6.1 Introduction

Amongst the various routes of drug delivery, oral route is regarded as the most preferred route offering numerous advantages including, convenience, compliance and cost effectiveness. The success of controlled release oral drug delivery systems lies in achieving therapeutic plasma drug concentration rapidly and maintaining it throughout the course of therapy via oral route. The controlled release powder for reconstitution (CRPFR) of Metoprolol Succinate (MS) and Metformin Hydrochloride (MH) were developed with an aim to overcome the problems of dose fluctuation and dosing frequency associated with their presently available conventional immediate release dosage forms and thus, to improve patient compliance for pediatric, geriatric and hospitalized patients. Pharmacokinetic studies were performed in rabbits in order to investigate the potential of developed formulation in mitigating the aforesaid drawbacks through maintaining therapeutic plasma drug levels for prolonged period.

6.2 Methods

6.2.1 Protocol approval

The study protocol (no. MSU/IEAC/2011/22) was approved by Institutional Animal Ethics Committee of M.S. University of Baroda, Vadodara, India, in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

6.2.2 Animals

Healthy male New Zealand white rabbits weighing 2.2 to 2.5 Kg were procured from Vaccine Institute, Gandhinagar, Gujarat and acclimatized to laboratory conditions for 1 week and fed a fixed standard diet. Good Laboratory Practice was followed for animal handling routines with strict monitoring of the environmental conditions.

6.2.3 Pharmacokinetic study of MS formulation

Prior to dosing, animals were randomly assigned to two treatment groups of six animals each and were fasted for 24h with free access to water. Since the marketed tablet formulation could not be administered intact (owing to its size; 17.3 x 8.7 mm) to the

rabbits, the pharmacokinetics of developed formulation was compared with drug solution. Group 1 animals were administered with plain drug solution and group 2 animals were administered with optimized formulation pellets, both with the help of an oral feeding tube and at a dose equivalent to 10.3 mg/Kg body weight of MS. Water was allowed ad libitum throughout the experiment. Blood samples (1 mL) were carefully withdrawn from the marginal ear veins at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 20 h post administration. The withdrawn blood samples were transferred to a series of graduated centrifuge tubes containing 0.1 mL of 100 IU heparin solution. The heparinized blood samples were centrifuged at 3600 rpm and 4ºC for 10 min (Remi Centrifuge, Mumbai, India). The supernatant plasma was transferred into another set of sample tubes and preserved below -20ºC until analyzed.

For drug quantification, plasma samples were defrosted to room temperature and filtered through 0.25 µm membrane filter. 200 µL of filtered plasma sample was mixed with 1 mL of acetonitrile and the precipitated plasma proteins were made to settle down by centrifugation at 4000 rpm for 10min. The supernatant acetonitrile solution was evaporated to dryness and the residue was dissolved with 300 µL of the HPLC mobile phase. The drug was then quantified using HPLC method [1] as described in section 3.3.3. The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (t_{max}) were directly obtained from the observed values. Other pharmacokinetic parameters including area under curve up to last sampling point (AUC_{last}), total area under curve up to infinity (AUC_{total}), mean residence time (MRT) and half life ($t_{1/2}$) were obtained using Kinetica v5.0 software from Thermo Fisher Scientific. Relative bioavailability (%) was also calculated by following formula,

$$F_{Relative} = \left(\frac{AUC_{total} \text{ of optimized pellets}}{AUC_{total} \text{ of plain drug solution}} \right) \times 100 \qquad (6.1)$$

6.2.4 Pharmacokinetic study of MH formulation

The methodology adapted for pharmacokinetic study of MH was also same as described in section 7.2.3 except that the formulations were administered at a dose equivalent to 25.7 mg/Kg body weight of MH and the MH present in plasma samples were quantified using HPLC method [2] as described in section 3.3.7.

6.3 Results and Discussion

6.3.1 Pharmacokinetic study of MS formulation

The estimated concentrations of Metoprolol Succinate in blood plasma at different sampling time points after oral administration of plain drug solution as well as optimized pellets are given in Table 6.3.1. Figure 6.3.1 represents the plasma drug concentration time profile graphically.

