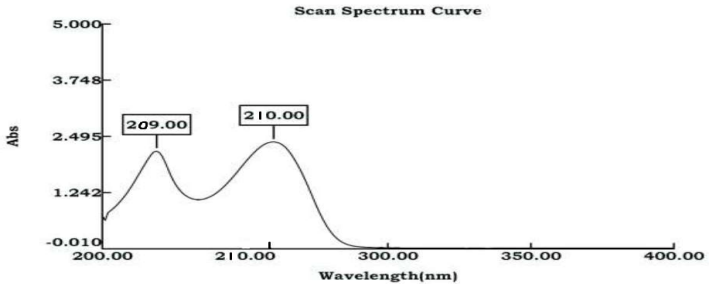

Figure 3: Scan spectrum of dimethyl fumarate

Table 1: Calibration table of Dimethyl fumarate

S. No.	CONCENTRATION (µg/ml)	ABSORBANCE			ABSORBANCE $\overline{X}$ ± S.D
		TRAIL 1	TRAIL 2	TRAIL 3	
1	0	0	0	0	0
2	5	0.141	0.140	0.141	0.141±0.015
3	10	0.259	0.258	0.258	0.258±0.023
4	15	0.387	0.390	0.388	0.399±0.025
5	20	0.510	0.510	0.511	0510±0.027
6	25	0.629	0.630	0.628	0.629±0.031
7	30	0.772	0.772	0.772	0.772±0.032
8	35	0.883	0.882	0.883	0.883±0.035

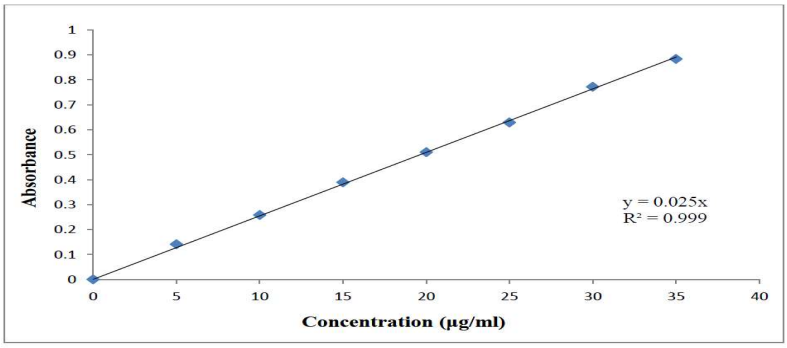

Figure 4

Table 2: *In vitro* release profile of dimethyl fumarate % drug released

S. No.	Time(mins)	% Drug release $\overline{X}$ ± S.D
1	0	0
2	5	10.8±0.013
3	10	21.5±0.012
4	20	34.4±0.017
5	30	47.3±0.021
6	45	78.2±0.025
7	60	83.6±0.031
8	90	89.4±0.032
9	120	95.8±0.035

Table 3: *In vitro* Drug Release Profile of Dimethyl fumarate delayed release capsules

Time (hrs)	% Drug released $\overline{X}$ ± S.D				
	F1	F2	F3	F4	F5
1	0	0	0	0	0
2	12.5±0.012	15.9±0.014	13.9±0.012	10.5±0.02	9.9±0.0016
3	24.6±0.014	32.3±0.015	29.3±0.025	19.7±0.022	16.7±0.027
4	42.7±0.021	59.2±0.014	52.3±0.028	37.8±0.032	28±0.031
5	78.8±0.032	85.6±0.023	87.6±0.035	64.7±0.035	85.8±0.035
6	88.9±0.027	87.7±0.035	93.5±0.039	82.8±0.036	95±0.038

