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COMPARATIVE STUDY ON THE PHARMACOKINETICS OF IBUPROFEN ALONE OR IN COMBINATION WITH PIPERINE AND ITS SYNTHETIC DERIVATIVES AS A POTENTIAL BIOENHANCER

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Keywords:

Ibuprofen (Ibu), Pharmacokinetics, Piperine, NSAID (Non-steroidal anti-inflammatory drugs), COX (Cyclo-oxygenase), Bioavailability

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ABSTRACT: Ibuprofen has a higher dose (2000-2400 mg/day) that leads to several adverse effects like stomach upset, headache, bloating, hyperacidity, diarrhea, nausea, constipation, mild heartburn, ringing in your ears, vomiting and blurred vision. Piperine is a natural alkaloid obtained from piper species, principally from *Piper longum* and *Piper nigrum* (Family: Piperaceae). Piperine demonstrates excellent bioavailability Enhancing activity of different xenobiotics. Here in this study, the synergistic effect of Piperine and its synthesized derivatives was studied. Ibuprofen (10 mg/kg), in combination with Piperine and its derivatives (5a-5d) (10 mg/kg, were administered to Wistar rats, and the pharmacokinetic parameters were estimated. Thus, the study concludes that the presence of 5a significantly increases the absorption and reduces the elimination of the drug from the body.

INTRODUCTION: Ibuprofen is a well-known medication under the class of nonselective anti-inflammatory drugs (NSAIDs), used for triggering inflammation, fever and spasms. Chemically, it belongs to the propionic acid derivatives ((2-RS)-1[4-(2-methyl propyl) phenyl] propionic acid). Ibuprofen, an initially identified propionic acid derivative to be announced as a better substitute for aspirin in 1969 [1]. Ibuprofen acts by blocking the nonselective cyclo-oxygenases.

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(COX-1 and COX-2) that results in inhibiting the synthesis of prostaglandins by prostaglandin synthase. However, Ibuprofen gives rise to block the production of thromboxane A2 from thromboxane synthase, which ultimately leads to the inhibition of platelet aggregation [2]. Dose approaching 175 mg/kg or more leads to an increase in risks of developing renal failure in dogs [3].

The inhibition of COX-1 presents unwanted effects on GIT [4]. A report submitted by the European medical agency reported that higher doses (400-800 mg thrice a day or more) of ibuprofen should be avoided to prevent the risk in patients having severe underlying heart/ circulatory conditions or with those who had a history related to stroke or heart attack [5-7].

The side effects of ibuprofen include stomach upset, headache, nausea, vomiting, bloating, gas, constipation, diarrhea, mild heartburn, ringing in the ears and blurred vision. Reportedly, a dose of 2400-3200 mg (upto 16%) causes an increase in the alanine transaminase (ALT) levels (theoretical level = mild or rarely >100 U/L). Ibuprofen overdose (above 5-10 gm), characterized by perturbation and numbness after 3 to 6 h of ingestion, followed by respiratory depression, coma, and lactic acidosis that can be fatal [8-10]. However, ibuprofen in combination with coumarin analogs and anticoagulants may cause platelet aggregation inhibition and perpetuated bleeding time. Thus, it is suggested that ibuprofen should be used with caution and the patient should be observed carefully when combined with an anticoagulant (warfarin or streptokinase) [11-13]. Another study conducted on pregnant women suggests that the chances of miscarriage 2 to 4 times higher in the human volunteers taking the ibuprofen medication than those who did not take any medication containing the NSAIDs. Intake of alcohol during the ibuprofen medication may increase the risk of stomach bleeding [12, 13].

Piperine is a naturally occurring alkaloid obtained from '*Piper longum* (long pepper), as well *as Piper nigrum* (black pepper), which belongs to the Piperaceae family. Piperine **Fig. 1** is a well-known bioavailability enhancer. Piperine has a significant bio enhancing activity in various drugs. Recently, Piperine has attained the interest over to its bioenhancing property and reduction in the toxicity. It has been proven that Piperine is an effective bioenhancer when combined with drugs having low bioavailability and a higher dose (*i.e*., Isoniazid, Bioperine®).

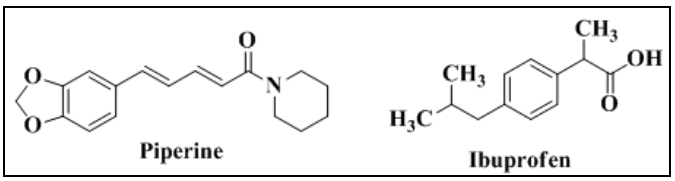

FIG. 1: CHEMICAL STRUCTURE OF PIPERINE AND IBUPROFEN

Piperine acts through several mechanisms, such as affecting the process of absorption, metabolism, and reducing the dose. Bioenhancers are advantageous in several ways, such as; they are non-toxic, effective in low dose, ease of formulation *etc*.[14, 15]. Herein, a validated bioanalytical method to determine ibuprofen and Piperine was developed, and the pharmacokinetic parameters of ibuprofen were estimated alone and in combination with Piperine and its analogs.

