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Simple bioanalytical method development and validation of micronised Domperidone 20 mg tablets using LCMS-MS and its pharmacokinetic application in Healthy Indian Volunteers

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The current investigation deals with an validated Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) analytical method for the quantification of micronised domperidone in plasma of human volunteers. The validation of LC-MS/MS method was accomplished by evaluating the inter-day and intra-day precision and accuracy in a linear concentration range of 3.33-100 ng/ml. The entire study was an attempt to evaluate the comparison index of the bioavailability study of micronised domperidone tablet formulation with that of conventional domperidone tablet containing 20 mg of domperidone. Both the formulations were given orally as a single dose cross over design. The washout period was taken as 1 week. A single-dose, two-sequence, two-treatment, two-period crossover Bioequivalence study of two formulation were performed on 12 Indian healthy male volunteer. The estimation of domperidone concentration in human plasma was determined by the validated LC-MS/MS method. The various pharmacokinetics parameters like peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}), area under the plasma concentration-time curve (AUC_{0-t}), area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), of both the formulations were evaluated and compared. The results evaluated by estimated pharmacokinetic parameters did not find any statistically significant difference between the two formulations. The relative bioavailability of micronized test formulation was found to be 104.62% to that of reference conventional formulation.

Key words: Bioequivalence, domperidone, liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis, pharmacokinetics.

INTRODUCTION

Gastroesophageal reflux disease (GERD) can be defined as one of the most common incident related to upset in

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the gastro intestinal system (Hosseini et al.,2017; Dent et al., 2005). The recent advancement of medical sciences has already explored several options to treat and manage GERD effectively. Among them Acid suppression is one of the best treatment strategy to counter GERD symptoms for this reasons, Acid suppressing agents proton pump inhibitors (PPIs) provides the rapid and smooth symptomatic relief and heals esophagitis in a high proportion of patients (Dent et al., 2005). Domperidone can be considered as one of the most effective antiemetic drug for the treatment of mild to severe GERD symptoms Domperidone has dual mechanism of action. Domperidone can act as prokinetic agent which can stimulate the sphincter muscle of duodenam and easily can induce the effect of prokinetic movement. In addition, domperidone can block D_2 receptor antagonist in chemo trigger receptor zone (CTZ). The problem associated with domperidone for the treatment of GERD is its less bioavailability Domperidone is a water insoluble drug, domperidone is one of the ideal candidate to increase the solubility and as well as bioavailability. Here we developed a sustained release formulation with micronised domperidone. The main objective of our research work is to access the bioavailability of sustained release formulation and compare it with conventional non micronised formulation. Bioequivalent study of water insoluble drug has been executed by several researchers but very few related to micronised formulation has been addressed in a proper scientific way. Thorough literature survey finds that several methods was performed for conducting bioequivalent study but an attempt was made here to perform bioequivalent study of micronised domperidoene formulation as compare to conventional domperidone dosage form (Toyama et al., 2015; Bhadoriya et al., 2018; Blandizzi et al., 2015; Censi et al., 2015; Benet, 2013; Rockville, 2001).The interesting research conclusion which may come out from this study can be described as whether micronised drugs formulated as sustained release matrix tablet dosage form are able to deliver better and sustained bioavailability.

MATERIALS AND METHODS

Chemicals reagents and drug product

Raw domperidone (API) was provided as gift samples as by Kusum Healthcare, Punjab, India. HPLC grade methanol and Ethyl acetate were procured from Merck India Pvt. Ltd. (Mumbai). Milli Q water purification system was installed to acquire High Performance Liquid Chromatography (HPLC) grade water. The human blank plasma sample with EDTA-K_3 anticoagulant was procured from Bioequivalence Study Centre, Jadavpur University, Kolkata, India. Test product: Tablet containing micronized Domperidone 20 mg and Reference product: Domstal, from Torrent Pharmaceutical Ltd, (Torrent House, Ahmedabad, India), containing domperidone 20 mg.

