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ORIGINAL ARTICLE

In vivo Pharmacokinetic evaluation of Lansoprazole-loaded Nanosuspension: As a proof of Enhanced Solubility and Absorption

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ABSTRACT
In this research, we presented the pharmacokinetic studies that were done on a nanosuspension that contained lansoprazole. The most optimized nanosuspension was subjected for in vivo evaluations. In order to better understand how the lansoprazole nanosuspension affects the drug's solubility and absorption properties, pharmacokinetic experiments were carried out. The preparation of the lansoprazole nanosuspension was done with the intention of achieving this goal.From various mobile phases tried, mobile phase containing. ACN: KH_2PO_4 Buffer (10 mM) (65:35 pH 4) was selected, since it gives sharp reproducible retention time for Lansoprazole.In calibration curve, the obtained graph was linear and hence we concluded that the developed method can be applied for the estimation of Lansoprazole in different systems.The peak plasma concentration (C_{max}) of lansoprazole in pure formwas found as 227.33 ± 26.135 ng/ml in 2 hr, while C_{max} of Lansoprazole nanosuspension formulation was found as 410.83 ± 10.962 ng/ml in 2 hr.Data on peak plasma concentrations indicate that a greater amount of lansoprazole is absorbed into the bloodstream from the nanosuspension formulation than from the pure drug. This finding is consistent with the hypothesis that the nanosuspension formulation reduces the crystallinity of the drug and causes it to undergo an amorphous phase transition. Maximum plasma drug concentration and area under the curve (AUC) were demonstrated by the optimized lansoprazole nanosuspension formulation, as indicated by the pharmacokinetic parameter. Pharmacokinetic parameters strongly suggested that nanosuspended Lansoprazole had improved bioavailability compared to the parent drug. One possible reason for the improved bioavailability of lansoprazole in the nanosuspension form is the drug's increased solubility in the delivery vehicle.

Keywords:*Lansoprazole; Nanosuspension; Pharmacokinetic; In vivo; Solubility*

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INTRODUCTION

Nanosuspensions are a type of drug delivery system in which a drug is suspended in a liquid medium in the form of very small particles, typically in the nanometer size range (typically below 1 micron). The small particle size of nanosuspensions can improve the bioavailability of a drug by increasing its solubility and stability, and by allowing it to bypass first-pass metabolism in the liver(1,2).Nanosuspensions can be produced by various techniques such as high pressure homogenization, ultrasonication, and microfluidization. These techniques are used to break down the drug particles to a smaller size and to create a stable suspension of the particles in the liquid medium.Nanosuspensions have been studied for a variety of drugs, including poorly soluble drugs, and have shown promise in improving the bioavailability and efficacy of these drugs. They have potential applications in various routes of administration, such as oral, parenteral, and topical(2–4).

In vivo pharmacokinetic (PK) studies are important in the pharmaceutical sciences because they provide information on how a drug behaves in living organisms, typically animals, and how it is processed by the body. This information is used to predict how the drug will behave in humans and to guide the design of clinical trials(5,6).PK studies provide crucial information on the absorption, distribution, metabolism, and elimination (ADME) of a drug, which can be used to optimize dosing regimens and to identify potential

drug-drug interactions. For example, PK studies can determine the maximum concentration of a drug in the blood (Cmax), the time it takes to reach maximum concentration (Tmax), and the drug's half-life ($t_{1/2}$). This information can be used to determine the appropriate dosing frequency and to identify potential toxicity issues(7,8).

*In vivo*PK studies also provide information on the pharmacokinetic properties of a drug, such as bioavailability, clearance, and volume of distribution, which are important for understanding the efficacy and safety of a drug.In addition, PK studies can help to identify potential metabolic pathways and metabolic enzymes involved in the metabolism of a drug, which can aid in the design of new drugs and in the optimization of existing drugs(9,10).In summary, *in vivo* PK studies are essential in the development of new drugs, as they provide critical information on the ADME properties and pharmacokinetics of a drug, which can be used to optimize dosing regimens, identify potential toxicity issues, and guide the design of clinical trials(11).

