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In vitro and *in vivo* evaluation of a sustained-release once-a-day formulation of the novel antihypertensive drug MT-1207

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Abstract

Hypertension is one of the most common chronic cardiovascular disorders. Sustained-release formulations are developed to maintain drug therapeutic levels throughout the treatment of hypertension, to promote patient compliance and improve patient outcomes. We have developed and tested in *in vivo* trials a once-a-day tablet formulation for the novel antihypertensive drug MT-1207. The tablets based upon a hydrophilic polymer matrix underwent post-compression parameter and physicochemical characterisations, along with *in vitro* drug release testing. The most promising formulation containing 31% w/w HPMC K15M gave a 24-hour release of MT-1207 with an almost constant release rate up to 20 hours. Follow in *in vivo* studies were carried out in Beagle dogs for the optimised sustained-release tablets in comparison to immediate-release tablets. The results showed that a sustained release of MT-1207 from the new formulation was achieved with a drug $t_{1/2}$ 2-2.5 times longer than the immediate-release tablets. Moreover, the $AUC_{0-\infty}$ values of both sustained- and immediate-release tablets were identical at the same dose of 30 mg, indicating that the same amount of drug was absorbed in each case. For treatments based upon MT-1207 this development is significant for future commercial exploitation via scale-up and further trials, and ~~for~~ improved patient outcomes.

Keywords: MT-1207, Sustained-release tablets, Hydrophilic matrix, Drug release, Pharmacokinetics

1. Introduction

Hypertension is the most common cardiovascular disease, with 1.39 billion adults worldwide having the condition in 2010 (Katzung et al. 2012; Mills et al. 2016). Many variables influence the prevalence of this disease, including race, age and education (Katzung et al. 2012). Hypertension presents a significant challenge in therapeutics as it is usually a lifelong disease that causes few symptoms until its advanced stage. There is a need for improved formulations that can make more effective use of current drugs, as even relatively small improvements in factors such as patient compliance and therapeutic efficacy are important and would have a very significant impact on human health.

There are many drugs that are used to treat hypertension through different mechanisms of action, such as beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, alpha-receptor antagonists, diuretics. It is mainly the level of blood pressure and the presence, and severity of, end organ damage that dictate the selection of a drug or drug combination for a patient. The potential use of multiple drugs targeting different receptors leads to the polypharmacy paradigm in the treatment of hypertension (Tsioufis and Thomopoulos 2017; Williams et al. 2018).

Since hypertension is a chronic disorder, a sustained concentration of the drug(s) is required throughout the time of treatment (Nair et al. 2010). Sustained-release formulations are frequently used to achieve this, where a portion of the drug is released immediately and the rest of the amount is released at a predetermined rate for an extended period of time (usually 12 to 18 hours) (Wen and Park 2010). For the extended-release enteric-coated tablets and capsules are the most common forms of such formulations, where the drug release starts in the neutral environment of the small intestine, rather than in the acidic environment of the stomach (Prakash and Markham 1999). In fixed-dose combination formulations, either the same drug is incorporated in both parts of the dosage form, i.e. the immediate-release and

sustained-release formulation parts or one drug is incorporated into an immediate-release part and a second into a sustained-release part (Alaux et al. 2000; Simon 2005; Wen and Park 2010).

The use of sustained-release formulations comes with advantages and certain disadvantages. Drug concentration can be maintained at an optimum level so that its therapeutic effect is prolonged (Klein 2002). This may result in an improved treatment efficiency with a less total amount of drug, less frequent administration, minimised adverse effects and enhanced patient compliance (Michelson 1991; Hutton and Morris 1992; Wagstaff and Goa 2001; Pieper 2002; Michel 2002; McCarberg 2007). In contrast, due to the use of higher amounts of the drug in the formulation, damage of the drug reservoir of a sustained-release formulation can result in dose dumping and a drug blood concentration above the toxic level (Wen and Park 2010).

Although there are many sustained-release pharmaceutical products available on the market, the mechanisms by which controlled release of a drug from a formulation can be achieved are relatively few. Dissolution-controlled formulations have the drug in a reservoir or dispersed in a matrix and the drug release rate is determined either by the dissolution rate and thickness of the polymer membrane, or the dissolution rate of the polymer matrix, respectively. Likewise, in a diffusion-controlled formulation, drug release is controlled by the rate at which the drug molecules diffuse through a polymer membrane (reservoir systems) or a matrix. The matrix can be formed *in situ* from a physical mixture of the drug and the excipients or be in the form of a solid dispersion where the matrix is usually formed by a soluble polymer (Shergill et al. 2016; Baghel et al. 2016). Additionally, osmosis-based formulation is an important category of controlled-release products, where drug release occurs at a rate dependent on the rate of water penetration through a semi-permeable membrane surrounding the drug core and on the thickness of the membrane. Zero-order

release of the drug can be achieved from these systems largely independent of environmental factors, such as temperature, pH and presence of food (Emara et al. 2012). Finally, ion-exchange-based formulations consist of ion-exchange resins that are water-insoluble polymers containing ionic groups. Drug molecules attach to the surface of the polymer through electrostatic interactions, provided that their charge is opposite to that of the polymer. The drug release takes place through replacement of the drug molecules with other ions with the same charge on the polymer surface (Malinovskaja et al. 2013). Here, we chose to exploit a sustained-release system based on a hydrophilic polymer matrix.

Hydrophilic polymer matrix systems have been extensively used in oral controlled-release delivery. The desired controlled-release profile is achieved easily through their use, they are cost-effective and have the United States (US) Food and Drug Administration (FDA) acceptance (Merchant et al. 2006). A typical hydrophilic polymer matrix system consists of drug, hydrophilic polymer(s) and other excipients distributed throughout the matrix. This system is dependent on polymer wetting, hydration and dissolution with regards to achieving controlled release of the drug (Merchant et al. 2006).

Many different hydrophilic polymers can be used to prepare controlled-release matrix systems. The most commonly used ones are cellulose ether derivatives which include hydroxypropylmethylcellulose (HPMC) (Melia 1991). HPMC is a partly O-methylated and O-(2-hydroxypropylated) cellulose (Rowe et al. 2009). Its hydration rate depends on the molecular structure and degree of substitution of these substituents. More specifically, the hydration rate of HPMC increases with increasing hydroxypropyl content (Dipti et al. 2014). It forms swellable matrices that lead to the formation of viscous solutions, instead of true gels (Aulton and Taylor 2017). High viscosity grades of HPMC can be used in tablets to retard drug release from matrices at a percentage of 10-80% w/w (Rowe et al. 2009).

MT-1207 hydrochloride is a novel antihypertensive drug that presents combinatory pharmacological actions. More specifically, it exhibits highly inhibitory activities simultaneously towards adrenergic a_{1A}, a_{1B} and serotonin (5-Hydroxytryptamine) 5-HT_{2A} receptors (2019 email from Shenyang Haiwang Biotechnology Co. Ltd to us; unreferenced). In a hypertension animal model, MT-1207 had an evident dose-effect relationship. It can reduce the blood pressure without accelerating the heart rate while improving the auditory brainstem response (ABR) and kidney protection functions. Therefore, it is considered promising and efficacious in the treatment of hypertension. It is expected to prevent the first-dose effect and postural hypotension effectively (2019 email from Shenyang Haiwang Biotechnology Co. Ltd to us; unreferenced). As is now well known, the drug is largely eliminated via hepatic clearance, mainly S-methylation. MT-1207 is a poorly soluble and highly lipophilic (logP of 4.15) basic compound with a pK_a value of 7.31. The most stable polymorph (anhydrous) has been chosen to be formulated in immediate-release tablets at a dose of 10 mg. Immediate-release tablets of MT-1207 hydrochloride were manufactured at an industrial scale by Shenyang Haiwang Biotechnology Co., Limited (Shenyang, China) and are being tested in terms of efficacy in phase II clinical trials. These tablets are designed to be administered three times a day. However, due to the challenges that come with hypertension, it is desirable to reduce the dosing frequency as much as possible to improve patient compliance.

In the present study, a new sustained-release matrix tablet formulation of MT-1207 hydrochloride was prepared. HPMC polymers of different viscosities were used as release retardants. Different tablet formulations were prepared using wet granulation and were characterised in terms of their physical properties and *in vitro* drug release. An optimised tablet formulation which provided a 24-hour release of MT-1207 in dissolution medium was

developed. Pharmacokinetic studies were performed in Beagle dogs, after oral administration of the optimised sustained-release and immediate-release tablets.

2. Materials and Methods

2.1 Materials

MT-1207 hydrochloride reference standard and micronised MT-1207 hydrochloride:colloidal silicon dioxide mixture with a mass ratio of 5:1 were donated by Shenyang Haiwang Biotechnology Co., Ltd (Shenyang, China). Sodium phosphate monobasic dihydrate, sodium acetate anhydrous, polyvinylpyrrolidone K30, hydroxypropylmethyl cellulose average molecular weight 86000 (K4M), hydroxypropylmethyl cellulose average molecular weight 90000 (K15M) and magnesium stearate were purchased from Sigma-Aldrich (Gillingham, United Kingdom). Alpha-D-lactose monohydrate, sodium dodecyl sulfate (SDS), hydrochloric acid 37%, methanol HPLC grade and propan-2-ol HPLC grade were purchased from Fisher Scientific (Loughborough, United Kingdom).

