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A Quantitative Analysis of Memantine in Human Plasma Using Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry

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Abstract: The aim of this study is to compare the single-dose oral bioavailability of memantine hydrochloride 10 mg tablets of Ranbaxy Laboratories Limited, with NAMENDA™ tablets (containing memantine hydrochloride 10 mg) of Forest Pharmaceuticals Inc. in healthy, adult, human subjects under fasting condition. The study was carried out as 2-way crossover design on 8 subjects in fasting and fed conditions. The plasma samples were obtained over a 72 h post dose in each period. Plasma memantine samples were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with positive ion electro spray ionization using multiple reactions monitoring (MRM). A sensitive, reproducible, accurate and validated LC-MS/MS method with limit of quantification (LOQ) 0.200 ng/mL was used to analyze memantine. Ln transformed AUC_{0-72} and C_{max} were assessed for bioequivalence using 90% confidence interval (CI). 90% confidence intervals for the ratio of test and reference (Ratio of least-squares mean) for ln-transformed AUC_{0-72} and C_{max} were within the regulatory acceptance criteria of 80-125%.

Keywords: Bioequivalence, Pharmacokinetics, Tablet, UPLC, LC-MS/MS, Memantine and Least square means

Introduction

Memantine, an amantadine derivative, chemically 3,5-dimethyladamantan-1-amine is a moderate-affinity, uncompetitive, voltage-dependent, NMDA-receptor antagonist with fast

on/off kinetics that inhibits excessive calcium influx induced by chronic over stimulation of the NMDA receptor. Memantine is indicated for the treatment of patients with moderate to severe dementia of the Alzheimer's type[1]. Memantine showed low to negligible affinity for GABA, benzodiazepine, dopamine, adrenergic, histamine and glycine receptors and for voltage-dependent Ca^{2+}, Na^{+} or K^{+} channels. Memantine also showed antagonistic effects at the 5HT3 receptor with a potency similar to that for the NMDA receptor and blocked nicotinic acetylcholine receptors with one-sixth to one-tenth the potency[2].

Memantine generally modified the progressive symptomatic decline in global status, cognition, function and behavior exhibited by patients with moderate to severe Alzheimer's disease[3].

Memantine is well absorbed after oral administration and has linear pharmacokinetics over the therapeutic dose range. Peak concentrations reached in about 3-7 hours. The mean volume of distribution of memantine is 9-11 L/kg and the plasma protein binding is low (45%).

Memantine undergoes little metabolism, with the majority (57-82%) of administered dose excreted unchanged in urine. Memantine is primarily eliminated by kidneys (75-90%) and remaining (10-25%) through bile and faeces. The drug undergoes both renal tubular secretion and reabsorption and has a terminal elimination half life of about 60-80 hours[4].

Experimental

A single oral dose of two different formulations of memantine hydrochloride 10 mg tablets of Ranbaxy Laboratories Limited with NAMENDA™ tablets (containing memantine hydrochloride 10 mg) of Forest Pharmaceuticals Inc. were administered to the subjects with 240 mL of drinking water at ambient temperature under fasting condition5.

Institutional review board, subject selection and safety analysis

This research was carried out in accordance with the basic principles defined in US 21 CFR Part 320, the ICH (62FR 25692, 09 May 1997), 'Guidance for Good Clinical Practice' and the principles enunciated in the Declaration of Helsinki[6]. The protocol and informed consent forms (ICFs) were reviewed and approved prior to study initiation by Institutional Review Board (IRB). All subjects read and signed the ICF prior to study initiation. This clinical trial was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice and relevant EU guidelines and also by considering the regulations for the protection of human subjects and their personal data.

