

PHARMACEUTICAL STUDIES ON FLASH TABLETS OF A HIGHLY SOLUBLE METFORMIN HYDROCHLORIDE

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ABSTRACT

Objective: Flash metformin hydrochloride (MH) was formulated in smaller dose compared to conventional oral tablets to obtain a rapid onset of action especially in severe cases of type 2 diabetes mellitus patients and this is due to its eventual absorption from mouth to blood stream compared to the poor bioavailability (50%) of conventional tablet (CT).

Methods: The flash tablets (FT) were prepared by dissolving the drug in an aqueous solution of highly water-soluble carrier (gelatin, glycine, and sorbitol). The mixture was dosed into the pockets of blister packs and then was subjected to freezing and lyophilization. The dissolution characteristics of MH from the FT were investigated and compared with the plain drug, the physical mixture (PM) and commercial tablets (CT1 and CT2).

Results: The in-vivo study was carried out using volunteers according to Helsinki Declaration and Resolution 8430 of 1933 by the Ministry of Social protection. Results obtained from dissolution studies showed that FT significantly improved the dissolution rate of the drug when compared with the plain drug, PM and commercial tablets (CT). More than 88% of MH in FT was dissolved within 2 min compared to only 41.98%, 56.12%, 10.65% and 6.88 for plain drug, PM, CT1 and CT2 respectively. Initial dissolution rate of MH in FT was almost two folds higher than plain MH. Physical states of MH, PM and FT were conducted through infrared spectroscopy (FTIR), x-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), and scanning electron microscope (SEM) to denote eventual solid state during the process.

Conclusion: The maximum concentration in blood plasma (C_{max}) was achieved in a short time (15 min) which reflects the higher bioavailability of FT as a result of flash absorption of the drug.

Keywords: Metformin hydrochloride, Flash tablets, Scanning electron microscope, Dissolution rate, Bioavailability.

INTRODUCTION

Metformin is a biguanide type insulin sensitizing drug used to treat type-2 diabetes [1]. It is used in drug discovery in vivo models to assess the anti-diabetic potential of other drugs. Metformin became commercially available in 1957. Therapeutic doses of metformin do not produce hypoglycemia, and it is a therapeutic advantage when compared with sulfonyl ureas [2]. Doses of 0.5-1.5 g have a bioavailability of 50%- 60% [3]. Absorption is slow and incomplete in the upper gastrointestinal tract, because of the high polarity and low liposolubility of the molecule. Metformin has a short biological half life of 1.5-4.5 h [4]. However, frequent dosing schedule and risk of gastrointestinal symptoms makes its dose optimization complicated. The maximum plasma concentration is reached after 2.5h, the drug being excreted through the urinary tract unaltered [5]. At intestinal pH between 7 and 8, metformin is mainly ionized ($pK_a=2.8$ and 11.5), which slows its absorption rate [6]. Metformin is rapidly distributed after absorption, and it is accumulated in the esophagus, stomach, duodenum, salivary glands, and kidneys [7]. It has neither binding to plasma proteins nor metabolism, and it undergoes renal excretion. The mechanism of action of the anti-diabetic agents used for the treatment of type 2 diabetes, includes increasing insulin release, increasing insulin sensitivity, controlling hepatic glucose release or inhibiting intestinal glucose absorption [8-12].

Often, therapy with insulin and OHAs become less effective in controlling hyperglycemia, particularly as a result of weight gain, worsening insulin resistance and progressive failure of insulin secretion due to glucose toxicity. Insulin therapy alone or with hypoglycemic agents can produce weight gain due to reducing glucose excretion [13-15]. Among commonly used OHAs, thiazolidinediones and sulphonylurea contribute to weight gain, whereas metformin causes weight loss and dipeptidyl peptidase-4 inhibitors are weight neutral [16,17]. Overall, there is a need for novel agents which can effectively control blood sugar level without producing weight gain or hypoglycemia. World Health Organization estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030

[18]. Non-insulin dependent (Type 2) diabetes mellitus is a heterogeneous disorder characterized by an underlying insufficiency of insulin. This insufficiency results from defective insulin utilization and can be corrected by administration of one or more of the currently available oral hypoglycemic agents [19].

MATERIALS AND METHODS

Materials

Metformin HCL, Phenformin, acetonitrile, Gelatin, Glycine, and Sorbitol, potassium dihydrogen phosphate, n-hexane, methanol were purchased from Sigma Chemical Co., St. Louis. All water used was distilled and de-ionized. All other chemicals were of reagent grade and used as received.