In this article, we have reported pharmacokinetic evaluations of lansoprazole-containing nanosuspension. The most optimized nanosuspension was subjected for *in vivo* evaluations. The formulation section of the present work is already under review in the *International Journal of Applied Pharmaceutics* (IJAP) and will be published soon. The lansoprazole nanosuspension was prepared with the aim to improve the solubility and absorption parameters of the drug, and the pharmacokinetic studies were performed to investigate the same.

MATERIAL AND METHODS

Determination of λmax of Lansoprazole

Approximately 50 mg of drug was taken in a 50 ml of volumetric flask. A stock solution was prepared by adding methanol as co-solvent and the volume was made with phosphate buffer pH 7.4. Stock solution ranged from 2-40 µg/ml prepared and absorbance's recorded at 200-400 nm(12).The lambda max of Lansoprazole was found to be 272 nm as shown in Fig.1. Therefore further screening was performed at 272 nm.

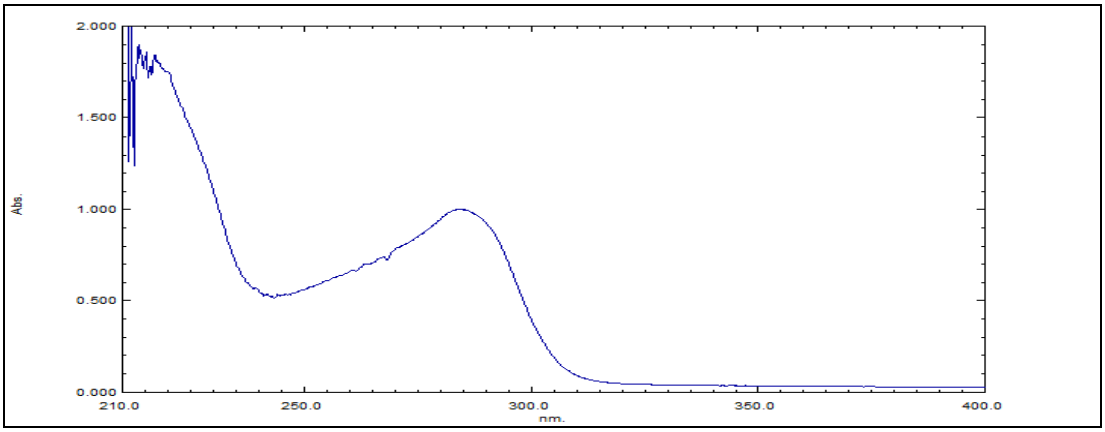

Fig. 1.UV Spectrum of Lansoprazole

Selection of Mobile Phase and Optimization

Accurately weighed quantity 5 mg of Lansoprazole was dissolved in methanol and volume was made up to 25 mL (200 µg/mL). The stock standard solution was diluted further with methanol to get final concentration of about 10 mg/mL of Lansoprazole. The methanol was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing Lansoprazole was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No. 42. The well resolved peaks with symmetry was tried to obtain by using various mobile phase compositions(13,14). The different mobile phase tried along with inference obtained from each trial are tabulated in Table 1. The obtained chromatograms are depicted in Fig. 2 to 6.

Table 1. The mobile phase trials and inference

Mobile phase	Inference
MeOH: H_2O (90:10)	No clear separation and poor resolution
MeOH: KH_2PO_4 Buffer (90:10)	No clear separation and poor resolution
ACN: KH_2PO_4 Buffer (10 mM) (80:20 pH 6)	No clear separation and poor resolution
ACN: KH_2PO_4 Buffer (10 mM)(70:30 pH 5)	Clear separation but poor resolution
ACN: KH_2PO_4 Buffer (10 mM) (65: 35pH 4)	Clear separation with good resolution

