Research Article

FORMULATION AND EVALUATION OF EXTENDED RELEASE DOSAGE FORM OF METFORMIN HYDROCHLORIDE USING A COMBINED HYDROPHOBIC AND HYDOPHILIC MATRIX: RATE OF *IN-VITRO* AND *IN-VIVO* RELEASE STUDIES

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ABSTRACT

Extended release formulation of metformin hydrochloride presents significant challenges due to its poor inherent compressibility, high dose and high water solubility. Extended release matrix tablets of metformin hydrochloride were formulated different combination of polymers in hydroxyl propyl methyl cellulose ($K_{100}M$) and ethyl cellulose (18 centipoise) (DRUG: HPMC: EC in the ratios of Formulations F_1 5:1:1:1, F_2 5:1.5:1.5 and F_3 5:2:2 respectively by direct compression method. The formulated powder blends were evaluated for compatibility (DSC), angle of repose, True density, bulk density, compressibility index and total porosity. The tablets were subjected to thickness, weight variation test, hardness test, friability test and drug content test. In-vitro release studies were carried out at pH 1.2 simulated gastric fluids for first 2h and followed by simulated intestinal fluid at pH 7.2 using the apparatus (basket) equipment as described in the USP dissolution monograph. The formulated powder blends showed satisfactory flow properties and drug content. The selected formulation further subjected to accelerated stability studies up to 12 and 6 months as per ICH guidelines at room and accelerated temperature and in-vitro and in-vivo release studies carried out formulation F_3 in Wistar albino rats to find out the reduction of blood glucose level using blood glucometer up to 10h. Tablet thus formulated provided extended release of metformin hydrochloride over a period of 12 h. Formulation F_3 was selected on the basis of t_{25}, t_{50} and t_{90} using ANOVA, paired t-test pharmacokinetic studies and compared with reference standard (marketed sustained release tablet) (F_4M).

Keywords: Metformin hydrochloride, matrix tablets, extended release, hydroxyl propyl methyl cellulose and ethyl cellulose.

INTRODUCTION

Metformin hydrochloride, an anti-diabetic drug lowers both basal and postprandial-elevated blood glucose in patients with non-insulin-dependent diabetes mellitus (NIDDM or type-II diabetes) whose hyperglycemia cannot be satisfactorily managed by diet alone. Some high incidence of concomitant GI symptoms, such as abdominal discomfort, nausea and diarrhea, many occur during the treatment. Administration of a extended release, once-a-day metformin hydrochloride dosage form could reduce the dosing frequency and improve patient compliance[1-2].

In spite of its favorable clinical response and lack of significant draw backs, chronic therapy with metformin hydrochloride suffers from certain problems of which the most prominent is the high dose (1.5 – 2.0 g/day) low bio-availability (60%) and high incidence of gastrointestinal tract (GIT) side effect (30%) case). Therefore, there were continued efforts to improve the pharmaceutical formulation of metformin hydrochloride in order to achieve an optimal therapy. These efforts mainly focus on extended release of drug including the sophisticated gastro retentive system [3-7].

Numerous studies have been reported in literature investigating the HPMC matrices to control the release of variety of drug from matrices[8-10]. Several authors have reported the use of ethyl cellulose matrices to control the release a variety of drugs [10-12]. Therefore, in this study, the hydrophobic (EC) and hydrophilic polymer (HPMC) alone/ in combination have been used as matrix material in order to get the required release profile of metformin hydrochloride.

MATERIALS

Metformin hydrochloride – USP was a gift sample from wockhard pharmaceuticals (Mumbai, India), hydroxyl propyl methyl cellulose (HPMC K100M) USP was obtained from shin-etsu, Chemicals Co.Ltd., (Tokyo, Japan). Ethyl cellulose (EC 18 centipoises) was procured from SD fine chemicals Ltd, (Mumbai, India). Microcrystalline cellulose powder I.P. was obtained from sigha ehichiro chemicals Pvt Ltd., (India), sodium chloride injection I.P. mound mettur pharmaceutical Ltd., (Tamilnadu, India). Alloxan, loba chemie (Bombay, India). All other chemicals and reagents used were of high analytical grade. Double distilled water was used for evaluation studies.

Machineries

Machineries and equipment used was tablet compression machine, (cadmech machinery Co. Pvt Ltd.,). UV-visible spectrophotometer, (Shimadzu 1700), six stage dissolution rate test apparatus IP/BP/USP, (tab machines), Monsanto hardness test apparatus, (Rollex pvt Ltd) India, B.S.Sieves, (Jaynant scientific) and tray dryer (Mumbai engineering works). Differential scanning colorimeter (perkin elemer DSC-7 model), Blood glucose monitoring system (smartcare TD-4227), saify traders (Indore, India).