Methods

Preparation of flash tablet by lyophilization technique (FT)

A 2% w/v solution of gelatin in water was prepared by first soaking the gelatin in water until complete hydration. The hydrated gelatin was stirred using a magnetic stirrer until a clear solution was obtained. Different weights of glycine and sorbitol were added to the gelatin solution while stirring until completely dissolved. Glycine (used to prevent shrinkage of the tablet during manufacturing) and sorbitol (used to impart crystallinity, hardness, and elegance to the tablet) are well-known and acceptable materials used in preparing freeze-dried tablets. The percentage excipient used was optimized during the formulation process to result in a strong and elegant tablet that could be handled with ease. An accurately weighed amount of MH powder (10 % w/v) was then dissolved in the aqueous solution of gelatin, glycine, and sorbitol. One milliliter of the resulting suspension was poured into each of the pockets of a tablet blister pack to result in MH dose of 100 mg in each tablet. The tablet blister packs, each containing 10 tablets, were then transferred to a deep freezer at -22°C for 24 h. The frozen tablets were placed in a lyophilizer for 24 h using a Novalyph-NL500 Freeze Dryer (Savant Instruments, Holbrook, NY) with a condenser temperature of -52°C and a pressure of 7×10^{-2} mbar. The FTs were kept in a desiccators

over calcium chloride (0% relative humidity) at room temperature until further used. Four blister packs containing a total of 40 tablets were produced in each run. The quantitative amounts of ingredients used in the preparation of FT are tabulated in table 1

Table 1: Qualitative amounts of the ingredients used in the preparation of FT

Ingredients	%w/v
Metformin HCL	10
Gelatin	2.5
Sorbitol	1, 1.5, 2
Glycine	1,1,1

Drug content

Eight randomly selected tablets (two from each pack) were assayed for drug content uniformity.

Preparation of physical mixture

MH was uniformly mixed with gelatin, glycine and sorbitol in the same percentage used in the FT using a mortar and pestle. The prepared mixtures were kept in a desiccator until used.

Infrared analysis (FTIR)

The x-ray diffraction pattern of MH plain drug, FT and PM were performed in infrared spectrophotometer (Gensis II, Mattson, (England). Radiation was provided by a copper target (Cu anode 2000W:1.5418 high intensity x-ray tube operated at 40 KV and 35MA). The monochromator was a curved single crystal (one PW1752/00). Divergence slit and receiving slit were 1 and 0.1 respectively. The scanning speed of goniometry (PW/050/81) USED WAS 0.02.20/5 and the instrument were combined with a Philips PM8210 printing recorder with both analogue.

X-ray Powder Diffraction Analysis (XRPD)

X-ray diffraction experiments were performed in a Scintag x-ray diffractometer (USA) using Cu K α radiation with a nickel filter, a voltage of 45 kV, and a current of 40 mA. Diffraction patterns for MH, FT, and PM were obtained.

Differential scanning calorimetry (DSC)

Samples were placed in Al pan and heated at rate of 50°C/min with indium in the reference pan, in an atmosphere of nitrogen up to a temperature of 400°C. The DSC studies were performed for MH, FT and PM.

Scanning Electron Microscopic Analysis (SEM)

Surface morphology of MH, FT as well as PM, was examined by SEM (Jeol JSM-6400, Tokyo, Japan). Photographs were taken at magnification of 1200.

Dissolution Study (DS)

The dissolution profiles of PM, FT, commercial tablet 1 (CT1), commercial tablet 2 (CT2) and the plain drug, were determined in a dissolution tester (VK 7000 Dissolution Testing Station, Vankel Industries, Inc., NJ) following the USP paddle method. All tests were conducted in 900 ml of distilled water maintained at $37 \pm 0.5^\circ\text{C}$ with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 100 mg. After specified time intervals, samples of dissolution medium were withdrawn, filtered, and assayed for drug content Spectrophotometrically at 232 nm after appropriate dilution with water.

Study subjects.

The study was conducted according to the Helsinki Declaration and Resolution 8430 of 1993 by the Ministry of Social Protection. It was also approved by the Ethics Committee of the School of Medicine, at Universidad de Antioquia. 20 adult male (volunteers) were recruited for this study; ages 25.2 ± 1.6 years; weight 68.5 ± 5.2 kg; height 1.65 ± 0.06 m. Subjects were divided into two groups; healthy group and type 2 diabetes mellitus group after having been medically examined and clinically tested, complete blood count, urinalysis,

blood biochemistry were normal, All volunteers were briefed on the bioavailability and pharmacologic studies details and they all agreed and signed a written informed consent. All volunteers were free to leave the study at any time.