MATERIALS AND METHODS:

Chemicals and Reagents: Chemicals and reagents used were procured from commercial sources such as Sigma Aldrich, St. Louis, MO, USA or Merck, India, Loba Chemie, India and Spectrochem Pvt. Ltd. India. Procured Chemicals and reagents are further used in the reaction without purification. The reaction was carried out using the oven-dried borosilicate glassware; solvents of anhydrous grade were used. All reactions were monitored continuously using TLC plates (Silica gel 60 GF_{254}) and were stained using the iodine indicator. Veego VMP-PM (digital melting point apparatus) was used to determine the melting point and was uncorrected; Fourier Transmission Infrared (FT-IR) spectrum for each of the synthesized compounds was recorded using KBr pelletson the 'Jasco FTIR 4100' instrument (cm^{-1}). Proton and Carbon-13 NMR spectra were obtained using DMSO-D_6 solvent using 'Bruker Avance –III (400 MHz) using tetramethylsilane as an internal standard. Chemical shifts expressed in parts per million (δ) and coupling constants (J) were expressed in Hz. The mass spectroscopic analysis was carried out using 410 Pros Tar Binary LC with 5000 MS IT PDA detector, Varian Inc., ESI-MS mass spectrometer. All the prepared final compounds were checked for purity (>95%) on HPLC.

Protocols for the Chemical Synthesis of Piperine Derivatives:

Synthesis of Piperic Acid: Piperine 1 gm (0.035 mmol), 20% ethanolic KOH solution taken in the 500 ml round bottom flask and was allowed to reflux for 48 h. The chemical reaction is monitored by thin-layer chromatography using the mobile phase (*n*-hexane: EtOAc (3:1 v/v)). Thereafter, the solvent decanted under reduced pressure; after filtration, the solution was acidified using hydrochloric acid (HCl), pH: <3). The yellow precipitate of piperic acid obtained and filtered in vacuum using Buchner funnel and the crude piperic acid was air-dried. The crude product was recrystallized using methanol yielding needle-shaped crystals [16, 17,] **Fig. 2**.

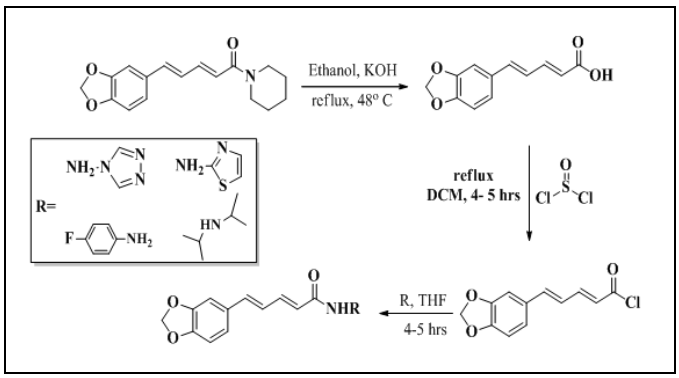

FIG. 2: SCHEME FOR THE SYNTHESIS OF PIPERINE DERIVATIVES

Preparation of Amide Derivatives of Piperine (5a-d): Piperic acid (500 mg; 2.29 mmol) dissolved in tetrahydrofuran (THF; 10 ml); thionylchloride (1 ml; 11.5 mmol) was appended, and the reaction mixture refluxed for 4 to 5 h. After five h, the reaction mixture was cooled to 25 °C and excess thionyl chloride was eliminated, leaving behind the acyl chloride as yellowish residue, which was further used in the preparation of amides without purification. The yellow residue was dissolved in THF (2-3 ml), appropriate amine/aniline was dissolved in a small amount of cooled THF and was kept in separate addition funnel and dropwise added to the acid chloride, the basic condition provided by adding triethylamine (TEA). The reaction mixture was allowed to stir at 60 °C for 4-5 h, and the completion was monitored by TLC using toluene: methanol (2:2 v/v) as the mobile phase to afford the precipitated amides (5a-d) which were filtered. The obtained crude product was recrystallized using EtOH: water (2:1 v/v).