Stability chamber environmental chamber. The Ileco, Chennai, India.

Animals

Wistar albino rats (200-250g) from Central Animal House, Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamilnadu, India were used in this study. The protocol for animal experiment was approved by Institutional Animal Ethics Committee which follows the norms of CPCSEA, India.

METHODS

Preparation of metformin hydrochloride extended release matrix tablets

Different tablet formulations (F_1 to F_3) were prepared by direct compression technique.[13] Ingredients required per tablet are given in Table no: 1 and tabulated as follows.

Table 1: Composition of tablet formulations F_1 to F_3

Ingredients (per tablet) mg	Formulations		
	F_1	F_2	F_3
Metformin hydrochloride	500	500	500
HPMC (K_{100} M)	100	150	200
Ethyl cellulose (18 cps)	100	150	200
Microcrystalline cellulose	75	75	75
Colloidal silicone dioxide (Aerosil)	0.006	0.006	0.006
Magnesium stearate	0.012	0.012	0.012

The metformin hydrochloride, HPMC (K_{100}M), EC (18 centipoises) and MCC powders were separately passed through mesh No.44. The powders were uniformly mixed in a double cone blender for 5 mins. Then the dried powders were lubricated with magnesium stearate and aerosil by mixing in a rapid mixer at slow speed for 5 mins, separately and compressed using 16/32 inch flat punches in cadmach tablet compression machine to get tablets.

Evaluation of powder blends

The formulated powder blends were evaluated for compatibility, angle of repose, bulk density, true density, percentage compressibility index and total percentage porosity [14-19].

Evaluation of tablets

The compressed tablets (formulations F_1 to F_3) and reference standard (F_4M) were tested for hardness, percentage friability, percentage weight variations and the percentage drug content [20-22].

In-vitro Release Studies

In-vitro dissolution studies were carried out using six stage dissolution rate test apparatus IP/BP/USP at 50 rpm. The dissolution medium consisted of simulated gastric fluid (pH 1.2 - acid buffer) (for first 2 h) and followed by in simulated intestinal fluid (pH 7.2 - Phosphate buffer) from 2 to 12 hours (900 ml), maintained at 37°±0.5°C[23-24]. Samples were withdrawn at predetermined time intervals and drug content was analyzed by UV visible spectrophotometer at 227.5 and 230 nm respectively compared with blank. The same procedure was followed to study the in-vitro release of metformin hydrochloride sustained release tablet (F_4M) (reference standard). All the release studies were conducted in triplicate and the mean values were plotted versus time with standard deviations less than 3 indicating the reproducibility of the results. Statistical calculation of ANOVA and t-test were used to find out best formulation [25-27].

In-vivo release studies

Diabetes was induced in healthy wistar albino rats of either sex weighing (200-250gm) by injecting a single Intraperitoneal injection of 150 mg/kg body weight of aloxan monohydrate. Blood glucose level was checked after 48h. Animal with blood glucose level greater than 250mg/dl were considered diabetic and were selected for our further study.

The rats were divided into 4 groups, each group having 6 rats and group-I animal served as normal control, they were not given any drug. The groups II, III and IV were diabetic rats. From the groups (II-IV), group II animal are diabetic control rats. The groups III and IV were given formulated metformin hydrochloride matrix tablet formulation F_3 and reference standard (F_4M) respectively in the form of suspension orally at a dose level of 450 mg/ kg body weight. On fasting blood samples were collected from the tail vein on 3rd day of each groups (I-IV) at 0, 1, 2, 4, 6, 8, 10 h, intervals. Glucose levels were estimated by using blood glucosemeter. Statistical comparisons with animal of non-treated groups of control I and II with treated groups were performed with student's t-test. Data's were expressed as mean ± standard error mean [28-32].

RESULTS AND DISCUSSION

Metformin hydrochloride is a highly water soluble drug. Its poor inherent compressibility coupled with high dose (500mg) posses a significance challenge for developing an extended release dosage form. For developing extended release matrix tablet with desirable drug release profile, cost effectiveness and broader regulatory acceptance combination of HPMC (K100M) and EC (18 CPS) was chosen as release controlling polymers.

Compatibility study of metformin hydrochloride by DSC

DSC thermograms of pure metformin hydrochloride, blend of polymer/polymers mixture with drug were determined (Figure: 1).