Clinical design and sample collection

The study was designed as an open label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, crossover bioavailability study comparing memantine hydrochloride 10 mg tablets of Ranbaxy Laboratories Limited with NAMENDA™ tablets (containing memantine hydrochloride 10 mg) of Forest Pharmaceuticals Inc. in healthy, adult, human subjects under fasting condition. A total of forty (40), 5 mL blood samples were collected in EDTA vacutainers during the course of the study through indwelling cannulae placed in forearm veins. The minimum blood sample required for analytical purpose is 5 mL. The blood samples collected pre-dose were, 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 48.0 and 72.0 hours post dose in each period. The pre-dose blood sample in each period will be collected within a period of 1.5 hours before dosing and the post-dose samples will be generally within 2 minutes of the scheduled time. The actual end-point time of collection of each blood sample was recorded.

Chemicals and reagents

Memantine was purchased from USP (Rockville, MD, USA), amantadine was procured from sigma aldrich (stienheim, Germany) and methanol was purchased from Qualigens fine chemicals (A division of GSK Ltd, Mumbai, India). Human plasma containing ethylene diamine tetra acetic acid (EDTA) was collected in-house which were free from HIV and Hepatistis. All other solvents & reagents of analytical grade were purchased from S.d. fine chem. Ltd (Mumbai, India).

Stock Solution, calibration curve (CC) and quality control (QC) sample preparation

Approximately 1000 µg/mL of stock solutions for memantine were prepared using water. Sub stocks of 2000,1520,1064, 638.4, 319.2, 191.52, 28.728 & 9.997 ng\mL solutions were used to prepare spiking standards. The calibration range selected to measure the expected sample concentrations was 0.2 to 39.682 ng/mL for memantine in EDTA plasma. Calibration curve consisted of single replicate of eight non-zero standards, with two standards below the low QC and two standards above the high QC. Quality control concentrations of 0.573, 15.150 and 29.135 ng/mL were prepared by spiking 100 mL of blank plasma with an appropriate memantine stock/substock solutions. In addition, precision & accuracy batches included lower limit of quantification (LOQ QC) samples. The spiking volume did not exceed 2% of the total calibration curve & quality control sample volumes. The QC samples were mixed and redistributed into 0.5 mL aliquots for storage at -15 ºC.

LC-MS/MS

Chromatographic separation was achieved by maintaining the column oven temperature at 35 ºC by using the Aquity BEH C18 column (Waters corporation, Milford, Massachusetts, USA; 2.1x50 mm, 1.7 µm) using the mobile phase comprised of acetonitrile - ammonium acetate buffer (pH 6.7; 2 m M) (90:10 v/v) which was pumped by using waters micro pump at a flow rate of 0.25 mL/min. The samples were loaded in the Waters Aquity UPLC autosampler and temperature of the auto sampler was set at 10 ºC. 5 µL of sample extract was injected and the eluent was monitored by tandem mass spectrometry with an electro spray ionization (ESI) interface of Quattra Premier (Waters ,UK). The chromatographic data was acquired and processed using MASSLYNX software Version 4.1. The positive ions were monitored in the multiple reaction monitoring (MRM) mode. The following ion transitions (*m/z*) were monitored 180.0 (parent) and 163.0 (product) for memantine and 152.0 (parent) and 135.1 (product) for amantadine

Bioanalytical sample preparation

An aliquot of human plasma containing both analyte and its internal standard (amantadine) was extracted by using solid phase extraction (MCX cartridges, 30 mg/1cc). 50 µL of internal standard stock dilution (amantadine, approximately 300.0 ng/mL) and 500 µL aliquot of each sample were added into polypropylene tubes and vortex. 200 µL of solution- 1 (hydrochloric acid solution; 4.3% v/v) were added and vortexed. The cartridges were conditioned with methanol (1 mL) and equilibrated with de-ionised water (1 mL). The prepared samples were then loaded onto cartridges and the cartridges were washed with 1 mL of solution-1 followed by washing with 1 mL methanol and finally eluated twice with 1 mL liquor ammonia solution (5%v/v). The eluates were evaporated to dryness at 50 ^{0}C under N_2 gas. Residues were then reconstituted in 500 µL of mobile phase.

