

# High - Performance Liquid Chromatography Method for the Quantification of Pantoprazole in Rat Plasma

R. Mohankandhasamy\*, J. Dharuman, I. Ponnilaravasan, M. Thiagarajan

*Department of pharmaceutical analysis, K.M.C.H College of pharmacy, kovai estate, kalapatti road, Coimbatore 641035, India.*

**\*Corres.author: thambiavn@gmail.com  
+91-9894808363**

**ABSTRACT:** A rapid, sensitive and selective HPLC method was developed for the determination of pantoprazole in rat plasma. In this method, pantoprazole was extracted from rat plasma using acetonitrile with Lansoprazole as internal standard. Analysis was achieved on a Phenomenex C<sub>18</sub> column with water (pH 7.0)-acetonitrile (55:45%, v/v) as the mobile phase. Enteric coated pantoprazole tablets from Pantocid (test, Sun pharmaceuticals, India) and from Pantop (reference, Aristo pharmaceuticals, India) were evaluated following 20mg oral dose to healthy rats. The pharmacokinetic parameters C<sub>max</sub>, AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, T<sub>max</sub> and t<sub>1/2</sub> 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. The analysis of variance (ANOVA) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range. Based on these statistical inferences it was concluded that the two formulations exhibited comparable pharmacokinetic profiles and that Pantocid is bioequivalent to Pantop.

**Key words:** pantoprazole, bioequivalence, pantocid, pantop, albino rat.

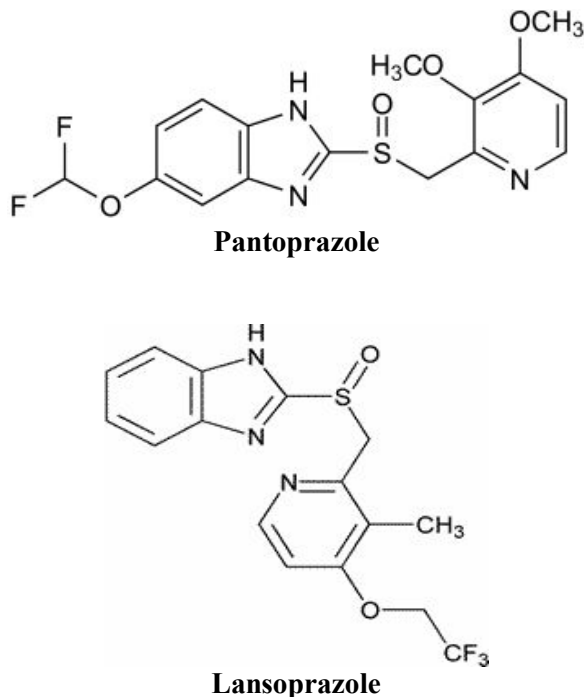
## 1. INTRODUCTION

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<sup>+</sup>,K<sup>+</sup>-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, thereby selectively affecting the acid

secreting parietal cells of the stomach. Therefore, the complete pharmacological and therapeutic effect for pantoprazole can only be achieved in the acid-secreting parietal cells<sup>1</sup>.

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 H<sub>2</sub> 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 is

known 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<sup>2</sup>.



**Fig.1:Chemical structures of Pantoprazole and Lansoprazole**

### 1.1 OBJECTIVE

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 albino rat plasma and then to compare the pharmacokinetic parameters statistically to evaluate the bioequivalence between the reference and test formulations of pantoprazole (40 mg) after a single oral dose in 12 albino rats.

## 2. MATERIALS AND METHODS

### 2.1 SUBJECTS

The study adheres to “Principles of Laboratory Animal Care” and is approved by the animal care committee IAEC/CPSEA-Institutional animal ethics /Committee for the purpose of control and supervision of experiments on animals. (KMCRET/ M.Pharm./2007).

Healthy Albino rats (both sex) of weight 150-250 ± 10grams were taken and grouped. Rats are divided in to two groups (12 rats/group), each group subdivided in to three subgroups A, B, C (4 rats/subgroup).The rats were taken from KMCH college of pharmacy animal house, which were quarantined a week before.

