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Improvement in Pharmacokinetic Parameters of Ibuprofen by Crystal Engineering Approach

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ABSTRACT: Purpose: The aim of present work was to improve the pharmacokinetic parameters like C_{max}, T_{max}, and AUC of Ibuprofen by crystal engineering approach. Methods: Ibuprofen crystals were prepared by conventional solvent evaporation process in presence of saccharin sodium excipient at room temperature. The Ibuprofen crystals were evaluated by *in-vivo* study and the results obtained were compared with the pure drug. Results: *In-vivo* pharmacokinetic study was carried out by using albino wistar rats which shows the improvement in 2.95 folds higher plasma concentration from Ibuprofen crystals compared to the plain drug. One group of rats treated with pure drug i.e., Ibuprofen suspension (STANDARD) while the other group of rats treated with Ibuprofen loaded crystals (TEST). The results revealed that the plasma concentration-time profile of standard group and test group depicted the C_{max} of 16181.12 ng/mL and 29585.68 ng/mL while T_{max} of 90 minutes and 30 minutes, and AUC found was 44816.86 ng*h/mL and 132080.30 ng*h/mL respectively. Conclusions: The crystal engineering approach can be used as a tool to improve the pharmacokinetic parameters of BCS Class-II drugs.

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INTRODUCTION

Ibuprofen is a water insoluble BCS Class II drug used as a potent non-steroidal anti-inflammatory drug (NSAID) which is a propionic acid derivative with analgesic activity. It is widely used clinically as an anti-inflammatory and antipyretic in the treatment of rheumatoid arthritis, osteoarthritis, and spondylitis [1-3]. As Ibuprofen is of BCS Class II drug having poor aqueous solubility [4-9] and higher permeability is selected for the preparation of crystals by crystal engineering approach which improves the aqueous solubility, *in-vitro* drug release rate [10] and ultimately it improves the pharmacokinetic parameters of Ibuprofen.

Ibuprofen is a Non-steroidal anti-inflammatory drug (NSAID). The exact mechanism of action of Ibuprofen is unknown but it is believed that its therapeutic action is because of cyclooxygenase-2 (COX-2) inhibition which reduces the inflammation, pain, fever, swelling and thromboxane A_2 which initiates the aggregation of platelets and lead to the formation of thrombus by lowering the synthesis of prostaglandins. It has antipyretic action by acting on the hypothalamus leading to an increased peripheral blood flow, vasodilation and subsequent heat dissipation [11].

Racemic mixture of Ibuprofen is administered which comprised of the R-enantiomer shows extensive inter conversion to the S-enantiomer *in-vivo* among which the later form is therapeutically more active than former one [12]. Ibuprofen is a non-selective COX inhibitor same as other NSAIDs like Aspirin and Indomethacin which inhibits both cyclooxygenases i.e., COX-1 and COX-2. The analgesic, antipyretic and anti-inflammatory action of Ibuprofen is owing to the inhibition of COX-2 while undesirable gastrointestinal ulceration is owing to the inhibition of COX-1 [13]. It has been observed that different NSAIDs shows different degree of analgesic, anti-inflammatory and gastric ulceration depends upon the inhibition of individual COX isoforms [14].

Pharmacokinetics of Ibuprofen includes rapid absorption through the gastrointestinal tract and peak plasma level is achieved within 1-2 hours after the oral administration of 200-400 mg dose and the concentration appears in the blood circulation is about 15-25 mg/mL. Ibuprofen has extensive protein binding capacity and is excreted via kidneys. The biological half-life is about 2-3 hours.

Ibuprofen is used primarily as an anti-inflammatory, to treat fever, mild to moderate pain after surgery, osteoarthritis, toothache, painful menstruation and headache [15]. It is used as an anti-inflammatory drug and it can be employed in juvenile as well as rheumatoid arthritis and also in pericarditis [16-18].