Bio analytical method validation

The validation of this procedure was performed in order to evaluate the method in terms of selectivity, sensitivity, linearity of response, accuracy, precision, recovery, stability, dilution integrity, matrix effect. The described LC-MS/MS method to quantify memantine in human

plasma was validated and found to be specific and sensitive for memantine. For selectivity six lots of plasma with EDTA as anticoagulant were evaluated and none showed significant interfering peaks at the retention time of memantine and amantadine. Figure 1 & 2 depicts chromatogram of blank and blank with internal standard for sensitivity the limit of quantification (LOQ) was 0.200 ng\mL. Figure 3 depicts chromatogram of LOQ and with internal standard from extracted plasma sample. The method was validated for the analytical range of 0.2 to 39.682 ng/mL and linearity was determined by weighted least square regression analysis of standard plot associated with eight point standard curve. Six sets of quality control samples at different concentrations levels of lower limit of quantification (LLOQ) 0.202 µg/mL and 0.573 ng/mL (LQC), 15.150 ng/mL (MQC) and 29.135 ng/mL (HQC) prepared in human plasma, were analyzed to ensure acceptable assay precision and accuracy.

The accuracy of the assay was defined as an absolute value of the calculated mean concentration of the quality control sample to their respective nominal values, expressed as percentage. The intra and inter batch accuracy using internal standard area ratio method ranged from 98.0 to 110.4% and 98.1 to 109.2% respectively. Deviation of the assay accuracies was well within +/-15.0% for the analyte.

The recovery was based on the comparison of the peak areas of extracted plasma QC samples at high, medium and low concentrations with un-extracted aqueous samples. The mean recovery of memantine and its internal standard from plasma was more than 65%. Memantine proved to be stable in plasma for at least three freeze and thaw cycles. The bench top, in-Injector and long term stability were found to be 7.25 hours, 54.25 hours and 95 days respectively, there was no matrix effect observed. Memantine met the acceptance criteria for dilution integrity as well. The method was meeting all the acceptance criteria as per USFDA method validation guidance[9]**.**

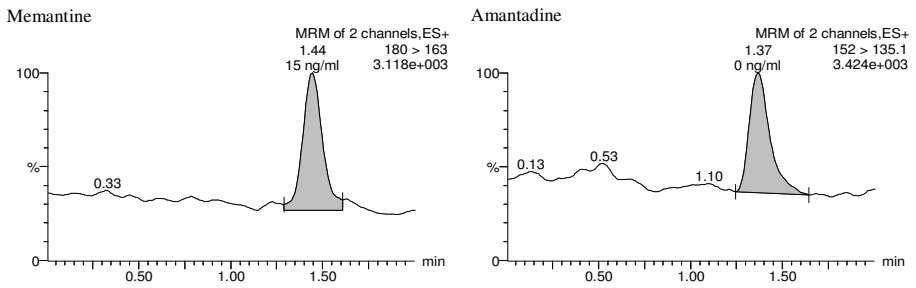

Figure 1. Representative chromatogram of memantine and its internal standard from extracted plasma blank.

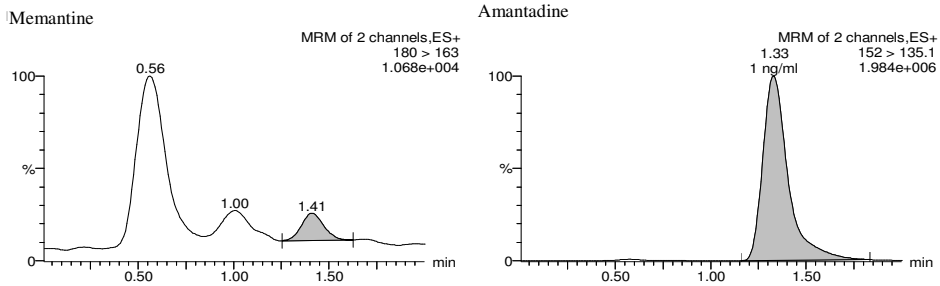

Figure 2. Representative chromatogram of memantine and its internal standard from extracted plasma blank + internal standard.

