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PHARMACOKINETIC STUDIES OF SECNIDAZOLE IN THE PRESENCE OF PIPERINE AND ITS SYNTHETIC DERIVATIVE

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Keywords:

Secnidazole, Piperine, Pharmacokinetics, Bioenhancing effect, HPLC, AUC

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ABSTRACT: Piperine has been widely used as a bioenhancer. Secnidazole is a long-acting 5-nitroimidazole analog. It is used in the treatment of amoebiasis, giardiasis, trichomoniasis and anaerobic bacterial infection. In this study derivative of piperine was synthesized and studied for its bio enhancing the effect and this effect was compared with the bioenhancing effect of piperine. The pharmacokinetic profile of Secnidazole alone and in combination with piperine and piperine derivative was investigated by validated bioanalytical HPLC method as per USFDA guidelines. It was observed that the synthesized derivative of piperine significantly improved the bioavailability of Secnidazole compared to piperine alone in Wistar rats. The N,N-diallyl-5-(benz)(1,3)dioxol-5-yl)penta-2,4-dienamide (DA) derivative showed 1.49 fold increase in the bioavailability of Secnidazole. After oral administration of Secnidazole alone and in combination with piperine and piperine derivative (0.035 mmol, 10 mg/kg) there was a significant increase in AUC and C_{max} of Secnidazole.

INTRODUCTION: Secnidazole, (1-(2-methyl-5-nitroimidazole-1-yl)propane-2-ol), is a long-acting 5-nitroimidazole derivative **Fig. 1** is used in the treatment of amebiasis, giardiasis, trichomoniasis and anaerobic bacterial infections [1]. It is used widely in the treatment of female genital infections [2, 3, 4]. It is observed that patients suffering from intestinal amoebiasis or giardiasis, clinical or parasitological, are cured 80% to 100% after treatment with 2 g of Secnidazole [5]. It is a high dose. It may be true that cure rate with such a high dose is high, but it also has larger side effects including dizziness, abdominal pain, urticaria, glossitis, stomatitis, taste disturbances, and paresthesis.

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Secnidazole induced acute pancreatitis has been reported in the literature after a single dose administration [6]. Piperine **Fig. 1** is a bioactive principal obtained from *Piper nigrum* and *Piper longum* (black paper) [7, 8].

The powder of black paper is used as a food additive in several countries [9, 10]. It has several properties such as antioxidant [11], anti-inflammatory [12], antihypertensive [13] and hepatoprotective action [14]. In the past several years it has generated considerable interest due to its bioenhancing effect and low toxicity in animals and humans. In this paper synthesis and pharmacokinetic properties of piperine, derivative are reported [15]. This is the first such report wherein the derivative of piperine was synthesized and studied for its bioenhancing effect. We report here, the scheme of synthesis, characterization of a synthetic derivative of piperine. The pharmacokinetic comparison of Secnidazole alone and after co-administration with piperine and the synthesized derivative is also reported. This is the first such study where the

effect of different amide substituent in piperine and its effect on the pharmacokinetics of Secnidazole reported.

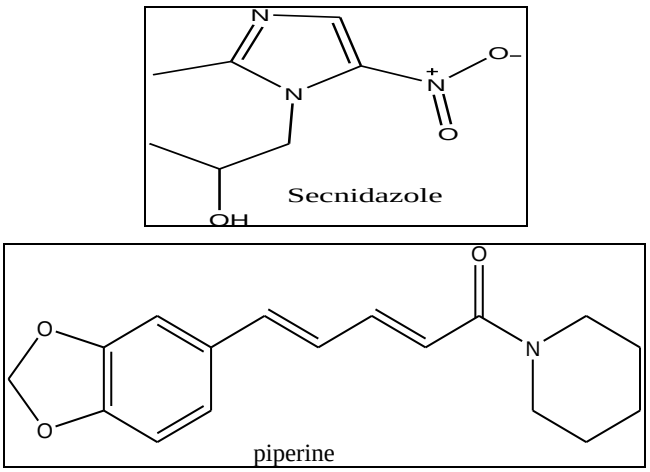

FIG. 1: STRUCTURES OF SECNIDAZOLE AND PIPERINE

MATERIALS AND METHODS:

Chemicals and Reagents: All reagents and chemicals were obtained from Aldrich Chemical Company. HPLC grade chemicals and reagents were used for HPLC analyses. They were purchased from Merck Specialities Pvt. Ltd., India. All the solvents used in reactions were distilled and dried before use. All reactions were monitored by TLC on 0.25 mm silica gel plates coated on the aluminum sheet (Merck). For vacuum evaporation rotavac of Kika Werke, HB4, Germany was used. IR spectra were recorded on JASCO, V-530 FTIR as potassium bromide pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometer operating at 400 MHz and 100 MHz respectively with TMS as the reference standard. DMSO was used as the solvent; chemical shifts are reported in δ values. Mass spectra were recorded on 410 Prostar Binary LC with 500 MS IT PDA detectors, Varian Inc. with (-) ESI mode.