MATERIALS AND METHODS

Materials

Ibuprofen (IBU) was gifted by Marksans Pharma Limited, Goa. Ketoprofen was gifted by Emcure Pharmaceuticals Limited, Pune. Potassium dihydrogen phosphate (KH_2PO_4) and Disodium hydrogen orthophsophate dihydrate (Na_2HPO_4) were purchased from SDFCL, Mumbai. Sodium Hydroxide (NaOH) and Sodium acetate trihydrate were procured from Rankem, New Delhi, India. Saccharin sodium dihydrate (SAC-Na) was gifted by Pure Chem. Pvt. Ltd., Ankleshwar, Gujarat. All other solvents, excipients and chemicals used were of analytical and HPLC grade (Merck Pvt. Ltd., Mumbai, India).

Crystal preparation by conventional solvent evaporation technique

Ibuprofen loaded crystals were prepared by solvent evaporation technique using Saccharin sodium excipient in molar proportion of (1:2) which formed hydrogen bonding with Ibuprofen which improved physicochemical properties of Ibuprofen like aqueous solubility, *in-vitro* dissolution rate [10] which may lead to improve pharmacokinetic parameters like C_{max}, T_{max} and AUC by *in-vivo* study which was investigated and presented in this work [19].

***In-Vivo* Pharmacokinetic Study For Ibuprofen loaded crystals**

Wistar albino rats (weighing approximately 210 ± 30 g) of either sex was used for the pharmacokinetic study of Ibuprofen pure drug and Ibuprofen loaded crystals. The animals were maintained in temperature and humidity-controlled room with a 12:12 h light: dark cycle and were supplied with food and water *ad libitum*. The animal requirement for pharmacokinetic study of Ibuprofen was approved by the Institutional Animal Ethics Committee (IAEC) with protocol number **IAEC/DPS/SU/1522** and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

The overnight fasted rats (~12 h; n=4) with free access to water were used for the experiment of standard and test groups. Two groups of rats were used among which one group was administered orally with aqueous suspension of Ibuprofen pure drug and another group was administered with Ibuprofen loaded crystals [01, 20-21].

Samples of the Ibuprofen pure drug (18 mg/kg) (obtained by converting human dose to rat dose [01, 22]) and Ibuprofen loaded crystals (18 mg/kg equivalent to Ibuprofen) were accurately weighed and separately dispersed into distilled water (3 mL) by mixing homogeneously for 30 s prior to dosing. Each formulation was administered separately to rats by oral gavage using an animal feeding needle.

Collection of Blood Samples

Under ether anesthesia, blood samples (0.5 mL) were collected via the retro-orbital plexus at pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 12 and 24 hours after oral administration into the polypropylene tubes containing K3EDTA solution as anticoagulant tubes. The plasma was harvested by centrifuging the blood at 15000 rpm for 10 min at 4 °C temperature using a cooling centrifuge (REMI, Mumbai, India) and stored frozen in Deep freeze (Remi Quick Freezer, Mumbai, India) at -80 ± 10 °C until analysis. Both group animals were allowed to feed 2 h post-dose.

An aliquot of 200 µL of thawed plasma samples were processed as shown in the following sample preparation sections.

Preparation of Calibration Curve of Ibuprofen in Rat Plasma (Spiked CC Standards)

Drug free rat plasma having K3EDTA as an anticoagulant was procured from Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. CC standards were prepared by spiking the respective CC spiking solutions in drug free K3EDTA rat plasma as shown in **Table 1**.

Quality Control (QC) Spiking Solutions

QC spiking solutions were prepared in Methanol using Drug Stock Solution (5 mg/mL) by serial dilution as shown in **Table 2**.

