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# Pharmacokinetic study of Piperine in wistar rats after oral and intravenous administration

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## Abstract

Purpose: To evaluate the potential of piperine as a therapeutic agent, we considered whole animal studies to characterize its pharmacokinetics (PK) in *Wistar* rats after oral and intravenous (i.v.) administration, using high performance liquid chromatography (HPLC). This study will enable in determination of piperine exposures needed to predict the dose regimen for clinical trials to test the proposed mechanism of action in enhancing the therapeutic efficacy of the concurrently administered drugs.

Materials and Methods: A single dose of piperine was administered intravenously (10 mg/kg) by jugular vein cannulation and orally (20 mg/kg) by oral gavage in male *Wistar* rats. Serial blood samples were collected and plasma piperine concentrations were determined using HPLC.

Results: After intravenous administration the apparent terminal half-life (7.999 hr), apparent steady state volume of distribution (7.046 L/kg) and total body clearance (0.642 L/kg/hr) were calculated. After oral administration the apparent terminal half-life (1.224 hr), apparent steady state volume of distribution (4.692 L/kg) and total body clearance (2.656 L/kg/hr) were calculated. The peak plasma concentration of piperine in plasma after oral administration was found to be 0.983 μg/ml, occurred approximately 2 hr post-dose. The $AUC_{(0-)}$ of Piperine after oral and intravenous administration in rats were found out to be 7.53 μg*hr/ml and 15.6 μg*hr/ml, respectively. The absolute oral bioavailability of piperine was found to be 24%.

Conclusion: From the results of the experiment, it can be concluded that piperine achieves extensive distribution because of its large volume of distribution in the body. These studies are useful in interpreting preclinical efficacy studies of Piperine & predicting human pharmacokinetic through scaling technique.

**Keywords:** Piperine; Pharmacokinetics; AUC; Bioavailability; HPLC; PDA detector.

## Introduction

Bioenhancers are molecules that are often used in the combination therapy to promote the biological activity or enhance the bioavailability of drugs. Biomolecules obtained from plant origin or from their semisynthetic derivatization have boosted the production of medicines [1].

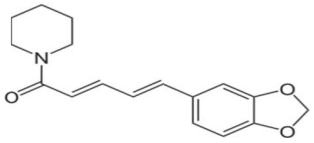

**Figure1:**1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine

Piperine (trans-trans-isomer of 1-piperonylpiperidine) (Figure 1) is one of the major alkaloidal constituent of black pepper (*Piper nigrum*) and long pepper (*Piper longum*), family *Piperaceae*. Black pepper is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning [2]. The structural and chemical of piperine is as shown in the figure 1. It is slightly soluble in water and more soluble in alcohol, ether and chloroform. It has the ability to increase the bioavailability of certain nutrients and drugs, such as: beta carotene, curcumin, selenium, pyrodoxine (Vitamin B6), glucose, and amino acids [3]. Black pepper is widely used in the Indian System of Medicine (Ayurvedic System) along with ginger to enhance the therapeutic efficacy of the concurrently administered drugs. Piperine has shown antioxidant, anti-platelet, anti-inflammatory, anti-hypertensive,

hepatoprotective, anti-thyroid, antidiarrhoeal, anti-asthmatic and also is a fertility enhancer [4-14]. An interesting observation is that the combination of piperine isolated from *Piper nigrum* with essential drugs, such as antibiotics, antihypertensive and antiepileptics as well as nutrient supplements, led to dose economy due to enhanced uptake, higher blood concentration and the drug being available for a longer duration in the body [15-19].

# Material and Methods

## Chemicals and reagents

Piperine was purchased from Sigma Aldrich Chemical Co., St. Louis, MO, USA. Methanol (MeOH), dichloromethane (DCM) and acetonitrile (ACN) were of HPLC grade purchased from Ranbaxy fine chemicals Ltd, Delhi, India. All other chemicals used in this study were of analytical grade obtained from Merck, Mumbai, India.

## Animals

Male *Wistar* rats of body weight 180 g - 200 g were obtained from central animal house, Indian Institute of Integrative Medicine (CSIR). The animals were fed on standard pellet diet (Ashirwad Industries, Chandigarh, India) and water ad libitum. The rats used in the present study were maintained in accordance with guidelines of the CPCSEA, India and the study approved by the ethical committee of IIIM, Jammu.

## Drug administration

Drug was administered via oral and intravenous routes in male *Wistar* rats.

