COMPARATIVE IN VIVO EVALUATION OF EXTENDED RELEASE PELLETS AND ORAL SOLUTION CONTAINING METHYLPHENIDATE HYDROCHLORIDE

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ABSTRACT

Methylphenidate Hydrochloride (HCl) is used to treat Attention deficit hyperactivity disorder (ADHD). Methylphenidate HCl Extended release (ER) pellets were successfully developed and optimized using wurster process. The present investigation aimed to evaluate the pharmacokinetic properties of Methylphenidate HCl ER pellets using rabbits as animal model. Blood samples were collected at various time intervals. The plasma concentration of Methylphenidate HCl was estimated by reverse phase liquid chromatography/ tandem mass spectroscopy (LC/MS/MS) method. The pharmacokinetic parameters were calculated from the plasma concentration of Methylphenidate HCl vs. time profile. The sustained T_{max} , lower C_{max} and prolonged Mean residence time (MRT) indicate an extended release of Methylphenidate HCl ER pellets compared to Methylphenidate HCl API. From the obtained results, it is concluded that the test formulation was provided extend the delivery of Methylphenidate HCl in desired rate.

Key words: Methylphenidate HCl, Extended release, Pellets, Pharmacokinetics, formulation variables, process parameters

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INTRODUCTION

Multiple-dose units have many kinetic and therapeutic advantages over single-dose sustained release units, such as improved bioavailability, easy administration, reproducible gastric residence time, low risk of dose dumping, low intra and inter subject variability, flexibility of blending of different release profile and divided into various dose strengths without formulation changes. The most commonly used pelletization techniques are Suspension/solution layering, extrusion spheronization and powder layering. However, suspension/solution layering (Wurster) technique is most preferable in the pharmaceutical industry owing to its advantages like continuous process, less manual interruption and batch to batch reproducibility [1-3].

Methylphenidate HCl is an amphetamine like CNS Stimulant, commonly used to treat Attention deficit hyperactivity disorder (ADHD) in children, adolescents and adults. Its hydrochloride salt is freely soluble in water, stable and well absorbed from the intestinal tract, with a short elimination half life (i.e. 3 to 4 h). These favorable properties combined with a low dose and also need to decrease the dosing frequency, make methylphenidate as an ideal

candidate for development of a new generation of modified release formulation, which offers the equivalent efficacy of repeated administration of immediate release product [4].

Impact of various formulation variables and critical process parameters on Methylphenidate HCl extended release (ER) pellets were statistically interpreted and significant variables were optimized in our earlier investigation [5-6]. The present study aimed to evaluate the pharmacokinetic parameters of optimized formulation of Methylphenidate HCl ER pellets.

MATERIALS AND METHODS

Materials

Methylphenidate HCl was obtained from RA CHEM Pharma Ltd., Hyderabad as gift sample, Sugar spheres (Arun pharma), Hypromellose (Dow chemical's), Povidone (BASF), Talc (Luzenac), Eudragit RSPO (Evonik), Eudragit L 30 D55 (Evonik), Triethyl citrate (Merck), Isopropyl alcohol (Avantor), Purified water and empty hard gelatin capsule shells size 1 (ACG) were used as received.

Methods

Preparation of Methylphenidate HCl ER Pellets

Methylphenidate HCl ER Pellets were prepared by employing bottom – spray fluid bed (Wuster) coating process (Glatt GPCG 1.1). The dosage form was designed to obtain the biphasic release profile from single population of pellets comprising immediate release and extended release portions. Dose was distributed among the two portions equally, i.e. 50% as immediate release portion and second part as extended release portion.