Rapid attainment of higher peak plasma drug concentration in case of plain drug solution (C_{max} 183.74 ± 31.19 ng/mL) could be attributed to rapid absorption of drug from its solution form. Rapid decline in plasma drug concentrations can also be seen in animals administered with plain drug solution. Optimized formulation showed significantly lower C_{max} (69.87 ± 10.68 ng/mL) as compared to plain drug solution ($p < 0.05$) and required significantly more time to reach C_{max} (t_{max} 3.0 h) as compared to plain drug solution (t_{max} 0.5 h). This could be due to a prolonged absorption at a slower rate which reflects controlled release behavior of the developed formulation after oral administration in rabbits

Table 6.3.1: Plasma concentration profiles of orally administered MS formulations in rabbits

Sampling Time (h)	Plain drug solution* (ng/mL)	Optimized pellets formulation* (ng/mL)
0	0	0
0.5	183.74 ± 31.19	21.48 ± 3.85
1.0	171.43 ± 26.65	32.93 ± 4.96
1.5	155.26 ± 22.47	53.24 ± 8.37
2.0	122.47 ± 18.58	64.18 ± 11.35
3.0	76.18 ± 13.37	69.87 ± 10.68
4.0	48.42 ± 8.91	66.76 ± 11.23
6.0	18.96 ± 3.58	61.73 ± 9.27
8.0	7.33 ± 1.24	51.96 ± 7.45
10.0	ND	46.54 ± 7.92
12.0	ND	33.89 ± 5.36
20.0	ND	21.31 ± 3.99
24.0	ND	10.28 ± 0.86

* Mean ± SD (n=6); ND, Not detected.

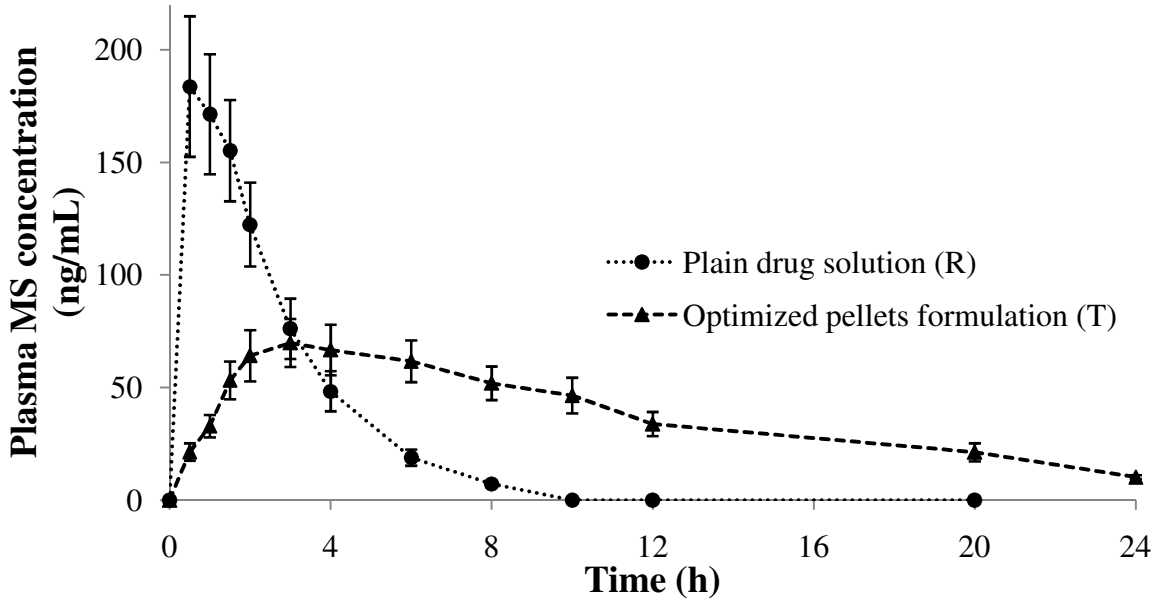

Figure 6.3.1: Plasma concentration profiles of orally administered MS formulations in rabbits

Mean plasma concentrations of MS at various sampling time points were used to generate pharmacokinetic parameters using kinetica software. The pharmacokinetic parameters were obtained (Table 6.3.2) using AUC calculation method under non compartmental analysis for extra vascular administration.