## Oral administration

For oral administration, 20 mg of the drug was triturated with gum acacia to form a fine homogeneous mixture in 10 ml of water. This drug was administered at a dose of 20 mg/kg (20mg/10ml suspension) to rats with the help of stainless steel oral gavage needle. The volume of drug administered was corresponding to their body weight (2 ml / 200 g body weight). Drug was administered via oral route with the help of rat cannula and syringe corresponding to the body weight of respective animal.

## Intravenous administration

For i.v. administration, 20 mg of drug was dissolved in 20 ml of 75% of polyethylene glycol 400 in normal saline to form a homogeneous solution (10 mg/10 ml). Each rat was cannulated under anaestheia. The jugular vein cannulation for i.v. administration and the carotid artery cannulation for blood sampling were done with a polyethylene tube while [20, 21]. Heparinized normal saline was used to prevent blood clotting in cannula. Piperine solution at a dose of 10 mg/kg was infused over 1 min via the jugular vein of rats. Volume of drug solution administered corresponding to the body weight of the animal i.e. for 200 g rat 2.0 ml of drug solution was injected).

## Preparation of plasma samples

Approximately 500 µL of blood sample was collected from each animal at respective sampling time post administration. In oral study the blood samples were collected from retro-orbital plexus of rats at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16 and 24 hr and incase of iv study the blood samples collected via carotid artery at 0.083, 0.5, 2, 4, 6, 8, 16 and 24 hr. Blood samples are collected in pre-labeled micro centrifuge tubes containing 50 µL of 5% EDTA at respective sampling time points. The blood samples centrifuged for 10 minutes at 5000 rpm to collect the plasma. Plasma samples (250 µl) extracted with 3 ml DCM. Concentration of Piperine in plasma was estimated by using high performance liquid chromatography, Shimadzu.

## HPLC Instrumentation

Chromatography was performed using a Shimadzu (Japan) HPLC system equipped with 600E HPLC pump, RP-18, 5 µm, 250 X4.6 mm (Supelco) column, an auto sampler and PDA detector. The detection of analyte was carried out at 340 nm and the column temperature was kept at 40 C. The separation was carried out with the mobile phase consisting of Methanol (HPLC grade) and Water (Milipore), 70:30 at a flow rate of 1.0 ml/min. Standard solution of Piperine was used for calibration curve. The retention time of Piperine was obtained to be 7.808 min.

## Calibration curve

The different concentration of piperine was prepared by dissolving 1 mg of piperine in 1 ml of methanol to yield a solution of concentration 1 mg/ml. The serial dilutions of the stock were prepared with methanol to yield a concentration range from 1 µg/ml to 250 µg/ml. A calibration curve was performed by the analysis of various concentrations. The concentration of sample was determined from the peak area by using the equation for linear regression obtained from the calibration curve.

## Recovery of Piperine from rat plasma matrix

Methanolic solution of Piperine (25 µl) of 100 µg/ml concentration was added to 250µl of plasma (in quadruplicates) in each labeled tube (except to the control tubes). Methanol (25 µl) was added to control tube for each set of solvent system. Tubes were vortexed for one minute on a vortex mixer. All tubes were incubated at 37 C for 30 minutes. The tubes were grouped into 3 different sets. Extraction of Piperine from plasma matrix was carried out using different solvent i.e. ethyl acetate, acetonitrile and dichloromethane 3 ml per tube and vortexed for 2 min at maximum speed. The tubes were centrifuged at 5000 rpm for 10 minutes at 20 C. The organic layers were decanted into separate set of pre-labeled tubes. The tubes were dried in solvent evaporator under 35 C. The residues left in the tubes were dissolved in 0.5 ml of mobile phase (Methanol and Water) in the ratio of 70:30. The tubes were vortexed for one minute on a vortex mixer. The samples were filtered into HPLC vials using 0.45 µm syringe filter and analysis done by HPLC. The maximum recovery of Piperine from the plasma matrix was achieved in dichloromethane (DCM) as mentioned in Table 1.



## Pharmacokinetic analysis

Plasma samples (250 µl) collected at different time points were added to respective pre-labeled tubes and extracted with dichloromethane as described in the recovery of piperine from rat plasma section. The samples were analyzed using HPLC for the estimation of Piperine concentration in the plasma at different time point post drug administration [22-25].

## Statistical analysis

Plasma concentration obtained at different time points were represented as Mean ± SEM. Different pharmacokinetic parameters were calculated using non-compartmental analysis by using the software PK-Solutions 2.0, USA.