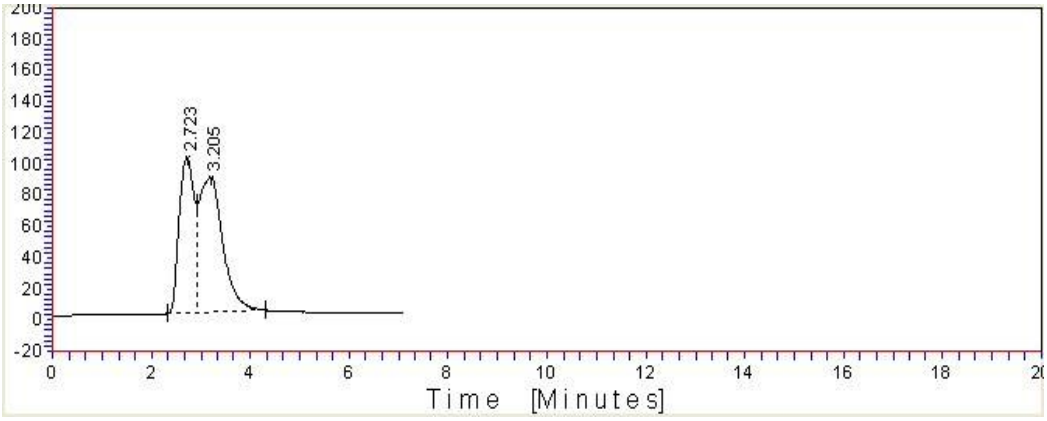

Fig. 2. The chromatogram obtained using MeOH: H_2O (90:10)

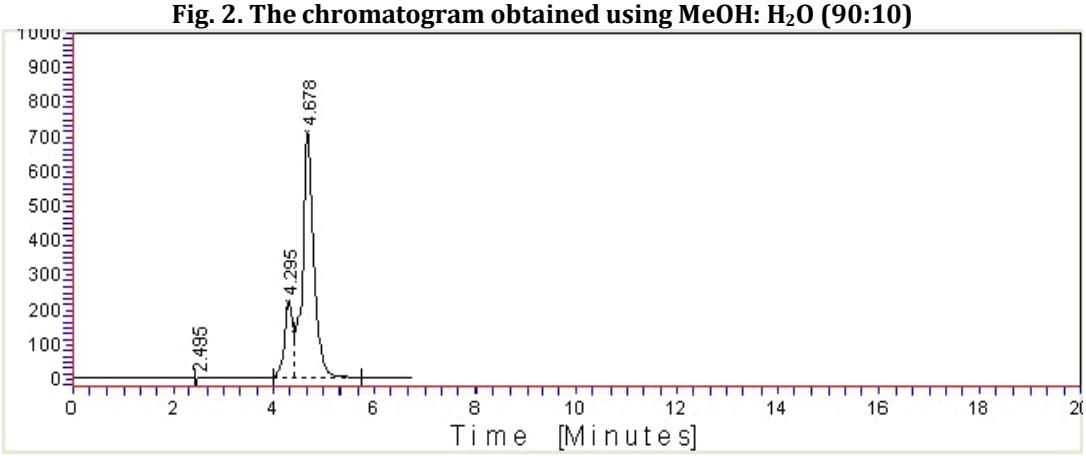

Fig. 3. The chromatogram obtained using MeOH: KH_2PO_4 Buffer (90:10)

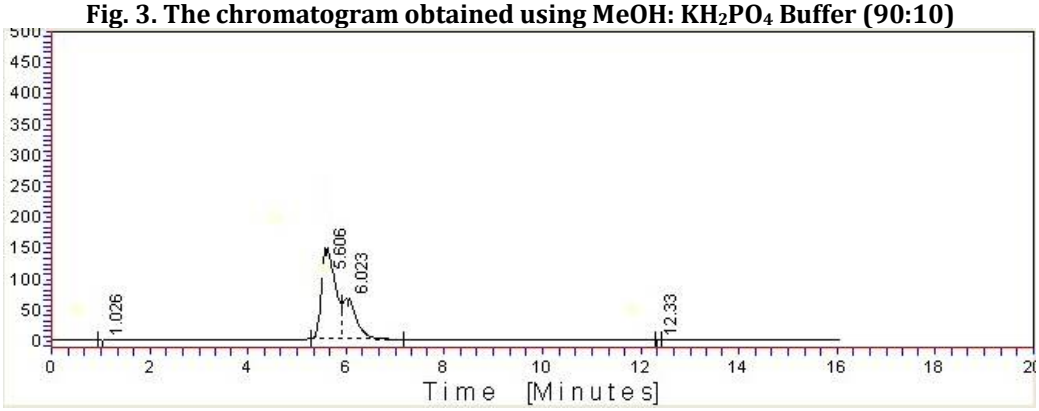

Fig. 4. The chromatogram obtained using ACN: KH_2PO_4 Buffer (10 mM)(80:20 pH 6)