General Methods:

Preparation of Piperic Acid from Piperine: To piperine (1g, 0.35 mmol), 20 ml of 20% ethanolic KOH was added and refluxed for 48 h. The completion of the reaction was monitored by TLC using mobile phase *n*-hexane: ethyl acetate (7:3 v/v). After completion of the reaction, ethanol was removed under reduced pressure to give yellow colored solid. This solid was dissolved in 20 ml of 10% sodium bicarbonate solution and shaken for 15 min. It was filtered, and the filtrate was acidified with conc. HCl (drop by drop) yielding a yellowish precipitate of piperic acid. This precipitate was filtered under mild vacuum and recrystallized from methanol (15 ml) to give yellow needles of pure piperic acid (750 mg, 75% yield), m.p. 216 ºC (Lit. 217-218 ºC) **Fig. 2** [16].

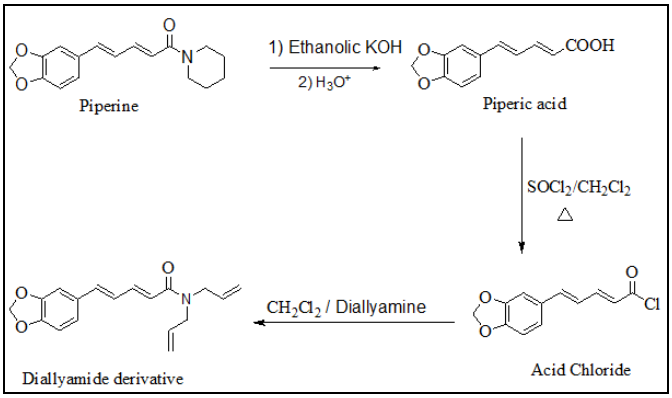

FIG. 2: SYNTHETIC SCHEME FOR PREPARATION OF DIALLYLAMIDE DERIVATIVE FROM PIPERINE

Preparation of Acid Chloride of Piperic Acid: To piperic acid (750 mg, 3.7 mmol) in dry dichloromethane (10 ml) was added freshly distilled thionyl chloride (0.4 ml) and the contents were refluxed for 3 h. Completion of the reaction was monitored by TLC using mobile phase n-hexane: ethyl acetate (7:3 v/v). Then excess thionyl chloride was removed under reduced pressure. The crude acid chloride was used immediately without further purification [17] **Fig. 2**.

Preparation of Diallylamide Derivative from Acid Chloride: To the acid chloride of piperic acid in dichloromethane (7.5 ml) was added diallyl amine (0.3 ml) dissolved in dichloromethane. The contents were stirred for 2 h, the reaction was monitored by TLC using mobile phase *n*-hexane: ethyl acetate (7:3 v/v). To the reaction mixture was added 15 ml water, it was carefully and thoroughly shaken for 5 min. The organic layer was separated, washed with water (2 × 10 ml), dried over anhydrous sodium sulphate and concentrated to give crude solid which was recrystallized from ethyl acetate/petroleum ether (1:4) to give colourless crystals of the diallylamide derivative (DA) **Fig. 2**, yield 82 %, R_f : 0.651, (mobile phase: *n*-hexane: ethyl acetate 7:3 v/v), mp 207-209 ºC. IR: cm^{-1} 3027 (aromatic C-H); 2543 (O-CH_2-O); 1675 (amide carbonyl); 1675 (C=N); 1601-1447 (C=C dine). ^{1}H NMR: 7.23 (d, J=5.6Hz, 1H,), 7.37 (dd, J=10.4Hz, 1H), 7.02 (dd, J= 10.4Hz, 1H), 6.98 (d, J=5.6Hz, 1H), 6.93 (d, J=6.8Hz, 1H), 6.90 (d,

J=8Hz, 1H), 5.95 (s, 2H), 5.91 (s, 1H), 4.58-4.89 (m, 2H), 4.23(d, J=13.2Hz, 4H), 4.01(dd, J=10.4Hz, 4H). ^{13}C NMR: 168.07, 148.58, 148.45, 140.25, 138.45, 128.50, 125.33, 123.55, 121.60, 108.98, 106.18, 101.11, 46.56, 42.88, 25.90, 24.63, 14.45, 14.33. MS: m/z- 296.09 ($C_{18}H_{19}NO_2$, 297.35, (-) ESI mode).