Pharmacokinetic Studies

The tested metformin hydrochloride in flash tablet (100 mg) and the reference Glycolphage formula CT1 (500 mg) were administered to 12 volunteers. The volunteers were recruited for this study (ages 26 ± 1.8 years; weight 68 ± 6.5 kg; height 1.7 ± 0.05) divided into two groups; group A (healthy volunteers) and group B (Type 2 diabetes mellitus volunteers) in a double blind, randomized, cross over design. The washout period was seven days. The volunteers were screened for vital signs, blood and urine analysis before enrolment. The tablets were administered without water in case of flash tablet and with 240 ml of potable water in case of reference tablet at ambient temperature. 7 ml of blood samples were withdrawn at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 18.0, 24.0 hours post dose. The samples were stored at -20°C for analysis. A concentration time curve was plotted and Area Under Curve (AUC) was calculated by linear trapezoidal rule (AUC_{0-inf}). Maximum Plasma Concentration (C_{max}) and Time to achieve the maximum concentration (T_{max}) was obtained directly from the concentration time curve without interpolation. All the pharmacokinetic and statistical data were calculated using the software *Kinetica*. (Innaphase, USA)

Analytical procedure and method validation

Metformin extraction from plasma was accomplished by the liquid-liquid extraction method proposed [20]. One half ml of plasma was vortex during 30 seconds in a screw-capped glass tube after adding 2 ml of acetonitrile to precipitate plasma proteins. After centrifugation (2500 rpm) for 5 minutes at 5°C , 2 ml of supernatant was transferred to another clean glass tube. The drug was extracted with 2 ml of the extraction solvent (n-hexan) and vortex for thirty seconds followed by centrifugation (2800 rpm) for 5 minutes. The organic phase was then transferred by aspiration to a clean glass tube. The extraction procedure was repeated with the remaining samples. A gentle air flow and a water bath were used to dry the organic phase. The residue was reconstituted in 400 μl of a mixture of KH_2PO_4 buffer 10 mM (pH 7.5) and acetonitrile (72:28, v/v), and filtered by using a vacuum pump. One hundred microlitres of the sample were injected directly into a chromatographic system. The HPLC system is comprised of an Agilent, model HP1100 (California, USA), pump, an Agilent diode array detector, and an auto-injector. The software package ChemStation (2000 Version) was used to control the chromatographic system. The Analytical column was a LiChrospher C18 RP-Select B (Agilent, 250 mm, 4 mm ID, 5 μm particle size). The mobile phase consisted of dihydrogen phosphate buffer 0.01M (pH 7.5) and acetonitrile (40:60 v/v), wave length was 232 nm. The flow rate was 1.5 ml/min.

Method 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 60-2500 ng/ml and subjecting 100 μl 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. Interday assay reproducibility was assessed over a period of 4 days at 100, 3000 and 4750 ng/ml concentration. Intraday analysis was determined upon replicate analysis of 8 check samples at same concentrations.

Pharmacokinetic analysis

Pharmacokinetic data were calculated by non-compartmental method. The maximum plasma concentration (C_{max}) and the time to reach it (T_{max}) were determined by inspecting each individual plasma level-time curves. The elimination rate constant (ke) was obtained by ln-linear regression of the terminal decay phase. The area under the plasma level-time curve (AUC_{0-24h}) and (AUC_{0-inf}) was

obtained by dividing the last plasma concentration by k_e , and adding this result to the AUC_{0-24h}.

Statistical analysis

In order to assess the effects of treatment, period, sequence of administration, and subjects, In-transformed data for AUC_{0-inf} and C_{max}, and non-transformed T_{max} were evaluated by means of analysis of variance (ANOVA) for the cross design (Statistica 6.0, Statsoft Inc, 2001).

RESULTS AND DISCUSSION

Preparation of flash tablet by lyophilization technique(FT)

The formulation containing glycine/sorbitol in a ratio of (1:1) depicted the highest drug content when compared with the ratios of (1:1.5 & 1:2), good formed tablets and the best elegance, therefore, the ratio (1:1) was selected to proceed the study.

Drug content

The mean % drug content was found to be 97.88% ± 1.40.

Infrared Analysis

Figure(1)depicted IR spectra of MH(a),PM(b) and FT(c).The IR spectra revealed no change in both functional group and fingerprint regions of drug,PM and FT. This indicates that there is no change in both chemical and physical properties of the drug after formulation with the tested excipients.

X-ray Powder Diffraction Analysis(XRPD)

Figure(2a) depicted the x-ray diffraction pattern of the pure drug. The drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The diffraction study of the PM(fig2b)of drug and excipients showed the peaks corresponding to the crystalline drug molecules present in the mixture, although their intensity was lower due to the high excipients–drug ratio employed. The diffraction pattern of the FT(fig2c) of drug showed absence, broadening, and reduction of major MH diffraction peaks indicating that mostly an amorphous form (disordered state) existed in FT. These results could explain the observed enhancement of rapid dissolution of MH in FT.