Chromatographic Conditions: Chromatographic analysis was performed using the Jasco HPLC system consists of connected to an auto-sampler (20 µl Rheodyne valve and 20 µl loop), pump (Jasco PU-2080 plus; PU- 2087 plus LC pumps). The column used was Chromatopak Peerless Basic C18 Column (250mm × 4.6mm × 5µm) connected to an auto-sampler (20 µl Rheodyne valve and 20 µl loop) guarded with Phenomenex C18 guard column, and the detector used was Jasco UV- 2075.

The software used Jasco-Borwin version 15, LC-NET II/ADC. Before beginning the analysis, ACN: Water (60: 40) mobile phase was filtered using a 0.22 µm filter and was ultrasonically degassed; the HPLC analysis was performed by pumping the mobile phase at a flow rate 1 ml/min. The HPLC run time kept 30 min **Table 1**.

TABLE 1: INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

S. no.	Test Conditions	Result
1	Elution	Isocratic
2	Wavelength	235 nm
3	Mobile phase	ACN: Water: Water: Formic Acid (60: 39:1 v/v)
4	Column	Chromatopak peerless basic C18 column 250 mm × 4.6 mm × 5 µm
5	Retention time	Piperine: 7.1
	min	Ibuprofen: 9.1
6	Flow rate	1 ml/ min
7	Run time	20 min

Calibration Standards and QC Samples: The stock solution of drugs contained 1 mgibuprofen and piperine in 1 mL acetonitrile (1000 µg/mL) and was stored at 4 °C until analysis. Then, serial dilutions from the prepared stock solution were prepared. An aliquant (200 µL) of each dilution spiked in blank plasma (200 µL) to yield the spiked standard solution in a series of 2-20 µg/mL to obtain the calibration curve. QC samples LOQ (2 µg/mL), MOQ (11 µg/mL), and HOQ (17 µg/mL) were prepared, and all solutions were stored at 4 °C and later used within 30 days.

Method Validation: The method was developed and validated in accordance with the guidelines provided by the United States Food and Drug Administration (USFDA) [18- 20].

Specificity and Selectivity: Selectivity and specificity of the developed method routinely estimated through the analysis of plasma samples from different sources. The selectivity of quality control samples studied by comparison of six batches of blank with spiked plasma samples containing the analyte.

Linearity and LOD & LLOQ: The calibration curve was the calibration curve was prepared by determining the ratio of area under the curve of peaks obtained using least-square linear regression.

The Limit of Detection (LOD) and Lower Limit of Quantitation (LLOQ) was estimated according to signal to noise ratio of (LLOQ = 10:1 and (LOD= 3:1), respectively.

Precision and Accuracy: Intraday precision and accuracy six replicates of quality control samples were analyzed at selected concentrations on the same day, and inter-day precision was performed on three consecutive days.

Stability: The experiment for the stability testing was conducted for the determination of the potential of ibuprofen in rat plasma matrix under short term stability testing at r.t. for 24 h followed by three cycles of freeze-thaw stabilityat r.t. For three consecutive days and long-term stability testing at the -80 °C for one month and the determination of stability concentrations was compared to nominal values.

Robustness: Robustness is routinely examined to analyze the capacity of the method to persist unchanged by deliberate changes in various parameters of the method. The changes were made in the wavelength (235 ± 2 nm) and the flow rate (1 ± 0.2 ml/ min).

Animals and Treatments: The Wistar rats (male; weight, 180-220 gm) procured from Agharkar Research Institute (ARI), Pune. The animal experiments performed according to the protocol provided by the Committee for the control and supervision of experimental animals (CPCEA guidelines) and procedures were approved by institutional animal ethics (IAEC), Pune (CPCSEA/PCH 01/2018-2019). Before beginning the experiment, animals were quarantined under-maintained conditions such as temperature (24 ± 1° C), Rh (45 ± 10%), and 12 hrs of the day and dark cycles each. Animals received food pallets (Navmaharashtra Chakan Oil Mil Ltd, Sangali, Maharashtra, India) and tap water *ad libitum*. After acclimatization, the health status of each animal was examined. Before beginning the experiments, animals fasted overnight

Single Oral Dosing: Animals were randomly segregated among six groups **Table 2** (*n*= 9). The control group was provided with plain distilled water, the second group of animals was administered with ibuprofen (10 mg/kg) and rest five groups of animals were doused with 10 mg/ kg of piperine and its synthetic derivatives, respect-tively, in suspension from using water (calculated in the equimolar basis fora dose of piperine).