Instrumentation and Chromatographic Conditions: Analysis was performed on HPLC system consisting of a pump (model Jasco PU-2080 plus PU- 2087 plus intelligent LC pumps) with autosampler injector. The detector used was UV (Jasco UV-2075 plus). The software was Jasco-Borwin version 15, LC-NET II/ADC. The column was Thermo hypersil GOLD C18 (250 × 4.6 mm, i.d., 5 µm) Thermo Technologies Corporation, Japan, protected with a Phenomenex C18 guard column. Before analysis, the mobile phase was filtered through a 0.22 µm filter and then degassed ultra-sonically for 30 min. The analyses were conducted at a flow rate of 1.0 mL/min, and the detection wavelength was 337 nm and the run time was about 25 min **Table 1**.

TABLE 1: MOBILE PHASE GRADIENT PROGRAM

S. no.	Time (min)	Mobile Phase	Ratio
1	0-10	Methanol : Water	75:25
2	11-20	Methanol : Water	20:80
3	21-25	Methanol : Water	75:25

Pharmacokinetics Studies: Wistar male rats weighing 270-300 g were purchased from the National Institute of Biosciences, Sinhgad Road, and Pune-51. During the experiment, rats were housed in standard housing conditions like the temperature of 25 ± 1 ºC, relative humidity of 45% -55% and 12h light: 12 h dark cycle. The experiments started after the animals were acclimatized for two weeks. They had free access to food pellets (Nav-Maharashtra Chakan Oil Mill Ltd., Sangali, Maharashtra, India) and tap water during the experiment. The animal facilities of the Department of Pharmacology at Poona College of Pharmacy are approved by the committee for purpose of control and supervision of experiments on animals.

The pharmacokinetic study protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (CPCSEA/PCT03/2016-2017).

Before initiating the pharmacokinetic study, animals fasted overnight. Seven groups were formed; each group contained 9 rats (n=9) [18]. For oral administration, the suspension of Secnidazole was prepared in water (10 mg/ml/rat) and suspension of piperine and DA was prepared in water (10 mg/ml/rat). After administration, about 1 ml blood sample was collected from each rat at a time interval of 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 h respectively by a retro-orbital puncture (ROP) and transferred into labeled tubes containing Ca-EDTA and shaken gently to prevent coagulation. The blood samples were collected then centrifuged at 10000 rpm for 10 min at 4 °C. Until the assay; the plasma samples were kept in the deep freezer at -78°C.

Preparation of Plasma Sample: Rat plasma, 200µl was transferred into a 1.5 ml Eppendorf tube. To this 200 µl of the precipitating agent was added (100% ACN) and vortexed for 5 min and the mixture was centrifuged at 10000 rpm for 20 min. The supernatant layer was transferred into another tube and reconstituted with 200 µl diluent and vortexed for 1 min. This was filtered through a 0.2µ syringe filter. A 20 µl of the filtered solution was injected onto the HPLC column [19, 20, 21].

Method Validation: The method validation was performed according to the United States Food and Drug Administration (USFDA) guidelines.

Selectivity: A selectivity study was designed to find out whether constituents and other substances existing in samples would interfere with the detection of Secnidazole and piperine. Selectivity was studied by comparing the chromatograms of six different batches of rat blank plasma with the corresponding spiked plasma.

Linearity, LOQ, and LOD: The linearity of calibration curves were determined by plotting the peak area ratio of Secnidazole to piperine versus the nominal concentration of Secnidazole. The lower limits of quantification (LOQ) and limits of detection (LOD) indicated that this method was sensitive for the quantitative evaluation of Secnidazole.

Precision and Accuracy: For evaluation of intra-day precision and accuracy, six replicate samples were analyzed at three concentration levels on the

same day. For the evaluation of inter-day precision and accuracy, six replicates of samples were analyzed at three concentration levels on three consecutive days.