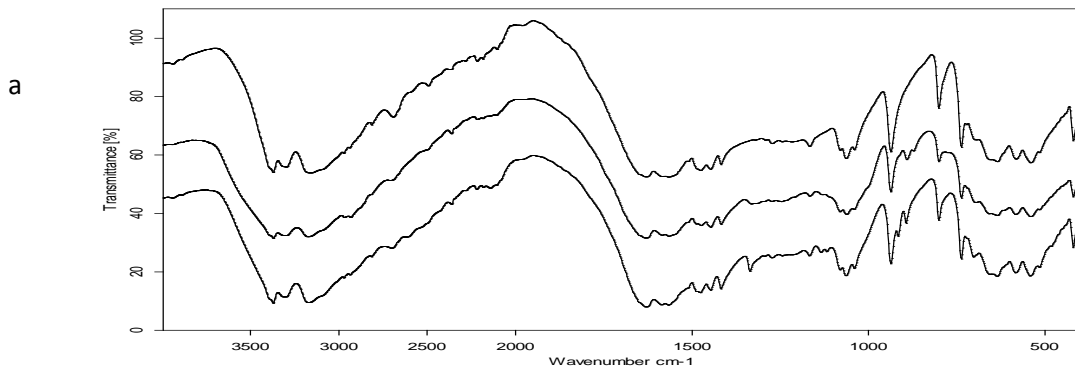


Fig. 1: FTIR of Plain MH(a), PM(b) and LT(c)

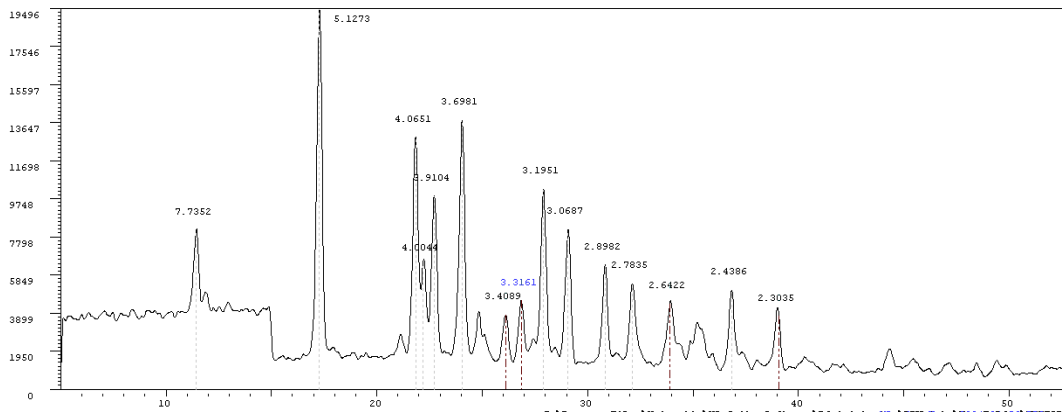


Fig. 2a: XRPD of Plain MH

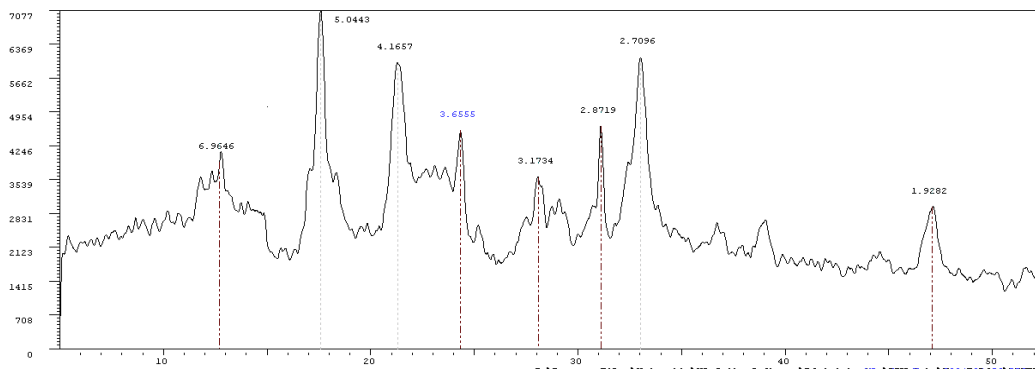


Fig. 2b: XRPD of PM

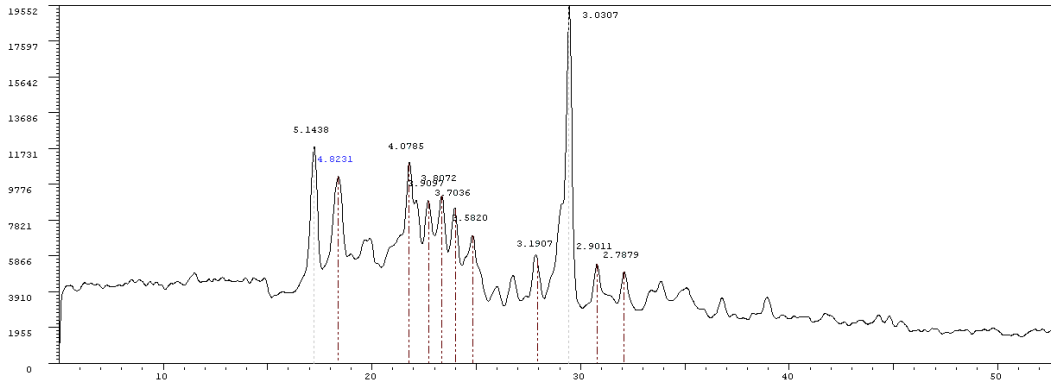


Fig. 2c: XRPD of FT

Differential Scanning Calorimetry Analysis (DSC)