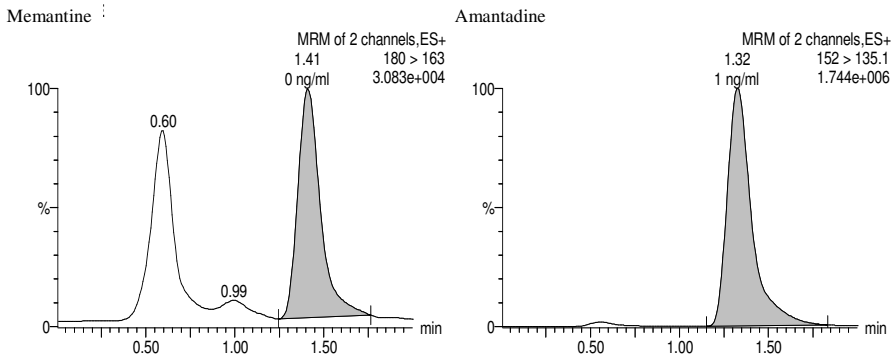

Figure 3. Representative chromatogram of memantine and its internal standard from extracted plasma at LLOQ concentration

Table 1. Intra & inter-batch precision and accuracy of memantine in human plasma.

QC Samples	LOQQC	LQC	MQC	HQC
Concentration, µg/mL	0.202	0.573	15.150	29.135
Intra-batch mean	0.2163	0.5878	15.6324	29.3571
Intra-batch % CV	5.3	5.2	2.8	2.9
Intra-batch % SD	0.01151	0.03071	0.44538	0.85763
Inter-batch mean	0.2128	0.5752	15.7237	29.6212
Inter-batch % CV	4.0	5.1	2.4	1.7
Inter-batch % SD	0.00861	0.02939	0.38516	0.51488

Statistical analysis

Appropriate statistical analysis for a two-way crossover design was performed to assess the bioequivalence. The analyses were performed using SAS software version 9.1 (SAS Institute Inc., USA). Analyses of variance (ANOVA) was performed on the log-transformed $AUC_{0\text{-}72}$ and C_{max} using a mixed effects ANOVA model using type III sum of square with the main effects of sequence, formulation and period as fixed effects and subject nested within sequence as a random effect. The sequence effect was tested at the 0.10 level of significance using the subjects nested within sequence mean square as the error term. All other main effects were tested at the 0.05 level of significance against the residual error (mean square error) from the ANOVA model as the error term. Ratio of means expressed as a percentage of the LSM for the reference formulations. Ratio of means was calculated using the LSM for log-transformed $AUC_{0\text{-}72}$ and C_{max} consistent with the two one-sided tests for bioequivalence[7] 90% confidence intervals for the difference between drug formulations LSM was derived from the analyses of log-transformed $AUC_{0\text{-}72}$ and C_{max}. 90% confidence interval for the ratio of the test and reference product averages (least square means) were calculated by first calculating the 90% confidence interval for the differences in the averages (arithmetic means) of the log-transformed data and then taking the antilog of the obtained confidence limits.

Memantine was readily absorbed from the gastrointestinal tract and was measurable at the first sampling time (0.5 h) in the majority of volunteers. Mean plasma concentration profiles of memantine attained after the administration of test and reference products are shown in Figure 4 and 5. Mean plasma concentration profiles of memantine were comparable and closely similar between the two formulations. The summary results of pharmacokinetic parameters are summarized in below Table 2.

