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Effect of Recrystallization Technique on Oral Bioavailability of Olmesartan

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ABSTRACT

Olmesartan medoxomil (OLM) is a widely prescribed anti-hypertensive agent with angiotensin II type I receptor antagonistic activity. OLM belongs to BCS class II having a low and variable oral bioavailability (BA) (29%) and its absorption is dissolution rate limited. Recrystallization of OLM from different organic solvents improved its aqueous solubility andthereby*in vitro* dissolution properties. In this investigation, oral BA of Olmesartan (OL) (parent drug molecule of OLM) from recrystallized products of OLM with acetonitrile and methanol (OACN and OMET respectively) solvents were evaluated in male Wistar rats. Also, a rapid, economical and reliable RP-HPLC-PDA method was developed for the estimation of OL in rat plasma samples and validated according to ICH guidelines. Chromatographic separation was achieved on an Agilent eclipse C_{18} column (150×4.6mm, 5μ) with a mobile phase composition of 10mM ammonium acetate - Acetonitrile (62:38%v/v) at a flow rate of 1.2 mL/min. The retention time of OL was found to be 2.3 min and showed good linearity (R^2>0.99) in the selected concentration range of 0.2-1.0μg/mL. A 1.8, 1.6 folds increase in C_{max} and a relative BA of 186, 160% were observed with OACN and OMET respectively, when compared to that of untreated OLM. Thus it can be inferred that recrystallization can be easily scalable and economical technique for enhancing the pharmaceutical properties like solubility, dissolution properties and oral BA of poorly water soluble drugs like OLM.

Key words: Bioanalytical method, Bioavailability, Male Wistar rats, Olmesartan, Recrystallization

INTRODUCTION

Bioavailability (BA) is the rate and extent at which a drug moiety enters systemic circulation, thereby gaining access to the respective site of action. In case of oral administration, the oral BA depends on numerous factors like the physicochemical properties of drug, various aspects of dosage form, the physiological aspects of GI tract, etc. Oral BA of drugs from solid dosage forms (tablets, capsules) depends mainly on solubility of drug particles in GI fluids and permeability of dissolved drug molecules across GI membranes [1].

Olmesartan medoxomil (OLM) is widely prescribed, potent oral antihypertensive agents with angiotensin II type I (AT_1) receptor antagonistic activity. It is a BCS class II molecule and was reported to have a lowand variable absolute oral bioavailability of about 29% owing to low solubility and interference with efflux pumps in GIT, and its absorption is majorly dissolution rate limited [2, 3].OLM is a prodrug and it rapidly undergoes de-esterification in GIT during absorption to free Olmesartan (OL)[4]. Hence, even though OLM is administered, the pharmacokinetics and other parameters *in vivo* were given for its parent drug, OL.

Several techniques were reported to increase dissolution properties of OL and thus enhancing its oral BA including formulation of self-microemulsifying drug delivery system (SMEDDS), reduction in particle size (nanosuspensions), etc[4-6]. Crystal morphology of the drughas a great effect on the physicochemical properties of the drug and many drug molecules exist in more than one crystal forms (polymorphism). Modifying and controlling the crystalline nature of a drug *via* recrystallization can improve several pharmaceutical properties of drugs like stability, solubility, rate of dissolution, etc. which in turn may affect the absorption and BA of the drug [7]. An earlier study on the evaluation of the effect of recrystallizationon various properties of OL inferred in significant improvement in aqueous solubility and *in vitro* dissolution properties of recrystallized products when compared to untreated OLM [7]. Since, orally administered drugs must dissolve in the aqueous medium of gastrointestinal tract prior to absorption, the improvement of the solubility and the rate of dissolution of poorly soluble drugs can be seen as first steps towards the improvement in oral bioavailability. *In vitro* dissolution tests seem to be sensitive and reliable for prediction of BA, yet *in vitro* testing cannot always predict the *in vivo* performance.