The DSC thermogram of MH showed three endothermic peaks at nearly 266.29°C, corresponding to its melting transition point, 345.22°C and 372.83°C (fig3a). The thermogram of the PM reflected

the endothermic peak shift in melting transition point at 101°C indicating that the crystalline state is reduced in the PM (fig3b). However, the melting transition endothermic peak was completely disappeared in FT, suggesting the minority of crystallinity and majority of amorphous state in the drug (fig 3d).

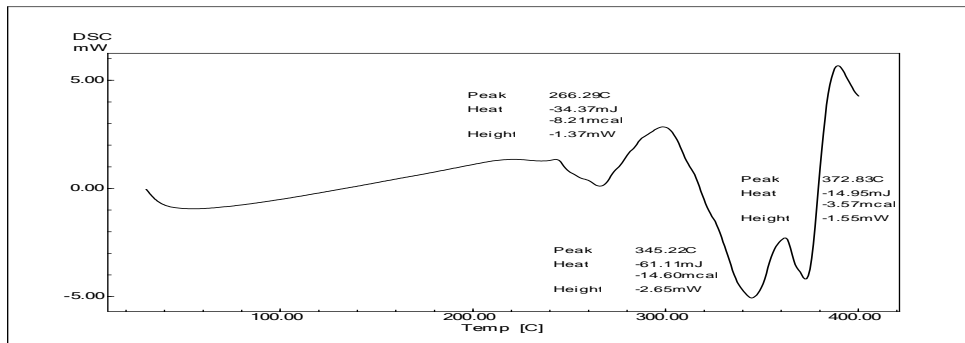


Fig. 3a: DSC of MH

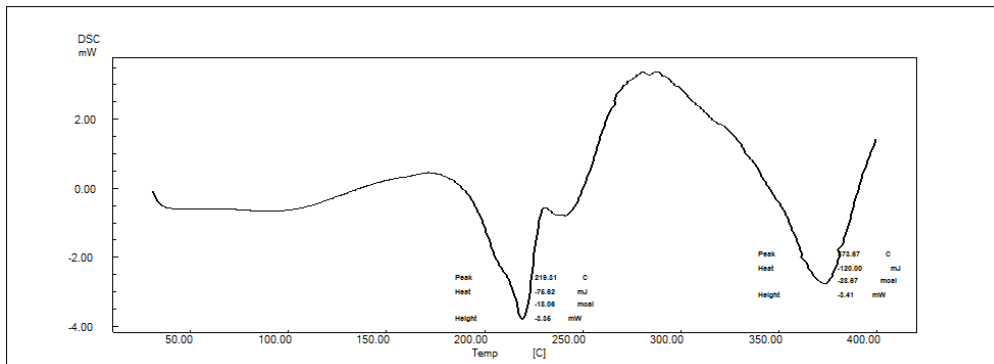


Fig. 3b: DSC of PM

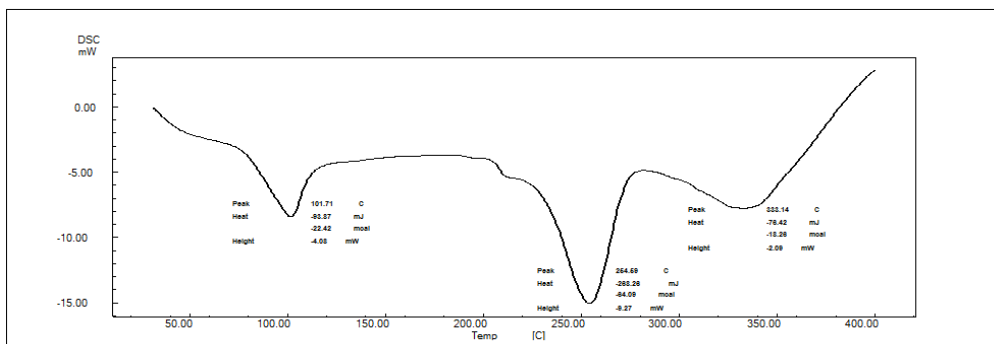


Fig. 3c: DSC of FT

Scanning Electron Microscopy(SEM)

Figure(4) depicted SEM micrographs of MH(4a), PM(4b) and FT(4c).The results showed that the drug has crystals of different shapes(feather, rod, finger like)(fig4a).Whereas the micrographs of both PM and LT revealed a lettuce shape matrix. This could therefore indicate that MH particle size has been reduced as result of disappearance of characteristic crystal shapes of the drug. This will accelerate dissolution.