Figure: 1 compatibility study of metformin hydrochloride and polymer(s) by differential scanning calorimetry (DSC)

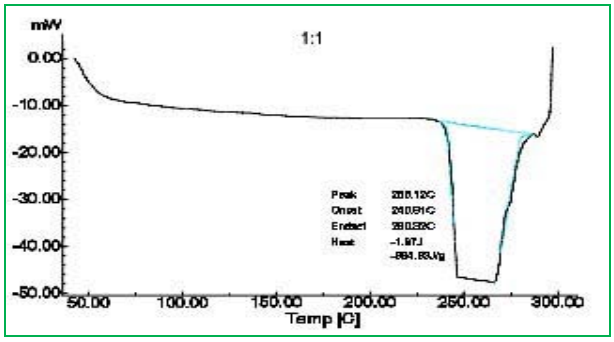

Fig: 1:1 metformin hydrochloride

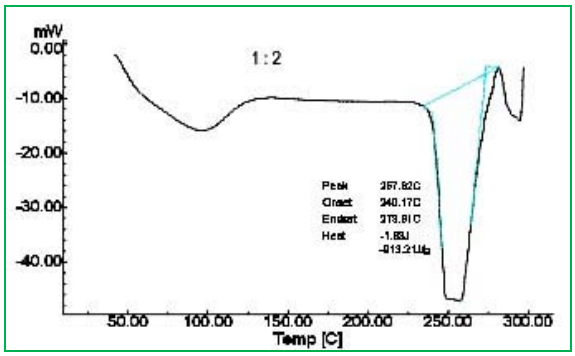

Fig: 1:2 metformin hydrochloride and hydroxyl propyl methyl cellulose

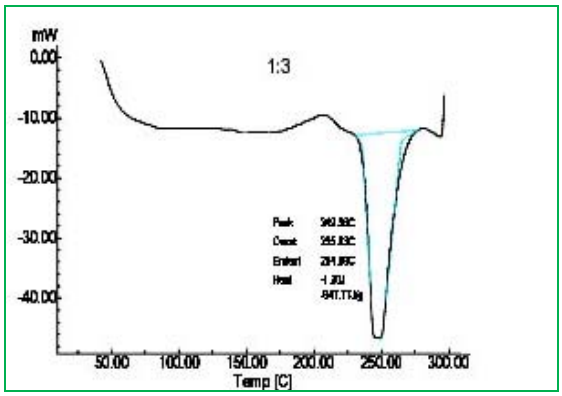

Fig: 1:3 metformin hydrochloride and ethyl cellulose

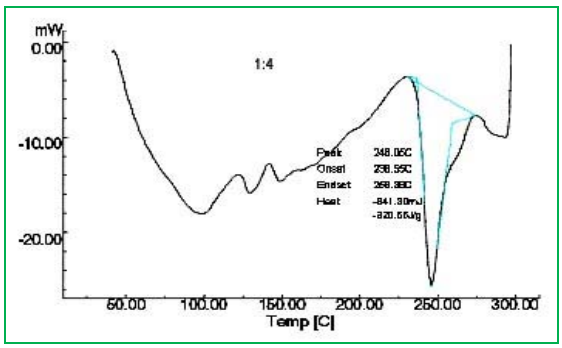

Fig: 1:4 metformin hydrochloride, hydroxyl propyl methyl cellulose and ethyl cellulose

The different in the peak areas in the thermograms of blends of drug in the polymer from that of pure drug is due to less quantum of drug in the blend. Absence of any new endothermic peak are disappearance of no shift of endothermic peak confirms that peak in thermo grams of pure drug and the blends of drug in the polymer confirms that there is no any interaction and hence the polymers are compatible with drug[14-15].

Evaluation of physical and chemical parameters of formulated powder blends

Physical parameters such as specific surface area, shape, hardness, surface characteristics and size can be significantly affect the rate of dissolution of drugs contained in a complex system. The formulated powder blends of different formulations (F1 to F3) were evaluated for angle of repose, true density, bulk density, compressibility index and total percentage porosity (Table No: 2).

The results of angle of repose (<30) indicated good flow properties of all the formulated powder blends except one formulation (F_1). The compressibility index value were recorded <15%, result in good to excellent flow properties in one formulation (F_3) supporting the angle of repose indicating good flow, which in rest of the formulations it can >15%. Formulated powder blends density; porosity and hardness are often interrelated properties and are likely to influence compressibility, porosity, dissolution profile and properties of tablets made from it. The percentage porosity value ranged from 24.31 to 31.25 indicating that the packaging of the powder blend may range from close to lose packaging and also confirming that the particle are not of greatly different sizes. Generally a percentage porosity value below 25% shows that the particles in the powders are of greatly different sizes and values greater than 48 % shows that particle in the powder are in the form aggregates of flocculates. All these results indicate that the formulated powder blends processed satisfactory flow properties and compressibility [16-19].