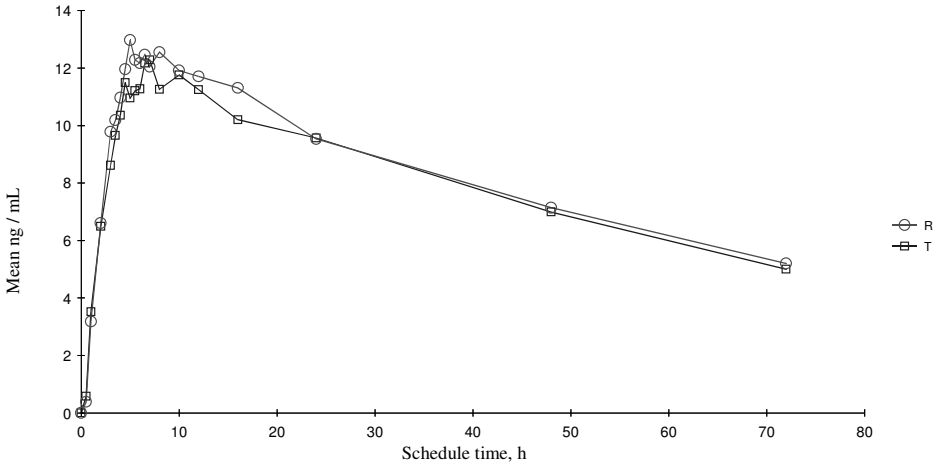

Figure 4. Linear plot of mean concentration *vs*. time of memantine in plasma.

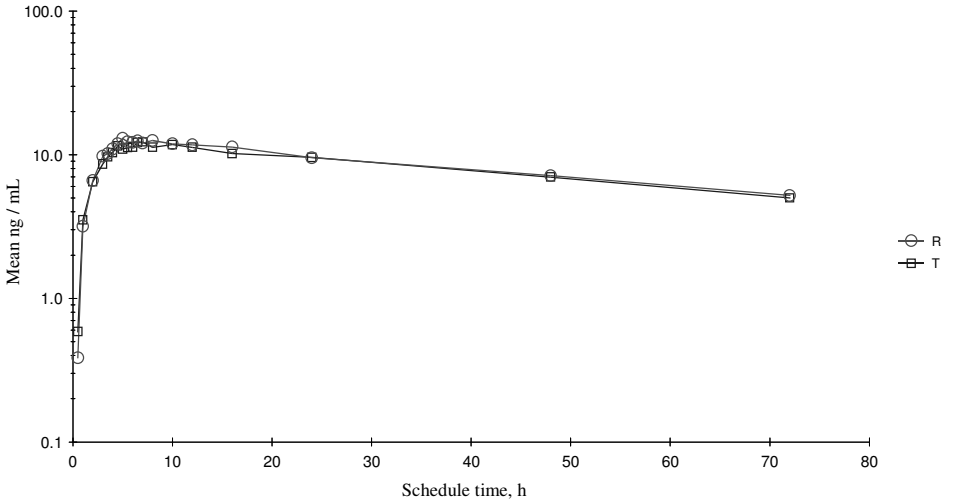

Figure 5. Log linear plot of mean concentration *vs*. time of memantine in plasma.

Table 2. Summary statistics of pharmacokinetic parameters for memantine in plasma.

	Test formulation T (mean ± sd)	Reference formulation R (mean ± sd)
T_{max}, h	6.878±1.7021	6.250±1.3093
C_{max}, ng/mL	12.7339±2.82226	13.6975±3.28078
AUC_{0-72}, ng/mL.h	580.1645±126.97002	599.1301±140.10662

The most important objective of this bioequivalence study was to assure the safety and efficacy of generic formulations. If two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available at the site of drug action, they are bioequivalent and thus considered therapeutically equivalent[8,9]. It is generally accepted that the standard equivalence range for basic pharmacokinetic characteristics such as AUC_{0-72}

and C_{max} is 0.80-1.25. Statistics analyses were performed on log-transformation pharmacokinetics parameter and the results are shown in Table 3. 90% confidence interval for the ratio of test and reference product averages (least-squares means) for AUC_{0-t} and $C_{max,}$ derived from the analysis of log transformed pharmacokinetic parameters, were well within the regulatory acceptance range 80-125% suggesting bioequivalence between memantine hydrochloride 10 mg tablet (Test Formulation T) developed by the Ranbaxy Laboratories Limited and namenda™ 10 mg tablets (Reference Formulation R) (containing Memantine Hydrochloride 10 mg) of Forest Pharmaceuticals Inc., subsidiary of forest Laboratories, Inc. St Louis, Missouri 63045 in fasting condition.