### 2.2 DRUGS AND REAGENTS

The test formulation was Pantocid<sup>®</sup>- pantoprazole 40mg tablets, (B.No: GK 60202 , from Sun pharmaceutical industries , India). The reference product was Pantop<sup>®</sup>- pantoprazole 40mg tablets, (B.No: 171F087, from Aristo pharmaceuticals pvt limited, India). Pantoprazole and Lansoprazole standard drugs were obtained from M/s. Burgeon pharmaceuticals, Chennai, India. HPLC-grade acetonitrile from Rankem (Mumbai, India), water from Qualigens (Mumbai, India) were purchased. All other chemicals were of analytical grade.

### 2.3 CHROMATOGRAPHY

The integrated HPLC (LC 20AT, Shimadzu corporation, Kyoto, Japan) was equipped with a Rheodyne injector with 20 µl 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., 5µ) at 30°C temperature. The mobile phase was a mixture of phosphate buffer (pH 7.0) /acetonitrile (55/45, v/v) pumped at a flow rate of 1.0mL/min. detection was set at a wavelength of 290nm<sup>6,7,8</sup>.

### 2.4 STUDY DESIGN

The dose of pantoprazole to rat was 20 mg/kg.10 tablets of pantop/pantocid which contain 40 mg 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. According to their body weight of each animal dose (20 mg/kg) of the solution was given through oral gavage.

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, 4.5and 5.0 hours) after dosing by tail vein puncture.

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<sup>3,4</sup>.

## 2.5 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<sup>5</sup>. The upper layer was collected and injected into HPLC.

## 2.6 PHARMACOKINETIC DATA ANALYSIS

The following parameters were assessed for the period of 0–5 hr. Maximum plasma concentration ( $C_{max}$ ), Time of maximum plasma concentration ( $T_{max}$ ), Area under plasma concentrations time curve AUC (0-t) for 0 to 12hrs, Area under plasma concentrations time curve AUC (0- $\infty$ ) for 0 to  $\infty$  hrs, Elimination half life ( $T_{1/2}$ ), and Elimination constant ( $K_{eli}$ ).

$C_{max}$  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<sub>0-t</sub> was calculated using the linear trapezoidal method. The terminal rate constant  $K_{eli}$  was calculated by applying a log-linear regression analysis.

## 2.7 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.

## 3. RESULTS AND DISCUSSION

### 3.1 SEPARATION

The following figures show typical HPLC chromatograms of sample analysis. No interfering peaks were observed for drug-free animal plasma. 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.

### 3.2 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  $y = 0.0008X - 0.032$ .

### 3.3 RECOVERY FROM PLASMA

The extraction efficiency of pantoprazole from rat plasma at the concentrations of 50, 100 and 200ng/ml was found to be 97.63%, 98.975% and 99.815% with precision of 2.275, 1.117, and 0.621. The mean recovery for internal standard was 98.806.

## 3.4 PHARMACOKINETIC ANALYSIS

The pharmacokinetic parameters of the samples were calculated manually and using graph pad version 5, Microsoft excel 2003software's. The elimination rate constant ( $\lambda_z$ ) 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 ( $t_{1/2}$ ) was calculated as  $0.693/\lambda_z$ . Time to peak plasma concentration ( $T_{max}$ ) and peak plasma concentration ( $C_{max}$ ) were read directly from the observed concentration versus time profiles. The area under the curve to the last measurable concentration ( $AUC_{0-t}$ ) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ( $AUC_{0-\infty}$ ) was calculated as  $AUC_{0-\infty} = AUC_{0-t} + C_t / \lambda_z$ , where  $C_t$  is the last measurable concentration<sup>9</sup>.

## 3.5 STATISTICAL ANALYSIS

For the aim of bioequivalence analysis between two formulations  $C_{max}$ ,  $AUC_0^t$  and  $AUC_0^\infty$  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 within 80% to 120% which is the range accepted by U.S Food and Drug Administration<sup>10</sup>.

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.