2.2 HPLC-UV method for the quantitation of MT-1207 samples

An HPLC-UV method was developed to quantify MT-1207 samples for solubility and dissolution testing. A Dionex Ultimate 3000 HPLC system was used (Thermo Scientific®, United Kingdom) with an ACE® Generix 3 C18, 150 × 4.6 mm, 3 µm column. The mobile phase consisted of 0.1 M sodium acetate buffer pH 4.0 and methanol at a ratio of 40:60 (% v/v). The mobile phase flow rate was set at 0.7 mL/min. The wavelength maximum of MT-1207 was found to be 230 nm, therefore the UV wavelength was set at that value. The injection volume was 20 µL. The retention time of MT-1207 was approximately 5 minutes. The quantitation of MT-1207 samples was carried out using a calibration curve which covered a concentration range of 1-50 µg/mL. The HPLC method was validated in terms of

linearity, the limit of detection (LOD) and limit of quantitation (LOQ) as per International Council for Harmonisation (ICH) and FDA guidelines. The method LOD was 0.1 µg/mL and the LOQ was 0.4 µg/mL. The validation of the HPLC method for specificity, accuracy, intra-day and inter-day precision was carried out along with that of the dissolution method.

2.3 Scanning electron microscopy (SEM) analysis of MT-1207 reference standard and micronised MT-1207

The MT-1207 hydrochloride reference standard and micronised MT-1207:silicon dioxide 5:1 mixture were examined by scanning electron microscopy (Philips® XL30 SEM, Netherlands) using 10 kV accelerating voltage. Before imaging, the samples were sputtered with gold using an SC7640 Sputter Coater (Polaron®, United Kingdom). Coated samples were then observed in the SEM at 10 kV under vacuum conditions at ambient temperature.

2.4 Solubility measurements of MT-1207:colloidal silicon dioxide 5:1 mixture

Solubility testing was carried out for MT-1207 reference standard and the micronised MT-1207:colloidal silicon dioxide 5:1 mixture. The aim was to determine the solubility of MT-1207 in different pH values and to define whether micronisation had an effect on the solubility of MT-1207 agent. The testing was carried out in hydrochloric acid pH 1.2 and 0.1 M phosphate buffer pH 6.8 simulating the pH values in the fasted stomach and small intestine, respectively. Solubility studies were carried out using the shake-flask method (Baka et al. 2008). A known excess quantity of the drug was weighed each time and dispersed in 5 mL of medium. Then, the dispersions were placed in a Stuart® orbital incubator and were shaken for 24 hours at 37˚C. Afterwards, 3 mL were placed in Sarstedt® tubes and centrifuged in an Eppendorf® Centrifuge 5810 R (2700 g, 15 minutes). A two-step dilution was carried out for the supernatant. For the samples in hydrochloric acid, 500 µL of the supernatant were each time diluted to 1 mL with water:methanol 50:50 (% v/v) and, then, 100

µL of the diluted solution were further diluted to 1 mL with water:methanol 50:50 (% v/v). For the samples in 0.1 M phosphate pH 6.8, 500 µL were diluted to 1 mL with methanol and, then, 100 µL from the diluted solution were further diluted to 1 mL with 0.1 M phosphate pH 6.8:Methanol 50:50 (% v/v). Each time, the final diluent was transferred into an amber HPLC vial for HPLC analysis.

2.5 Preparation of MT-1207 tablet formulations

The HPMC matrix tablets were prepared using a wet granulation method. The micronised MT-1207:silicon dioxide mixture was geometrically diluted with alpha-D-lactose monohydrate and then the rest of the excipients were added. Mixing was carried out for 15 minutes manually. The powder mixture was then transferred into a beaker and the required amount of isopropyl alcohol (IPA) was added as droplets under manual mixing that was conducted using a spatula. IPA has low toxicity in humans and is, therefore, classified as Class 3 solvent, as per United States Pharmacopeia (USP) <467> (USP 2017). Afterwards, there was a wet massing time of 30 seconds. The wet mass was manually pressed through a 12-mesh stainless steel sieve. The wet granules were then left in the fume hood to dry for two hours and were then placed in the tray drier for two hours at 60˚C. The dried granules were then sieved through a 25-45-170-mesh sieve array and the few fines (particle size < 90µm) were discarded. Magnesium stearate was added as a lubricant in the granulated mixture and mixing was carried out for 5 minutes. The granules were then compressed into tablets using a Piccola rotary tablet press machine (Riva®, Argentina). 8 mm flat-faced round punches and dies were used during the compression process. The compression force applied during the preparation of the different tablet formulations was 6.5 kN. The target tablet weight was 224.5 mg for formulations F1-F4 and 226.5 mg for formulation A1. Formulation F1 contained 18% w/w HPMC K4M, while formulations F2, F3 and F4 contained 18% w/w, 22% w/w and 31% w/w HPMC K15M, respectively. The compositions of the different tablet

formulations can be seen in Table 1. Three different batches of the optimised formulation were prepared and characterised to ensure the reproducibility of the manufacturing method. Finally, formulation A1 used in the pharmacokinetic study was prepared based on the optimised formulation containing 30 mg MT-1207, instead.

[Table 1]

2.6 Flowability characterisation of the tablet blends

The flow properties of the granulated mixture are most important. The flowability directly affects the filling uniformity of the die in the tabletting machine and, therefore, the corresponding uniformity of tablet weight and drug content (USP 2016). As per the USP, the flowability was assessed in terms of bulk/tapped density, compressibility index, Hausner ratio and angle of repose.

The bulk/tapped density testing was carried out using tapped density tester (Copley®, Nottingham, United Kingdom) as per USP <616> (USP 2015). Each time, a certain amount of granulated mixture was weighed and then added inside a 100-mL graduated measuring cylinder. The initial volume (bulk volume, V_0) was recorded. Then, the mixture was tapped 10, 500 and 1250 times and the respective volumes were recorded (V_{10}, V_{500}, V_{1250}). If the difference between V_{500} and V_{1250} was not more than 2 mL then V_{1250} was considered as the final tapped volume (USP 2015). The V_0 and V_{1250} were then used to calculate the compressibility (Carr) index and Hausner ratio. These values were used to characterise the flowability of the different mixtures.

The a~~A~~ngle of repose testing was also used to characterise the flowability of powder mixtures. The fixed funnel method was carried out, as per USP <1174> (USP <1174> 2012). Each time, an amount of powder was added inside a funnel and left to flow through it. As the tip of the powder cone reached the bottom of the funnel, the funnel was carefully moved

upwards. When the diameter of the cone reached slightly more than 6 cm the diameter of the base and the height of the cone were measured using a ruler. These values were then used to calculate the angle of repose. Based on the value of the angle of repose, the flowability of the mixture was characterised, as per USP (USP <1174> 2012).

2.7 Hardness and friability of tablets

The HPMC matrix tablets prepared were assessed in terms of their hardness using a C50 Tablet Hardness tester (Engineering Systems (Nottm)®, Nottingham, United Kingdom). The test was carried out as per USP <1217> (USP <1217> 2012). 10 tablets were tested for each formulation batch. Each time, the tablet dimensions (diameter, thickness) were measured. Then, the tablet was placed in the hardness tester and the hardness value was recorded in kg units. Afterwards, the tablet tensile strength was calculated, as per USP (USP <1217> 2012).

The friability of the HPMC matrix tablets was assessed, as per USP <1216> (USP <1216> 2012). The tablets were weighed before being placed into the friability tester (Erweka®, Heusenstamm, Germany). The friabilator was set at a revolution rate of 25 rpm for 4 minutes. The tablets were then removed from the device and were smoothly wiped using a brush. Then, they were weighed and the % friability was calculated. As per USP, the friability should be less than 1.0%.

2.8 Uniformity of weight

The tablet weight variation was assessed, as per USP <905> (USP 2016). 10 tablets were weighed individually for each formulation. The mean tablet weight was calculated and expressed as % of the target tablet weight. Then, the acceptance value was calculated. The tablet weight variation was considered to be within the acceptable range set in USP if the acceptance value was less than 15.

2.9 Differential scanning calorimetry analysis of MT-1207 and the sustained-release tablets

The thermal behaviours of MT-1207 hydrochloride and pulverised tablets were evaluated using DSC Q2000 (TA® Instruments, Centennial Park, United Kingdom). Samples (1-5 mg) were each time weighed and sealed inside an aluminum pan with pierced aluminum lids. The analysis took place over a temperature range of 40-240˚C at a rate of 5˚C/min in an atmosphere of nitrogen, the flow rate of which was set at 50 mL/min. The analysis for the API took place over a temperature range of 40-300˚C to spot any potential thermal degradation at temperature values beyond 240˚C.

2.10 Fourier-Transform Infrared (FTIR) spectrophotometric studies

Fourier-Transform Iinfrared (FTIR) spectra were obtained for MT-1207 hydrochloride and the sustained-release tablets that were prepared using Cary 630 FTIR spectrophotometer (Agilent Technologies®, Craven Arms, United Kingdom). Each time, the solid sample was placed on the lid and scanned at the range 4000-650 cm^{-1}.

2.11 X-ray powder diffraction (XRPD) analysis of MT-1207 and the sustained-release tablets

The diffractograms of MT-1207 hydrochloride and pulverised tablets were obtained using an X'Pert Pro MPD X-ray diffractometer (Malvern Panalytical®, Malvern, United Kingdom). Cu K-alpha1 radiation was used. The samples were scanned at an angle range of 4-50˚(2θ) with a step size of 0.01˚(2θ) at 40 mV, 40 mA and a sample rotation speed of 15 rpm.

