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Method Development and Validation of HPLC for the Determination and Quantification of Pantoprazole

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ABSTRACT
A rapid, sensitive and selective HPLC method was developed for the determination of pantoprazolein. Analysis was achieved on a Phenomenex C18 column with water (pH 7.0)-acetonitrile (55:45%,v/v) as the mobile phase. Enteric coated pantoprazole tablets from Pantocid and from Pantop were evaluated following 20mg oral dose. The pharmacokinetic parameters C max, AUC0-t, AUC0-∞, T max and t1/2 were determined from plasma concentration time profile of both formulations and found to be in good agreement with previously reported values. The calculated pharmacokinetic parameters were compared statistically to evaluate bioequivalence between the two formulations.
Keywords: *pantoprazole, bioequivalence, pantocid, pantop.*

INTRODUCTION

The drug pantoprazole sodium sesquihydrate (5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole sodium sesquihydrate) is used in the treatment of gastroesophageal reflux disease (GERD), reflux esophagitis, gastric ulcers, duodenal ulcers and Zollinger-Ellison Syndrome. Pantoprazole accumulates in the acidic compartment of the parietal cells after absorption and is protonated by specific action on the proton pumps of the parietal cells.
Pantoprazole is a proton pump inhibitor, i.e., it inhibits specifically and dose proportionally H^+,K^+ - ATPase, the enzyme which is responsible for gastric acid secretion in the parietal cells of the stomach. Pantoprazole exerts its full effect in a strongly acidic environment (pH<3) and remains mostly inactive at higher pH values, there by selectively affecting the acid complete pharmacological and therapeutic effect for pantoprazole can only be achieved in the acid secreting parietal cells.
By means of a feedback mechanism this effect is diminished at the same rate as acid secretion is inhibited. As with other proton pump inhibitors and H2 receptor inhibitors, treatment with pantoprazole causes reduced acidity in the stomach causing a reversible increase in gastrin in proportion to the reduction in acidity. Since pantoprazole binds to the enzyme distal to the cell receptor level, it affects hydrochloric acid secretion independently of stimulation by other substances like acetylcholine, histamine, and gastrin. The same effect is observed following oral or intravenous administration. In general, preparation of pantoprazole sodium sesquihydrate and certain of its polymorphic forms isknown in the art. However, it is also known that different polymorphic forms of the same drug may have substantial differences in certain pharmaceutically important properties. Therefore, there is a continuing need for new methods of preparation.
The present study was conducted to evaluate the bioequivalence of two brands of pantoprazole 40-mg tablets. Although several studies have been published regarding pantoprazole pharmacokinetics, very few of them have focused on the proof of bioequivalence between two formulations. This present study, therefore, aims to develop the bioanalytical method for the estimation of pantoprazole in the pharmacokinetic parameters statistically to evaluate the bioequivalence between the reference and test formulations of pantoprazole (40 mg) after a single oral dose.

MATERIALS AND METHOD

Drugs and Reagents

The test formulation was Pantocid®- pantoprazole 40mg tablets, (Sun pharmaceutical industries , India). The reference product was Pantop®- pantoprazole 40mg tablets,(Aristo pharmaceuticals

pvt limited, India). Pantoprazole and Lansoprazole standard drugs were obtained from M/s. Burgeon pharmaceuticals, Chennai, India. HPLC-grade actonitrile from Rankem (Mumbai, India), water from Qualigens (Mumbai, India) were purchased. All other chemicals were of analytical grade.

Chromatography

The integrated HPLC (LC 20AT, Shimadzu corporation, Kyoto, Japan) was equipped with a Rheodyne injector with 20 l loop, a SPD-M20A Prominence Diode array detector system. The separation of compounds was made on a Phenomenex – Luna, C18 (250 x 4.6 mm i.d., 5) at 30 ºC temperature. The mobile phase was a mixture of phosphate buffer (pH 7.0) /actonitrile (55/45, v/v) pumped at a flow rate of 1.0mL/min. detection was set at a wavelength of 290 nm.

Study Design

The dose of pantoprazole was 20 mg/kg.10 tablets of pantop/pantocid which contain 40mg dose of pantoprazole was crushed and weight equivalent to 40 mg was dissolved in a 5 mL volumetric flask with sufficient water. The volumetric flask was made up with water. For the analysis 2mL venous blood samples were withdrawn at specified pre-determined time intervals (0, 0.416, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 hours). The blood samples were immediately transferred to the collection tube (containing Disodium EDTA), and shaken well and centrifuged using ultra cooling micro centrifuge at 5000 rpm/sec to separate plasma. The separated plasma samples were transferred to a labeled air tight sample tubes and kept in deep freezer for further analysis.

Sample Processing

The protein precipitation method was used for extraction from plasma. A 0.2mL of plasma 0.05 mL of standard solution, 0.05 mL of internal standard solution and 0.1 mL of acetonitrile was spiked and then centrifuged for 15 minutes at 5000 rpm. The upper layer was collected and injected into HPLC.

Pharmacokinetic Data Analysis

The following parameters were assessed for the period of 0–5 hr. Maximum plasma concentration (Cmax), Time of maximum plasma concentration (Tmax), area under plasma concentrations time curve AUC (0- t) for 0 to 12hrs, Area under plasma concentrations time curve AUC (0-∞) for 0 to ∞ hrs, Elimination half life (T ½), and Elimination constant.