Drug loaded pellets were prepared by spraying the aqueous drug dispersion over non pariel seeds (Sugar spheres (20#- 25# ASTM)) employing wurster process (Bottom spray fluid bed coating technology). The drug dispersion was coated on to sugar spheres using 1.0 mm of spray nozzle with a spray rate of 2-6 g/min, 0.8-1.0 Kg/cm² of atomization air pressure, 45-60 cfm of air volume and 38-42°C as product temperature. The drug dispersion was sprayed until get desired weight gain. The drug loaded pellets were dried for 10 minutes at 38-42°C. Further, aqueous seal coating dispersion was coated on to the drug loaded pellets employing similar process parameters except 40-45°C as product temperature. Seal coated pellets were dried for 10 minutes at 40-45°C. Hydro alcoholic (IPA : Water 80:20) ER coating solution was coated over the seal coated pellets using wurster process at a spray rate of 4-8g/min & 34-38°C as product temperature. The ER coated pellets were dried for 15 minutes at 34-38°C. Further, the aqueous enteric coating dispersion was coated on to the ER coated pellets at 28-32°C as product temperature and at a spray rate of 2-6g/min. Enteric coated pellets were subjected for drying at 35°C for 15 minutes. Finally, immediate release portion of drug dispersion was coated over the enteric coated pellets using similar process parameters as that of earlier. IR drug loaded pellets were dried for 10 minutes at a temperature of 38-42 °C. Final pellets were sifted through #16-#20 ASTM mesh to separate the fines and agglomerates and collect the desired portion. The composition of the optimized formula described in Table 1.

Table1. Composition of the Methylphenidate HCl ER Pellets

S.no.	Ingredient	mg/capsule
I	Core	
1	Sugar Spheres (#20-#25)	150.25
II	Drug Loading	
2	Methylphenidate HCl	20.00
3	Povidone	3.64
4	Hypromellose (Pharma coat 606)	7.27
5	Polyethylene glycol 6000	1.09
6	Talc	1.82
7	Purified water	Q.S
III	Seal Coating	
8	Hypromellose (Pharma coat 606)	18.18
9	Polyethylene glycol 6000	1.82
10	Talc	1.82
11	Purified Water	Q.S
IV	Extended Release Coating	
12	Ammonio Methacrylate Copolymer Type B (Eudragit RSPO)	12.73
13	Triethyl citrate	2.55
14	Talc	3.20
15	Isopropyl alcohol	Q.S
16	Purified Water	Q.S
V	Enteric Coating	
17	Methacrylic Acid- Ethyl Acrylate Copolymer (1:1) Dispersion 30% (Eudragit L 30 D-55)	72.73
18	Triethylcitrate	14.55
19	Talc	18.18
20	Purified Water	Q.S
VI	Drug Loading	
21	Methylphenidate HCl	20.00
22	Povidone	3.64
23	Hypromellose (Pharma coat 606)	7.27
24	Polyethylene glycol 6000	1.09
25	Talc	1.82
26	Purified Water	Q.S.
	TOTAL	363.63

Evaluation of Methylphenidate HCl ER Pellets**Assay**

Methylphenidate HCl ER Pellets equivalent to 20mg of Methylphenidate HCl were transferred into 100mL volumetric flask, added diluent ((Methanol: Acetonitrile: pH 4.0 Sodium acetate buffer at a ratio of 4:3:3) and sonicated for 15 minutes to dissolve, made the volume up to the mark with diluent. Transferred 10mL of this solution to 20mL volumetric flask and made the volume up to the mark. The solution was filtered through 0.45µ nylon membrane filter [7].

The following chromatographic conditions were employed for analysis:

Column : Kromosil 60, CN 250 mm X 4.6 mm, 5µm or its equivalent

Injection volume: 50µL

Flow rate : 1.5mL/min

Detector : UV, 210nm

Run time : 10 minutes

Calculations:

Assay of Methylphenidate HCl:

$$= \frac{A_T}{A_S} \times \frac{W_S}{100} \times \frac{10}{20} \times \frac{100}{W_T} \times \frac{20}{10} \times \frac{P}{100} \times A.W = \text{mg/capsule}$$

Assay of Methylphenidate HCl

$$\% \text{ of label amount} = \frac{\text{Assay of Methylphenidate HCl}}{\text{Label claim}} \times 100$$

Where,

A_T = Peak area of Methylphenidate HCl obtained from the Sample Solution.

A_S = Average Peak area of Methylphenidate HCl obtained from the standard Solution

W_S = Weight of Methylphenidate HCl working standard taken in mg

W_T = Weight of sample taken in mg

P = Potency of Methylphenidate HCl working standard used (on as is basis)

A = Average weight of the fill contents of capsules in mg.