Hence, in the present investigation, the oral BA of pure OL and its recrystallized products (OACN and OMET) were evaluated using male Wistar rats. Such enhancement in oral BA was represented in terms of improvement in various pharmacokinetic parameters like C_{max}, AUC, etc. Several analytical methods were reported in literature for estimation of OL alone and in combination with other drugs in bulk and pharmaceutical dosage forms [8-11]. It was also aimed to develop and validate a rapid, economical and sensitive RP-HPLC-PDA bioanalytical method for the estimation of OL in rat plasma samples.

MATERIALS AND METHODS

Materials

OLM and OL were provided by Aurobindo Pharma Ltd (Hyderabad, India). Acetonitrile, Ammonium acetate, Ethanol, Methanol, were purchased from Loba Chemie Pvt, Ltd., (Mumbai, India). Male Wistar rats were obtained

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from Mahaveer enterprises (Hyderabad, India). All the chemicals and reagents used in HPLC studies are of HPLC grade.

Methods

Analytical Method

The assay of OL in plasma samples was performed on a Shimadzu Prominence HPLC system equipped with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20ACHT auto sampler with 200µL loop volume, programmable variable wavelength PDA detector SPD-M20A VP. Data acquisition and processing was carried out using LC-Solution software. The proposed bio analytical method was validated for linearity, system suitability, stability, intra-day and inter-day precision, accuracy, limit of detection and limit of quantification parameters according to ICH guidelines[12].

Preparation of OLrecrystallization products

Two grams of OLM was added to 5mL of a specific pure organic solvent (acetonitrile and methanol) in a 15 mL beaker and heated slowly to 45°C to afford a supersaturated solution. The resulting mixture was then cooled down to room temperature. The resulting recrystallized drug was then collected, dried at 40°C for 15min, and passed through a #80 sieve to obtain a product of uniform particle size. The powdered drug was packed in glass bottles and stored in a desiccator until use[7].

Oral BA study in rats

Male Wistar rats, weighing 200–220g at the start of each experiment, were obtained from Mahaveer enterprises, Hyderabad, India. Rats were acclimated to the colony for 7 days before the start of experiments. Standard rat chow (Harlan) and filtered water (0.22 µm) were available continuously and were maintained on a light/dark cycle in which the lights were on from 0600 to 2000 hours. All animal studies were carried out in accordance with institutional guidelines approved by the IAEC. The test compounds, OLM and recrystallized products (OACN and OMET) were suspended in 0.5 % Natrosol 250 HX (hydroxy ethyl cellulose) at room temperature 30 minutes before administration. The OLM and its recrystallized products were administered at a dose of 10mg/kg. Administration was performed by oral gavage and the animals were offered water 1h p.a. and standard laboratory diet 2h p.a. ad libitum.

Blood samples (~0.2 mL) were withdrawn (into 2mL collection tubes containing a drop of heparinized saline) at different time intervals from tail vein and after the sampling, the volume was replaced with same volume of heparinized saline. The animals were offered water 1h p.a. and standard laboratory diet 2h p.a. ad libitum. Plasma from these blood samples was obtained by centrifuging at 5,000 rpm for 10 min and samples were stored at -20°C until analysis by HPLC.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed using non-compartmental methods. The maximum plasma concentration (C_{max}) and time to reach C_{max}(T_{max}) were determined by direct observation of the plasma concentration-time profiles. The elimination rate constant (K_{el}) was obtained from the terminal slope using regression analysis, and the half-life ($t_{1/2}$) of the drug was calculated by the relationship of $0.693/K_{el}$. The area under the plasma concentration-time curve to the final measurable sample ($AUC_{0\text{-}10}$) was calculated by the trapezoidal rule and extrapolated to infinity ($AUC_{0\text{-}\infty}$), with the final observed plasma concentration divided by K_{el}. The relative bioavailability (F) of the products was calculated as percentage AUC of recrystallized OLM to that of pure OLM.