Extraction Recovery: The extraction recoveries were evaluated by comparing peak areas obtained from the spiked samples before extraction with those after extraction at corresponding concentrations. The extraction recoveries of secnidazole were determined by analyzing three replicates of samples at three concentration levels.

Stability: The stability experiments were performed to evaluate the stability of Secnidazole rat plasma under the following conditions: short-term stability at room temperature for 24 h; and three freeze-thaw (room temperature) cycles on consecutive days. All stability tests in plasma were performed by analyzing three replicates of samples at three concentration levels. The determined concentrations were compared with the nominal values.

RESULTS AND DISCUSSION:

Method Validation:

Selectivity: A selectivity study was designed to investigate whether constituents and other substances existing in samples would interfere with the detection of Secnidazole, DA, and piperine. The retention time of Secnidazole, DA and piperine were found to be 7.2 min, 19.5 min, and 17.3 min respectively. The detection of Secnidazole and piperine was selective with no interference **Fig. 3** and **Fig. 4**.

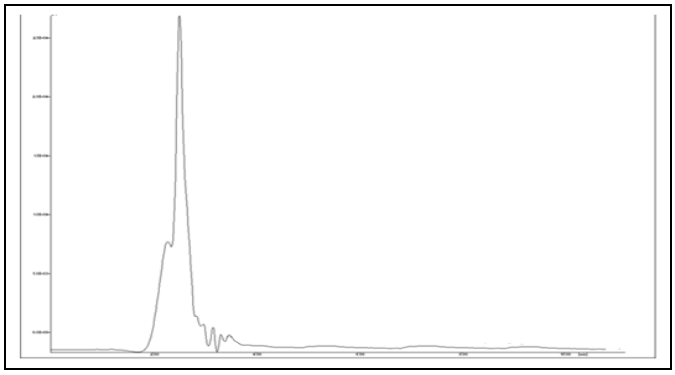

FIG. 3: CHROMATOGRAM OF THE BLANK PLASMA

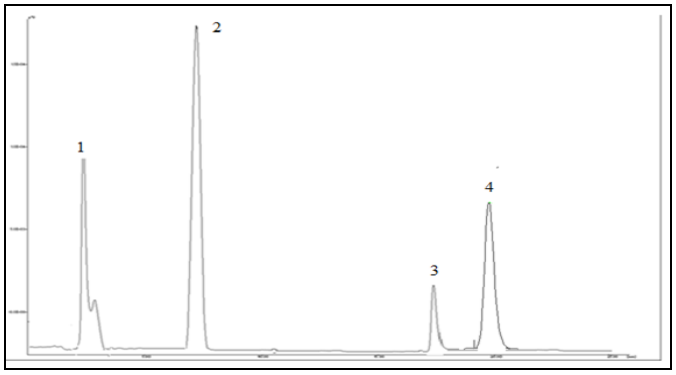

FIG. 4: CHROMATOGRAMS OF SECNIDAZOLE (2), PIPERINE (3) AND DA (4) IN PLASMA (1)

Linearity: Secnidazole and DA were found to be linear in the range of 1-10 µg/mL and 2-10 µg/mL respectively in rat plasma. The slopes, intercepts, and correlation coefficients of the regression equations were determined. The typical equation for the standard curve was y = 9299.5x + 7494.2 (r^2=0.9992), y=136329x+914322 (r^2 = 0.998) for Secnidazole and DA simultaneously. Deviations were within ±15% for all regression equations. LOD and LOQ for Secnidazole and DA were found to be 0.33 and1.00 µg/mL, and 0.593, 1.797 µg/mL respectively **Fig. 3** and **Fig. 4**.