Evaluation of formulated tablets

The tablets of different formulations (F_1 to F_3) and reference standard (F_4M) were evaluated for various parameters viz., hardness, friability, percentage weight variation and percentage drug content. The results of these parameters are given in Table No: 2. the results are comparable with the standard products (F_4M) and also confirm with the official and OPPI standard for tablets [20-22].

In-vitro release studies

Results of the in-vitro release studies [23-24] of various formulations designed and manufactured along with reference standard formulations (a marketed sustained release product) are presented in Table No: 3. The graphical representation of the data presented in the figure: 2 [20-23].

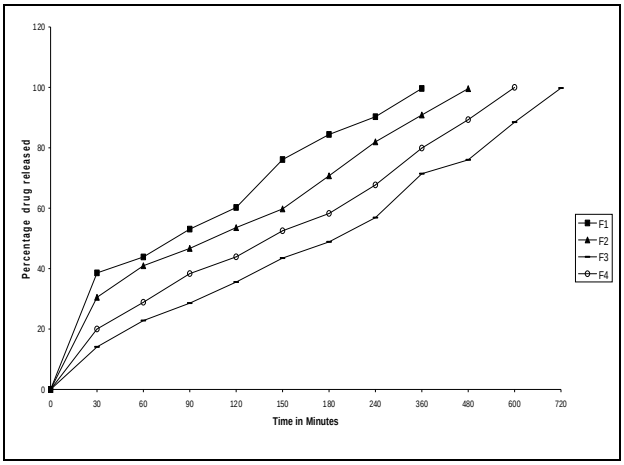

Figure 2: plot of In-vitro release profile in simulated gastric fluid (pH 1.2 - acid buffer) (for first 2 h) followed by simulated intestinal fluid (pH 7.2- phosphate buffer) for Metformin hydrochloride matrix tablet formulation F_1 to F_3 and reference standard (F_4M).

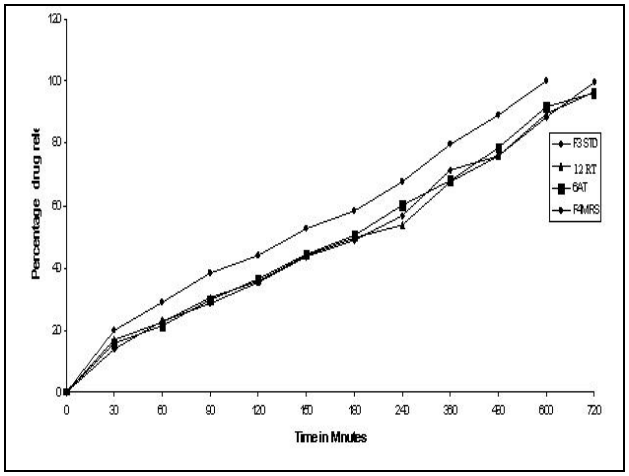

Figure 3: plot of stability studies on in-vitro release profile at room temperature (25°C±2°C and 60% ±5% RH) and accelerated temperature (40°C±2°C and 75% ±5% RH) in simulated gastric fluid (pH 1.2) (for first 2 h) followed by simulated intestinal fluid (pH 7.2) for Metformin hydrochloride matrix tablet formulation F_3 for 12 and 6 months respectively.

The plot of cumulative percentage In-vitro drug release profile of metformin hydrochloride from 3 formulations F_1, F_2 and F_3 made with different concentration and combination of hydroxy propyl methyl cellulose (HPMC) (K_{100}M) ethyl cellulose (EC) in simulated gastric fluid (pH 1.2 - acid buffer) (for first 2 h) followed by simulated intestinal fluid (pH 7.2- phosphate buffer) for 2 to 12 h is shown in figure: 2. It is found that the cumulative percentage drug release of the formulation, F_1 is faster than formulations F_2 and F_3, with formulation F_3 showing the slowest release. Release profile of F_3 is comparable to marketed sustained release products (F_4M) (reference standard). So, it can be inferred that the proportion of HPMC (K100M) is increased to release is retarded and Drug: HPMC: EC ratio of 5:2:2: is found to be optimum for comparable release profile with reference standard (Table No: 3)

Stability studies on In-vitro release

The selected formulation F_3 was subjected up to 12 and 6 months stability study as per ICH guidelines at room temperature (25^0C ± 2^0C at 60% ± 5% RH) and accelerated condition (40°C ±2°C at 75%±5% RH) respectively to find out the effect of aging on release pattern [33]. The result of the stability study does not indicate any significant alteration in the in-vitro release pattern of the drug from the tablets. The results are furnished (Table No: 4 & 5) and presenting graphically (Figure: 3).