Instrumentation

The LC system was purchased from Shimadzu (Kyoto, Japan). API 2000 triple quadrupole mass spectrometer (MDS Sciex, Canada) with electrospray ionization (ESI) source was used for detection of the compound. Data acquisition was done with Analyst 1.4.1. software. Chromatographic separation was performed on a standard C8 column, 50 mm x 3 mm, 3 µm i.d (Phenomenex, USA).

Products studied

The following test and reference products were used in the present study. Test product: Tablet containing micronized domperidone 20 mg and reference product: Domstal, from Torrent Pharmaceutical Ltd, (Torrent House, Ahmedabad, India), containing domperidone 20 mg.

Chromatographic conditions

The entire chromatographic analysis was executed at ambient atmospheric temperature with a runtime 5 min. The injection volume was taken as 20 µl. The composition of water: methanol (2: 98, *v/v*) was used as mobile phase containing 0.5% formic acid with a flow rate of 1 ml min^{-1}. The column oven was kept at 23°C. While the temperature of auto sampler was maintained at 10°C. The mass spectra of the compounds were acquired by using Electrospray ionization (ESI) with multiple reactions monitoring (MRM) technique. The entire ionization of the drug was accomplished in positive ionization mode. The important tuning parameters were calculated and optimized by injecting 100 ng mL^{-1} of standard solution containing all two drugs including internal standard. The validation parameters like sensitivity, accuracy, precision, stability, recovery, reproducibility and system suitability were measured in accordance with the US-FDA bioanalytical method guidelines (Bhadoriya et al., 2018).

Study design

The whole bioequivalent study was executed under fasting conditions as a two-sequence two-period crossover study. The study design was based on free randomization. The drug was administered with single-dose. Between the two periods minimum one week of dosing interval was taken as a washout period (Blandizzi et al., 2015) prior to the study. The experimental protocol was reviewed and approved by Institutional Ethical Committee (ICE) of Jadavpur University, Kolkata, India. The volunteers were enrolled after thorough investigation including medical history, vital parameters of physical examination, laboratory investigation, drug screening, ECG and HIV/hepatitis status. An experience clinical pharmacologist was actively present throughout the study to guide and monitor the entire study.

Drug administration and blood sample collection

12 non-smokers healthy Indian male volunteers were screened for the study. The age of the volunteers were between 19 to 33 years (29 ± 3.49) with a standard body mass index between 19 to 27 (24.66 ± 3.76). The whole study was executed under the regulation and guidance issued by U.S. Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EAMP) from time to time (Censi et al., 2015; Benet, 2013). A pre planed blood sampling schedule was designed to evaluate the rate and extent of absorption in such a manner so that all the important
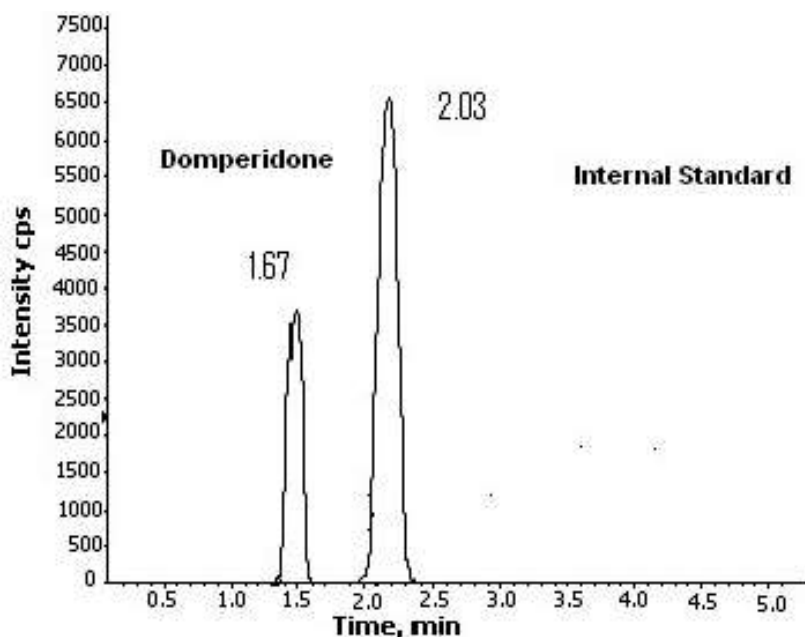
Figure 1. Retention time of Domperidone and internal standard.

pharmacokinetic parameters can be calculated properly (Rockville, 2001) Committee for Proprietary Medicinal Products(CPMP) (1991).