2.12 In vitro release of MT-1207 from sustained-release tablets

In vitro dissolution studies were carried out using a USP II dissolution apparatus (Copley®, Nottingham, United Kingdom) equipped with paddles. The apparatus was set at a

constant rotation speed of 75 rpm. Dissolution studies were carried out in 900 mL of 0.1 M phosphate buffer pH 6.8 with 0.2% w/v SDS at 37 ± 0.5˚C. Sinkers were used to prevent floating of tablets and their adhesion to the bottom of the vessels. Sampling was carried out manually at certain time intervals (0.08, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours), followed by centrifugation of the samples (2300 g, 10 minutes). Then, each time 1 mL from the supernatant was transferred into an HPLC vial and 20 µL injected into the HPLC for analysis. The *in vitro* release study was carried out in triplicate for each formulation.

2.13 Swelling and erosion studies

Swelling and erosion studies were carried out for the F4 tablets in dissolution medium, 0.1 M phosphate pH 6.8 with 0.2% w/v SDS. The USP II Dissolution Apparatus was used. Each time, the pre-weighed tablet (initial weight, W_0) was immersed in 900 mL dissolution medium within a dissolution vessel. The temperature was maintained at 37 ± 0.5˚C. The paddle speed was set at 75 rpm. At certain time intervals (1, 2, 4, 6, 8, 12, 16, 20 and 24 hours) the tablet was taken out of the dissolution vessel and excess water was carefully plotted from its surface. The wet tablet was weighed and the wet weight value, W_t, was obtained. The % swelling index of the tablets was calculated using Equation 1:

$$\% \text{ Swelling index} = (Wt - W_0) \times 100/W_0 \qquad \text{(Equation 1)}$$

The same tablets that were used to calculate the swelling index were also used to calculate the % erosion value. After weighing, the wet tablets were placed inside an oven and the temperature was set at 60˚C. The tablets were each time dried until constant weight. The

dry weight value, W_f, was recorded. The % erosion of the tablets at each timepoint was calculated using Equation 2:

$$\% \, Erosion = (W_0 - W_f) \times 100/W_0 \qquad \text{(Equation 2)}$$

All experiments were done in triplicate and the results were recorded as mean ± standard deviation.

2.14 Kinetics and mechanism of drug release

Based on the results obtained from *in vitro* dissolution testing for the optimised formulation F4, the release kinetics of MT-1207 from that formulation were studied. For this purpose, the data obtained from the *in vitro* drug release testing were plotted in various kinetic models. Those were first-order (Equation 3) as log percentage of MT-1207 remaining undissolved vs time, zero-order (Equation 4) as the cumulative amount of drug released vs time and Higuchi's models (Equation 5) as cumulative percentage of MT-1207 released vs square root of time. Finally, the data were plotted using the Hixson-Crowell cube root law to evaluate the drug release with changes in the particle/tablet surface area and diameter (Equation 6) (Merchant et al. 2006).

$$LogQ = LogQ_0 - kt/2.303 \qquad \text{(Equation 3)}$$

where Q is the amount of the drug in the tablet at time t, Q_0 is the total amount of the drug and k is the first-order constant.

$$C = k_0 t \qquad \text{(Equation 4)}$$

where C is the concentration of the drug at time t and k_0 is the zero-order constant.

$$Q = kt^{1/2} \qquad \text{(Equation 5)}$$

where Q is the cumulative % of drug released at time t and k is a constant the value of which depends on the system design variables (Merchant et al. 2006).

$$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = k_{HC} \times t \qquad \text{(Equation 6)}$$

where Q_t is the amount of drug released in time t, Q_0 is the initial total amount of drug in the tablet and k_{HC} is the rate constant for the Hixson-Crowell equation which refers to the plot of the cube root of the drug remaining in the tablet vs time.

The mechanism of drug release from F4 tablets was determined using the Korsmeyer-Peppas model (Korsmeyer et al. 1983). The data from the *in vitro* dissolution testing were plotted in the Korsmeyer-Peppas equation (Equation 7) as log cumulative percentage of drug released vs log time. The exponent n was calculated by measuring the slope of the straight line.

$$M_t/M_\infty = kt^n \quad \text{(Equation 7)}$$

where M_t is the amount of drug released at time t, M_∞ is the total amount of drug and k is a kinetic constant the value of which is characteristic of the drug/polymer system and n is an exponent that is used to characterise the mechanism of drug release (Korsmeyer et al. 1983). For cylinder tablets, $n = 0.45$ corresponds to drug release controlled by Fickian diffusion, $0.45 < n < 0.89$ corresponds to anomalous (non-Fickian diffusion) which refers to a combination of Fickian diffusion and polymer relaxation mechanisms, while $n > 0.89$ defines super Case-II transport where drug release is controlled by polymer relaxation (Peppas 1985; Siepmann and Peppas 2001; Nguyen et al. 2020).

2.15 In vivo animal study

An *in vivo* animal study was carried out as per the principles of the Declaration of Helsinki in Beagle dogs (body weight 10 ± 2 kg) for MT-1207 30 mg sustained-release (A1) and 10 mg immediate-release tablets. Eight healthy beagle dogs were used, half male and half female. According to gender and weight, they were randomly divided into two groups, 4 in each group (2 males and 2 females). After fasting for 12 hours (h), the two groups of animals were given MT-1207 tablets orally. In the first half of the first group (Group 1-A), the sustained-release tablets were administered once a day, one tablet at a time, and blank blood was taken before administration. After administration, at 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h and 32 h timepoints approximately 1.5 mL of venous blood were each time collected. In the second half of the first group (Group 1-B) immediate-release tablets were administered three times a day, one tablet at a time, with an interval of 8 hours. Blank blood was collected before administration and then at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h after the first administration (second administration), 8.25 h, 8.5 h, 9 h, 11 h, 13 h, 16 h (third

dose), 16.25 h, 16.5 h, 17 h, and 24 h. Approximately 1.5 mL of venous blood were collected at each timepoint. Two groups of animals were cross-administered with another formulation after 7 days of wash-out period. In the second group (Group 2), the immediate-release tablets were administered once a day, three tablets at a time, and blank blood was taken before administration, and at 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 8 h, and 24 h timepoints. Approximately, 1.5 mL of venous blood was collected at each timepoint. In all the cases, each sample was placed in a test tube containing heparin, centrifuged and the plasma was separated and stored at -20˚C. MT-1207 in plasma samples was determined using UPLC-MS/MS.

2.16 Bioanalytical method

MT-1207 in plasma samples was determined by a validated UPLC-MS/MS method using verapamil hydrochloride as an internal standard. Each time 10 µL of plasma sample were pipetted in 1.5 mL Eppendorf® tube. 200 µL of verapamil hydrochloride 2 ng/mL in acetonitrile were added and vortex was carried out for 5 minutes. Centrifugation was then carried out at 15000 rpm for 5 minutes and 100 µL of supernatant were collected for UPLC-MS/MS analysis. The ion source was an electrospray ionisation source (ESI). A positive ion scanning method was used for detection. The solvent gas (nitrogen) flow rate was 1000 L/h, the temperature of the solvent gas was 500˚C, and the capillary voltage was 3.0 kV. The scanning method was Multiple Response Monitoring (MRM). The cone voltage was set at 40 V, while the collision energy was 20 eV. For quantitative analysis, the ion pairs used had m/z 393.26 → 274.04 (MT-1207) and m/z 455.25 → 156.06 (internal standard). The samples were applied to an ACQUITY Ultra Performance Liquid Chromatography system with Xevo TQ-XS Triple Quadrupole Mass Spectrometer with operating software MassLynx V4.2 (Waters Technology Limited Company). The column used was an ACQUITY UPLC BEH C18 liquid chromatography column (2.1 × 50 mm, 1.7 µm). The mobile phase consisted of

0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B). Verapamil hydrochloride was used as the internal standard for determination. The gradient elution was: 0-1.2 min: 20-45% B, 1.2-1.5 min: 45-95% B, 1.5-1.8 min: 95% B, 1.8-2.5 min: 95-20% B. The flow rate was set at 0.5 mL/min. The column temperature was set at 45˚C.

Quantification was carried out using a calibration curve. 10 mg MT-1027 were accurately weighed and dissolved in DMSO to prepare a stock solution of MT-1207 with a concentration of 10 mg/mL. This was gradually diluted with a 50% acetonitrile solution to obtain standard working solution concentrations of 10, 20, 40, 100, 200, 400, 1000, 2000, 4000 ng/mL. The concentrations of the quality control samples were 30, 320, 3200 ng/mL. To prepare a quality control working solution 10 µL were added to 190 µL Beagle dog blank plasma. Vortex mixing was carried out to obtain the standard curve with drug concentration values of 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL and plasma with drug concentration values of 0.5, 1.5, 16, and 160 ng/mL. 20 µL of medicated plasma were transferred into a 1.5 mL Eppendorf® tube and 200 µL of 2 ng/mL verapamil hydrochloride in acetonitrile were added. Vortex was carried out for 5 minutes, followed by centrifugation at 15,000 rpm for 5 minutes. 100 µL of supernatant were collected for UPLC-MS/MS measurement. The lower limit of quantitation (LLOQ) was 0.500 ng/mL. The linear regression coefficient of the standard curve was around 0.9978. The accuracy and precision values of the quality control samples were 101.0% and 3.2% CV, respectively.