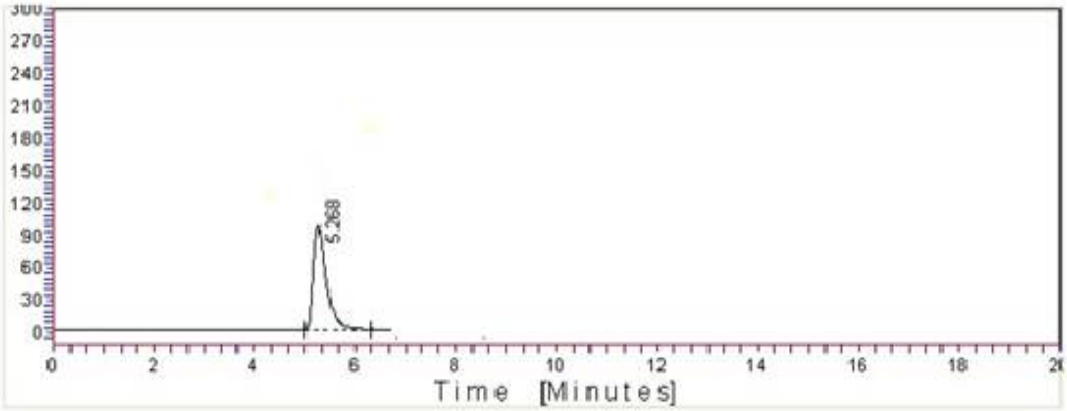

Fig. 5. The chromatogram obtained using ACN: KH_2PO_4 Buffer (10 mM)(70:30 pH 5)

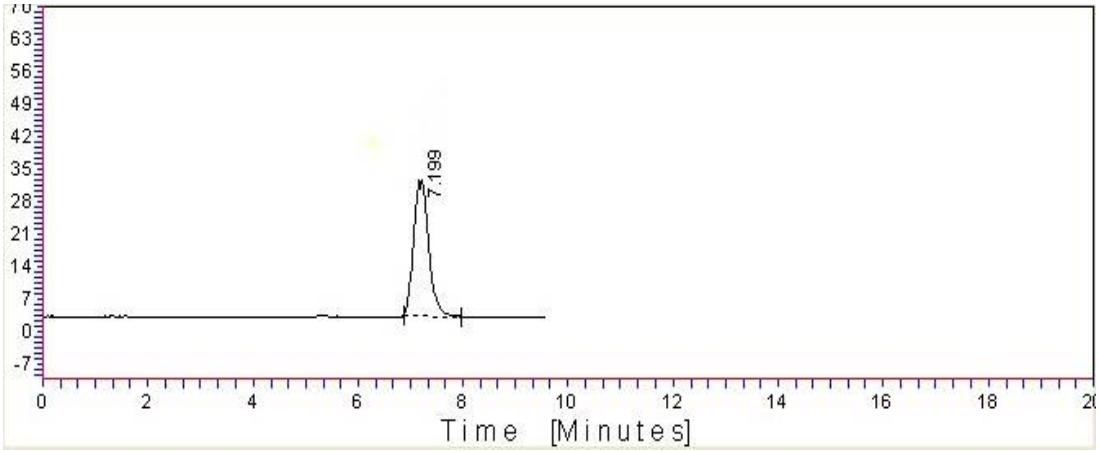

Fig. 6. The chromatogram obtained using ACN: KH_2PO_4 Buffer (10 mM) (65: 35pH 4)

From various mobile phases tried, mobile phase containing. ACN: KH_2PO_4 Buffer (10 mM) (65:35 pH 4) was selected, since it gives sharp reproducible retention time for Lansoprazole. The following chromatographic conditions were established by trial and error and were kept constant throughout method.