Table 4: Mathematical model fitting of invitro release data of dimethyl fumarate delayed release capsules

s.no	Formulation code	Zero Order		First Order		Higuchi model		Korsmeyer peppas model	
		K_o	R^2	K_1	R	K_H	R	N	R
1	F1	2.632 ±0.012	0.961 ±0.013	0.051 ±0.012	0.915±0 .01	13.82 ±0.013	0.952±0 .02	0.732±0 .08	0.905± 0.06
2	F2	2.87 ±0.013	0.975 ±0.015	0.057 ±0.015	0.945±0 .06	15.32 ±0.019	0.975±0 .05	0.657±0 .09	0.953± 0.09
3	F3	3.185 ±0.023	0.982 ±0.016	0.075 ±0.019	0.925±0 .09	16.72 ±0.024	0.975±0 .06	0.681±0 .05	0.934± 0.03
4	F4	1.432 ±0.035	0.972 ±0.023	0.028 ±0.023	0.938±0 .04	10.65 ±0.034	0.962±0 .09	0.687±0 .08	0.901± 0.02
5	F5	1.643 ±0.037	0.978 ±0.032	0.041 ±0.032	0.954±0 .02	12.45 ±0.035	0.966±0 .05	0.712±0 .02	0.926± 0.05

Table 5: Evaluation of enteric coated tablets which placed in capsules

S. No.	Parameters	Results ($\overline{X}$ ± S.D)
1	Bulk density	0.541 ±0.01
2	Tapped density	0.927 ±0.014
3	Compressibility index	8.43± 1.62
4	Hausner's ratio	1.09± 0.01
5	Weight variation	5.5%
6	Friability	0.283± 0.08

DISCUSSION

Characterization of DMF delayed release capsules:

The enteric coated tablets in capsules prepared were compiled with the In-house specifications mentioned. These values of percentage of drug content indicated that the drug was uniform and resistant to acidic environment in the batch of enteric coated tablets in all cases. From the results above, these values of percentage of drug content indicated that the drug was uniform and resistant to acidic environment in the batch of enteric coated pellets in all cases.

Drug coating:

Drug coating was performed on the core tablets by using suspension layering technique. The lab scale batches (n=5) with batch size of 3000 capsules were developed using different binders, namely HPMC 5cps and HPC and varying binder concentrations. The drug coated tablets were analyzed for their *invitro* drug release in buffer (6.8pH phosphate). As per In- house specifications, the *invitro* drug release of 90% or above in 15 minutes is considered as the optimized formula. About 72% of drug release was seen in F1 formulation in 15 minutes, but this formulation was not

taken up for further experiment because of processing problems and aggregate formation, which led to decreased drug release. Thus problem was addressed by optimizing the binder concentration. The concentration of binder was then reduced to 12% from16% in F2 formulation to address the processing problems encountered in F1. Although the percentage drug release was improved compared to F1 formulation, the processing problems were not overcome. When the binder concentration was further reduced to 8% in formulation F3, it was observed that the lump formation could be avoided but the required binding capacity was not observed in the tablets. In these formulations the percentage drug release was found to be 86% in 15 minutes. F4 formulation was found to achieve 88% drug release by using combination of binders. No significant change in drug release was observed compared to F3 formulation. The F3 formulation using methacrylic acid copolymer A was found to have achieved a drug release of 95% in 15 minutes and the desired drug release profile was achieved. Hence this formulation was chosen as the optimized formulation to be taken up for further coating stages.

Enteric coating*:* Enteric coating was optimized by comparing the parameters like assay, acid resistance and dissolution of the enteric coated tablets with that of reference. Enteric coating formulations were optimized based on the above results mentioned

Effect of Enteric Coating: methacrylic acid copolymer A was used as enteric polymer in f1 formulation which showed very rapid

drug release compared to the reference. So in and F2 & F3formulations another enteric polymer methacrylic acid C was used. The drug release was found to be rapid in F2. Rapid release was observed with barrier coating B1 and slow release was observed with barrier coating B2.

Effect of Solvents: In F3 formulation, combination of methacrylic acid, metyl methacrylate in 1:1 ratio and solvent Triethyl citrate & isopropanol (1:1) and barrier coating B2 was taken. Here the drug release profile obtained was close to reference. In F4 formulation, the same formula with another solvent polysorbate-80 & water (1:1) was taken and checked for drug release profile. Reduced drug release was observed. in this formulations of 5 the F3 and F5 were showed release near to the reference.