Statistical Analysis of the data

All the data was given as mean ± SD. Statistical analysis of the data was carried out with a one-way ANOVA analysis (Fisher's Least-Significant-Difference post hoc test)using SYSTAT 13 software (Systat Software Inc., CA, USA). Statistical significance was checked at a threshold of $p<0.05$.

RESULTS

Bio analytical method

Chromatographic separation of OL in plasma samples was achieved on an Agilent eclipse C_{18} column (150 × 4.6mm, 5µ) at ambient temperature. The mobile phase consisted of Acetonitrile and 10mM ammonium acetate (38:62 %v/v), pumped at a flow rate of 1.2 mL/min. The separation was carried out using 10µL injection volume with acetonitrile:10mM ammonium acetate (38:62 v/v) as diluent. The total runtime was set at 5 min while the retention time of OL was found to be 2.3 min. Quantification of OL was carried out by PDA detector set at 254 nm. The optimized method using above LC conditions was validated according to ICH guidelines [12].

Extraction of OL from Plasma:

OL was extracted from plasma by adding 500µL of acetonitrile and centrifuged at 10,000 rpm for 10 min. The supernant was collected and evaporated under nitrogen flow and finally the residue was reconstituted with 200µL of diluent (acetonitrile: ammonium acetate -30:70 % v/v). The extraction efficiency of the method was found to be more than 66%.

Method validation:

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Specificity and selectivity

Selectivity of the method was demonstrated by comparing the chromatograms of the blank sample of the plasma (spiked with acetonitrile in place of drug) and OL standard (1 µg/mL). From the chromatograms it was clearly evident that no interference peak was present at the retention time of OL i.e. 2.3 min. The same was confirmed by peak purity index values (≥0.999).

Linearity

The linearity of this method was evaluated by linear regression analysis, which was calculated by least squares method in the concentration range of 0.2-1.0µg/mL both in plasma and diluent (**Fig 1,2**). The coefficient of regression for the calibration curves was found to be 0.986 and can be concluded that there was an excellent correlation between peak area and analyte concentration. The data was given in **Table 1**.

Table 1.Validation data of OL RP-HPLC-PDA method

Validation data of OL	
Linearity(n=3)	
Range	0.2-1 µg/mL
Regression equation	y = 7790x + 2071.6
Regression coefficient (R^2)	R=0.987
Correlation coefficient (R)	R^2=0.996
LOD	0.005 µg/mL
LOQ	0.015 µg/mL
Precision and Accuracy	**Mean Recovery (% RSD)**
Intra-day (n=6)	
0.2	99.91 (1.17)
0.4	99.35 (1.89)
0.8	99.67 (2.1)
1.0	100.13 (1.67)
Inter-day (n=9)	
0.2	100.32 (1.91)
0.4	99.34 (1.91)
0.8	96.67 (0.98)
1.0	98.67 (1.41)

Precision and Accuracy

Precision and accuracy were studied by covering low, medium and high concentrations (0.2, 0.4, 0.8 and 1µg/mL) of OL within the range of linearity. The %RSD for OL was found to be less than 2 for the four concentrations with respect to both Intra-day and inter-day (3 consecutive days).

Accuracy of the method was determined by recovery studies. Statistical evaluation revealed that %RSD of the drug at different concentration levels for six injections was less than 3. Precision and accuracy data were shown in **Table 1.**

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ values were determined by the formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves), given in **Table 1**.

Systemsuitability

System suitability was established by injecting six replicates of standard sample at various volumes (0.2-1.0µL) and the parameters (mean [RSD]) like plate number (N = 4312 [0.01]), tailing factor (T= 1.67 [0.19]) and retention time (R_t = 2.3 [0.013]) and peak symmetry of samples were studied. All the specifications were found to be within limits.