TABLE 2: GROUP OF ANIMALS

S. no.	Group (*n*= *9*)
1	Vehicle Control
2	Ibuprofen
3	Ibu + pip
4	Ibu + 5a
5	Ibu + 5b
6	Ibu + 5c
7	Ibu + 5d

Blood samples were collected by retro-orbital venous plexus (ROP) method from each group on the various time points as 0 min (15 min - 24 h) one rat at a time from each group, respectively. The Ca-EDTA tubes were used for the collection of blood samples, and gentle shaking was followed to prevent blood coagulation. The blood samples were centrifuged using a microcentrifuge (Centrifuge 5420, Eppendorf, USA) at 10,000 × for 20 min, at 4°C. The whole process has been performed in triplicate.

Preparation of Plasma Samples: The extracted plasma sample (200 µL) was fetched in the Eppendorf tube. To this extracting solvent (200 µL; ACN) was appended, vortexed for 5 min further centrifuged at 4 °C (10,000 × for 15 min) after centrifugation method, the supernatant was collected in a separate Eppendorf tube and reconstituted using 100% acetonitrile (200 µl) followed by vortexing for 2 min. Before injection into the HPLC system, the sample was filtered using a nylon syringe and injected into the loop of the system. Separation of the compound(s) achieved using the Chromatopak Peerless Basic C18 column.

RESULTS AND DISCUSSION:

Characterization of Synthesized Compounds:

(2E, 4E) – 5 - (Benzo[d][1, 3] Dioxol – 5 - yl) – N -(1H - 1, 2, 4 – triazol - 1 - yl) Penta - 2, 4 - Dienamide (5a): Yield: 85.7%, melting point: 230-231 °C, FT-IR ($\nu_{max,}$cm^{-1}): 3443.28 (-NH), 3018.09 (aromatic-CH), 2911.02 (O-CH_2-O), 1694.16 (-C=O), 1602.56 (Methylene dioxyl group), 1602.66 (diene), 1494.56 (C=C). ^{1}H NMR (400 MHz, DMSO-D_6) δ: 5.93 (d, J = 15.7 Hz, 1H), 6.15 (d, J = 10.8 Hz, 2H), 6.87-7.03 2H, 6.90 (dd, J = 8.4, 0.4

Hz), 6.95 (dd, *J* = 17.7, 10.2 Hz), 7.14 (d, *J* = 17.7 Hz, 1H), 7.25 (dd, *J* = 1.9, 0.4 Hz, 1H), 7.39 (dd, *J* = 15.7, 10.2 Hz, 1H), 7.58 (dd, J = 8.4, 1.9 Hz, 1H), 8.15 (d, J= 1.7 Hz, 2H,). ^{13}C NMR (DMSO-D_6) *δ*: 165.51, 157.75, 149.05, 148.48, 144.48, 142.25, 141.92, 130.93, 125.46, 124.41, 123.11, 109.09, 106.34, 101.87. Mass Spectrum EI (-ve) averaged mode: m/z 283.9 (M^+ $C_{14}H_{12}N_4O_3$).

(2E, 4E) - 5 - (Benzo [d] [1, 3] Dioxol - 5 - yl) - N - (Thiazol - 2 - yl) Penta - 2, 4-Dienamide (5b): Pale yellow powder, Yield: 92.7%, R_f value: 0.51[toluene: methanol (3:1)], melting point: 178-180°C, FT-IR KBr (ν_{max} cm^{-1}): 3160 (-NH Stretch), 2882.09 (-CH_2), 1673.91 (C=O), 1609.31 (Methylene dioxyl group), 1609.90 (diene), 1490.70 (C=C).^{1}H NMR (400 MHz, DMSO-D_6) *δ*: 12.24 (s, 1H), 7.53 – 7.34 (m, 2H), 7.29 (s, 1H), 7.16 (d, *J* = 19.3 Hz, 1H), 6.87 (dd, *J* = 56.6, 31.0 Hz, 5H), 6.35 (d, *J* = 14.5 Hz, 1H), 6.02 (s, 2H), 5.94 (d, *J* = 12.7 Hz, 1H). ^{13}C NMR (400 MHz DMSO-D_6) *δ*: 163.85, 158.62, 148.58, 148.41, 143.59, 140.84, 138.26, 130.99, 125.26, 123.74, 121.83, 114.06, 108.91, 106.28, 101.78. Mass spectrum EI (-ve) averaged mode: m/z 301.06 (M^+ $C_{15}H_{12}N_2O_3S$).