2.17 In vitro and in vivo data analysis

The peak plasma concentration of MT-1207 (C_{max}), the time to reach C_{max} (T_{max}) and the area under the concentration-time curve from 0 hours to 24 hours (AUC_{0-24h}) and to infinity ($AUC_{0-\infty}$) values were calculated from the plasma concentration vs time profile using WinNonlin® version 6.4. Furthermore, the half-life ($t_{1/2}$), clearance (CL), apparent volume of

distribution (V_d), and mean residence time (MRT) values of MT-1207 after administration of the tablets following the different dosing regimens were also calculated using WinNonlin® version 6.4. Finally, the bioequivalence between the sustained-release and immediate-release tablets was investigated by calculating the ratio of $AUC_{0\text{-}24h}$ values of the sustained-release tablet dosing regimen and the two separate immediate-release tablet dosing regimens.

T-test and one-way analysis of variance (ANOVA) were carried out for the statistical analysis of results using Microsoft Excel® software. A statistically significant difference was reflected by a P value less than 0.05.

3. Results and Discussion

3.1 Scanning electron microscopy and solubility characterisation of MT-1207

Solubility testing was carried out in hydrochloric acid pH 1.2 and phosphate buffer pH 6.8 to obtain information on the solubility of the drug at different pH values. MT-1207 was more soluble in hydrochloric acid than in phosphate buffer (Table S1). Since the latter was chosen as the dissolution medium in the present study, a small amount of SDS was added into phosphate buffer to ensure sink conditions during dissolution testing.

There was no statistically significant difference between the recorded solubility values of non-micronised MT-1207 and micronised MT-1207:silicon dioxide mixture in the different media (t-test, $p > 0.05$) (Table S1). These results agree with literature findings since particle size reduction of drugs at this scale was not expected to lead to increased aqueous solubility unless it caused amorphisation of the API (Jinno et al. 2006; Loh et al. 2015). Particle size reduction could, however, enhance the dissolution rate of MT-1207, due to increased drug surface area for interaction with liquid media (Chu et al. 2012; Marinko and Zámostný 2020). In Figure 1, it can be seen that the drug crystals have an elongated shape. The long-axis particle size values of the non-micronised drug and micronised drug that was

used to make the tablets were (11.97 ± 7.42) µm and (4.02 ± 1.90) µm, respectively (n=100 particles for each batch). The particle size distribution was narrower in the micronised batch than in the non-micronised drug and was used for the preparation of both immediate and sustained release tablets.

[Figure 1]

3.2 Characterisation of flowability and compressibility of the tablet powder blends

Before compression, the granulated powder blends were assessed in terms of compressibility and flowability through bulk/tapped density and angle of repose testing, as per USP.

The flowability of a powder blend is most important since it determines whether the filling of the powder in the dies of the tabletting machine is uniform, thus ensuring uniform tablet weight and dimensions and drug content uniformity (Abd-Elbary et al. 2012). The flowability of a powder blend can be assessed through Carr index, Hausner ratio or angle of repose. Compressibility is also important since it defines the ability of a powder to compress upon application of pressure (Jamzad and Fassihi 2007). Carr index is a good indicator of the powder flowability and compressibility (Carr 1965b). Therefore, a Carr index value between 5% and 16% is desirable to achieve both good flowability and compressibility (Jamzad and Fassihi 2007). F1, F3, F4 and A1 powder blends had Carr index values of 14.7, 15.4, 12.2 and 13.3%, respectively (Table S2), which indicates a combination of good flowability and compressibility. The F2 powder blend, however, had a relatively high compressibility index value of 23.5%, but still, its flowability was characterised as fair, which ensured its free flow in the feeder of the tabletting machine and the dies.

Hausner ratio is another measure of the flow properties of a powder blend (Hausner 1967). It provides information on the extent of the powder densification upon compression.

Powders with a higher tendency to densify have higher Hausner ratio values. All powder blends had Hausner ratio values that indicated fair to good flowability and were in complete alignment with the respective Carr index values. For each tablet batch, both of these values were calculated through bulk/tapped density testing, as per USP. The Hausner ratio values ranged between 1.14 and 1.18 for the powder mixtures of formulations F1, F3, F4 and A1 (Table S2) which confirmed their good flowability and compressibility, along with the respective Carr index values. F2 powder blend, however, gave a relatively high Hausner ratio of 1.31, but, still, its flowability was characterised as fair and in alignment with the respective Carr index value.

The angle of repose can also be used to characterise the flow properties of solids. It is a characteristic related to powder cohesion or resistance to movement between particles (USP <1174> 2012). High angle of repose values correspond to high powder cohesiveness and, thus, poor flow properties. The angle of repose values for the F1, F3, F4 Repeat 2, F4 Repeat 3 and A1 powder mixtures were 34.2˚, 33.2˚, 38.7˚, 35.6˚ and 36.4˚, respectively (Table S2). These test findings align with the bulk/tapped density results for flowability of the blends. In F2 and F4 Repeat 1 batches, there was a deviation of the angle of repose value from bulk/tapped density results. For F2 the angle of repose value (39.0˚) defined a good flowability, while the rest of the results indicated a fair flowability. For F4, the angle of repose value, 42.0˚, defined a passable flowability of the powder whilst the bulk/tapped density results indicated a good flowability of the same blend. Such subtle variations can occur within these empirical type tests but they remain broadly consistent.

3.3 Post-compression parameters of tablet formulations

The hardness of the prepared tablet formulations was assessed (Table S3). Increasing HPMC content and viscosity grade from formulations F1 to F4 resulted in higher hardness

values. This could be due to the binding properties of HPMC, so increasing its content probably resulted in stronger solid bridges within the tablets and, thus, higher hardness (Chowhan 1980; Dipti et al. 2014). The hardness test was also carried out for F4 Repeats 2 and 3 (Table S3). There was no statistically significant difference in tablet hardness between the three F4 batches (one-way ANOVA, $p > 0.05$). Also, the hardness of A1 tablets bound to be used for animal studies was similar to that of F4 tablets (one-way ANOVA, $p > 0.05$).

All tablet batches gave friability values that were well within the acceptable range of 1.0% set in USP (Table S4). This confirmed their good quality and their ability to withstand the expected, abovementioned mechanical shocks.

All tablet batches gave acceptable mean weight values and acceptance values, with regards to uniformity of weight (Table S5). Therefore, for all formulations, the tablets had a uniform weight of drug substance per weight of dosage unit.

3.4 Differential scanning calorimetry (DSC) analysis

The thermal behaviour of an MT-1207 reference standard and that of the tablet formulations was assessed using DSC.

DSC thermograms of MT-1207 and the different formulations can be seen in Figures 2 and 3. MT-1207 hydrochloride crystal (Figure 2) showed a sharp endothermic peak at around 210˚C which was attributed to its melting point, while degradation seemed to take place at around 275˚C. In the thermograms of the tablet formulations (Figure 3) different peaks were noted. The peaks at around 150-160˚C correspond to the dehydration of alpha-D-lactose monohydrate towards beta-lactose and melting point of PVP K30 and the peak at around 220˚C corresponds to the melting point of beta-lactose. A relatively broad endothermic peak was seen at the temperature range 178-197˚C. This is proposed to correspond to the melting point of the MT-1207 hydrochloride crystal. A broadening of the peak was noted, though,

along with a reduction in the heat of fusion, compared to the reference standard. This could potentially be due to the mixing process which results in lower purity of each component, thus leading to lower melting point of the drug crystal (Smith 1982; Dürig and Fassihi 1993; Pani et al. 2011). Additionally, the strong physical interactions taking place between the drug and the excipients during mixing, granulation and, particularly, compression into tablets could significantly contribute to the broadening of the MT-1207 endothermic peak (Mura et al. 1995). Finally, the dispersion of the drug crystal in the HPMC and PVP K30 polymer matrix could account for the reduction in both the melting point and the heat of fusion of the drug (Mura et al. 1998).

[Figure 2]

[Figure 3]

3.5 Fourier-Transform Infrared (FTIR) analysis

In Figure 4 the Fourier-Transform Infrared (FTIR) spectra of MT-1207 hydrochloride reference standard (Figure 4A), HPMC K15M (Figure 4B), colloidal silicon dioxide (Figure 4C) and pulverised tablets (Figure 4D) can be seen.

FTIR characterisation is useful in determining whether chemical reactions between the API and the excipients occur during mixing, granulation and tabletting (Pani et al. 2011). The chemical structure of MT-1207 does not contain functional groups that would readily react with the excipients. The peaks in the 2800-3000 cm^{-1} region (Figure 4A) that correspond to aliphatic and aromatic C-H stretch can also be seen unchanged in the pulverised tablet spectrum (Figure 4D). Furthermore, the peaks in the 1250-1300 cm^{-1} region that correspond to C-N stretch can be seen in ~~both~~ the spectra of both the MT-1207 reference standard and pulverised tablets. Finally, the fingerprint region of the pulverised tablets is a simple mixture of the fingerprint regions of MT-1207 hydrochloride (Figure 4A), colloidal silicon dioxide

(Figure 4B) and HPMC K15M (Figure 4C) with no additional peaks observed. These results illustrate that there were no significant chemical interactions between MT-1207 and the excipients.