Dissolution Study(DS)

The dissolution profiles of MH in the PM,FT,MH,CT1,CT2 and drug powder alone in distilled water at 37°C are shown in Fig.5 More than 88% of MH in FT was dissolved within 2 min compared to only 41.98%,56.12%,10.65% and 6.88 for plain drug, PM, CT1 and CT2 respectively. Initial dissolution rate of MH in FT was almost two fold higher than plain MH powder alone during 2 min.

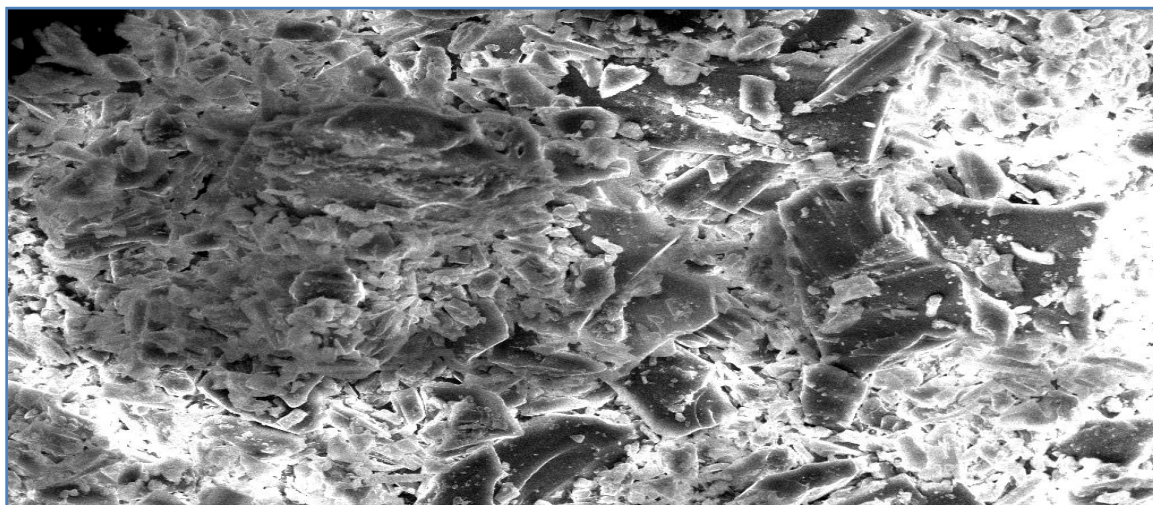


Fig. 4a: SEM of MH

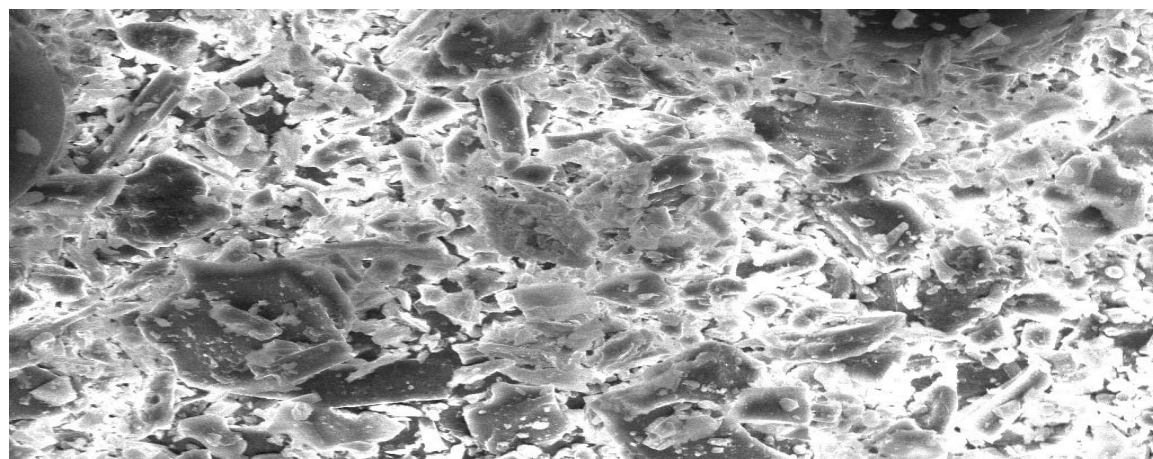


Fig. 4b: SEM of PM

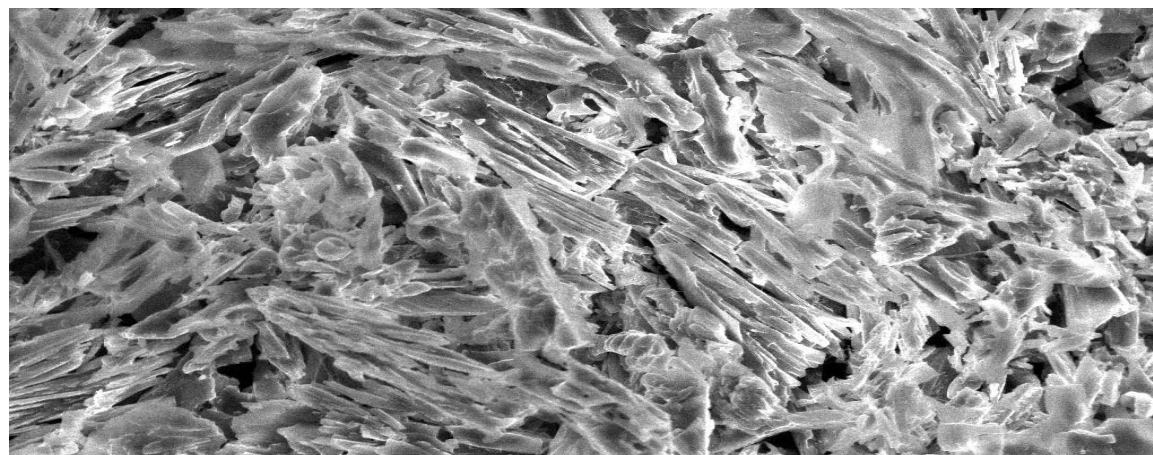


Fig. 4c: SEM of FT

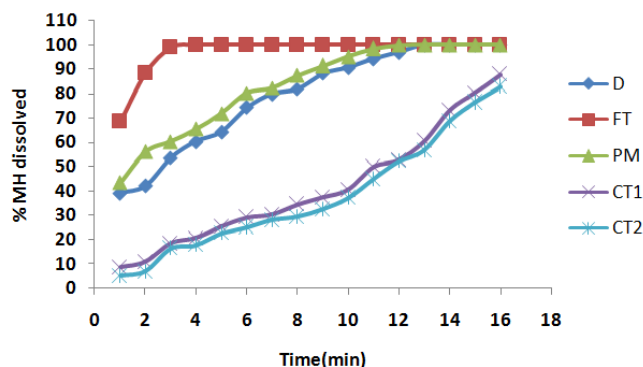


Fig. 5: Dissolution pattern of MH in LT, PM, CT1, CT2 and plain MH in distilled water at 37±0.5oC

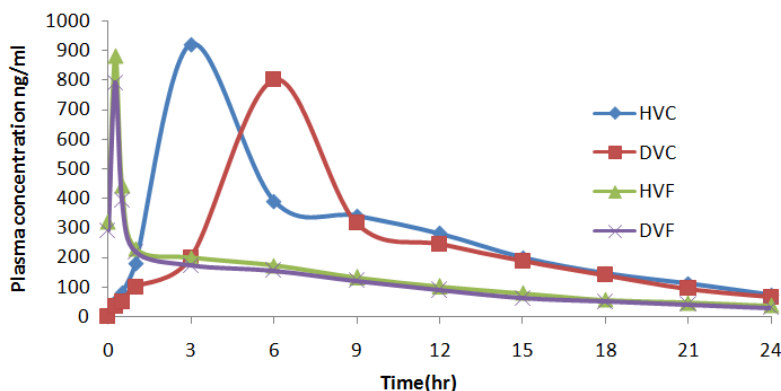


Fig. 6: Comparison of mean plasma metformin concentration of two treatments after a single oral administration of test and reference product (tablet 500 mg) in six healthy and six Diabetic volunteers.