Column : Grace 4.6 (id) x 250 mm
Particle size packing : 5 µ
Stationary phases : C18 Grace
Mobile phase : ACN: KH_2PO_4 Buffer (10 mM) (65:35 pH=4.0)
Detection wavelength : 272 nm
Flow rate : 1 mL/min.
Temperature : Ambient
Sample size : 20 µL

Calibration Curve of Lansoprazole by Developed Method

Accurately weighed quantity 10 mg of Lansoprazole was dissolved in mobile phase and volume was made up to 100 ml mark (100 µg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations. The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 2-20 µg/ml of Lansoprazole were injected and peak area was recorded. The concentrations of drug and peak areas are tabulated in Table 2. The graph plotted as the concentration of the drug Vs peak area depicted in Fig. 7. The obtained graph was linear and hence we concluded that the developed method can be applied for the estimation of Lansoprazole in different systems.

Table 2. The concentrations and peak area

Sr. No.	Conc. (µg/ml)	Peak Area
1	2	121.474
2	4	211.506
3	6	312.765
4	8	432.969
5	10	552.62
6	12	663.281
7	14	739.893
8	16	848.216
9	18	938.769
10	20	1015.9

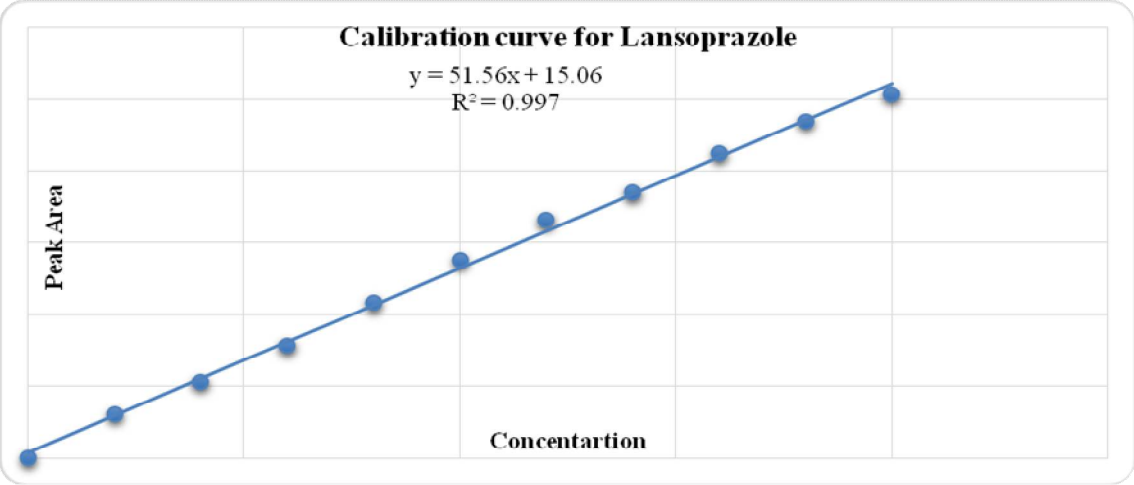

Fig. 7. The calibration curve of Lansoprazole

In vivo Pharmacokinetic Study

On the basis of evaluation parameter, the formulation that showing promising evaluation results in term of its drug solubility and dissolution rate, such optimized Nano suspension formulation of lansoprazole was chosen to conduct the in-vivo study and compare it with pure lansoprazole. In vivo pharmacokinetic study was carried out on Albino Wistar rats according to the guidelines of the CPCSEA, after approval of study(15). The ethical approval number was MET-IOP-IAEC/2022/2023/22 which was approved by MET'S Institute of Pharmacy Nashik, Maharashtra 422 003, India.The study was conducted on healthy male and femaleAlbino Wistar rats weighing between 250 and 300 grams. The rats were randomly separated into two groups, each with six rats (n=6). Before the test, all rats were fasted overnight with free access to water. Lansoprazole oral bioavailability was tested at a dose of 10 mg/kg of body weight. The drug sample was made by suspending it in 1 mL of, 1 percent w/v aqueous sodium carboxyl methyl cellulose and diluting it with water to produced 1 mg/mL concentration. Aqueous solution of pure Lansoprazole was administered orally to one set of animals, which was treated as a control using an oral feeding sonde, based on their body weight(16).The second group of animals was considered as test and given the selected optimized Lansoprazole nanosuspension formulation at the same dose. At specific time intervals of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 hours, 0.5 mL of blood was taken from the postorbital vein sinus into a micro centrifuge tube treated with EDTA. The plasma was separated by centrifugation at 3000 rpm for 10 minutes, then treated with a small amount of acetonitrile before centrifugation at 3000 rpm for another 10 minutes. 50µL of plasma were combined with 0.5 ml of mobile phase. The 20 µL sample was injected into a C18 column (Grace 4.6 (id) x 250 mm) at a flow rate of 1 mL/min and examined by HPLC method(17,18). The study design and dosing details are depicted in Table 3.