Cumulative percentage release versus time plot drug release data from selected formulation F_3 after 6 months exposure to stability testing condition at room temperature and accelerated condition as per ICH guidelines in simulated gastric fluid (pH 1.2-acid buffer) (for first 2h) followed by simulated intestinal fluid (pH 7.2 phosphate buffer). The release data were shown in Table No: 4 and 5 and depicted in figure: 3. There is no significant difference in the in-vitro release formulation F_3 and reference standard (F_4 M) before and after stability studies.

All the In-vitro release data is applying by ANOVA [25-27], from the ANOVA table (Table No: 6 and 7) values, F calculated value is 0.007 and F table value is 2.27 at room temperature and F calculated value is 0.004 and F table values is 2.23 at accelerated condition were determined. It was inferred that F calculated value is less than F table value. There is no significance difference in the stability studies of the formulation. Therefore we can conclude formulation F_3 was selected as best formulation.

Comprehensive data of in-vitro release rate studies was showing t_{25} (time of 25% drug release), t_{50} (time of 50% drug release) and t_{90} (time of 90% drug release) values were determined of formulation F_3 at room and accelerated condition was shown in Figure: 4 and 5, (Table No: 8).

All the data of t_{25}, t_{50} and t_{90} in-vitro release rate study value is applying paired t-test (Table No: 9). There is no significant difference between room and accelerated condition at $p<0.001$ for formulation F_3. So, the formulation F_3 was selected as best formulation.

***In-vivo* Studies**

From the t-test, comparison of formulation F_3 and reference standard (F_4M), t calculated value > F table value (2.38>2.34), therefore rejected the null hypothesis. There is a significant difference between F_3 and F_4 M at $P < 0.001$ [29-32].

Compared to reference standard (F_4M) the formulation F_3 was superior one to produce maximum extended release to lower the blood glucose level in animal at the tested dose level (Table No: 10).

Table 2: Physical and chemical parameters of formulated metformin hydrochloride powder blends and compressed tablet formulations (F_1 to F_3) and reference standard (F_4M)*

Evaluation parameters	Formulations			
	F_1	F_2	F_3	F_4M
Angle of repose	30.43±0.11	25.02±0.38	22.94±0.12	-
True volume	4.40±0.01	5.10±0.01	5.68±0.02	-
Bulk density (gm/ml)	0.67±0.00	0.63±0.00	0.54±0.00	-
Compressibility Index (%)	15.93±2.97	16.67±3.61	12.68±0.05	-
Porosity (%)	27.26±0.08	22.68±0.05	18.33±0.12	-
Hardness (kg/cm^2)	8.67±0.57	7.67±0.57	8.67±0.58	8.00±1.00
Friability (%)	0.53±0.03	0.41±0.06	0.38±0.02	0.39±0.07
Weight variation (%)	0.79±0.11	0.79±0.13	0.73±0.13	0.51±0.06
Drug content (%)	99.10±0.20	99.40±0.76	100.20±0.53	100.40±0.35

*All values are mean ±S.D for n=6

Table 3: Comparative In-vitro release profiles on metformin hydrochloride formulations ($F_1$to F_3) and reference standard (F_4M)*

Time (h)	pH	F_1	F_2	F_3	F_4M
0.30	pH 1.2 (Simulated gastric fluid)	38.60±2.13	30.49±1.35	14.10±1.11	19.98±0.02
1.00		43.86±1.71	40.95±2.32	22.78±0.52	28.86±1.40
1.30		53.08±1.47	46.68±2.36	28.60±0.95	38.36±1.75
2.00		60.25±0.55	53.59±3.46	35.53±0.79	43.90±2.69
2.30	pH 7.2 (Simulated intestinal fluid)	76.08±3.38	59.79±1.91	43.49±0.67	52.51±1.32
3.00		84.4±2.77	70.73±1.66	48.85±0.44	58.27±2.12
4.00		90.26±1.46	81.95±3.37	56.86±1.14	67.72±2.69
6.00		99.62±0.62	90.85±2.22	71.43±0.79	79.90±0.78
8.00		-	99.57±0.57	76.03±1.67	89.32±2.12
10.00		-	-	88.48±1.92	100.01±0.45
12.00		-	-	99.78±0.17	-

*All values are mean ±S.D and % RSD for n = 6

Table 4: Stability studies of in-vitro release profiles on tablet formulation F_3 at room temperature (25°C ± 2°C at 60% ±5% RH) in the period of six months*