(2E, 4E) – 5 - (Benzo [d] [1, 3] Dioxol – 5 - yl) - N-(4 - Fluorophenyl)penta - 2, 4 - Dienamide (5c): Colorless crystals; Yield 82%; R_f: 0.651, [mobile phase: n-hexane: acetone (2:3 v/v)]. Melting point -157-158 °C; FT-IR (KBr) (cm^{-1}): 3224.52(-NH), 1699.94 (-C=O), methylene dioxyl group (1617.02), C=C (1488). ^{1}H NMR (DMSO-D_6) *δ*: 9.901(s, NH exchangeable), 8.036(s, 1H), 7.327(d, *J*= 14.4Hz, 1H), 7.263(d, *J*= 9.2Hz, 1H), 7.17(d, *J* = 14.8Hz, 1H), 7.003-6.91(m, 4H), 6.503 (dd, *J*= 16Hz, 1H), 6.062(s, ,1H), 5.946 (dd, *J*= 16Hz, 1H). ^{13}C NMR (DMSO-D_6) *δ*: 79.11, 79.44, 79.77, 101.83, 106.22, 108.94, 108.98, 115.79, 115.98, 121.58, 123.50, 123.58, 124.18, 124.79, 125.31, 125.44, 126.82, 126.93, 131.17, 139.72, 140.27, 142.03, 145.10, 148.45, 148.58, 164.69, 168. Mass spectrum (EI (-ve) averaged mode): m/z 312.10 (M^+ $C_{18}H_{14}FNO_3$).

(2E, 4E) - 5 - (Benzo[d] [1, 3] Dioxol 5 - yl) - N, N – Diisopropyl Penta - 2, 4-Dienamide (5d): Yield 79%, R_f: 0.681, [mobile phase: n-hexane: ethyl acetate 7:3 v/v], Melting point: 196-198 °C. FT-IR (anhydrous KBr ν_{max} cm^{-1}): 3036 aromatic (C-H); 2547 (O-CH_2-O); 1675 C=C (diene); 1601-1447 C=C (benzene ring),1047 (C=N). ^{1}H NMR (DMSO-D_6) *δ*: 1.21 (d, *J* = 6.8 Hz, 12H), 4.16 (sept, *J* = 6.8 Hz, 2H), 5.96 (d, *J* = 15.8 Hz), 6.14 (2H, d, *J* = 10.8 Hz), 6.86-7.01 (2H, 6.90 (dd, *J* = 8.4, 0.4Hz), 6.94 (dd, *J* = 17.7, 10.2 Hz, 1H,), 7.12 (d, *J* = 17.7 Hz, 1H), 7.24 (dd, *J* = 1.9, 0.4 Hz, 1H), 7.35 (dd, *J* = 15.8, 10.2 Hz, 1H), 7.58 (dd, *J* = 8.4, 1.9 Hz, 1H). ^{13}C NMR (400 MHz DMSO-D_6) *δ*: 168.08, 148.57, 148.45, 145.07, 140.25, 125.32, 123.55, 121.60, 114.27, 108.99, 106.18, 101.90, 101.83, 48.76, 46.56, 19.46, 18.98, 11.50, 10.57. Mass Spectrum (EI (-ve) averaged mode) m/z-300.99 ($C_{18}H_{23}NO_3$, 301.17).

Method Validation:

Selectivity: There was no interference observed at the retention time (R_t) 7.10 min. for piperine and 9.10 min. for ibuprofen. **Fig. 3** illustrates the selective separation of ibuprofen and piperine. **Linearity, LLOQ and LOD:** The calibration curve was plotted ranging 2-20 µg/ml and was linear for the analysis of ibuprofen and piperine. Determination of slope, regression equation, and intercepts was carried out using least square regression. The typical regression equation was y= 2978.8 - 502.5 (r^2 = 0.993) **Table 3**, **Fig. 5**.