Table 3. Comparison T *vs.* R.

	Ratio of Least Square Means, 90% CI	Power	Intra subject CV, %
AUC_{0-72}, ng/mL.h	97.08 (92.13-102.30)%	99.94	5.4
C_{max}, ng/mL	93.33 (88.24-98.71)%	99.91	5.8

Results and Discussion

Total 8 subjects were enrolled and all subjects completed all periods of the study, pharmacokinetic and statistical analysis was performed on the completed subjects. The test and reference products were well tolerated by the study subjects as there were no serious adverse events. Both the periods of the study were conducted under identical conditions in all study related activities.

Memantine was readily absorbed from the gastrointestinal tract and was measurable at the first sampling time (0.5 h) in the majority of volunteers. Plasma concentrations profiles of memantine attained after the administration of test and reference products were shown in Figure 4 and 5. Mean plasma concentration profiles of memantine were comparable and closely similar between the two treatments. The pharmacokinetic parameters of memantine for the test and reference treatments were shown in Table 2. Peak concentrations of 12.7339±2.82226 ng/mL and 13.6975±3.28078 ng/mL for test and reference respectively for memantine were attained at 6.878±1.7021 and 6.250±1.3093 hours after administration of test (T) and reference (R) products respectively. In addition, the sample size of 8 subjects was close enough to fetch more than 99% power and to detect the difference of at least 20% in AUC_{0-72} and C_{max} between the two treatments. The intra subjects CV was around 5.8 and 5.4 for C_{max} and AUC_{0-72} suggesting the low variability of the drug. The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available at the site of drug action, they are bioequivalent and thus considered therapeutically equivalent. It is generally accepted that the standard equivalence range for basic pharmacokinetic characteristics such as AUC_{0-72} and C_{max} is 0.80-1.25. Pharmacokinetics parameter estimates were statistically analyzed following log transformation and the results of statistical analysis were shown in Table 3. Geometric mean ratios and 90% confidence intervals for AUC_{0-72} and C_{max} for memantine were 97.08 (92.13-102.30)% and 93.33(88.24-98.71)% respectively and the intra-subject CV (%) for AUC_{0-72} and C_{max} for memantine was 5.4 and 5.8 respectively. Memantine hydrochloride 10 mg tablet developed by Ranbaxy Laboratories Limited, India is therefore bioequivalent to namenda 10 mg tablets Forest Pharmaceuticals Inc., subsidiary of forest Laboratories, Inc. St Louis, Missouri 63045.

Conclusion

Memantine was determined in plasma and other biological fluids by several methods such as gas chromatography-mass spectrometry (LOQ- 0.5 ng/mL; RT-3.6 mins)' high pressure liquid chromatography coupled to fluorimetric detection (LOQ 0.6 ng/mL; RT 7 min). Our method had a suitable LOQ of 0.2 ng /mL along with the very short retention time of 2 minute was useful for analyzing more than 500 samples in a single day which is the most advantageous aspect in comparison to other analysis techniques available in published literature. Our method employs a very simple cost effective liquid extraction procedure finally requiring 5 µL injection volumes resulting in high throughout bioanalysis technique. The current investigation demonstrates that the two tablet formulations of memantine displayed similar rate and extent of bioavailability under fasting condition. The test/reference ratio (Ration of Least Square Means) and its 90% confidence interval for ln-transformed $AUC_{0\text{-}72}$ and C_{max} were entirely within the bioequivalence acceptance range of 80%-125%. The plasma concentration profiles of memantine from the test formulation and reference formulation were identical and the efficacy resulting from their pharmacokinetics profiles will also be considered identical. As a result memantine 10 mg tablet developed by Ranbaxy Laboratories Limited, India is bioequivalent to namenda 10 mg tablets of Forest Pharmaceuticals Inc., subsidiary of forest Laboratories, Inc. St Louis, Missouri 63045 in fasting condition and may be used interchangeably in medical practice.

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