In vitro dissolution profiles of F1-F5 formulations and Reference, the results indicated that the dissolution profile of F5 formulation is found to be similar to that of the reference standard and therefore chosen as the optimized formulation. The percentages of Dimethylfumarate released from F5 and the reference in 0.1 N HCl at 120 min are 1.0% and 1.3% respectively. From the results, it is clearly evident that F5formulation has shown highest similarity factor f2, compared to other formulations, which indicates that the closeness between reference and test is more and hence this formulation (F5) is considered as Optimized formulation.

FT-IR:- of pure drug and Formulation, the FT-IR spectrum of the formulation showed the presence of the drug in its active form without alteration of its chemical structure. The following important FTIR bands of the drug remain intact in both the spectra of the drug and formulation1760–1690:- C=O stretch carboxylic acids 1710–1665:- C=O stretch α,β –unsaturated aldehydes, ketones, 1680–1640:- –C=C– stretch alkenes, 1320–1000:- C–O stretch alcohols, carboxylic acids, esters, ethers 1715:- C=O stretch ketones, saturated aliphatic. These characteristic bands are present in both the FTIR spectrum, confirming the presence of the drug in its original structure in the formulation and preserving drug efficacy.

Scanning electron Microscopy: figures of optimized formulation (F5), the coated tablets appeared the average size of the tablets was 534 µm. Results of measurements such as bulk density, tapped density, compressibility index and Hausner's ratio are represented in the table. From the results Optimized formulation F5 showed good flowable properties.

Kinetic models for Optimized formulation: Kinetic models for Reference capsules, it was observed that formulation was following First order kinetics and it complies with reference as mentioned in the

table below. The formulation shows highest R2 for first order kinetics.

***Invitro* dissolution profile:** of optimized formulation at 40oC/ 75% RH and also there was no significant change observed in case of Assay and Acid resistance when compared with Initial samples. Thus, it means that formulation F3, F5 was found to be stable.

***In Vivo* Pharmacokinetic Study:**

The pharmacokinetic study was approved by the Chalapathi Institute of Pharmaceutical Sciences (approval number:CIPS/06/1987/2021). Twelve rabbits weighing 2.25 ± 0.22 kg (divided into two groups) were fasted overnight. The optimized formulation and marketed formulation (tablet) were administered orally via gastric intubation. The first group received marketed formulation and second group received delayed release capsules (F). Rabbits were kept in rabbit restainers during blood sampling. Blood samples were collected from ear vein at pre determined intervals of 1, 2, 4, 8, 12, 16, 20, and 24hr into heparinized tubes. The collected blood samples were centrifuged at 3500rpm for 5mins. The collected plasma was stored at 4ºC and frozen at -71ºC until further analysis. A sensitive HPLC method was used for analysis of plasma. Pharmacokinetic analysis was done by using software PK solutions 2.0 TM.

Stability:

The optimized formulation was kept under accelerated storage conditions 40 ± 2 °C and 75 ± 5% relative humidity according to ICH guidelines using a stability chamber (Thermolab, Mumbai) for a period of three months. The samples were withdrawn at predetermined time intervals and evaluated for drug content and physical parameters.

CONCLUSION:

The present study showed that the anti inflammatory drug Dimethyl fumarate (DMF) can be formulated in the capsule form. FTIR spectrum analysis indicated that there were no interactions between the dimethyl fumarate and other reagents. The particle size, dissolution, solubility of dimethyl fumarate capsules can be altered by using different preparation methods. The % drug release of formulations F3, F4, F5 was obtained as 93, 87 & 95 respectively. The evaluation studies like weight variation, content uniformity, disintegrations were in the correct limits. The average diameter, % drug release was found to be good for the formulation F5 compared with other formulations. Hence formulation F5was selected for *in vivo* study. Finally it was proved that of formulation F5 was better as compared to formulation F3.