Total of 13 blood samples were collected from each volunteers at various interval of time including 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 24.0 and 48.0 h in the sterile EDTA added centrifuge tubes. After the centrifugation the plasma was separated entirely and stored at a temperature of -20°C.

Pharmacokinetic analysis

Noncompermental pharmacokinetic model were used here to calculate various pharmacokinetic parameters of domperidone. The peak plasma drug concentration (C_{max}) and time to reach peak plasma concentration (t_{max}) were calculated directly from the results obtained after the analysis of the drug. The elimination half-life ($t_{1/2}$) was calculated by using the formula of $0.693/K_{e.}$, where K_e is considered as apparent elimination rate constant calculated from the slope of the terminal log linear phase. Area calculation of trapezoidal rule was implemented to find $AUC_{0-t.}$. $AUC_{0-\infty}$ was also estimated according to the following standard formula:

$$AUC_{0-\infty} = AUC_{0-t} + C_{last} / K_e,$$

where C_{last} is the last quantifiable plasma concentration (US Food and Drug Administration, 2017)

Statistical analysis

Pharmacokinetic parameters of each subject were studied thoroughly on the basis of statistical approach. Bioequivalence study parameters like AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values were compared as primary variables. Statistical tool of analysis of variance (ANOVA), including treatment, period and subject were

applied for these primary parameters and also for log-transformed values of these parameters. The statistical approach of bio-equivalence analysis was done according to guidance of Committee for Proprietary Medicinal Products (Censi et al., 2015). The experimental test formulation was considered to be bioequivalent to reference formulation when 90% confidence interval (CI) for the ratio between each pharmacokinetic parameters of test and reference was found to be within the fixed equivalence range of 80-125% (Nation and Sansom, 1994).

RESULTS

The developed bioanalytical method used for the estimation of domperidone in biological matrix was found to be accurate and sensitive. The peaks of domperidone and Internal standard both were found to be well resolved. No interference was observed in the chromatogram of blank plasma sample during. During LC-MS/MS analysis (Figure 1). The retention time (RT) of domperidone and Internal Standard was found to be at 1.67 and 2.03 min respectively. The lower limit of quantification (LLOQ) for domperidone in plasma was noted as 3.33 ng /ml. The peak area ratio (domperidone: Internal standard) between concentration and was found to be linear within the range of 3.33 ng/ml to 500 ng/ml (r^2=0.9998). Stability, absolute recovery, within-day and between-day, precision and accuracy was estimated for three different quality control points at low, medium, and high levels (5, 50 and 80 ng/ml). Mean drug plasma concentration at various interval of time after oral

Table 1. Mean (±SD, n = 12) pharmacokinetic parameters of 20 mg domperidone tablet for test and reference preparation.

Parameter	Test	Reference	90% CI (Log-transformed data)
AUC_{0-t} (ng. h /ml)	510.55±24.25	488.05±33.52	0.99007 - 1.00296
$AUC_{0-\infty}$ (ng. h /ml)	563.69±31.25	545.98±36.95	0.98942 - 1.00080
C_{max} (ng/ml)	78.32±53.30	73.17± 51.55	0.99527 - 1.00836
t_{max} (h)	0. 800± 0.150	0.798±0.04	
K_e (h^{-1})	0.15±0.008	0.17±0.004	
$t_{1/2}$ (h)	4.62±0.306	4.081± 0.295	

AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, t_{max}, K_e, and $t_{1/2}$ are the area under the plasma concentration-time curve upto 48h, area under the plasma concentration-time curve upto infinity, maximum plasma concentration, time to reach maximum plasma concentration, elimination rate constant, and half-life of a drug, respectively.
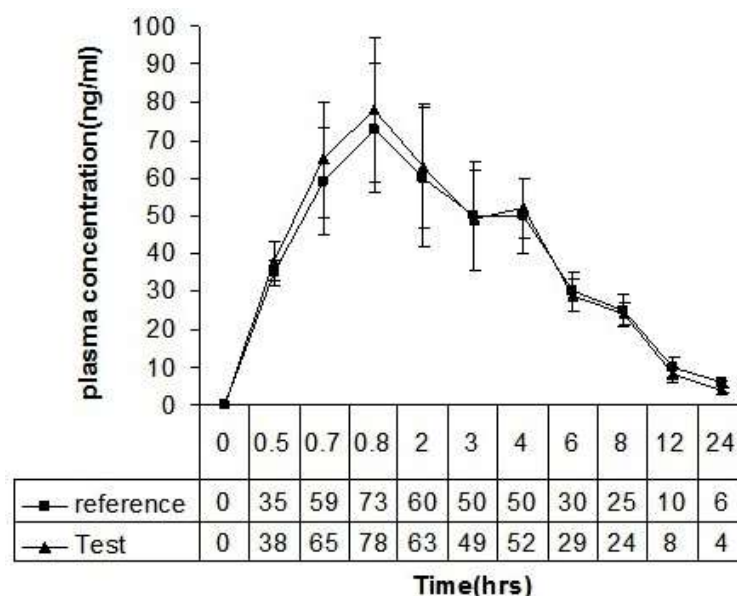

Figure 2. Mean (± SD, n=12) plasma concentration-time profiles after administration of test and reference preparations in healthy Indian subjects [-■- is test formulation graph and -●- is reference formulation graph obtained by plotting time (h) on X-axis and plasma concentration (ng/ml) on Y-axis.

administration of reference and test products to healthy volunteers are depicted in Table 1. The comparison of all the major pharmacokinetic parameters for the drugs including ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were obtained within the range of 0.80-1.25 at 90% confidence interval.

DISCUSSION

The above described bioanalytical method used for estimation of domperidone in plasma matrix was found to be very simple, robust, accurate and sensitive. The entire therapeutic window was covered by the linearity range achieved for this assay (3.33 to 500 ng/ml). The peak of drug domperidone and Internal Standard were well resolved as shown in Figure 2. Throughout the whole experimental study, domperidone was found to be stable in biological matrixes. Final mean recovery of three different quality control sample for three freeze and thaw cycles was found to be 87.60% and coefficient of variation (CV) was noted as 4.26%.

The elimination half-life ($t_{1/2}$) of domperidone in various formulations was found to be in the range 4.21 to 5.02 h. For this reason, one-week wash out period was sufficient

between the two phases. Peak drug plasma concentration (t_{max}) was observed at 0.8 h after drug administration, and the last samples were sufficient for calculating at least 80% of $AUC_{0-\infty}$. After oral administration of reference drug the peak plasma concentration C_{max} was found to be 73.17±21.55 ng/ml at the time 0.798± 0.04 h (t_{max}). For the test preparation peak plasma concentration (C_{max}) was found to be 78.32±23.30 ng/ml at the time 0.800±0.150 (t_{max}). AUC_{0-t} of the test and reference were found to be 510.55±24.25 ng h/ml versus 488.05±33.52 ng h /ml respectively and $AUC_{0-\infty}$ of the test and reference were found to be 563.69±31.25 ng h /ml versus 545.98±36.95 ng h /ml respectively. On the basis of calculation of comparison of the AUC_{0-t} for domperidone after single dose administration, the relative bioavailability of the test preparation was 105% to that of reference preparation.

The objective of the bioequivalence study is to confirm interchangeability between a test (innovator sample) and a generic drug (reference) formulation on the basis of efficacy and safety. When a pharmacological effect of certain drug is difficult to estimate, the plasma levels of a drug may be utilized as an indicator of clinical activity. For this reasons, domperidone plasma concentration obtained in this bioequivalent study suggest an equal clinical efficacy of the two brands tested and provide pharmacokinetic data from Indian healthy volunteers.

Conclusion

The 90% CI of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of domperidone of these two preparations was found to be in acceptable range as mentioned earlier. There was no statistically significant difference for the treatment values. Both formulations were equal in terms of rate and extent of absorption. Consequently bioequivalence between two formulations can be concluded.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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