HVC: healthy volunteers taken conventional tablet; DVC: dia

Table 2: Pharmacokinetic parameters after administration of 500 and 100 mg of Metformin in Healthy and Type 2 Diabetes Volunteers (n=12)

Pharmacokinetic parameter	HVC(SD)	DVC(SD)	HVF(SD)	DVF(SD)
C _{max} (ng/ml)	610.11±196.34	523.22±154.62	180.45±148.31	165.66±139.6
T _{max} (h)	3±0.62	6±0.98	0.25±0.11	0.25±0.13
AUC _{0-Tmax}	2015.67±344.13	1652.28±412.34	581.34±20.44	528.44±19.63
AUC ₀₋₂₄ , ng,h/ml	2836.16±344.36	2194.08±288.25	1893.28±166.6	1707.11±144.12
AUC _{0-inf} (ng,h/ml)	5369.30±961.27	3490.30±879.25	2098.41±669.46	1776.71±166.31
t _{1/2} (h)	9.9±0.56	7.7±0.46	4.33±0.61	3.1±0.52
k _e (h ⁻¹)	0.07±0.021	0.09±0.03	0.16±0.032	0.23±0.041

Pharmacokinetic study

The mean venous plasma concentration-time profile of metformin after oral administration of 500 mg and 100 mg are shown in Fig. 6. The pharmacokinetic parameters derived from the analysis are listed in Table 2.