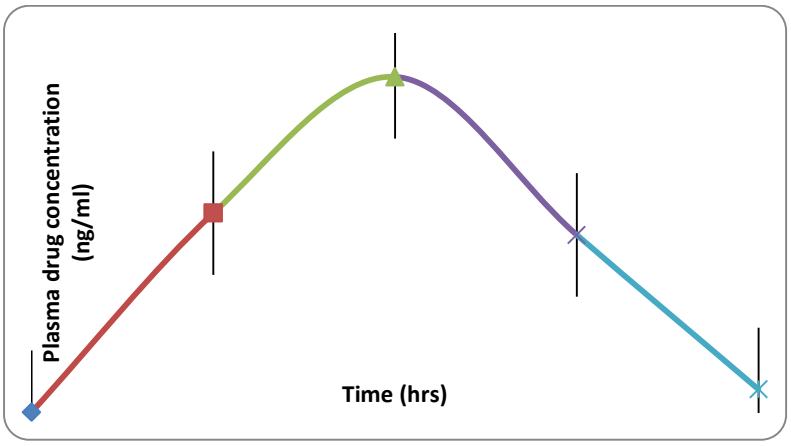

Figure 5: Plasma drug concentration vs Time profile of Marketed formulation

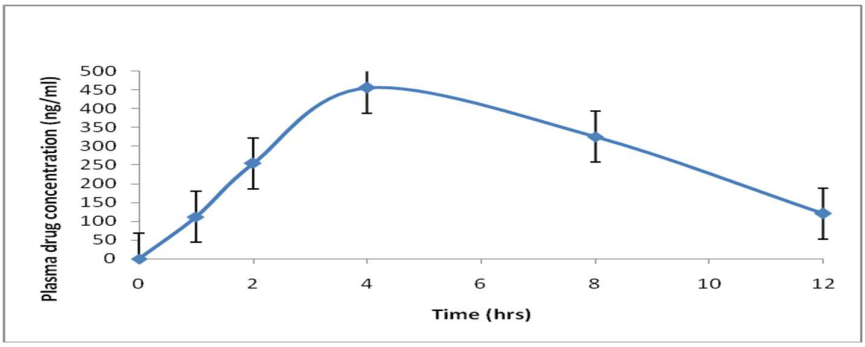

Figure 6: Plasma drug concentration vs Time profile of optimized formulation (F5)

Table 6: Pharmacokinetic parameters from plasma concentration- time profile:

Parameter	Marketed formulation	Optimized formulation (F5)
C_{max}(ng/ml)	324 ±22.13	441 ±24.35
T_{max} (hrs)	2 ±0.12	4 ±0.35
K_E (hr^{-1})	0.3465 ±0.87	0.088 ±0.14
$T_{1/2}$ (hr)	2 ±0.45	7 ±0.68
AUC (ng hr/ml)	3125 ±147.89	5489 ±385.41
MRT	2.6 ±2.3	8.7 ±3.65

In case of marketed conventional tablet, dimethyl fumarate was detectable in blood within 30 min after its oral administration in rabbits. The absorption was rapid with conventional tablets as indicated by low t_{max} value (2 h) in comparison with optimized formulation (F5) which exhibited delayed absorption as demonstrated by high t_{max} (4 h) values. C_{max} value of optimized formulation was high compared with marketed conventional tablet. In comparison, formulation F5 exhibited low elimination rate constant and high values of mean residential time (MRT). The low area under the curve (AUC) was observed with conventional tablets whereas the extended-release formulation showed high AUC values indicating increased bioavailability of the drug in the matrix tablet.

Accelerated stability studies conducted for the optimized batch of delayed release

capsules (F5) showed no change in drug appearance and assay after storage at 40 °C for 3 months. The drug content was 97% at the end of 90 days and appearance was unchanged indicating that the optimized formulation is fairly stable at accelerated storage condition.

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