Table 3. The study design and dosing details

Drug Dose	10 mg/kg											
Stock Solution	1 mg/ml											
No. of animals	12 (Every group contain 6 animals)											
Wt. of animals (g)	Group I (Control) Pure Lansoprazole						Group II (Test) Optimized Nanosuspension					
	281	296	298	276	285	278	286	288	300	285	280	300
Dose Volume (mL)	2.8	3	3	2.8	2.9	2.8	2.9	2.9	3	2.9	2.8	3

Pharmacokinetic Parameters Estimation and Statistical Analysis

The drug concentration in plasma Vs. time was plotted as a results of the HPLC analysis. The PK Solver computer software was used to calculate non-compartmental pharmacokinetic parameters such as T_{max}, C_{max}, and AUC. The AUC values for each curve were determined using the trapezoidal rule and extrapolation to infinity from time zero to the last data point. The relative bioavailability was calculated using the AUCo-∞values obtained from the curve. The results of *in vivo* investigations are shown as mean standard deviation. Graph Pad Prism 5.0 software was used to perform statistical significance tests. A one-way ANOVA was used to compare the variables. Significance was defined as a P-value of less than 0.05(19).

RESULTS AND DISCUSSION

The rate and amount of a drug goes to the general circulation is known as bioavailability. For both new drug products and their generic equivalents, bioavailability studies are critical during the drug development process. Bioavailability is a key component in the performance and quality of a dosage form, and it can have a major effect on the drug's safety and efficacy. It is also beneficial to analyzed drug activity *in vivo*. The AUC is a useful metric for determining how much unmodified medication makes it into systemic circulation. Bioavailability studies are generally single dose comparisons of test dosage form with standard dosage in normal healthy animals in fasting state. Generally, these studies are performing in two ways cross over fashion were all the subjects receive both the test and standard formulation on different days. Fasting should occur for at least 12 hrs prior to dosing(20). Blood sampling must be frequent enough to design adequately the absorptive phase of the plasma concentration time course. Non-compartmental (model independent) or compartmental approaches are used for pharmacokinetic study. The calculation of total drug exposure is critical in non-compartmental pharmacokinetic studies. Area under curve (AUC) approaches employing the trapezoidal rule are commonly used to estimate total drug exposure. Based on the optimization study, an optimized Lansoprazole nanosuspension formulation, was chosen for *in-vivo*study. The non-compartmental pharmacokinetic specifications of lansoprazole were determined, after dosing via oral route pure Lansoprazole (Control) and chosen optimized nanosuspension formulation (Test) to Wister rats by HPLC method. The chromatogram obtained from serum sample of rats is depicted in Fig. 8.