In vitro drug release studies

The Methylphenidate HCl ER pellets equivalent to 40mg Methylphenidate HCl were accurately filled into size 1 hard gelatin capsules and evaluated for in vitro drug release studies, which were performed using USP Type I dissolution test apparatus. The volume of the dissolution medium was 500ml with a stirring speed of 100 rpm, and the temperature was maintained at 37°C±0.5°C. These conditions were kept constant for all dissolution studies. The study was carried out in 0.01N HCl for 2 h followed by pH 6.0 Phosphate buffer at 1, 2, 3, 4, 6, 8 and 10 h. 10ml of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium [8]. The collected samples were filtered through 0.45µ nylon membrane filter and analyzed to assess the % drug dissolved by employing same chromatographic conditions as that of assay.

The % labeled amount of Methylphenidate HCl dissolved at respective time intervals (Dn) was estimated from following formulae:

$$= \frac{A_T}{A_S} \times \frac{W_S}{100} \times \frac{5}{25} \times \frac{500}{LC} \times \frac{P}{100} \times 100 = \text{-----} \%$$

Where,

A_T = Peak area of Methylphenidate HCl obtained from the Sample Solution.

A_S = Average Peak area of Methylphenidate HCl obtained from the Standard Solution

W_S = Weight of Methylphenidate HCl working standard taken in mg

P= Potency of Methylphenidate HCl working standard used (on as is basis)

LC = Label claim

Calculate the correction factor (CFn) at each time point by using the following formula:

$$CFn = \frac{Dn}{500} \times 10$$

Drug release kinetics

The drug release kinetics and mechanism from the formulations were studied by fitting the data obtained from the in vitro drug release study into several mathematical equations [9].

Stability studies

The optimized formulation of Methylphenidate HCl ER pellets were filled into hard gelatin capsules and subjected for stability studies according to guidelines of international conference of harmonization (ICH), at an accelerated (40°C/75%RH) and long term (25°C/60%RH) stability conditions [10].

In-vivo evaluation of Methylphenidate HCl ER capsules

Pharmacokinetic parameters

Parallel design was selected for the study. Healthy albino male rabbits of weighing (2.0-2.5Kg) were divided into 2 groups, each consisting of 6 animals. Reference (Methylphenidate HCl API, as solution) and Test formulation (Methylphenidate HCl ER pellets, which were filled into capsules of around 13mg to mimic the actual dosage form) were given orally via silicone rubber gastric intubation tube to the first group and second group respectively. All the rabbits were housed in individual cages at room temperature, fasted prior to the 12 hours of drug administration and have access to water and food after 4 hours of dosing throughout the study period. The study was approved by the Institute Animal Ethical Committee (IAEC) with IAEC.1032/PO/Re/s/07/CPCSEA/002/2015.

Blood samples of approximately 2 mL were collected through marginal ear vein at Pre dose, 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24 and 28 hours. All the blood samples were collected into K₂EDTA coated tubes. Samples were centrifuged at 4000 rpm for 5 minutes. A simple protein precipitation method was used for the extraction of Methylphenidate HCl from the rabbit plasma samples. Reverse phase liquid chromatography/ tandem mass spectroscopy (LC/MS/MS) method was used for estimation of Methylphenidate HCl from plasma (Figure 1) [11]. Pharmacokinetic parameters such as T_{max} , C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and Mean residence time (MRT) of

Methylphenidate HCl were estimated by non-compartmental method using Win-Nonlin version 6.2 (Pharsight Corporation, Mountain View, CA).

Fig.1: Standard LC/MS Spectra of Methylphenidate HCl

RESULTS AND DISCUSSION:

Preparation of pellets

Methylphenidate HCl ER pellets was prepared by employing wurster process. The prepared pellets were evaluated for stability stuides and in vivo studies.