# Results

## Calibration curve

The linear regression equation analyte was Y = 0.000143719x + 0.232802 with ($r^2$ = 0.999994) (Figure 2). The lower limit of quantification and the limit of detection were determined to be 0.965 µg/ml and 0.318 µg/ml respectively. The linear range for piperine was adequate for this method to be used in the current pharmacokinetic studies.

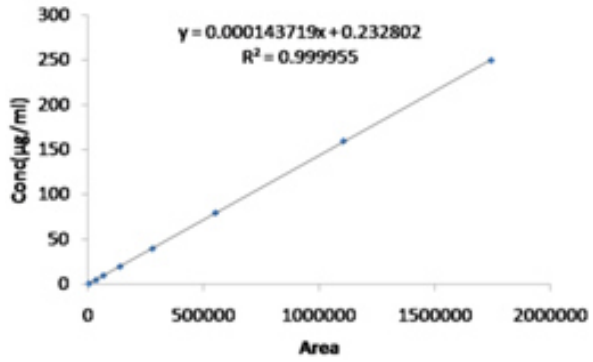


**Figure 2**: Calibration curve of standard piperine concentration ranging from 1 µg/ml to 250 µg/ml.

## Recovery

The mean percentage recovery of Piperine with different solvents i.e. ACN, DCM and ethyl acetate was 63.07%, 74.5% and 35.43% respectively (Table 1). The solvent dichloromethane (DCM) shows maximum recovery of Piperine hence it was chosen for extraction of piperine from plasma matrix obtained after oral and intravenous administration.

**Table 1:** Optimization of HPLC conditions for Piperine

| Extracted Solvent | % Recovery |
|---|---|
| ACN | 63.07±4.56 |
| DCM | 74.5±9.83 |
| Ethyl acetate | 35.43±9.5 |

## Pharmacokinetics studies and Data analysis

The concentration of Piperine present in plasma at different time intervals after oral and intravenous administration of drug in rats were shown in Fig. 3 and Fig. 4. After oral administration the maximum concentration of Piperine in plasma was found to be 0.983 µg/ml at 2 hr. The $AUC_{(0-)}$ of Piperine after oral and i.v. administration in Wistar rats were found to be 7.53 µg*hr/ml and 15.6 µg*hr/ml, respectively (Table 2 and Table 3). The plasma concentration vs. time curve of Piperine in Wistar rats for oral and intravenous administration demonstrated in Figure 3 and Figure 4 respectively. The pharmacokinetic parameters obtained were: $T_{1/2}$ – 1.224 hr, $T_{max}$ - 2.0 hr, $C_{max}$ – 0.983 µg/ml and Area Under Curve, $AUC_{(0-)}$ – 7.53 µg*hr/ml for oral administration and $T_{1/2}$ – 7.997 hr, and Area Under Curve, $AUC_{(0-)}$ – 15.6 µg*hr/ml for intravenous administration. The bioavailability of the compound was found to be 24.1%.

**Table 2:** The pharmacokinetic parameters of Piperine in Wistar rat plasma after oral administration.

| PK parameters (PO Study) | |
|---|---|
| $T_{1/2}$ (hr) | 1.224 |
| $T_{max}$ (hr) | 2.0 |
| $C_{max}$ (µg/ml) | 0.983 |
| $AUC_{(0-t)}$ (µg*hr/ml) | 7.48 |
| $AUC_{(0-)}$ (µg*hr/ml) | 7.53 |
| Vd (l/kg) | 4.692 |
| Cl (l/hr/kg) | 2.656 |

**Table 3:** Pharmacokinetic parameters of Piperine in Wistar rat plasma after intravenous administration

| PK parameters (IV Study) | |
|---|---|
| $T_{1/2}$ (hr) | 7.997 |
| $C_0$ (µg/ml) | 2.9 |
| $AUC_{(0-t)}$ (µg*hr/ml) | 14.6 |
| $AUC_{(0-)}$ (µg*hr/ml) | 15.6 |
| Vd (l/kg) | 7.046 |
| Cl (l/hr/kg) | 0.642 |