Time (h)	pH	initial	1st Month	3rd Month	6th Month	9th Month	12th Month
0.30	pH 1.2 (Simulated gastric fluid)	17.09±0.37	16.02±0.75	16.42±1.95	15.97±2.90	15.93±1.63	16.89±1.89
1.00		25.65±1.24	25.07±1.46	23.80±2.11	22.06±2.21	22.90±2.39	22.75±1.19
1.30		31.51±0.95	31.55±1.22	31.07±1.34	30.71±2.17	29.37±1.78	30.56±0.56
2.00		37.00±0.96	31.55±1.22	36.95±1.25	37.09±1.38	36.07±2.28	35.88±0.91
2.30	pH 7.2 (Simulated intestinal fluid)	42.67±0.81	45.63±1.27	43.28±2.18	43.22±2.99	43.25±1.35	43.95±1.71
3.00		46.84±1.80	47.95±1.08	49.16±1.33	50.77±1.80	49.26±1.31	49.72±2.21
4.00		54.56±1.21	53.82±1.79	54.86±2.39	55.62±1.17	54.63±1.92	53.97±1.80
6.00		70.67±1.40	76.61±2.10	68.72±2.42	62.64±2.69	65.48±2.43	67.74±3.11
8.00		80.56±1.30	81.35±1.11	77.69±3.41	74.99±4.65	77.19±3.32	75.83±1.75
10.00		89.97±0.80	87.63±0.93	88.79±3.42	88.48±2.86	89.01±1.63	89.39±3.77
12.00		99.74±0.72	99.52±0.65	99.35±0.31	98.02±0.56	97.65±0.70	96.74±0.33

* All values are mean ± SD and % RSD for n=6

Table 5: Stability studies of in-vitro release profiles on tablet formulation F_3 at accelerated temperature (40°C ± 2°C at 75% ±5% RH) in the period of six months*

Time (h)	pH	1st Month	2nd Month	3rd Month	4th Month	5th Month	6th Month
0.30	pH 1.2 (Simulated gastric fluid)	17.21±3.02	15.86±1.64	17.64±2.00	15.32±1.33	15.22±2.69	15.7±2.69
1.00		22.65±3.56	21.98±3.00	22.56±1.30	21.51±1.04	21.91±2.51	21.50±1.03
1.30		27.88±1.65	29.56±1.73	29.16±1.11	28.16±0.41	25.31±1.21	29.68±1.19
2.00		36.26±2.08	36.58±3.02	35.85±1.66	35.33±1.67	37.02±2.39	36.42±1.90
2.30	pH 7.2 (Simulated intestinal fluid)	42.64±2.99	40.84±1.50	43.23±2.07	42.98±3.71	42.60±2.19	44.42±2.95
3.00		49.18±2.12	51.58±3.00	48.67±1.49	49.24±1.74	49.89±1.14	50.53±2.70
4.00		56.85±2.33	58.11±2.19	56.21±3.31	59.2±4.38	57.7±1.08	60.27±2.63
6.00		69.31±2.31	72.11±1.84	70.44±2.19	70.60±1.91	68.02±2.10	67.97±3.49
8.00		74.76±3.67	78.22±1.49	78.78±1.74	76.86±2.19	77.85±3.05	78.60±4.07
10.00		84.83±1.38	87.75±4.02	87.11±3.29	88.25±2.98	90.80±1.49	91.79±1.78
12.00		99.87±0.39	99.48±0.67	99.41±0.43	97.69±0.44	97.29±0.87	96.46±0.34

* All values are mean ± SD and % RSD for n=6

Table 6: ANOVA table of comparative in-vitro stability studies after six month at accelerated temperature (40°C ±2°C at 75 % ± 5% RH) for formulation F_3

Source of Variation	Sum of square	Degree of freedom	Mean sum error	F calculated value	F table
Between formulations	26.62	5.00	5.32		
Within formulations	44089	60.00	734.81	0.007	2.37
Total	44115	65.00			

Table 7: Stability studies on in-vitro release profile of t_{25}, t_{50}, and t_{90} on formulation F_3 at room temperature and accelerated temperature in the period of 12 and 6 Months* respectively.

Period in Month	Room temperature (25°C±2°C at 60%±5% RH)			Period in months	Accelerated temperature (40°C±2°C at 75%±5% RH)		
	t_{25} (h)	t_{50} (h)	t_{90} (h)		t_{25} (h)	t_{50} (h)	t_{90} (h)
0 Month	0.56	3.12	10.01	1st month	1.12	3.09	10.45
1st Month	0.58	3.25	10.22	2nd month	1.26	2.57	10.18
3rd Month	1.06	2.59	10.18	3rd month	1.16	3.09	10.20
6th Month	1.08	2.58	10.26	4th month	1.28	3.08	10.20
9th Month	1.13	3.04	10.06	5th month	1.27	3.06	9.58
12th Month	1.12	3.09	10.08	6th month	1.18	2.59	9.15