## 4. SUMMARY

A rapid, sensitive and selective HPLC method for the determination of pantoprazole in rat plasma was developed and validated. Sample preparation was assured by one-step protein precipitation method. Separation occurred on a Phenomenex C<sub>18</sub> RP column (5  $\mu$ 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  $\mu$ L plasma samples. This method was successfully applied to the bioequivalent study of pantoprazole in rats for two formulation, reference (pantop) and test (pantocid).

For bioequivalence study, parameters like  $C_{\max}$ ,  $T_{\max}$ ,  $AUC_0^t$ ,  $AUC_0^\infty$ ,  $K_{el}$  and  $T_{1/2}$  are compared by statistical analysis. The maximum concentration ( $C_{\max}$ ) obtained in two brands pantocid and pantop were 987.39 and 1169.23 ng/ml respectively. The half life ( $T_{1/2}$ ) of pantoprazole for pantocid and pantop were calculated and found to be 1.621 and 1.651 hours. Area under the curve  $AUC_0^t$  of pantocid and pantop was calculated as 1930.65 and 2202.85 ng hr/ml and ng hr/ml respectively and  $AUC_0^\infty$  was calculated to be 2115.62 and 2622.2 ng hr/ml. Elimination rate constant ( $k_{el}$ ) 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  $C_{\max}$ ,  $AUC_0-t$ , and  $AUC_0-\infty$ , is 80% to 120%<sup>10</sup>.

## 5. CONCLUSION

The statistical comparison of  $AUC_0-t$ ,  $AUC_0-\infty$ ,  $T_{\max}$  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  $AUC_0-t$ ,  $AUC_0-\infty$  and  $C_{\max}$  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.

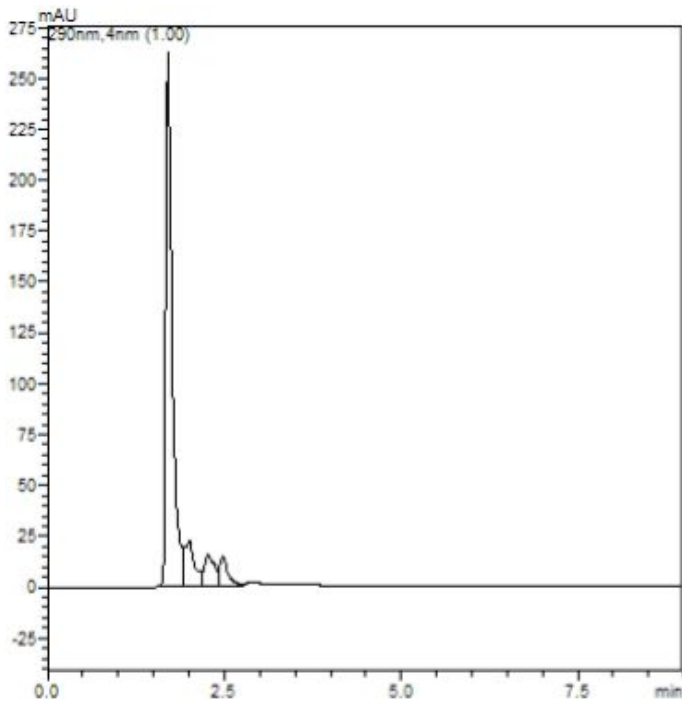
The method has proven to be simple, specific, sensitive, precise and accurate and is suitable for pantoprazole quantification in plasma samples from bioequivalence, and pharmacokinetic studies in healthy albino rats. It was successfully applied to a bioequivalence study of two drug products containing pantoprazole.