Evaluation of pellets

The results obtained from the micromeritic properties indicated that optimized formulation shown good flow properties according to USP limits, which are crucial to attain uniformity of dosage units while capsule filling. Assay of the formulation was observed as 100.2%. Drug release profile of the optimized formulation was comparable with the marketed formulation (Figure 2) and the similarity factor (F_2) was found as 66.8. The dissolution data fitted into various kinetic models, the obtained results concluded that the drug release followed the first order kinetics as r^2 values were higher for first order model (0.962) than zero order model (0.768). The n value is less than 0.45 (0.261); hence the mechanism of drug release was fickian diffusion.

Fig.2: Dissolution profile of the Reference listed drug (RLD) vs. optimized formulation

Stability studies

Results obtained from the stability studies (Table 2 & 3) shown that there was no significant change in description, assay, and dissolution profile at both accelerated and real time stability conditions for 6 months

Table 2. Stability results of optimized formulation at Accelerated ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ & 75%RH) storage conditions

Parameter	Testing Frequency				
	Initial	1 Month	2 Month	3 Month	6 Month
Description	White to off-white spherical pellets filled in white opaque capsule				
Assay	100.2	99.9	99.7	99.3	99.1
Dissolution profile					
2	49.2 ± 1.3	49.6 ± 1.5	49.4 ± 1.9	49.5 ± 2.1	49.1 ± 1.8
4	72.8 ± 0.9	72.3 ± 1.1	72.2 ± 1.6	71.7 ± 1.8	71.2 ± 1.5
6	82.5 ± 0.8	83.4 ± 0.9	84.1 ± 0.8	83.9 ± 1.1	83.6 ± 1.1
8	93.3 ± 0.9	94.2 ± 0.5	93.5 ± 0.9	93.1 ± 0.7	92.1 ± 0.6

Table 3. Stability results of optimized formulation at real time ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ & 60%RH) storage conditions

Parameter	Testing Frequency		
	Initial	3 Month	6 Month
Description	White to off-white spherical pellets filled in white opaque capsule		
Assay	100.2	99.8	99.5
Dissolution profile			
2	49.2 ± 1.3	49.1 ± 1.7	49.2 ± 1.9
4	72.8 ± 0.9	72.4 ± 1.1	71.8 ± 1.4
6	82.5 ± 0.8	83.1 ± 0.5	83.6 ± 1.1
8	93.3 ± 0.9	93.4 ± 0.7	93.1 ± 0.4

Pharmacokinetic parameters

The plasma concentration time profile of Methylphenidate HCl solution and Methylphenidate HCl ER capsules is depicted in Figure 3. The pharmacokinetic parameters observed from test formulation and reference is represented in Table 4. The pharmacokinetic data subjected t-test and very significant differences ($p < 0.05$) were noticed for $C_{\max}$, AUC and MRT except $T_{\max}$. From the obtained results, it was concluded that the test formulation exhibits better extension of the plasma concentration compared to drug solution (Reference).

Fig. 3: Plasma concentration vs. Time profiles of Methylphenidate HCl solution (REF_MPH) and Methylphenidate HCl ER Capsules (TEST_MPH)

Table 4. Summary of pharmacokinetic parameters of reference (Methylphenidate HCl solution) and test (Methylphenidate HCl ER Capsules)

S. No.	Pharmacokinetic Parameters	Reference	Test formulation
1	C_{max} (ng/ml)	12.825 ± 0.64	11.700 ± 0.572
2	T_{max} (h)	1.00 ± 0.00	1.00 ± 0.00
3	AUC_{0-t} (h*ng/ml)	53.106 ± 2.248	165.392 ± 6.509
4	$AUC_{0-\infty}$ (h*ng/ml)	58.474 ± 3.215	191.200 ± 8.266
5	MRT (hr)	3.072 ± 0.110	10.372 ± 0.386

CONCLUSION

Methylphenidate HCl ER pellets generating a biphasic release profile from single core were successfully fabricated by fluid bed coating technology. The physico chemical properties are satisfactory. The drug release profile is similar to the marketed formulation. The pharmacokinetic parameters shown that the optimized formulation exhibits extended plasma concentration. As the optimized formulation is stable and bioavailable, it can be used as an alternative to the marketed formulation.

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