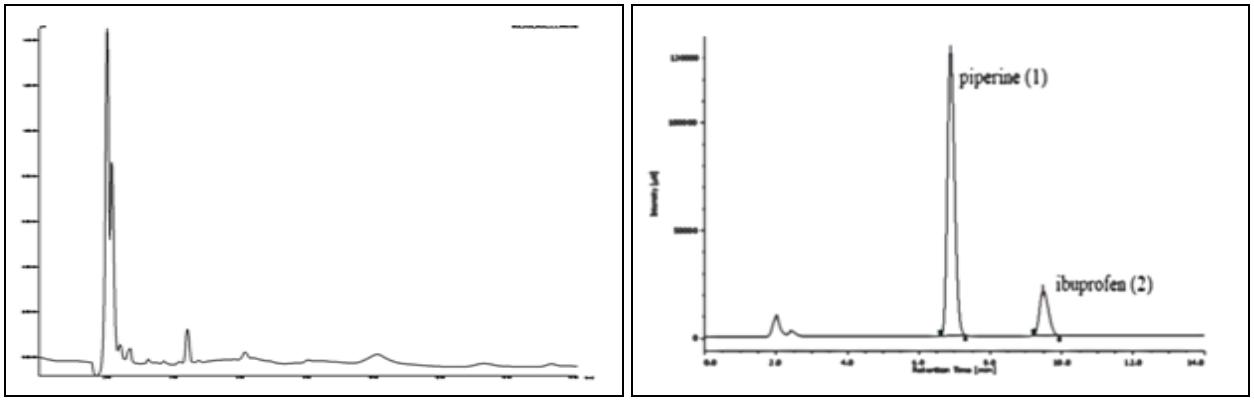

FIG. 3: CHROMATOGRAMS OF BLANK RAT PLASMA AND IBUPROFEN AND PIPERINE

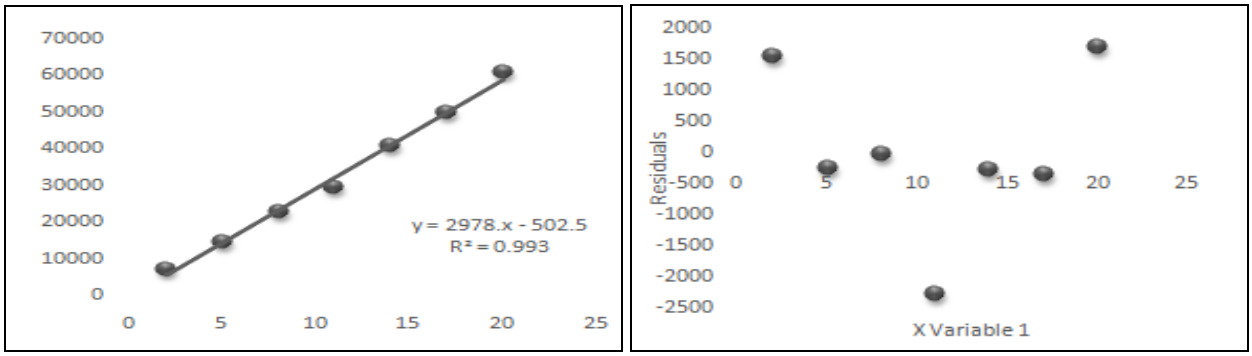

FIG. 4: LINEARITY DATA AND CONCENTRATION V/S RESIDUAL PLOT FOR IBUPROFEN FOR RAT PLASMA

TABLE 3: LINEARITY DATA FOR IBUPROFEN

S. no.	Validation Parameters	Ibuprofen
1	Linearity range	2-20 µg/ ml
2	Correlation co-efficient (r^2)	0.9938
3	Intercept	502.5
4	Slope	2978.8
5	S_{yx} [b]	1455.0
3	Regression equation	y = 2978.8x – 502.5
4	Limit of detection (LOD)	1.61 µg/ ml
5	Limit if quantitation (LLOQ)	4.88 µg/ ml

Accuracy and Precision: Precision (Intra-day and Inter-day) and accuracy were estimated using three QC samples (LOQ (2 µg/ ml), MOQ (11 µg/ ml), and HOQ (17 µg/ ml). At all levels, the accuracy was found to be under standard range(85-115%) along with the % RSD, which was found less than 15% indicates that the developed method was sufficiently reproducible and accurate for the detection of ibuprofen and piperine **Table 4**.

TABLE 4: ACCURACY AND PRECISION DATA OF IBUPROFEN IN RAT PLASMA (N = 6)

Analyte		Spiked (µg/ml)	Area (mAu) ± SD	% RSD	Measured Conc (µg/ml)	% Accuracy
	Intra-day	2	6062.51 ± 13.23	0.21	2.14 ± 0.01	115.854
		11	29307.43 ± 248.18	0.84	10.93 ± 0.04	91.307
Ibuprofen		17	49358.02 ± 126.14	0.25	16.85 ± 0.28	98.93
	Inter-day	2	6122.95 ± 18.35	0.29	2.16 ± 0.01	113.035
		11	29169.34 ± 16.84	0.05	9.96 ± 0.04	90.88
		17	49453.43 ± 105.25	0.21	16.88 ± 0.12	99.34

Stability: The drug found stable under the stability testing conditions (reconstituted solutions of test samples at 24 h at 20° C) in plasma at the initial concentration and followed by the freeze-thaw cycles found within the range of ± 15% RSD. Additionally, the processed samples were found stable at a storage temperature of -80° C for one month, **Table 5**.