[Figure 4]

3.6 X-ray powder diffraction (XRPD) analysis

The diffractograms of MT-1207 hydrochloride reference standard and formulations F1 and F4 were acquired and can be seen in Figures 5A and 5B, respectively. The peaks of MT-1207 anhydrous crystal are assigned in Figure 5C. The diffractograms of the sustained-release tablet formulations were identical. In Figure 5C, it can be seen that the peaks of the F4 diffractogram over the angle(°2theta) values of 11.5, 15 and 18 corresponded to the respective peaks of the MT-1207 hydrochloride reference standard, while all the other peaks of the F4 diffractogram corresponded to the peaks of alpha-D-lactose monohydrate. The reduction in peak height and broadening of the MT-1207 peaks in the F1 and F4 diffractograms (Figures 5B and 5C) can be attributed to the mixing of the drug with the rest of the excipients, most of which are amorphous. This resulted in a lower percentage of the API in the mixture analysed and, therefore, to broader and less intense peaks. All the peaks present in the diffractogram of F4 could be attributed to either MT-1207 anhydrous crystal or alpha-D-lactose monohydrate. Therefore, it can be asserted that within the sensitivity of the analysis that the drug was present in the tablets solely in the form of the anhydrous crystal.

[Figure 5]

3.7 In vitro release of MT-1207 from sustained-release tablets

The *in vitro* release profiles of the immediate-release tablets and the different sustained-release tablet batches can be seen in Figure 6. HPMC has been used as a release retarding polymer due to its ability to swell when in contact with water. In the present study,

HPMC polymers of different molecular weight and viscosity values were used in the different formulations. HPMC K4M was the initial choice of release retarding polymer. In the literature, this viscosity grade of HPMC has been effective in retarding the *in vitro* release of different drugs from sustained-release formulations (Abd-Elbary et al. 2012; Hiremath and Saha, 2008; Savaşer et al. 2013). However, when HPMC K4M was used as a sole release retardant at a percentage of 18% w/w (formulation F1) it was unable to significantly retard the release of MT-1207. For this reason, in the rest of the batches, HPMC K4M was replaced by HPMC K15M, a polymer of higher molecular weight and viscosity.

[Figure 6]

In Figure 6 it can be seen that a sustained release of MT-1207 was achieved from tablets with HPMC K15M. In the literature, it has been noted that drug release rate from matrix tablets decreases with increasing HPMC concentration and viscosity, due to increasing strength and thickness of the gel layer that is formed upon swelling of the polymer and the diffusion of the drug through the polymer chains being retarded (Jamzad and Fassihi 2006; Hiremath and Saha 2008). Lactose, in contrast, promotes drug release through promoting matrix hydration, gel formation and free volume (Gao et al. 1996). Increasing the concentration of HPMC K15M from F2 to F4 resulted in a slower release of MT-1207 which was maintained linear over a longer time. The results of the present study are, therefore, fully aligned with the literature (Jamzad and Fassihi 2006). The desired release profile of the drug was acquired from formulation F4 (31% w/w HPMC K15M), reaching approximately 93% release after 24 hours of dissolution with the linearity of release being maintained for 20 hours. This is most desirable since it could potentially ensure constant, effective levels of MT-1207 in plasma following absorption of the drug through the gastrointestinal tract (Merchant et al. 2006). Therefore, formulation F4 was selected as the optimised formulation and for *in vivo* studies. Furthermore, no statistically significant difference was noted between

the release profiles from the three different F4 batches (one-way ANOVA, $p > 0.05$) (Figure S1). Therefore, it was concluded that the manufacturing process and the formula gave rise to tablets with reproducible properties. Finally, formulation A1 was prepared based on F4 with a change in the drug dose to 30 mg. There was no statistically significant difference in the *in vitro* release profile of MT-1207 between the F4 and A1 formulations (t-test, $p > 0.05$). Therefore, the properties of F4 and A1 tablets were identical.

3.8 Swelling and erosion study

The swelling and erosion behaviour of tablets is most important since it can affect drug release kinetics. In Figure 7, the results from the swelling and erosion studies of F4 tablets can be seen. The tablets showed significant swelling in the dissolution medium with the tablet weight increasing by 170.6% w/w after 8 hours of dissolution. A rapid swelling phase was initially noted, since 110.3% w/w swelling took place within 2 hours of dissolution, followed by a relatively slower increase between 2 and 8 hours of dissolution. After 8 hours of dissolution, the tablet erosion reached 64.0% w/w indicating that most of the tablet mass had eroded at the time where maximum swelling of the tablets was achieved. After that timepoint, a noteworthy reduction of the erosion rate was recorded, possibly because maximum swelling was reached and therefore the thickness of the hydrogel and the viscosity of the surrounding viscous solution were maximised. These results, along with the *in vitro* release findings, indicate that diffusion and swelling may have contributed, to some extent, to the release of MT-1207 from the F4 tablets, but drug release was predominantly erosion-controlled.

[Figure 7]

3.9 Kinetics and mechanism of MT-1207 release from formulation F4

Through the selection of an appropriate molecular weight of HPMC in the matrix and the rate of matrix hydration, the rate of polymer disentanglement can be controlled. Therefore, the mechanism of drug release is dependent on the sum of drug diffusion and polymer relaxation and erosion. The release profile of F4 tablets was plotted against different models to elucidate the kinetics of drug release, while the Korsmeyer-Peppas model was used to obtain information on the mechanism of drug release. All models were used to analyse the data of up to 60% of the release curve (Peppas 1985; Saurí et al. 2014).

The release data of formulation F4 were plotted in zero-order, first-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models. The regression coefficient values of the different model plots can be seen in Table ~~1~~2 for both formulations. The data fitted best to the zero-order model (R^2 = 0.9992). When the data were plotted against the Korsmeyer-Peppas model, the *n* value (calculated from the slope of the curve) was 0.99~~8~~ (Table 2) which corresponded to a zero-order mechanism of release of MT-1207 from F4. Therefore, it can be assumed that both the kinetics and mechanism of the release of MT-1207 from F4 tablets were zero-order. The kinetic analysis findings were aligned with the swelling and erosion study results which, being well correlated with the *in vitro* release profiles, also indicated an erosion-controlled drug release from the F4 tablets.

[Table ~~1~~2]

~~**[Table 2]**~~

3.10 In vivo animal study

The sustained-release and immediate-release tablets of MT-1207 were cross-administered orally in four Beagle dogs. Figure 8 illustrates the pharmacokinetic profiles of MT-1207 in the dog plasma after oral administration of one MT-1207 30 mg sustained-

release tablet, three 10 mg immediate-release tablets with 8-hour interval between each and three 10 mg immediate-release tablets administered at one time. The pharmacokinetic parameters recorded on the respective dosing regimens in Beagle dogs can be seen in Table 3. A sustained pharmacokinetic profile of MT-1207 can be seen after oral administration of the sustained-release tablet, compared to the pharmacokinetic profiles of the IR tablets. There was a statistically significant decline (t-test, $p < 0.05$) in the C_{max} value from 62.5 ng/mL (recorded in 3 × 10 mg IR o.d.) to 37.01 ng/mL for 30 mg SR o.d. This, along with the increase recorded in T_{max} value from 1 and 1.63 hours (10 mg IR t.i.d. and 3 × 10 mg IR o.d., respectively) to 4.38 hours in 30 mg SR indicates a sustained release of the drug, along with no evidence of dose dumping after the oral administration of sustained-release tablets (Defang et al. 2005). Hence, HPMC K15M was able, as indicated in the *in vitro* data, to provide a sustained release of MT-1207 from the tablets *in vivo*. The drug level remained at similar concentration values for about 3 hours (3-6 hours after administration) before declining, which indicates the prevalence of drug elimination over absorption. With regards to the extent of absorption, it was noted that in all the above-mentioned cases the $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$ values recorded were statistically the same (one-way ANOVA, $p > 0.05$) (Table 3). In addition to this, the bioequivalence between the sustained-release and immediate-release tablets was investigated by calculating the $AUC_{0\text{-}24h}$ ratio of the sustained-release tablet dosing regimen and the immediate-release tablet dosing regimens. As per the EMA guidelines, the prerequisite for bioequivalence is that the $AUC_{0\text{-}t}$ ratio of the test and reference products should fall within the range 80.00-125.00% (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr 2010). The $AUC_{0\text{-}24h}$ ratios of the sustained-release tablet and the t.i.d. and o.d. immediate-release tablet dosing regimens were 98.12% and 90.05%, respectively. Based on these findings, it can be assumed that the whole amount of drug was released from the

sustained-release tablets *in vivo* and was absorbed to the same extent as when the same dose was administered in immediate-release tablets, irrespective of the dosing regimen.

[Figure 8]

The distribution of MT-1207 in the body is an important parameter considered during pharmacokinetic analysis. The apparent volume of distribution, V_d, reflects the space of the body seemed to be occupied by the drug (Shargel et al. 2012). V_d increases with increasing distribution of the drug to peripheral tissues. The mean residence time, MRT, reflects the average time during which the drug resides in the body. In the present case, even though the SR tablet showed the highest value for the apparent volume of distribution which was about 2.5 times higher than the IR tablet, there seemed to be no statistically significant difference (one-way ANOVA, $p > 0.05$) between the V_d values of MT-1207 recorded in SR o.d., IR t.i.d. and 3 × IR o.d (Table 3). Furthermore, with regards to MRT, the value obtained for 3 × IR o.d. increased more than 2 times for the same dose of MT-1207 administered as a sustained-release tablet (Table 3) and the difference between the two values was deemed to be statistically significant (t-test, $p < 0.05$). This indicates that the time during which the drug resides in the body was increased significantly when MT-1207 was administered in the form of a sustained-release tablet, compared to immediate-release tablets. There was a statistically significant difference in MRT between all three groups (one-way ANOVA, $p < 0.05$).