C_{max} and T_{max} were obtained directly from the concentration-time curve data. The area under the concentration – time curve from time zero to time of last quantifiable concentration AUC 0-t was calculated using the linear trapezoidal method. The terminal rate constant Keli was calculated by applying a log-linear regression analysis.

Statistical Analysis

A one-way ANOVA was performed with respect to relevant pharmacokinetic variables using an analysis of variance with subject, treatment and period effects with the raw data.

RESULTS AND DISCUSSION

Separation

The following data show typical HPLC chromatograms of sample analysis. No interfering peaks were observed. The retention times of pantoprazole and lansoprazole were around 5.3 and 7.4 min, respectively good separation and baselines with low background were observed. The peaks of interest were well resolved and there was no interference from endogenous plasma substances.

Linearity

The calibration curves were linear over the range of 100 to 500 ng /mL. The correlation coefficient(r) for pantopraole was above 0.9974 over the concentration range used. The mean linear regression equation of calibration curve for the analyte was y =0.0008*X*- 0.032.

Pharmacokinetic Analysis

The pharmacokinetic parameters of the samples were calculated manually. The elimination rate constant (lz) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($t1/2$) was calculated as 0.693/lz.Time to peak plasma concentration (Tmax) and peak plasma concentration (Cmax) were read directly from the observed concentration versus time profiles. The area under the curve to the last measurable concentration (AUC0-t) was calculated by the linear trapezoidal

rule. The area under the curve extrapolated to infinity (AUC0-∞) was calculated as AUC0-∞ =AUC0-t + Ct /z, where Ct is the last measurable concentration.

Statistical Analysis

For the aim of bioequivalence analysis between two formulations C_{max}, t AUC.0 and AUC considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA) and calculating 90% confidence interval (CI) of the ratio test/reference (T/R) using log transformed data. Statistical significance of variations in the different formulations was tested according to an ANOVA test by the PRISM program. The products were considered bioequivalent if the difference between the two compared parameters was statistically insignificant ($P > 0.05$) and 90% confidence intervals for these parameters fell with in 80% to 120% which is the range accepted by U.S Food and Drug Administration .The p value obtained from one-way ANOVA and t-test were found to be 0.0792. Hence there is no significant difference between two products.

SUMMARY

A rapid, sensitive and selective HPLC method for the determination of pantoprazole was developed and validated. Sample preparation was assured by one-step protein precipitation method. Separation occurred on a Phenomenex C18 RP column (5 m, 25 cm x 4.6 mm ID) with a mobile phase of 0.4 % triethylamine (pH 7.0) and acetonitrile(55:45% v/v) and detection at 290 nm. The standard curve was linear ($r > 0.9974$) over the concentration range of 100 – 500 ng/mL. The lower limit of quantification for pantoprazole was 10.0 ng/mL using 20 L plasma samples. This method was successfully applied to the bioequivalent study of pantoprazole in two formulation, reference (pantop) and test (pantocid). For bioequivalence study, parameters like Cmax, Tmax, t AUC.0 , ¥ 0 AUC , Kel and T1/2 are compared by statistical analysis. The maximum concentration (Cmax) obtained in two brands pantocid and pantop were 987.39 and 1169.23 ng/ml respectively. The half life (T1/2) of pantoprazole for pantocid and pantop were calculated and found to be 1.621 and 1.6 hours. Area under the curve t AUC.0 of pantocid and pantop was calculated as 1930.65 and 2202.85 ng hr/ml and and ng hr/ml respectively and 0 AUC was calculated to be 2115.62 and 2622.2 ng hr/ml. Elimination rate constant (kel) was calculated for pantocid and pantop from the slope of log concentration versus time curve with regression analysis. Elimination rate constant was found to be 0.5273 and 0.4836 hr-1. The p value obtained from one-way ANOVA and t-test were found to be 0.0792, which shows there is no significant difference between two products.
The most important objective of any bioequivalence study is to assure the safety and efficacy of the test and reference products. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent. It is generally accepted that equivalent range for basic pharmacokinetic parameters, such as Cmax, AUC0-t, and $AUC0$-∞, is 80% to 120%10.

Table: 1 Pharmacokinetic parameters of two tablet formulations of Pantoprazole

S.No.	Parameters	Pantocid (test product)	Pantop (reference product)
1	AUC 0 – t (ng. hr/ml)	1930.65	2202.85
2	AUC 0 – ∞ (ng. hr/ml)	2115.62	2622.2
3	C max (ng /ml)	987.39	1169.23
4	T max (hrs)	1.621	1.651
5	K eli (hrs -1)	0.5273	0.4836
6	T ½ (hrs)	1.314	1.433

CONCLUSION

The statistical comparison of AUC0-t, AUC0-∞, T_{max} and C_{max} clearly indicated no significant difference in the two formulations of pantoprazole 40- mg capsules. 90% confidence intervals for the mean ratio (T/R) of AUC0-t, AUC0-∞ and Cmax were entirely within the Food and Drug Administration acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that Pantop 40mg tablet is bioequivalent to Pantocid 40mg tablet, and that the two products can be considered interchangeable in medical practice. The method has proven to be simple, specific, sensitive, precise and accurate and is suitable for pantoprazole quantification in

plasma samples from bioequivalence. It was successfully applied to a bioequivalence study of two drug products containing pantoprazole.

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