TABLE 5: STABILITY STUDIES OF IBUPROFEN IN RAT PLASMA (N =3)

Analyte	Conc (µg/ml)	Benchtop stability			Freeze-thaw stability		
		Area (mAu) ± SD	% RSD	% Accuracy	Area (mAu) ± SD	% RSD	% Accuracy
Ibuprofen	2	6213.76 ± 18.61	0.298	109.86	6127.87 ± 18.56	2.16	108.39
	11	28866.48 ± 297.92	1.032	89.945	29168.8 ± 202.58	9.99	90.87
	17	48648.66 ± 285.29	0.586	97.726	48756.2 ± 0.47	16.65	97.95

TABLE 6: ROBUSTNESS OF IBUPROFEN (N = 3)

Wavelength				Flow Rate			
λ_{max} (nm)	Conc (µg/ ml)	Area (mAu) ± SD	% RSD	Rate (ml/ min)	Conc (µg/ ml)	Area (mAu) ± SD	% RSD
233	2	6908.96 ± 77.98	1.128	0.8	2	7075.96 ± 81.9272	1.157
	11	29316.2 ± 434.302	1.481		11	28919.3 ± 406.419	1.405
	17	29316.2 ± 434.302	0.654		17	48496.3 ± 735.488	1.516
235	2	7071.19 ± 68.836	0.973	1	2	7042.33 ± 120.063	1.704
	11	29176.9 ± 193.249	0.662		11	28826.5 ± 324.082	1.124
	17	49163.6 ± 462.673	0.941		17	49344.4 ± 403.183	0.817
237	2	7254.17 ± 53.737	0.740	1.2	2	7184.3 ± 17.7995	0.247
	11	29372.7 ± 379.934	1.293		11	29246.7 ± 233.49	0.798
	17	49050.3 ± 171.091	0.348		17	49055.5 ± 157.997	0.322

Robustness: The estimation of robustness or ruggedness of the method was carried out in the rat plasma using three QC samples in **Table 6**. At all levels, ruggedness was found under the percentage RSD range of < 15%, indicates the robustness of the developed method for the identification of ibuprofen.

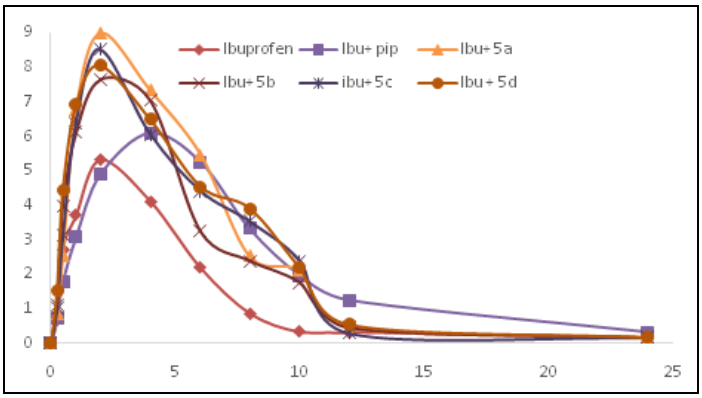

FIG. 5: OVERLAY OF MEAN PLASMA CONCENTRATION-TIME PROFILES OF IBUPROFEN ALONE OR IN COMBINATION WITH PIPERINE AND ITS SYNTHETIC DERIVATIVES THROUGH ORAL ROUTE AT THE DOSE OF 10 mg/kg BODY WEIGHT

Statistical Analysis: Plasma Concentration obtained at different time points and these concentrations were represented as Mean ± SD. Different pharmacokinetic parameters were calculated using non- compartmental analysis (NCA) in PKsolver software 2.0 USA. Pharmacokinetic parameters and the drug- plasma concentration prior to and after the administration of piperine were compared by applying the student's t-test. The results of the study considered statistically significant ($p < 0.05$).

Application of Bioanalytical Method on the Pharmacokinetic Study: To study the effect of piperine and its analogs on the pharmacokinetic parameters of ibuprofen was carried out by successfully applying the developed and validated method.

TABLE 7: EVALUATION OF PK PARAMETERS OF IBUPROFEN ALONE AND IN COMBINATION WITH PIPERINE AND ITS SYNTHETIC DERIVATIVES (MEAN ± SD, N = 9) RESPECTIVELY