The half-life, $t_{1/2}$, of MT-1207 was prolonged to 3.44 hours when the sustained-release tablet was administered in the dogs, 2 to 2.5 times higher than when three immediate-release tablets administered at the same time (Table 3). When immediate-release tablets were administered at 8-hour intervals the MT-1207 $t_{1/2}$ was similar to that recorded after administration of the sustained-release tablet. With regards to clearance, there was no

statistically significant difference (one-way ANOVA, $p > 0.05$) between the clearance values that were recorded in Beagle dogs for the three different dosing regimens (Table 3).

Hepatic metabolism is generally higher in Beagle dogs than in humans (Boxenbaum 1982; Shargel et al. 2012). Phase I clinical trials were carried out for MT-1207 at a certain dose range. At the 30 mg dose, the $t_{1/2}$ value of MT-1207 was 7.29 hours in humans (2020 email from Shenyang Haiwang Biotechnology Co. Ltd to us; unreferenced). Considering that formulation A1 gave a $t_{1/2}$ in Beagle dogs that was 2-2.5 times higher than that of the immediate-release tablets, the formulation could be considered as a once-a-day treatment, provided that a similar increase in $t_{1/2}$ occurs in humans.

[Table 3]

4. Conclusions

In the present study, a new sustained-release tablet for the novel antihypertensive drug MT-1207 was successfully developed. HPMC K15M was able to provide a sustained release of MT-1207 *in vitro* and in an animal model. Amongst the different formulations, the optimised formulation (F4) containing 31% w/w HPMC K15M was chosen for a study in Beagle dogs since it provided a 24-hour *in vitro* release of MT-1207 that demonstrated a sustained release of the drug. The half-life of the drug was extended, while the amount of drug absorbed was the same as that of the immediate-release tablets. Based on the pharmacokinetic data from Beagle dogs and phase I clinical trials, this formulation can be considered for once-a-day treatment of hypertension, an advance which could have significant commercial and patient health benefits.

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Declaration of interest

The authors declare that Shenyang Haiwang Biotechnology Co., Limited which funded the present study is the patent holder of MT-1207 agent.

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Ingredients (mg)	Formulation batch				
	F1	F2	F3	F4	A1
MT-1207 Hydrochloride	20	20	20	20	30
Alpha-D-lactose monohydrate	140	140	130	110	100
Polyvinylpyrrolidone K30	20	20	20	20	20
HPMC K15M	-	40	50	70	70
HPMC K4M	40	-	-	-	-
Colloidal silicon dioxide	4	4	4	4	6
Magnesium stearate	0.5	0.5	0.5	0.5	0.5
Tablet weight	**224.5**	**224.5**	**224.5**	**224.5**	**226.5**

Models	Kinetic constant	Regression coefficient
First-order	0.142	0.9835
Zero-order	0.001	0.9992
Higuchi	22.479	0.9849
Hixson-Crowell	0.103	0.9689
Korsmeyer-Peppas		
Kinetic constant	Regression coefficient	Exponent (*n*)
4.586	0.9985	0.98

Table 3: Pharmacokinetic parameters of MT-1207 after oral administration of a single MT-1207 30 mg sustained-release tablet (30 mg SR o.d.), 10 mg immediate-release tablets (one tablet at a time) three times a day (10 mg IR t.i.d.) and three 10 mg immediate-release tablets administered at the same time (3 × 10 mg IR o.d.) to Beagle dogs (n=4).

Dosing Regimen	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (h*ng/mL)	$AUC_{0\text{-}\infty}$ (h*ng/mL)	V_d (L/kg)	CL (L/h/kg)	MRT (h)
30 mg SR o.d.	3.44±1.49	4.38±2.14	37.01±8.29	213.6±59.8	222.1±56.4	723.3±448.1	140.9±31.1	6.40±1.09
10 mg IR t.i.d.	3.47±1.49	1.00±0.71	32.22±23.28	217.7±81.6	229.7±86.7	540.1±247.0	116.8±57.3	13.05±3.77
3 × 10 mg IR o.d.	1.69±0.20	1.63±0.25	62.50±18.65	237.2±53.8	250.8±57.8	299.7±58.8	124.1±26.5	2.84±0.27

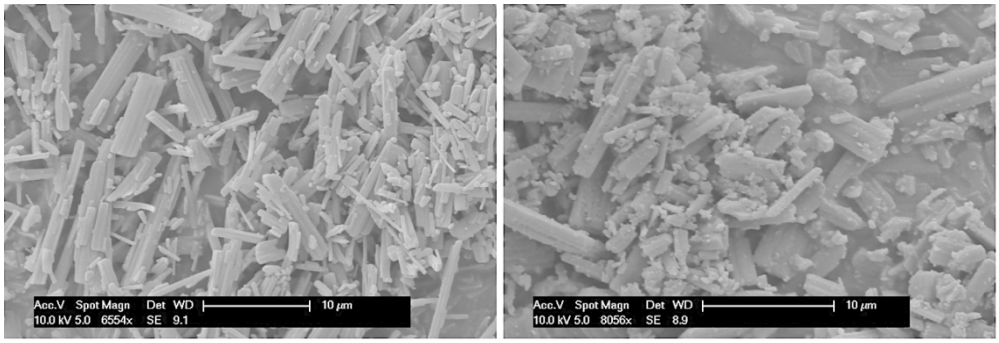

Figure 1: SEM image of (A) MT-1207 reference standard and (B) micronised MT-1207:silicon dioxide 5:1 mixture.

32x12mm (1000 x 1000 DPI)

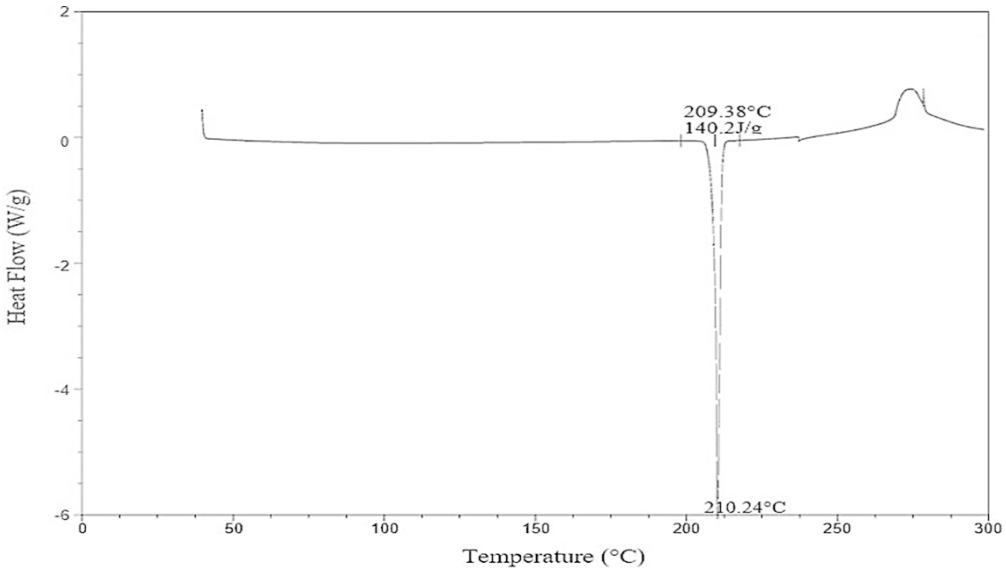

Figure 2: DSC thermogram of MT-1207 hydrochloride reference standard.

32x18mm (1000 x 1000 DPI)

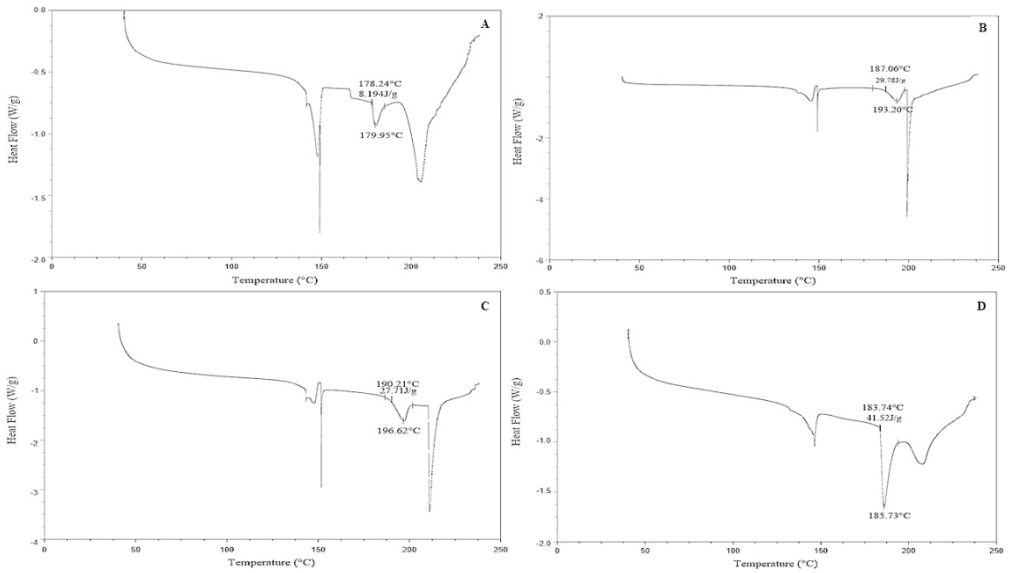

Figure 3: DSC thermograms of (A) Formulation F1, (B) Formulation F2, (C) Formulation F3 and (D) Formulation F4.