Stabilityof solutions

The short-term stability of the plasma samples was determined by analysing the samples kept at room temperature at different time intervals up to 24hrs. Also, the freeze-thaw stability of plasma samples was assessed over 3 consecutive cycles of freezing (24h) and thawing (24h), followed by extraction procedure and analysed. The variation in assay values at different time intervals were found to be less than 3% of the initial zero time, indicating that the samples were stable over the entire duration of experiment.

Bioavailability (BA) Studies in Rats

A parallel study design was adopted since it was practically impossible to give each rat the five different treatments in crossover with long enough wash out periods to prevent any sequence effect (**Table 2**). Mean plasma concentration-time profiles for OL and its recrystallized products (OACN and OMET) were presented in **Fig. 3**. Pharmacokinetic parameters such as C_{max}, T_{max}, $AUC_{0\text{-}10\ h}$ and $AUC_{0\text{-}\infty}$ for all the treatments were computed and

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given in **Table 3** and the statistical analysis of the parameters using one way ANOVA analysis (using Fisher's LSDpost hoc Test) was given in **Table 4**.

Table 2. The dose groups and numbers assigned to rats in a parallel study design

Group	Formulation	Dose [mg/kg]	Animal No.
G 1	OL	10	101-104
G 2	OACN	10	201-204
G 3	OMET	10	301-304

Table 3. Pharmacokinetic parameters of OL and its recrystallized products (n=4)

Treatment	C_{max} (µg/mL)	T_{max} (hrs)	AUC_{0-10} (µg.hr/mL)	$AUC_{0-\infty}$ (µg.hr/mL)
OL	1.56 ± 0.14	2.0 ± 0.00	7.27 ± 0.67	7.56 ± 0.68
OACN	2.65 ± 0.16	2.0 ± 0.00	13.40 ± 1.0	13.93 ± 1.13
OMET	2.37 ± 0.13	2.0 ± 0.00	11.78 ± 0.52	12.47 ± 0.13

Table 4. Statistical summary of pharmacokinetic parameters for OL (Fischer's LSD post hoc Test)

Groups compared	C_{max} (µg/ml)	AUC_{0-10} (µg.hr/ml)	$AUC_{0-\infty}$ (µg.hr/ml)
OL vs. OACN	S (2.31E-04)	S (1.62E-04)	S (1.4E-04)
OL vs. OMET	S (0.001)	S (0.001)	S (0.001)
OACN vs. OMET	NS (0.118)	NS (0.088)	NS (0.126)

*S = significant, NS = not significant; Values in parentheses were *p* values

DISCUSSION

The developed bioanalytical method showed good correlation between the peak area and concentration of the drug under developed LC conditions. A linearity range of 0.2-1.0 µg /mL for OL was established. The differences of less than 3.0 % in RSD for both intra- and inter-day data reflect the high degree of precision of the method. The developed bioanalytical method was successfully applied for quantifying OL in rat plasma samples.

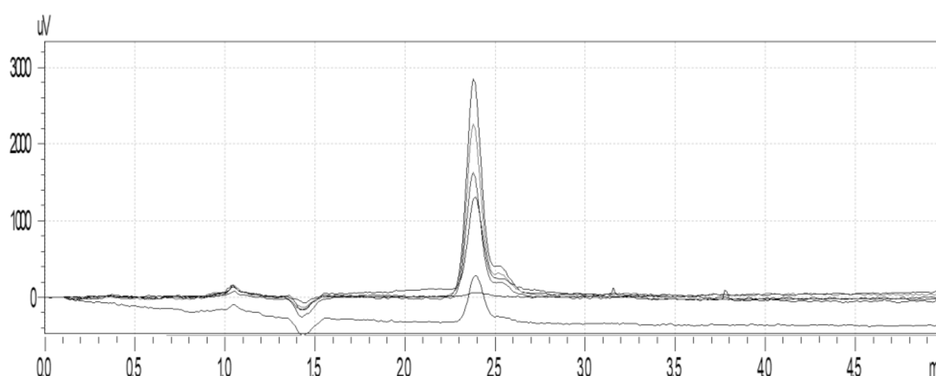

Figure 1. Ovelay chromatograms of OL at different concentrations in diluent (0.2-1µg/mL)