**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	$AUC_{0-\infty}$ (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	$K_{el}$ ( $hrs^{-1}$ )	0.5273	0.4836
6	$T_{1/2}$ (hrs)	1.314	1.433

**Table 2: Statistical analysis of log-transformed data**

1way ANOVA					
1	Table Analyzed	One-way ANOVA data			
2					
3	One-way analysis of variance				
4	P value	0.0792			
5	P value summary	ns			
6	Are means signif. different? (P < 0.05)	No			
7	Number of groups	2			
8	F	0.07375			
9	R squared	0.009134			
10					
11	Bartlett's test for equal variances				
12	Bartlett's statistic (corrected)	0.1165			
13	P value	0.7328			
14	P value summary	ns			
15	Do the variances differ signif. (P < 0.05)	No			
16					
17	ANOVA Table	SS	df	MS	
18	Treatment (between columns)	92290	1	92290	
19	Residual (within columns)	10010000	8	1251000	
20	Total	10100000	9		



**Figure 2: HPLC Chromatogram of blank Plasma**

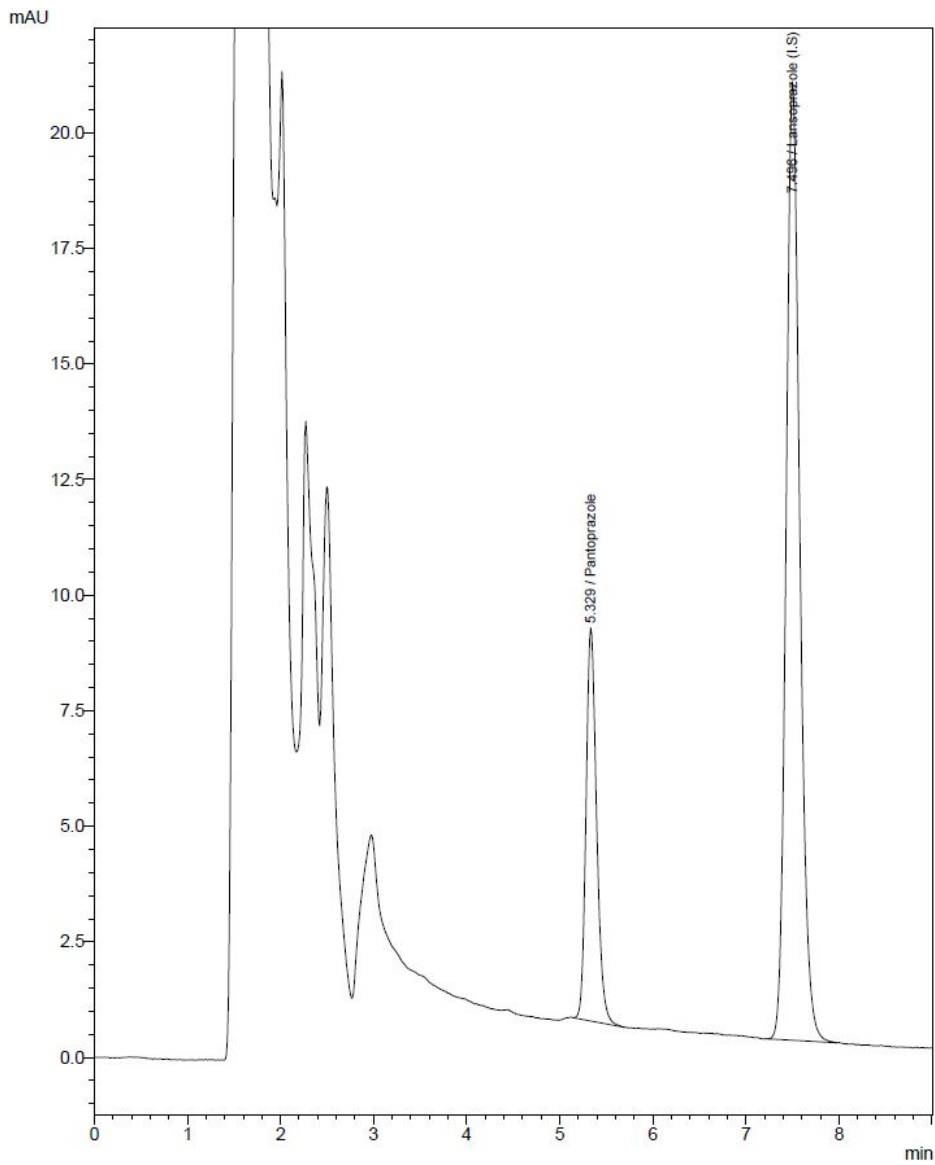


Figure 3: Animal plasma sample spiked with 400ng of Pantoprazole and I.S

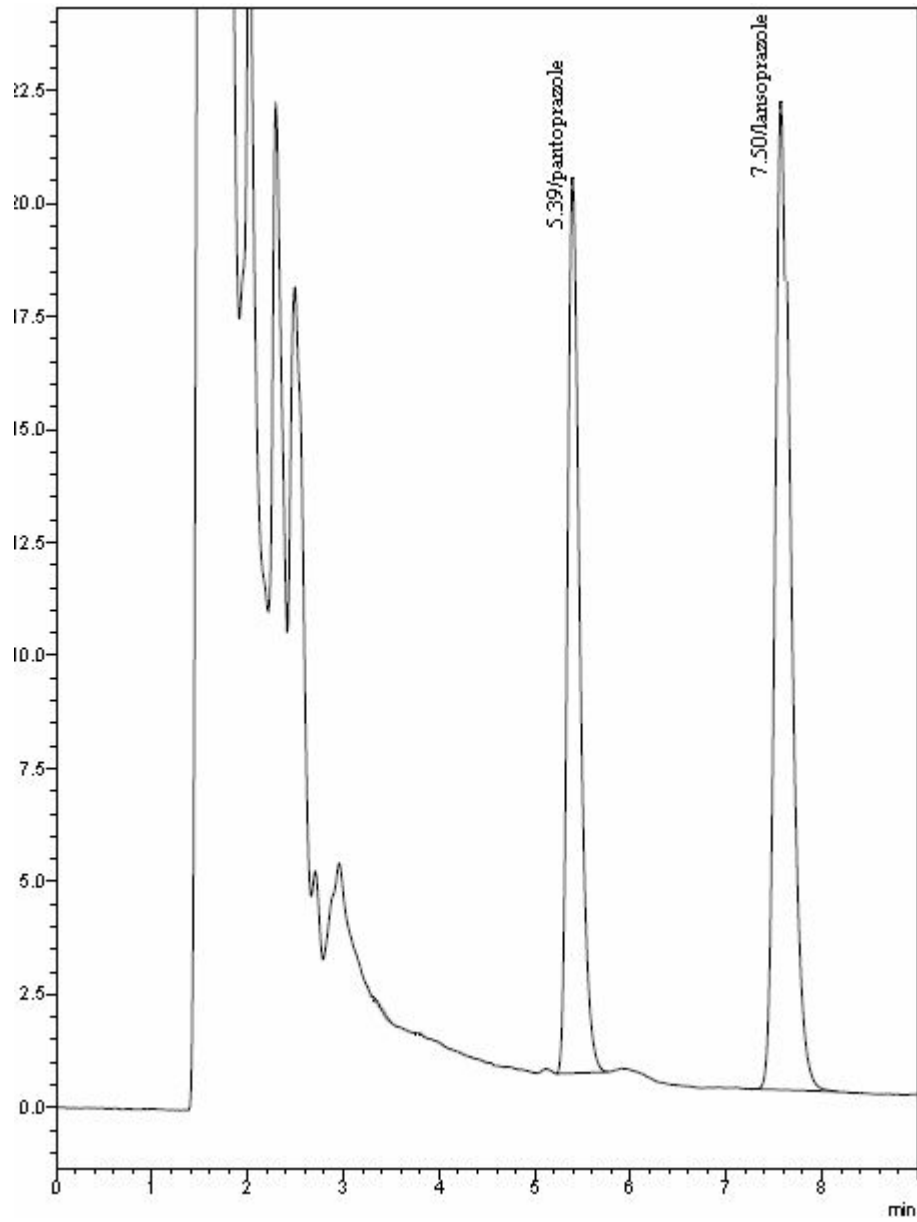


Figure 4: Collected plasma sample of Pantoprazole after 2.5 hr of PANTOP and spiked I.S