32x18mm (1000 x 1000 DPI)

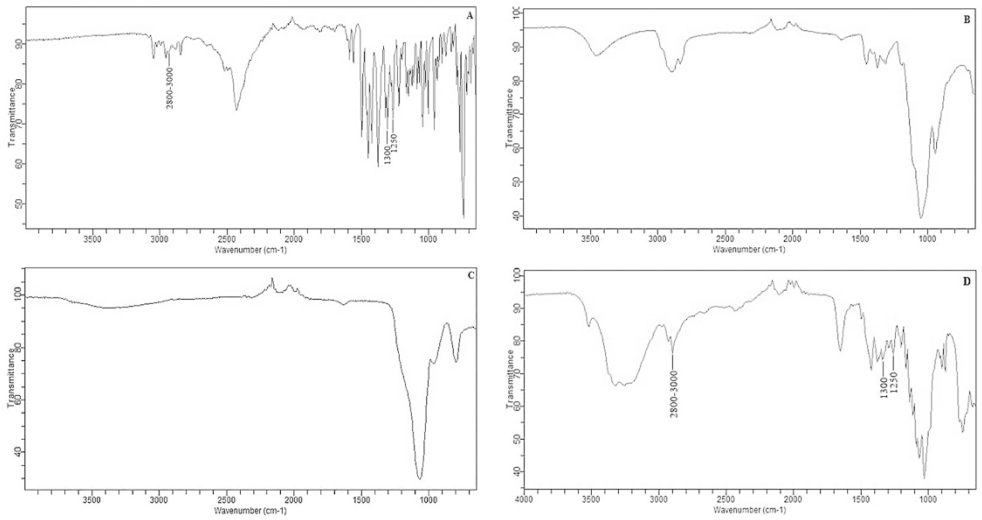

Figure 4: Fourier-Transform Infrared (FTIR) spectra of (A) MT-1207 hydrochloride reference standard, (B) HPMC K15M, (C) colloidal silicon dioxide and (D) pulverised tablets [Transmittance vs Wavenumber (cm^{-1})].

51x26mm (1000 x 1000 DPI)

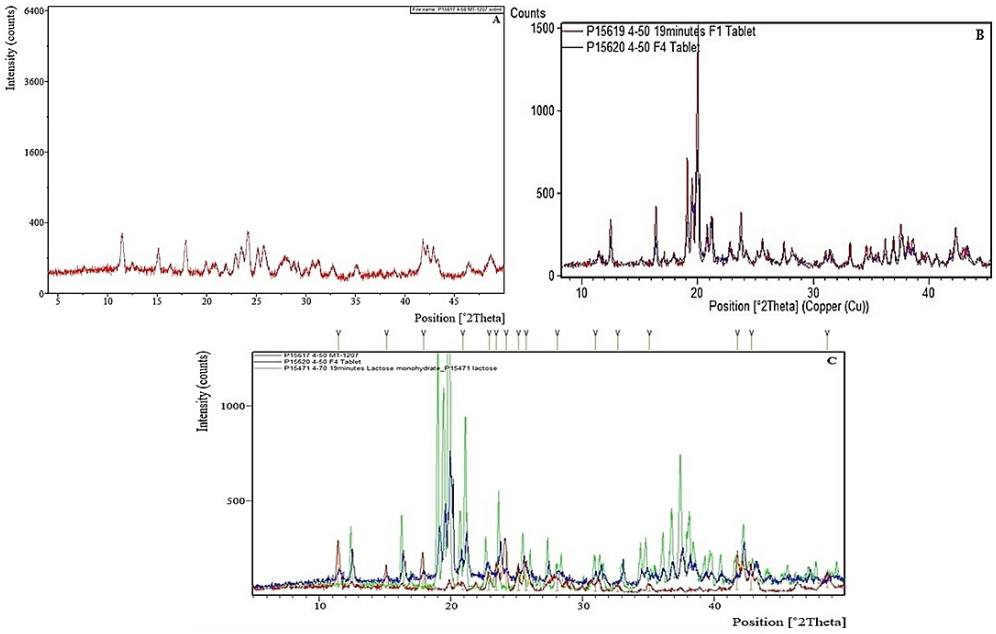

Figure 5: Diffractograms of (A) MT-1207 hydrochloride reference standard, (B) formulations F1 (red) and F4 (blue) and (C) MT-1207 hydrochloride reference standard (red), F4 (blue) and alpha-D-lactose monohydrate (green) combined [Intensity (Counts) vs Position(°2Theta)].

27x17mm (1000 x 1000 DPI)

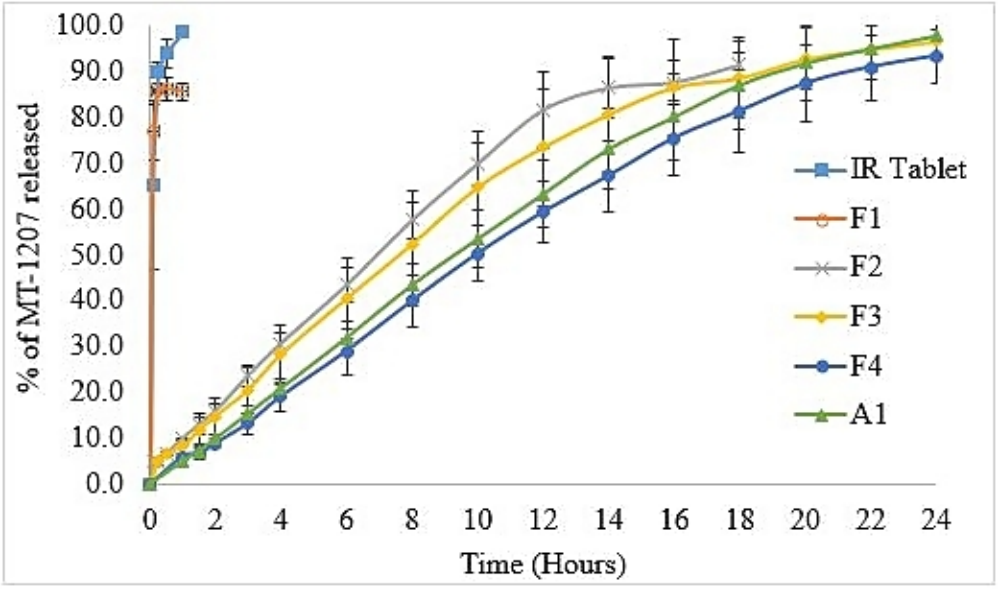

Figure 6: *In vitro* release profiles of MT-1207 from immediate-release tablets, formulations F1, F2, F3, F4 and A1 in 0.1 M phosphate pH 6.8 with 0.2% w/v SDS (n=3). The results are presented as mean ± standard deviation.

13x7mm (1000 x 1000 DPI)

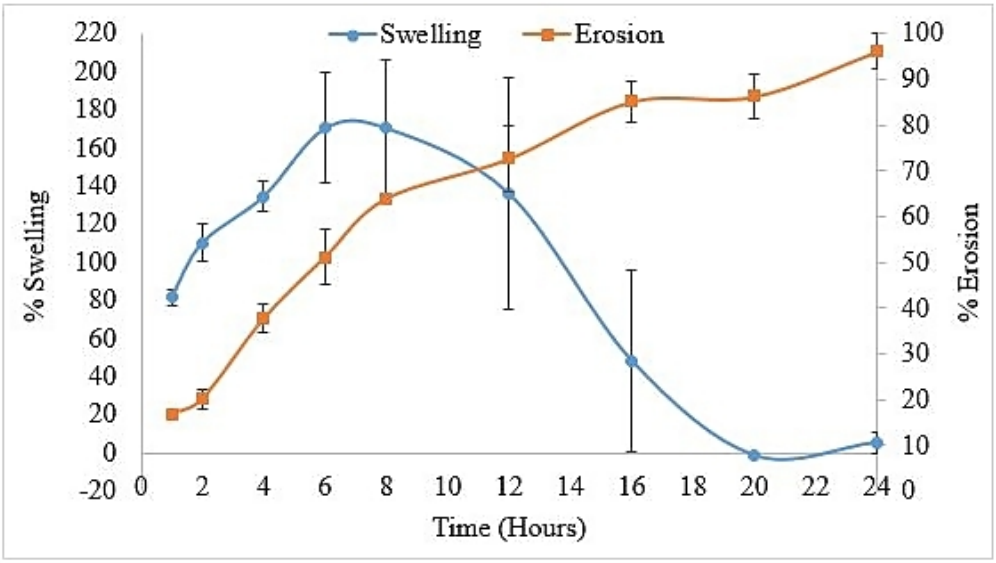

Figure 7: *In vitro* swelling and erosion profiles of formulation F4 in 0.1 M phosphate pH 6.8 with 0.2% w/v SDS (% swelling and % erosion vs time, n=3). The results are presented as mean ± standard deviation.