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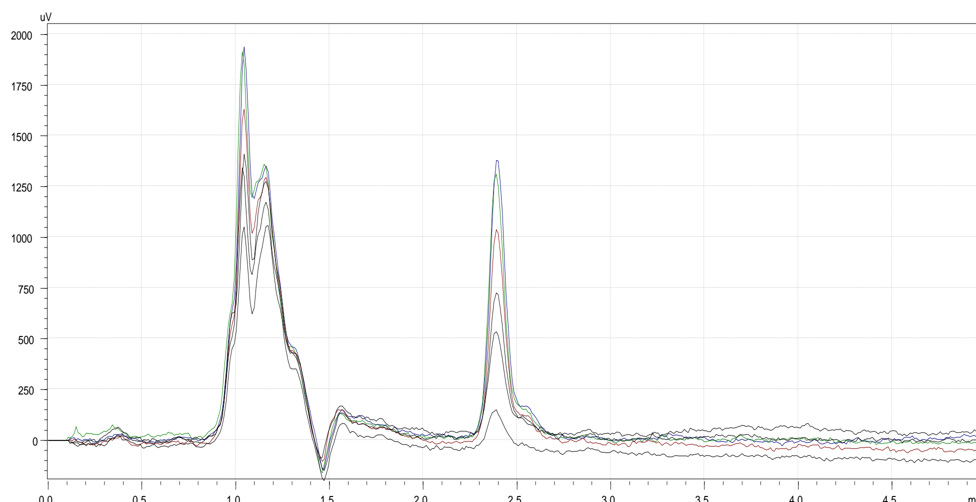

Fig 6.13: Overlay chromatograms of OL at different concentrations in plasma (0.2-1µg/mL)

OLM is a BCS class II drug, with dissolution of drug in GI fluids as the rate limiting step for absorption and several strategies reported in literature for the enhancement of its dissolution and oral BA. Chirag R *et al.*, 2010, prepared solid self-microemulsifying drug delivery systems (S-SMEDDS) of OL with a view to enhance its aqueous solubility and oral BA. They inferred in a significant enhancement in the in vitro dissolution of OL from S-SMEDDS with 99.02% release within 15 min [4]. Kang MJ *et al.*, 2011, reported a 2.7 fold increase in the oral BA of OL in rats with higher Cmax and shorter Tmax by formulating self-microemulsifying drug delivery systems (SMEDDS) with a mean droplet size of 200 nm [5].

Hetal PT *et al.*, 2011, tried nanosuspensions of OL using media milling technique followed by its lyophilisation, and reported a significant enhancement in the in vitro dissolution and diffusion (using dialysis tubing) rates and approximately 2 folds increase in the amount of OL permeated at the end of 6 h in *in vitro* intestinal (male Wistar rats) permeability studies when compared to plain drug [6].

Recrystallization is a simple and very economical physical modification technique that affects the physical and physicochemical properties of drugs such as melting point, solubility, true density, tabletability, etc. which in turn alters the pharmaceutical properties of drugs like rate of dissolution, rate and extent of absorption, etc.In our earlier paper, we observed that recrystallization of OLM using different solvents like methanol, ethanol, acetonitrile, acetone, etc. resulted in different polymorphs of OLM with altered physical characteristics, as confirmed by SEM, DSC and Powder X-RD Studies. It was found that the aqueous solubility of OL increased by about 14-17 orders of magnitude after recrystallization with different solvents and that the various *in vitro* dissolution parameters like drug percent dissolved at 10 min (DP_{10}) and 120 min (DP_{120}), dissolution efficiency at 20 min (DE_{20}) improved significantly for some recrystallized products when compared to pure OLM [7].