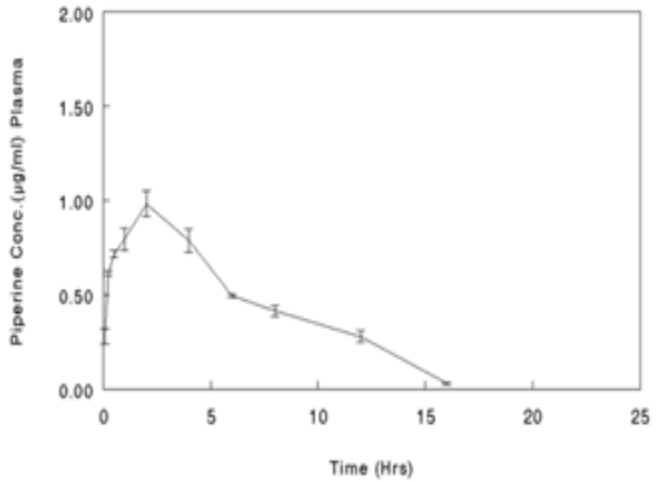


**Figure 3:** Plasma concentration versus time curve of piperine in Wistar rats after oral administration.

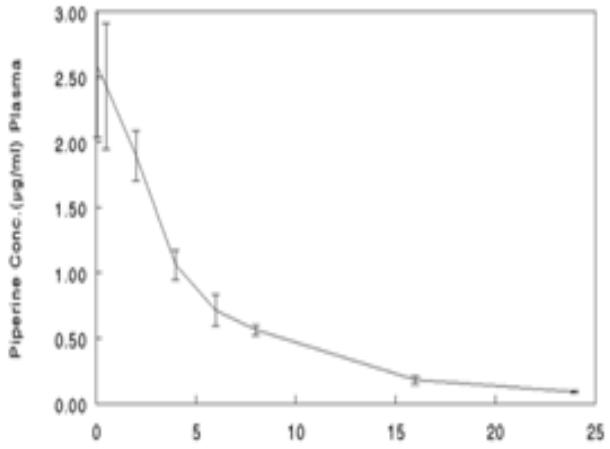


**Figure 4:** Plasma concentration versus time curve of piperine in Wistar rats after intravenous administration.

## Discussion

Administration of a drug helps in achieving the specific intended therapeutic goal. The therapeutic efficacy of a drug depends on the amount of the drug and its residence in sufficient amount at the site of action. But, the amount of the drug at the site of action depends on amount of the drug absorbed into the systemic circulation. For many drugs, the therapeutic efficacy is not as expected as it to be due to its poor bioavailability. Incomplete oral bioavailability includes poor dissolution or low aqueous solubility, poor intestinal membrane permeation, degradation of the drug in gastric or intestinal fluids, and presystemic intestinal or hepatic metabolism. Bioavailability is affected by gastric emptying time, intestinal transit time, blood flow through gastrointestinal transit, gastrointestinal contents and pre-systemic metabolism through luminal enzymes, gut wall enzymes, bacterial enzymes, and hepatic enzymes. Some drugs show poor oral bioavailability because a drug must not only penetrate the intestinal mucosa, it must also run the gauntlet of enzymes that may inactivate it in gut wall and liver [26 - 28].

The present study described the pharmacokinetic study of piperine in Wistar rats after oral and intravenous administration. The present pharmacokinetic study of piperine was divided into three segments viz. the optimization of the recovery solvent, the oral and the intravenous PK study. The recovery study was done with the use of three solvents i.e. Acetonitrile, Ethyl acetate and Dichloromethane 3ml each. Dichloromethane has shown maximum percentage recovery of piperine for which it was the choice of recovery solvent for oral and intravenous PK studies. Oral pharmacokinetic study was conducted in *Wistar* rats for sampling time with a dose of 20 mg/kg according to body weight of animal through oral gavaging. After 16 hrs of post oral administration, the drug concentration was below the detection level in the samples. The intravenous PK study was conducted for sampling time points with a dose reduced to 10 mg/kg according to the body weight of animals via jugular vein cannulation. The $AUC_{(0-)}$ of Piperine after oral and i.v. administration in Wistar rats were found to be 7.53 µg*hr/ml and 15.6 µg*hr/ml, respectively. The absolute fraction of the drug absorbed (bioavailability) was found to be 24.1%.

## Conclusion

In a drug discovery and development program, availability of accurate pharmacokinetic and metabolic data is must. Early pharmacokinetic and metabolic evaluation is very important to obtain optimal pharmacological properties of a new chemical entity (NCE). Many of the failures in drug development program are due to their undesirable pharmacokinetic properties, such as too long or too short $t_{1/2}$, poor absorption and extensive first pass metabolism. To ensure the success of a drug's development, it is essential that a drug candidate has good bioavailability and a desirable $t_{1/2}$. Therefore, an accurate estimation of the pharmacokinetic data will guide drug development. The various data obtained from the present pharmacokinetic study of Piperine are useful in interpreting the preclinical efficacy studies of Piperine & predicting human pharmacokinetic through scaling technique.

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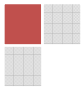

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