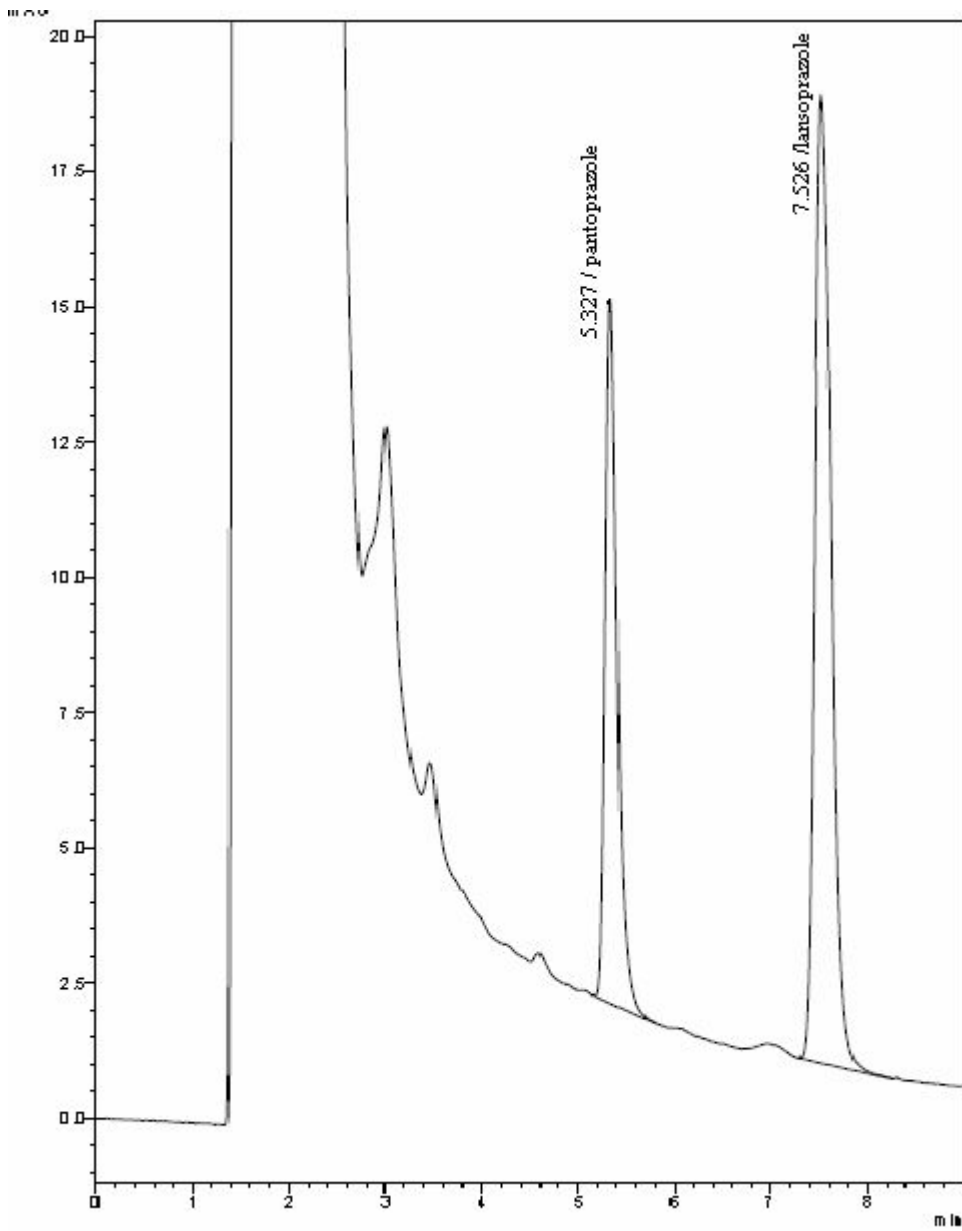


Figure 5: Collected plasma sample of Pantoprazole after 2.5 hr of PANTOCID and spiked I.S