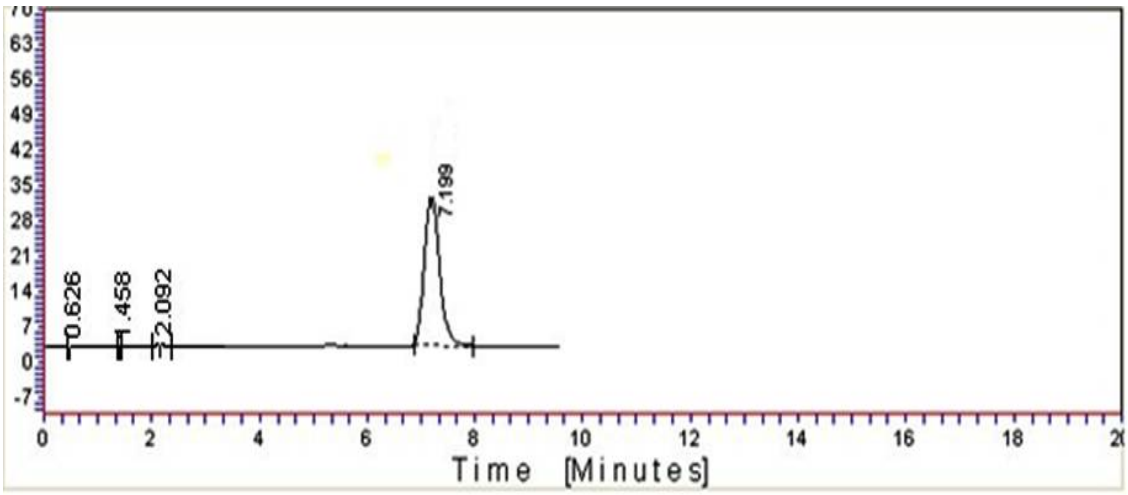

Fig. 8. The chromatogram obtained from serum sample of rats

The peak plasma concentration (C_{max}) of lansoprazole in pure form was found as 227.33 ± 26.135 ng/ml in 2 hr, while C_{max} of Lansoprazole nanosuspension formulation was found as 410.83 ± 10.962 ng/ml in 2 hr. The peak plasma concentration data suggests that absorption of drug in plasma from nanosuspension formulation is more and rapid as compare to pure drug, which suggest the reduction in crystallinity and amorphous conversion of Lansoprazole in nanosuspension. The AUC_0-t for pure and nanosuspension formulation of drug was found to be 1545.16±135.09 and 2765.87±160.57 ng/ml*h, while AUC_0-∞ was found to be 2113.69±222.12 and 3637.92±461.26 ng/ml*h,respectively. The pharmacokinetic parameter indicated the maximal plasma drug concentration and AUC was shown by optimized lansoprazole nanosuspension formulation. Pharmacokinetic parameter clearly suggested the boosted bioavailability of Lansoprazolein nanosuspension form, when compared with its original form. Increased solubility of drug in the nanosuspension form can be a cause for boosted bioavailability lansoprazole. The resultant pharmacokinetic values were tested for significance using one-way ANOVA. P value lesser than 0.05 was regard as significant. The *in vivo* plasma drug concentration for control and test and different pharmacokinetic parameters of pure Lansoprazole and optimized nanosuspension are shown in Table4 and 5, respectively. The plasma concentration profile of pure Lansoprazole and optimized nanosuspension is illustrated in Fig. 9.

Table 4.*In vivo* plasma drug concentration in control and test animals

Time (hr)	Concentration in ng/ml	
	Control(Pure Lansoprazole)	Test(Optimized Nanosuspension)
0.5	85.33 ± 26.15	140.33 ± 17.96
1	116.83 ± 17.82	231.50 ± 13.70
1.5	168.33 ± 16.69	318.66 ± 18.27
2	224.66 ± 29.79	402.16 ± 21.71
3	202.16 ± 36.16	371.50 ± 30.69
4	173.83 ± 21.91	306.33 ± 25.31
6	135.66 ± 26.00	256.50 ± 33.13
8	107.5 ± 13.64	186.16 ± 26.28
10	86.66 ± 14.89	143.16 ± 27.98
12	68.83 ± 13.16	116.33 ± 23.79

Values are expressed as mean±SD (n=6)

Table 5. Pharmacokinetic criterion of Pure Lansoprazole and Optimized Nanosuspension

Pharmacokinetic Parameter	Pure Lansoprazole	Optimized Nanosuspension
T_{max} (h)	2 ± 0.408	2 ± 0.516
C_{max} (ng/ml)	227.33 ± 26.135	410.83 ± 10.962
$t_{1/2}$ (h)	5.63 ± 0.861	5.07 ± 0.947
AUC $_{0\text{-}t}$ (ng/ml*h)	1545.16 ± 135.09	2765.87 ± 160.57
AUC $_{0\text{-}inf.}$ (ng/ml*h)	2113.69 ± 222.12	3637.92 ± 461.26
MRT (hr)	9.26 ± 1.227	9.51 ± 1.244