CONCLUSION

Flash tablet has both higher dissolution and oral bioavailability due to expected flash dissolution in the saliva and avoidance of the hepatic effect conversely to commercial conventional dosage forms; therefore, it would be possible to formulate MH in lyophilized tablets having an eventual decreased therapeutic dose resulting in reduced side-effects encountered with MH therapy such as gastrointestinal disturbance.

REFERENCES

1. Krentz A J, Bailey C J: Oral antidiabetic agents: current role in type 2 diabetes mellitus, *Drugs*.2005;65:385–411.

2. Dunn CJ, Peters DH: Metformin: A review of its pharmacological properties and therapeutic use in non insulin-dependent diabetes mellitus. *Drugs*.1995;49:721-749.
3. Scheen AJ : Clinical pharmacokinetics of metformin. *Clin Pharmacokinet*.1996;30: 359-71.
4. Dunn CJ, Peters DJ: Metformin. A review of its pharmacological properties and therapeutic use in non-insulin dependent diabetes mellitus. *Drugs*.1995;49:721-49.
5. Sweetman SC. Martindale: the complete drug reference. 34th.ed. London: Pharmaceutical Press; 2005. p2756.
6. Pentikainen PJ: Bioavailability of metformin. Comparison of solution, rapidly dissolving tablet and three sustained release products. *Int J Clin Pharmacol Ther Toxicol* 1986;213-20.
7. Hale TW, Kristensen LP, Hackett LP, Kohan R, Ilett KF: Transfer of metformin into human milk. *Diabetologia*.2002;24: 1509-14.
8. Wagman AS, Nuss JM : Current therapies and emerging targets for the treatment of diabetes. *Current Pharmaceutical Design*. 2001;7:417-450.

9. Dell'Aglio D M, Perino LJ, Kazzi Z, Abramson J, Schwartz MD: Acute Metformin overdose: examination serum pH, lactate level and metformin concentrations in survivors versus no survivors: a systematic review of the literature. *Ann Emerg Med.*2009;54: 818-823.
10. Kazory A, Walsh K, Harman E, Talor: Is metformin safe in patients with mild renal insufficiency? *Diabetes Care.* 2007;30:444.
11. Schwartz S, Fonseca V, Berner B, Cramer M, Chiang YK: Efficacy, tolerability and safety of novel once-daily XR metformin in patients with type 2 diabetes. *Diabetes Care.* 2006;29:759-763.
12. Najib N, Idkaidek N, Beshtawi M, Bader M, Admour I: Bioequivalence evaluation of two brands of metformin 500 mg tablets (Dialon & Glucophage) in healthy human volunteers. *Biopharm Drug Dispos;* 2002;23:301-306.
13. Buse J: Combining insulin and oral agents. *Am J Med.* 2000;108(Suppl.6a):23S-32S.
14. Wilding J: Thiazolidinediones, insulin resistance and obesity: finding a balance. *Int J Clin Pract.*2006;60:1272-80.
15. Makimattila S, Nikkila K, Yki-Jarvinen H: weight gain during insulin therapy with and Causes of without metformin in patients with type II diabetes mellitus. *Diabetologia.* 1999;42:406-12.
16. Bolen S, Feldman L, Vassy J: Systematic Review: Comparative Effectiveness and Safety of Oral Medications for Type 2 Diabetes Mellitus. *Ann Intern Med.* 200;147:386-99.
17. Hermansen K, Kipnes M, Luo E: Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, in patients with type 2 diabetes mellitus inadequately controlled on glimepiride alone or on glimepiride and metformin ; *Diabetes Obes Metab.* 2007;9:733-45.
18. Ritu B, Rajat R, Harsha V, Pradeep S, Bharat C: Formulation and Characterization of Sustained Release Matrix Tablet of Metformin Hydrochloride. *International Journal of Pharma Recent Research.*2009;1(1):49-53.
19. Howida K, Ahmed M, Attia, M. Ghorab M: Biopharmaceutical evaluation of formulated metformin/ rosiglitazone tablets. *Drug Discoveries & Therapeutics.*2010;4(2):100-108
20. Yuen KH, Peh KK: Simple high-performance liquid chromatographic method for the determination of metformin in human plasma; *J Chromatogr B Biomed Appl.* 1998; 710:243-46.