*All values are mean for n = 6

Figure 4: Histogram of stability studies on In-vitro release profile at room temperature (25°C±2°C at 60% ±5% RH) of t_{25}, t_{50}, and t_{90} values for metformin hydrochloride matrix tablet formulation F_3 for 12 months

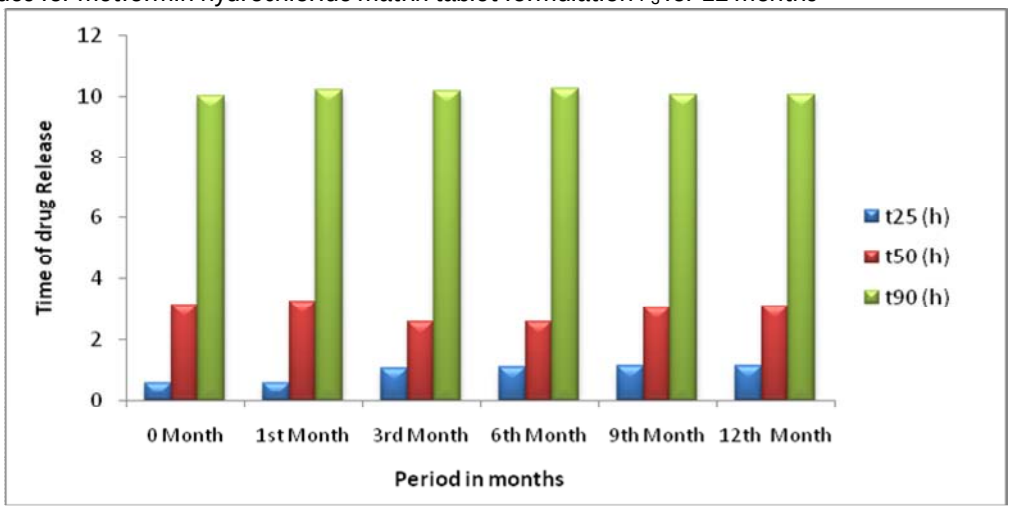

Figure 5: Histogram of stability studies on In-vitro release profile at accelerated temperature (40°C±2°C at 75% ±5% RH) of t_{25}, t_{50}, and t_{90} values for metformin hydrochloride matrix tablet formulation F_3 for 6 months.

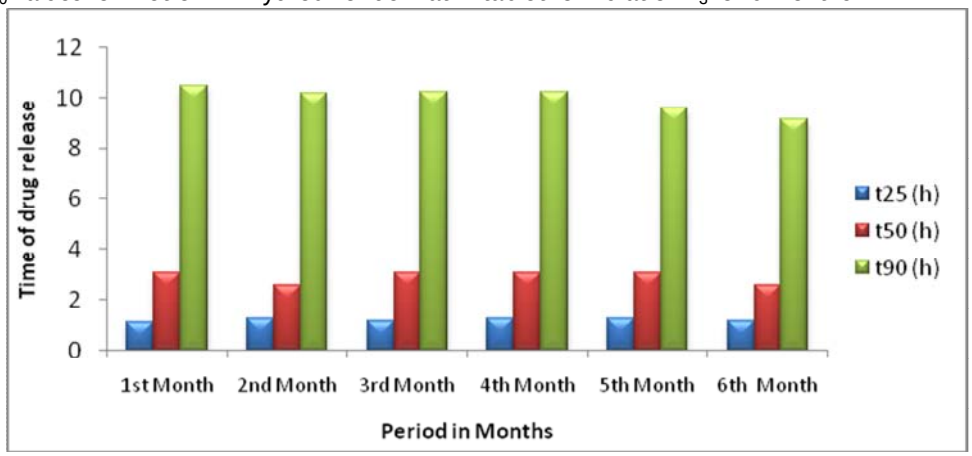

Table 8: Comparative rate of in-vitro release profile of t_{25}, t_{50} and t_{90} at room temperature (RT) (25°C ±2°C at 60% ± 5% RH) and accelerated temperature (AT) (40°C ±2°C at 75% ± 5% RH) for 12 and 6 months of stability studies using paired t-test for formulation F_3 respectively

Statistical parameter	Temperature and Rate of release					
	RT t_{25} (h)	AT t_{25} (h)	RT t_{50} (h)	AT t_{50} (h)	RT t_{90} (h)	AT t_{90} (h)
Mean	0.92	1.21	2.95	2.91	10.14	10.02
Variance	0.07	0.00	0.08	0.07	0.01	0.15
Degree of freedom	5.00		5.00		5.00	
t statistics	-2.71		0.16		0.77	
P(T<=t) one - tail	0.02		0.44		0.24	
t critical one - tail	2.02		2.02		0.02	
P(T<=t) two - tail	0.04		0.08		0.48	
t critical two - tail	2.57		2.57		2.57	