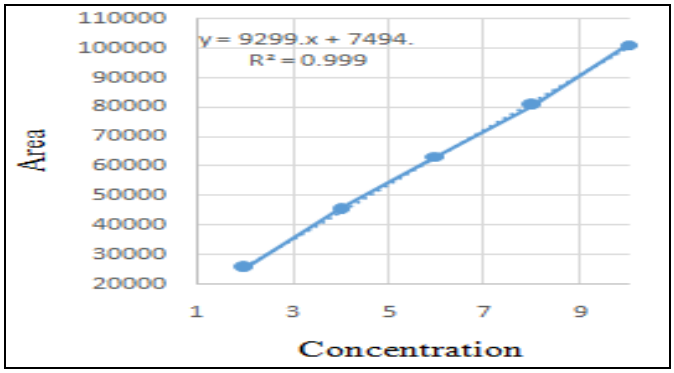

FIG. 5: CALIBRATION CURVE FOR SECNIDAZOLE

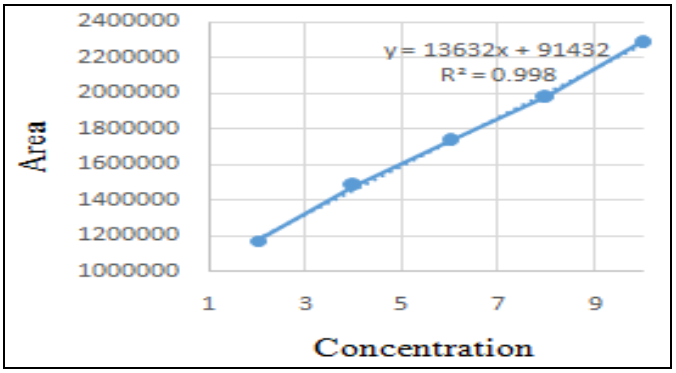

FIG. 6: CALIBRATION CURVE FOR DA

Precision: The intra-day and inter-day precisions and accuracies of rat plasmas were evaluated at three concentrations (2, 6 and 10 µg/mL). The results are summarized in **Table 2**. At all levels, the

accuracy with a CV less than 20% was obtained indicating that the method is reliable and reproducible for determination of Secnidazole and DA in rat plasma.

Recovery: The extraction recovery results of Secnidazole are summarized in **Table 2**. The extraction recoveries of Secnidazole and DA from the low, medium, and high QC samples were within the range of 90 to 108% **Table 3**.

Stability: Benchtop stability and freeze-thaw stability of the spiked quality control samples was determined during three freeze-thaw cycles stored at -78 °C.

Stability was assessed by comparing the freshly spiked quality control samples. The concentration changes related to the nominal concentration were less than 20%, indicating no significant substance loss during the storage **Table 4**.

TABLE 2: THE INTRA- AND INTER-DAY PRECISIONS AND ACCURACIES OF SECNIDAZOLE IN RAT PLASMA

Analyte		Spiked (µg/ml)	Area (mAu)	% CV
	Intra-day	2	52791.66	1.398036
		6	70704.81	0.891201
Secnidazole		10	105608.1	1.821971
	Inter-day	2	52588.89	1.689074
		6	71052.25	1.086283
		10	106719.2	1.803216
DA	Intra-day	2	1194359	1.80478
		6	1746557	3.23505
		10	2312797	1.6631
	Inter-day	2	1218413	4.57968
		6	1733144	3.7978
		10	2286273	2.71755

TABLE 3: RECOVERIES OF SECNIDAZOLE FROM RAT PLASMA

Analyte	Conc.	% Amount added	Total amount Present (µg/mL)	Mean (mAu)	Amount recovered (µg/mL)	% recovery
Secnidazole	LLOQ	80	3.6	122341	2.5	99
	(2µg/ml)	100	4	163003	4.1	102
		120	4.4	170750	4.4	100
	MOQ	80	10.8	194557	10.5	97
	(6µg/ml)	100	12	210909	11.9	99
		120	13.6	223188	13.0	95
	HOQ	80	18	282348	17.9	99
	(10µg/m)	100	20	307087	20.3	101
		120	22	327170	22.3	101
DA	LLOQ	80	3.6	1824240	2.4	95
	(2µg/ml)	100	4	2303871	4.1	104
		120	4.4	2411607	4.5	103
	MOQ	80	10.8	3629032	9.78	90
	(6µg/ml)	100	12	414160	12.29	102
		120	13.6	4537971	14.23	104
	HOQ	80	18	5148151	18.95	105
	(10µg/m)	100	20	5540797	21.62	108
		120	22	5757946	23.09	104

TABLE 4: RESULTS OF SHORT-TERM STABILITY AND FREEZE–THAW CYCLES OF SECNIDAZOLE IN RAT PLASMA

Analyte	Concern	Bentch top stability			Freeze-thaw stability		
		Mean (mAu)	% CV	% Accuracy	Mean (mAu)	% CV	% Accuracy
Secnidazole	LLOQ (2 µg/mL)	26608	1.77	102.76	24286	10.8	90.28
	MOQ (6 µg/mL)	65893	6.79	104.66	4618	6.7	109.7
	HOQ (10 µg/mL)	91654	11.04	90.49	2661	2.6	101.1
	LLOQ (2 µg/mL)	1161112	11.87	90.51	1424765	4.52	93.60
DA	MOQ (6 µg/mL)	1629341	4.423	87.41	1897985	7.71	90.19
	HOQ (10 µg/mL)	2393447	2.185	108.49	2914900	8.71	91.71