Values are expressed as mean±SD (n=6)

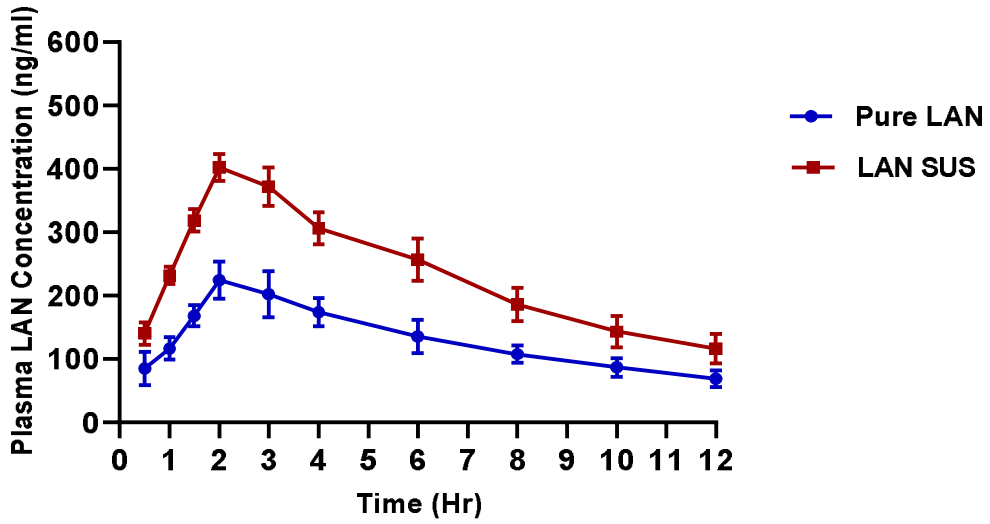

Fig. 9.The plasma concentration profile of pure Lansoprazole and optimized nanosuspension

CONCLUSION

In this article, we have reported pharmacokinetic evaluations of lansoprazole-containing nanosuspension. The most optimized nanosuspension was subjected for *in vivo* evaluations. The formulation section of the present work is already under review in the *International Journal of Applied Pharmaceutics* (IJAP) and will be published soon. The lansoprazole nanosuspension was prepared with the aim to improve the solubility and absorption parameters of the drug, and the pharmacokinetic studies were performed to investigate the same. The lambda max of Lansoprazole was found to be 272 nm and therefore further screening was performed at 272 nm. From various mobile phases tried, mobile phase containing. ACN: KH_2PO_4 Buffer (10 mM) (65:35 pH 4) was selected, since it gives sharp reproducible retention time for Lansoprazole. In calibration curve, the obtained graph was linear and hence we concluded that the developed method can be applied for the estimation of Lansoprazole in different systems. The PK Solver computer software was used to calculate non-compartmental pharmacokinetic parameters such as T_{max}, C_{max}, and AUC. The AUC values for each curve were determined using the

trapezoidal rule and extrapolation to infinity from time zero to the last data point. The peak plasma concentration (C_{max}) of lansoprazole in pure form was found as 227.33±26.135 ng/ml in 2 hr, while C_{max} of Lansoprazole nanosuspension formulation was found as 410.83±10.962 ng/ml in 2 hr.The peak plasma concentration data suggests that absorption of drug in plasma from nanosuspension formulation is more and rapid as compare to pure drug, which suggest the reduction in crystallinity and amorphous conversion of Lansoprazole in nanosuspension. The pharmacokinetic parameter indicated the maximal plasma drug concentration and AUC was shown by optimized lansoprazole nanosuspension formulation. Pharmacokinetic parameter clearly suggested the boosted bioavailability of Lansoprazolein nanosuspension form, when compared with its original form. Increased solubility of drug in the nanosuspension form can be a cause for boosted bioavailability lansoprazole.

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Authors Contributions
All the authors have contributed equally.

Conflict of Interests
All the authors confirm that there is no conflict of interest.

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