Since, it was found that both solubility and *in vitro* dissolution profiles of OLM improved significantly by recrystallization, there is good probability that the recrystallized products also have improved absorption (BA). Consistent with that idea, in the present investigation, *in vivo* oral BA studies were carried out in male Wistar rats as animal models and the plasma –concentration time profiles were shown in **Fig. 2**.

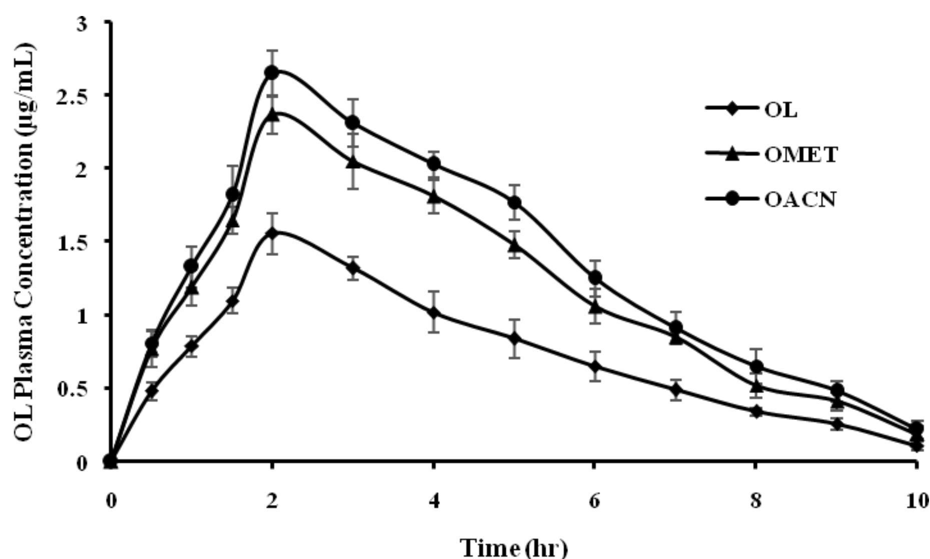

Figure 2. Mean comparative plasma concentration - time profiles of OL and recrystallized products (n=4)

The various pharmacokinetic parameters viz. C_{max}, T_{max},AUC_{0-10}, $AUC_{0-\infty}$, etc. of pure OL and its recrystallized products (OACN, OMET) were computed and compared (**Tables 3,4**). When compared to the pure OL, the C_{max} values for recrystallized products (OACN and OMET) increased by 1.8 and 1.6 foldsrespectively. A similar trend was also observed with $AUC_{0-\infty}$ values. Recrystallization of OLusing the selected solvents significantly improved the pharmacokinetic parameters considered ($p<0.05$) except for the T_{max} values which remained constant for pure and recrystallized OL. However, among the two recrystallized products (OACN and OMET), significant variation was not observed with pharmacokinetic parameters ($p>0.05$). Moreover, Relative BA of recrystallized products with respect to pure OLwere found to be 186% and 160% for OACN and OMET respectively.

Overall, it can be inferred that recrystallization under different conditions has significantly improved the oral BA of OL and is relatively very economical and easily scalable technique when compared to other techniques reported previously in literature like formulation of SMEDDS and nano suspensions, etc. Thus, it may be beneficial to develop solid oral dosage forms of OLM using its recrystallized products for enhanced BA and thereby, therapeutic efficacy.

CONCLUSION

Our investigation proves the potential of recrystallization, a physical modification technique, in enhancingthe oral BA of a poorly soluble drug, OLM. It was observed that the acetonitrile recrystallized product of OLM (OACN) showed a relative BA of about 190% compared to pure OLM. It may therefore be concluded that recrystallization is a very simple, economical technique that can be easily applied on commercial scale, capable of full automation of process, for improving various properties of poorly water soluble drugs like solubility, dissolution parameters, oral BA and overall ADME behaviours *in vivo* after administration.

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