Parameters	Units	Value					
		Ibuprofen	Ibu + Pip	Ibu + 5a	Ibu + 5b	Ibu + 5c	Ibu + 5d
$t_{1/2}$	h	2.172 ± 0.012	2.995 ± 0.15	1.953 ± 0.53	2.389 ± 0.23	1.561 ± 0.78	1.420 ± 0.052
T_{max}	h	2.00	4.000	2.000	2.000	2.000	2.000
C_{max}	µg/ml	5.320 ±0.07	6.101 ± 0.11	8.990 ± 0.272	7.643 ± 0.40	8.518 ± 0.24	8.053 ± 0.18
AUC $_{0\text{-}t}$	µg/ml*h	27.331 ± 0.21	45.024 ± 1.78	55.075 ± 4.25	47.154±2.90	52.078±1.60	54.147 ± 0.72
AUC $_{0\text{-}infobs}$	µg/ml*h	28.352 ± 0.80	53.153 ± 13.27	56.404 ± 3.97	48.732±2.73	52.76 ± 1.96	55.284 ± 0.64
AUMC $_{0\text{-}infobs}$	µg/ml*h	118.74 ± 4.35	393.35 ± 259.1	265.06±25.51	226.90 ± 8.7	250.54±11.9	265.78 ± 1.11
MRT	µg/ml*h^2	4.188 ± 0.04	6.938 ± 2.68	4.694 ± 0.145	4.659 ± 0.08	4.747 ± 0.06	4.808 ± 0.037
K_{el}	h	0.14	0.06	0.11	0.37	0.131	0.122
Fold increased in AUC as compared to Ibuprofen alone	-	-	1.64	2.01	1.72	1.90	1.98

Time Vs Concentration (n=3), AUC: Area under the Curve, t½: Half-Life, Cmax: Maximal Observed Concentration, MRT Mean Residence Time, **p< 0.001 control vs piperine and ** p<0.03 control vs. derivatives

Later, rats were grouped into groups in reference to pharmacokinetic parameters. Drugs were given to animals, as described in **Table 7**.

Pharmacokinetic parameters of ibuprofen alone, as well as when combined combination with piperine and its synthetic analogs were estimated. **Table 7** described the peak plasma concentration (C_{max}), peak time (T_{max}), half-life ($t_{1/2}$), Area under the curve (AUC), and rate of elimination (K_{el}), Mean residence time (MRT) **Table 7** and **Fig. 7**.

DISCUSSION: Natural products have gained great focus in the last two decades. Herbal medicines are available in the form of alternative medicine and as health supplements. These natural molecules have a

significant role when combined with therapeutic agents as a lead molecule, prodrug and excipients and, most importantly, as bioavailability enhancers. A bioenhancer is a molecule that enhances the bioavailability of drugs with lower absorption without exhibiting its pharmacological effect.

Piperine, a natural alkaloid, is well-known for its bio-enhancing effect at a given dose. Piperine acts as a bioavailability enhancer by inhibiting or by inducing the drug-metabolizing enzyme (CYP450), by enhancing the absorption and by prolonging the elimination time, therefore be influenced by piperine when combined with the therapeutic agents' results into the lowering of the therapeutic dose.

Non-steroidal Anti-inflammatory agents are used in pain management, reduce fever, and in preventing thrombosis. Higher doses of NSAIDs were found to decrease the inflammation. World Health Organization has included ibuprofen under the class of essential medication list. The mechanism of action of ibuprofen reveals that ibuprofen works by significantly blocking the production of prostaglandin (response to injury and illness).

The higher dose of ibuprofen (800 mg thrice a day) includes various adverse effects related to the higher dose. In the present study, piperine and its synthetic derivatives in combination with ibuprofen given to the rats, and the pharmacokinetic parameters were estimated; results reveal that the faster the absorption rate and delays the elimination of the drug.

Herein, a validated RP- HPLC method was developed, and the pharmacokinetic parameters of ibuprofen were studies. Results suggest that the dose of ibuprofen can be reduced by combining it with piperine. One of the synthetic derivatives of piperine shows a significant increase in the area, as compared to ibuprofen alone also delays the elimination rate. Therefore, the dose can be reduced.

CONCLUSION: In concussion, a precise and validated RP- HPLC method was developed to determine ibuprofen, and the USFDA guidelines were followed for the validation of the method developed. Thus, it can be concluded that the developed bioanalytical method was sufficiently selective, reproducible, precise, and robust for the analysis of ibuprofen in the biological matrix (rat plasma). Pharmacokinetic study reveals that the AUC for ibuprofen alone and the administration of piperine along with ibuprofen increase the rate and extent of the absorption of ibuprofen ($P< 0.01$). The observed pharmacokinetic data after oral administration of ibuprofen along with piperine and its synthetic derivatives. There is a significant increase in C_{max} (5.32 ± 0.079 µg/ml to 6.101 ± 0.116), AUC (27.331 ± 0.212 to 45.024 ± 1.786), and decrease in the elimination rate (0.14 ± 0.01 to 0.06 ± 0.02) whereas, one of the synthetic derivative (5a) shows increase in AUC (27.331 ± 0.212 to 55.075 ± 4.25), C_{max}(5.320 ±0.079 to 8.990 ± 0.272) and K_{el}(0.11 ± 0.06). The derivative (5a) shows a 2.01-fold increase in AUC. The possible mechanism of piperine and its derivatives to enhance the bioavailability by increasing the absorption and by reducing the elimination rate of ibuprofen.

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