Table 6.3.2: Pharmacokinetic parameters of orally administered MS formulations

Pharmacokinetic Parameters	Plain drug solution	Optimized pellets formulation
C_{max} (ng/mL)	183.74	69.87
T_{max} (h)	0.5	3.0
AUC_{last} (ng*h/mL)	533.19	902.64
AUC_{total} (ng*h/mL)	548.09	1020.88
MRT (h)	2.58	12.35
$t_{1/2}$ (h)	1.41	7.97
$F_{Relative}$ (%)	100	186.26

The AUC_{last} and AUC_{total} values for optimized pellets formulation (902.64 and 1020.88 ng*h/mL, respectively) were significantly higher than the same for plain drug solution (533.19 and 548.09, respectively) advocating significant improvement in extent of drug absorption from optimized pellets formulation as compared to plain drug solution. An almost two fold rise in bioavailability also reflected the improvement in extent of drug

absorbed. A significant increase in MRT and $t_{1/2}$ in case of optimized pellets formulation (as compared to plain drug solution) further signified the maintenance of plasma drug levels for prolonged period of time.

6.3.2 Pharmacokinetic study of MH formulation

The estimated concentrations of Metformin hydrochloride in blood plasma at different sampling time points after oral administration of plain drug solution as well as optimized pellets are given in Table 6.3.3. Figure 6.3.2 represents the plasma drug concentration time profile graphically.

Table 6.3.3: Plasma concentration profiles of orally administered MH formulations in rabbits

Sampling Time (h)	Plain drug solution[#] (ng/mL)	Optimized pellets formulation[#] (ng/mL)
0	0	0
0.5	168.37 ± 29.48	60.24 ± 11.43
1.0	345.92 ± 63.46	92.43 ± 16.63
1.5	554.48 ± 97.37	168.71 ± 31.29
2.0	647.56 ± 103.89	225.45 ± 39.72
3.0	805.65 ± 125.61	297.82 ± 54.76
4.0	699.38 ± 112.34	401.29 ± 39.61
6.0	439.72 ± 76.33	489.36 ± 98.38
8.0	328.87 ± 49.91	427.72 ± 84.52
10.0	161.63 ± 31.64	376.54 ± 71.73
12.0	58.89 ± 9.58	274.37 ± 52.87
20.0	3.28 ± 0.36	162.28 ± 29.45
24.0	ND	92.45 ± 18.48

[#] Mean ± SD (n = 6); ND, Not detected.

Early attainment of higher peak plasma drug concentration in case of plain drug solution (C_{max} 805.65 ± 125.61 ng/mL) could be attributed to faster absorption of drug from its solution form. Rapid decline in plasma drug concentrations can also be seen in animals administered with plain drug solution.