14x8mm (1000 x 1000 DPI)

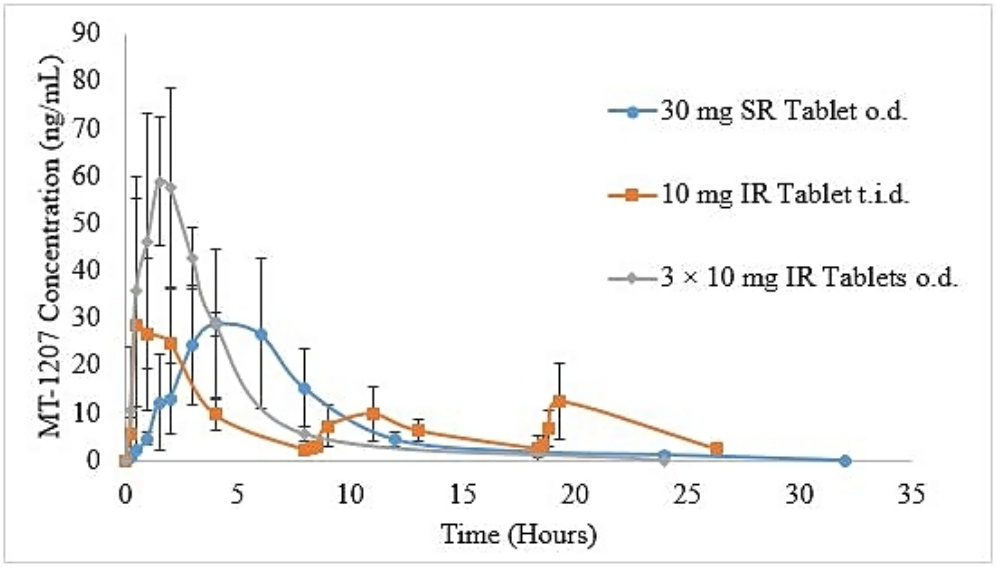

Figure 8: Plasma concentration of MT-1207 in Beagle dog after oral administration of 30 mg SR tablet o.d., 10 mg IR tablet t.i.d. and 3 × 10 mg IR tablet o.d. The results are presented as mean ± standard deviation.

14x7mm (1000 x 1000 DPI)

Supplementary materials for

In vitro and *in vivo* evaluation of a sustained-release once-a-day formulation of the novel antihypertensive drug MT-1207

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Table S1: Mean solubility values of non-micronised MT-1207 hydrochloride reference standard and micronised MT-1207 hydrochloride:colloidal silicon dioxide 5:1 mixture in hydrochloric acid (HCl) pH 1.2 (n=9) and 0.1 M phosphate buffer pH 6.8 (n=9). Results are expressed as mean ± standard deviation.

Table S2: Flowability characterisation results.

Table S3: Tablet hardness testing results (n=10 tablets for each formulation). Results are presented as mean ± standard deviation.

Table S4: Tablet friability results.

Table S5: Uniformity of weight results (n=10 tablets per formulation).

Figure S1: *In vitro* release profiles of MT-1207 (% of MT-1207 released vs time) from the three F4 batches in 0.1 M phosphate pH 6.8 with 0.2% w/v SDS (n=3).

Table S1: Mean solubility values of non-micronised MT-1207 hydrochloride reference standard and micronised MT-1207 hydrochloride:colloidal silicon dioxide 5:1 mixture in hydrochloric acid (HCl) pH 1.2 (n=9) and 0.1 M phosphate buffer pH 6.8 (n=9). Results are expressed as mean ± standard deviation.

Sample Name	MT-1207 Solubility (mg/mL)
Non-micronised MT-1207 hydrochloride in HCl pH 1.2	1.22 ± 0.05
Micronised MT-1207 hydrochloride:colloidal silicon dioxide 5:1 mixture in HCl pH 1.2	1.34 ± 0.08
Non-micronised MT-1207 hydrochloride in 0.1M phosphate pH 6.8	0.01
Micronised MT-1207 hydrochloride:colloidal silicon dioxide 5:1 mixture in 0.1M phosphate pH 6.8	0.03 ± 0.01

Table S2: Flowability characterisation results.

Tablet Formulation	Compressibility (Carr) Index (%)	Hausner Ratio	Angle of repose (°)	Flowability (as per USP)
F1	14.7	1.17	34.2	Good
F2	23.5	1.31	33.2	Fair
F3	15.4	1.18	38.7	Fair
F4	12.2	1.14	42.0	Passable to Good
F4 Repeat 2	12.9	1.15	39.0	Good
F4 Repeat 3	14.0	1.16	35.6	Good
A1	13.3	1.15	36.4	Good

Table S3: Tablet hardness testing results (n=10 tablets for each formulation). Results are presented as mean ± standard deviation.

Tablet Formulation	Diameter (mm)	Thickness (mm)	Hardness (kg)	Tensile Strength (kPa)
F1	8.01 ± 0.03	3.51 ± 0.03	9.18 ± 0.86	2057.2 ± 186.7
F2	7.98 ± 0.04	3.49 ± 0.02	10.79 ± 0.50	2413.7 ± 114.0
F3	7.99 ± 0.03	3.52 ± 0.04	11.69 ± 0.58	2595.9 ± 134.6
F4	7.99 ± 0.03	3.50 ± 0.07	13.89 ± 1.70	3100.2 ± 336.6
F4 Repeat 2	8.00 ± 0.03	3.50 ± 0.05	14.94 ± 1.41	3332.0 ± 287.4
F4 Repeat 3	7.99 ± 0.03	3.50 ± 0.02	13.18 ± 1.51	2943.7 ± 339.6
A1	7.98 ± 0.04	3.52 ± 0.06	14.81 ± 0.40	3295.0 ± 125.2

Table S4: Tablet friability results.

Tablet Formulation	% Friability
F1	0.18
F2	0.09
F3	0.06
F4	0.01
F4 Repeat 2	0.01
F4 Repeat 3	0.14
A1	0.04

Table S5: Uniformity of weight results (n=10 tablets per formulation).

Formulation	Mean Tablet Weight (% of label claim)	Standard Deviation	Acceptance Value
F1	100.4	2.8	6.7
F2	100.1	3.8	9.2
F3	99.9	0.8	2.0
F4	100.2	1.4	3.4
F4 Repeat 2	99.8	1.3	3.1
F4 Repeat 3	99.6	3.8	9.2
A1	100.0	1.2	9.2

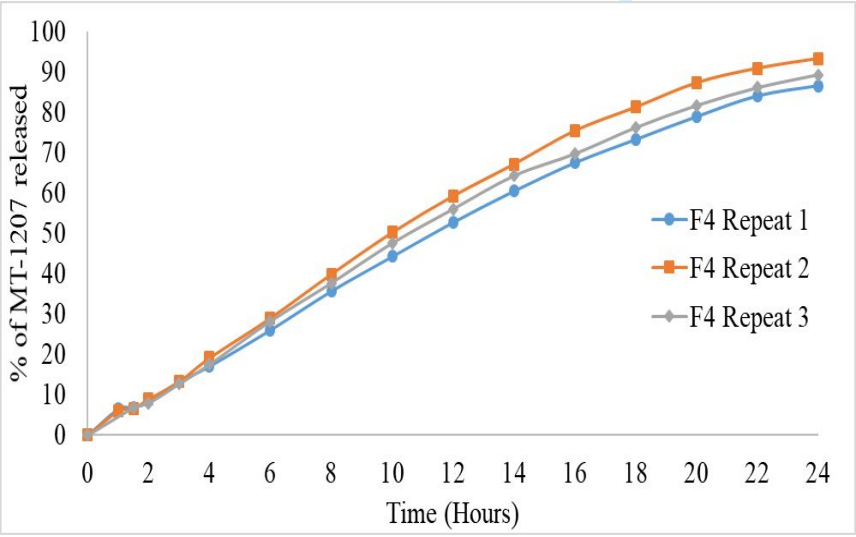
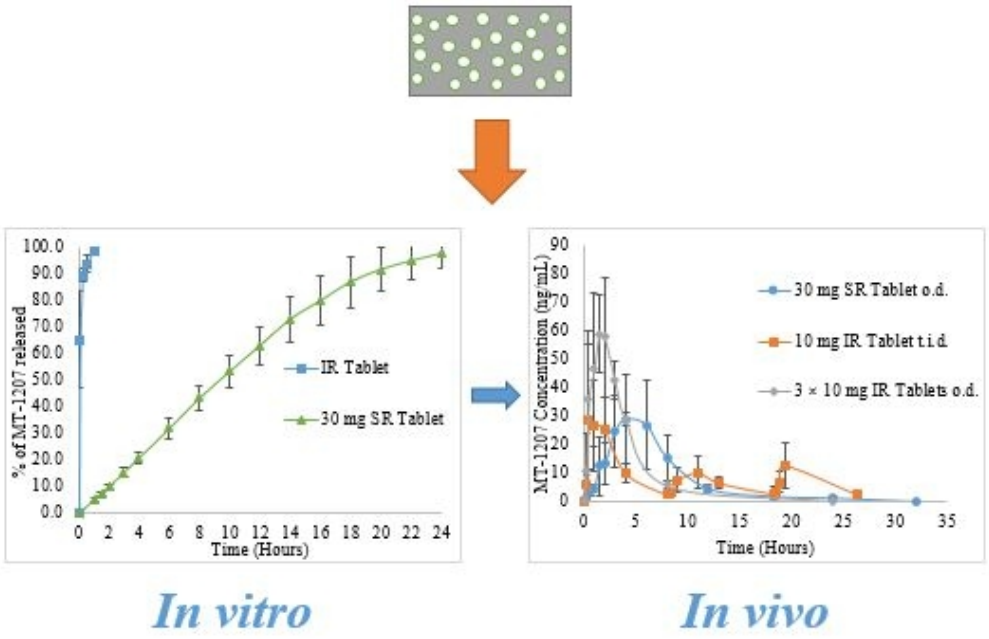


Figure S1: *In vitro* release profiles of MT-1207 (% of MT-1207 released vs time) from the three F4 batches in 0.1 M phosphate pH 6.8 with 0.2% w/v SDS (n=3).



139x89mm (96 x 96 DPI)