Analysis of Plasma Samples: The plasma samples were analyzed by the developed and validated HPLC method. C_{max} and T_{max} were obtained by extrapolating the AUC value in the linearity graph of Secnidazole and DA **Table 5**. Average area under the curve (AUC) was calculated and a graph

of average AUC *v/s* time interval was plotted **Fig. 7**. **Table 5** Pharmacokinetic parameters following oral administration of Secnidazole alone and in combination with piperine and piperine DA respectively.

The diallylamide substitution (DA) showed a considerable increase in AUC and C_{max} of Secnidazole compared to Secnidazole alone and after co-administered with piperine and alone.

TABLE 5: ANALYSIS OF PLASMA SAMPLES

Name (Dose: 10mg/kg)	AUC 0-t (µg/mL*h)	$t_{1/2}$ (h)	C_{max} (µg/mL)	MRT0-t (h)	Fold increase in AUC as compared with Secnidazole alone
Sec	236.361	9.358	50.292	6.22	-
Sec + Pip	256.886	10.570	66.229	6.41	1.086836
Sec+ DA	391.562	9.1004	92.641	6.22	1.656625

AUC: Area under the curve, $t_{½}$: Half-life, t_{max} Time taken to reach the maximum concentration, C_{max} The maximal observed concentration, MRT- Mean residence time

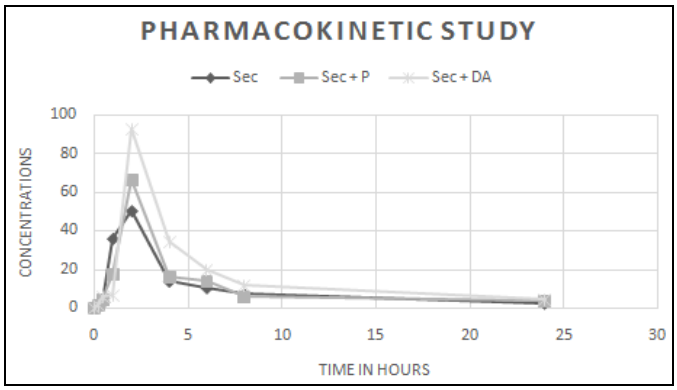

FIG. 7: MEAN PLASMA CONCENTRATION-TIME CURVES OF SECNIDAZOLE ALONE AND IN COMBINATION WITH PIPERINE

Pharmacokinetic Analysis: Pharmacokinetic parameters were determined by a noncompartment model by using software PK Solver. Maximum plasma concentrations (C_{max}), and the time to reach the maximum concentration (t_{max}) were the values observed in Wistar rat. The terminal phase rate constant (Kel) was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration versus time curve using least square regression analysis. The terminal phase half-life ($t_{1/2}$) was calculated as 0.693/Kel. The absorption rate constant (Ka) was calculated by the residual method and the absorption half-life ($t_{1/2}$) as 0.693/Ka. The area under the plasma drug concentration-time curve from time zero to the last quantifiable concentration *versus* time curve (AUC0–12h) was calculated by the trapezoidal rule.

Mean residence time (MRT), the volume of distribution (Vd) and clearance (Cl/F) were also calculated. The peak plasma concentration (C_{max}) and the time for reaching C_{max} (T_{max}) were obtained directly from the experimental data.

Statistical Analysis: Plasma drug concentrations, as well as the pharmacokinetic parameters before and after piperine administration, were compared by paired Student's t-test. A value of $p<0.05$ was considered statistically significant.

CONCLUSION: Piperine is a widely used bioenhancer and shows bio-enhancing property on many drugs. It was selected as bioenhancer for the present work, and its diallyl amide derivative was prepared to compare the bio-enhancing effect with piperine. The pharmacokinetic study demonstrated that piperine did increase the Secnidazole concentration in plasma, retarded its clearance and increases the volume of distribution. However, the diallylamide (DA) derivative was much superior to piperine for this parameter.

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CONFLICT OF INTEREST: All authors declare that they have no conflict of interest.

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