Table 9: t - test descriptive statistics for determination of blood glucose level by animal studies.*

Group	Treatment (dose, mg/kg)	Blood sugar in mg/dl (h) ±SEM						
		0	1	2	4	6	8	10
I	Normal (control)	99.67 ±2.26	100.12 ± 2.02	97.13 ± 1.89	102.44 ± 2.10	110.39 ± 2.31	95.91 ± 1.76	103.33 ±1.74
II	Diabetic control (Alloxan) (150)	502.17 ±1.38	509.06 ± 1.42	517.77 ± 1.33	522.88 ± 1.02	531.12 ± 1.18	536.46 ± 1.21	544.17 ±0.99
III	Formulation F_3 (450)	494.83 ±3.16	484.00 ±3.38	458.00 ±3.46	427.33 ±3.14	378.50 ±4.03	321.67 ±3.76	291.33 ±2.01
IV	Reference standard F_4M (450)	499.83 ±1.66	492.17 ±2.01	469.17 ±2.55	436.50 ±3.91	390.33 ±3.91	333.00 ±3.76	339.67 ±3.95

*All values are mean ± standard mean error for n = 6

Pharmacokinetic Studies

Five groups of Rabbit each contains three were used for the pharmacokinetic study. The groups were designated as follows

Group 1-Treated with Reference Standard (RS)

Group 2-Treated with Marketed product (F12M)

Group 3-Treated with Formulation F3 Dose: 400mg/kg

All the rabbits were fasted overnight. After collecting the zero hour blood sample of 1 ml (blank), the product in the study was administered orally in a capsule shell with 10 ml of water.

No food or liquid other than water was permitted until 4 hours following administration of the product. Blood sample were collected at 0.5, 1, 2, 4, 6 and 8 hr intervals from the marginal ear vein into heparinized tubes.

Table 10: Mean plasma drug concentration of metformin hydrochloride matrix tablet formulation of reference standard, F4M, F3

Parameters	RS	F4M	F3
Cmax (mcg/ml)	2.15	1.69	1.75
Tmax (hr)	2.00	2.00	2.00
AUC $^{0-t}$ (mcg-hr/ml)	4.06	5.37	5.46
Kel (hr $^{-1}$**)**	0.39	0.27	0.24
$t_{½}$ **(hr)**	1.77	2.56	2.97

Analytical procedure

The plasma was separated immediately by using cold centrifuge at 3000 rpm for 15 minutes and plasma was stored at -20°C until analysis.

Plasma Metformin concentration were determined by an HPLC method applying a Shimadzu HPLC system and Li chrospher 100 RP-18 column. The detection was at 234nm and phenformin was applied as the internal standard.

The mobile phase consisted of 0.01M Na_2HPO_4 solution (pH = 6.5), methanol and acetonitrile (20:3:6, v/v). The quantification limit was 100 ng/ml.

Sample extraction

100µl of metformin hydrochloride solution of appropriate concentration and 100µl of phenformin hydrochloride solution (20µg ml^{-1}) were added to 900 µl of drug free plasma contained in a clean 5ml Ria Vial and was properly mixed.

To this 50µl of protein precipitating agent (perchloric acid: acetonitrile 50%v/v each) was added and was vortexted for 30 seconds.

After centrifugation at 3000 rpm for 10 minutes, 700µl of the supernatant was evaporated to dryness at 45°C under nitrogen. The residue was reconstituted in 100µl of mobile phase and 20µl of this was injected to the HPLC system.

Method of validation

The linearity of the method was investigated by serially diluting a stock solution of metformin (in methanol; 1.0 mg/ml) with drug free plasma to concentrations in the range 30-5000 ng/ml and subjecting 100µl of each of these solutions to the proposed assay method.

Calibration curves were constructed by plotting the ratio of peak height of metformin to phenformin (Internal Standard) against the concentration of metformin added.

Analyte recovery was determined by comparing the ratio of peak height of metformin to internal standard for the standard preparations against those of same preparations in mobile phase.

CONCLUSION

Based on In-vitro and In-vivo data's which were statistically analysed by ANOVA and paired t-test. Formulation F_3 was found to have a selective drug release pattern among the formulations prepared. The values were compared with reference standard (F_4M) and were subjected to short term accelerated stability study to find out the effect of aging on release pattern. The result of this study does not indicate any significant alteration in the in-vitro release pattern of the drug from matrix tablet. Formulation F_3 was found to be stable on storage and does not exhibit any alteration in its release pattern. Hence it was concluded that, formulation F_3 was selected as best formulation.

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