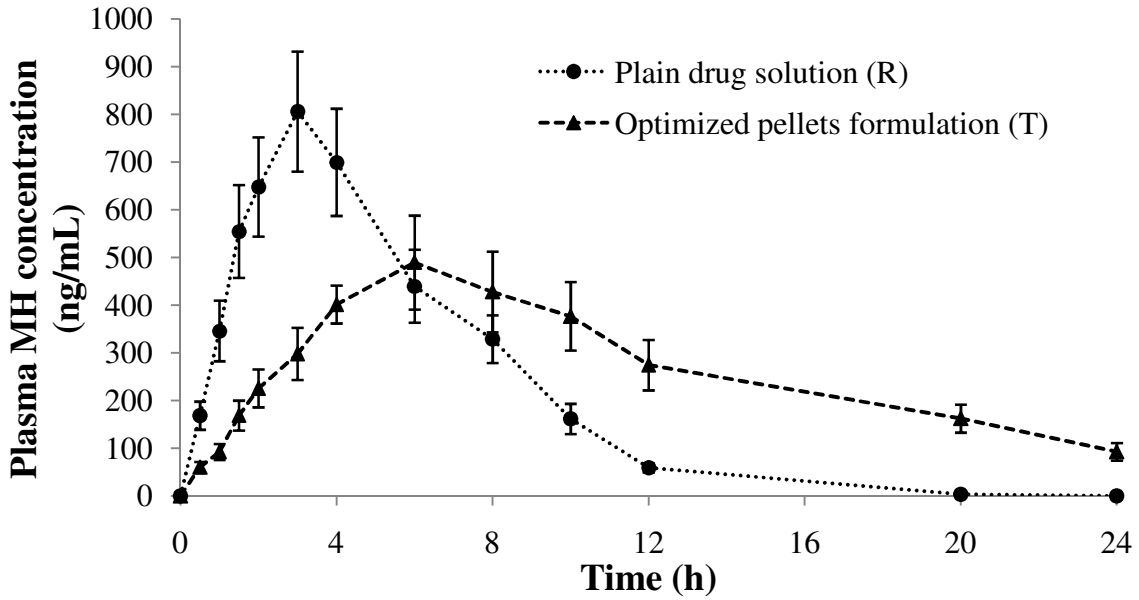

Figure 6.3.2: Plasma concentration profiles of orally administered MH formulations in rabbits

Optimized formulation showed significantly lower C_{max} (489.36 ± 98.38 ng/mL) as compared to plain drug solution ($p < 0.05$) and required significantly more time to reach C_{max} (t_{max} 6.0 h) as compared to plain drug solution (t_{max} 3.0 h). This could be due to a prolonged absorption at a slower rate which reflects controlled release behavior of the developed formulation after oral administration in rabbits. Mean plasma concentrations of MH at various sampling time points were used to generate pharmacokinetic parameters using Kinetica software. The pharmacokinetic parameters were obtained (Table 6.3.4) using AUC calculation method under non compartmental analysis for extra vascular administration.

Table 6.3.4: Pharmacokinetic parameters of orally administered MH formulations

Pharmacokinetic Parameters	Plain drug solution	Optimized pellets formulation
C_{max} (ng/mL)	805.65	489.36
T_{max} (h)	3.0	6.0
AUC_{last} (ng*h/mL)	4884.90	6287.25
AUC_{total} (ng*h/mL)	4893.43	7315.81
MRT (h)	5.11	13.82
$t_{1/2}$ (h)	1.8	7.7
$F_{Relative}$ (%)	100	149.50

The AUC_{last} and AUC_{total} values for Optimized pellets formulation (6287.25 and 7315.81 ng*h/mL, respectively) were significantly higher than the same for plain drug solution (4884.90 and 4893.43, respectively) advocating significant improvement in extent of drug absorption from optimized pellets formulation as compared to plain drug solution. An almost 1.5 fold rise in bioavailability also reflected the improvement in extent of drug absorbed. A significant increase in MRT and $t_{1/2}$ in case of optimized pellets formulation (as compared to plain drug solution) further signified the maintenance of plasma drug levels for prolonged period of time.

6.4 Conclusions

From the above results it was evident that the developed formulations of both MS and MH were able to sustain the drug release for 24h *in vivo.* Hence, the developed MUPS formulations of MS and MH can be potentially useful in clinical treatment of hypertension and diabetes, respectively. Thus, these formulations hold promise as better alternative to the existing solid dosage forms. However, further examinations in human beings under clinical conditions are essential for their commercialization.

REFERENCES

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[2] L.-D. Hu, Y. Liu, X. Tang, Q. Zhang, Preparation and in vitro/in vivo evaluation of sustained-release metformin hydrochloride pellets, European journal of pharmaceutics and biopharmaceutics, 64 (2006) 185-192.