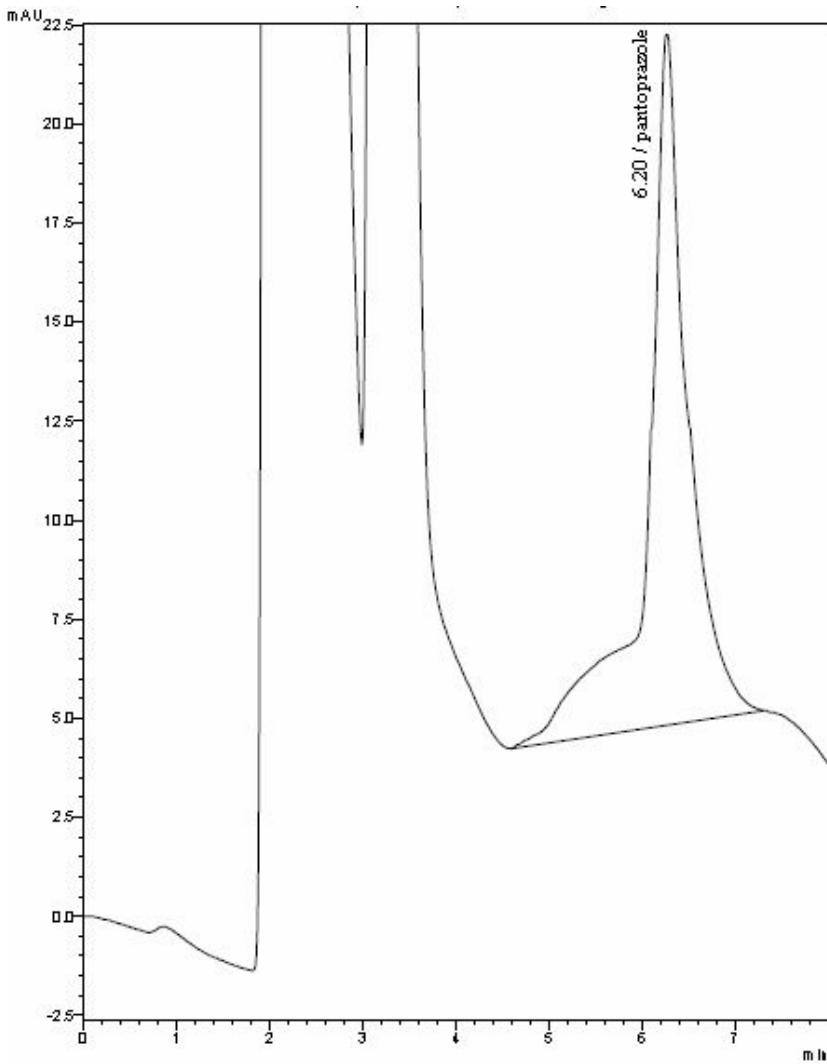


Figure 6: spiked plasma sample at LLOQ (10ng/mL)

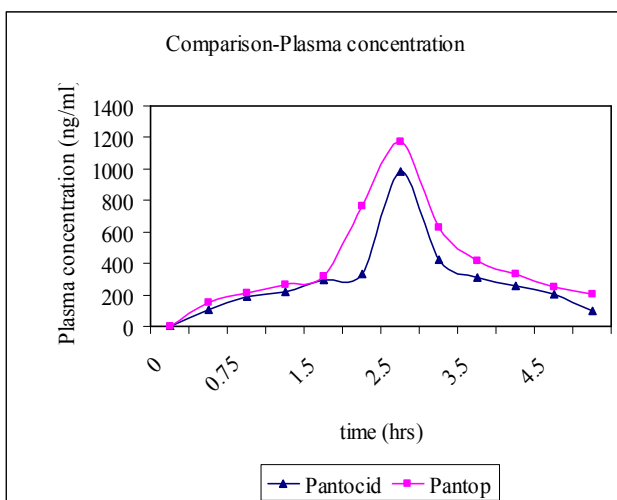


Figure 7: Concentration vs time profile over 5 hr of pantoprazole in animal plasma after receiving single dose of 40mg dose of pantoprazole

## 6. REFERENCES

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