Equipment Conditions

Chromatographic Conditions

The concentration of Ibuprofen in rat plasma of both groups were determined by HPLC (LC20AD, Shimadzu Corporation, Japan) analysis consisted of binary gradient system with Photon Diode Array (PDA) Detector and Lab Solution Software 5.72 B (Shimadzu Corporation, Japan). HPLC

system equipped with binary pump (LC-20AD) along with an auto sampler (SIL-HTC) was used to inject 10 µL plasma of the samples, processed as described in the extraction procedure section**,** onto a chromatographic column. The chromatographic conditions for Ibuprofen are shown in **Table 3**.

Data from the plasma samples of both groups were used to plot curves for the estimation of concentration of Ibuprofen in plasma with time and used for the comparison of pharmacokinetic parameters of Ibuprofen loaded crystals with Ibuprofen pure drug.

Extraction Procedure

Procedure for Extracted Sample Preparation

Note: 500 µL of respective spiking solution was spiked into tube containing 9500 µL of rat plasma & vortex to mix.

Step 1. Retrieve the required number of CC standards and QC samples from the deep freezer, thaw them at R.T. and vortex the tubes to mix. Transfer 0.2 mL of sample into pre-labelled tube.

Step 2. Add 50 µL of ISTD dilution to all the samples except STD Blank and vortex for about 15 seconds.

Step 3. Add 50 µL of 0.1 N Hydrochloric acid in water solution to all the samples and vortex for about 15 seconds.

Step 4. Add 1.2 mL of Ethyl acetate, cap the tubes and vortex all the samples on cyclo mixer, for 10 minutes.

Step 5. Extracted samples were centrifuged at 10000 RPM, at 10 ± 2 °C for 10 minutes.

Step 6. 1.0 mL of supernatant was transferred into pre-labelled tubes and evaporated to dryness under vacuum by using an vacuum oven set at 40 ± 5 ^{o}C.

Step 7. After drying, samples were reconstituted with 100 µL of reconstitution solution and vortexed for about 30 seconds.

Step 8. Reconstituted samples were transfer into pre-labeled auto sampler vials, arrange them in the auto sampler and inject by using HPLC-PDA.

Aqueous Sample Preparation

1. Take 400 µL of reconstitution solution in pre-labelled tubes.
2. Add 500 µL of ISTD dilution, vortex to mix.
3. Add 100 µL of respective spiking solution and vortex to mix.
4. Appropriate volume of samples was transfer into pre-labelled auto sampler vials and injected by HPLC-PDA.

Post Spiked Sample Preparation for Recovery Experiment for Drug and ISTD

Step 1. Retrieve the required number of STD BL from the deep freezer, thaw them at R.T. and vortex the tubes to mix. Transfer 0.2 mL of sample into pre-labelled tube.

Step 2. Add 50 µL of Methanol to all the samples, vortex for about 15 seconds.

Step 3. Add 50 µL of 0.1 N Hydrochloric acid in water solution to all the samples and vortex for about 15 seconds.

Step 4. Add 1.2 mL of Ethyl acetate to all samples and vortex for about 15 seconds.

Step 5. Centrifuge the samples at 10000 RPM, at 10 ± 2 °C for 10 minutes.

Step 6. 1.0 mL of supernatant was transferred into pre-labelled tubes and evaporated to dryness under vacuum by using the vacuum oven set at 40 ± 5 ^{o}C.

Step 7. After drying, samples were reconstituted with 100 µL of respective aqueous solution (Vial) and vortexed for about 30 seconds.

Step 8. Transfer samples into pre-labeled auto sampler vials, arrange them in the auto sampler and inject by using HPLC-PDA.

Data processing

The chromatograms were acquired by using Lab Solution Software 5.72 B supplied by Shimadzu. The calibration curve was plotted as the peak area ratio (Drug/ISTD) on Y-axis Vs the nominal concentration of Drug on the X-axis. The concentrations of the unknown samples were calculated by using linear regression equation with $1/C^2$ weighting factor.

Pharmacokinetic parameters

Pharmacokinetic parameters C_{max} and T_{max} were calculated using plasma concentration versus time profile. The area under the plasma concentration versus time curve from zero time to the last experiment point, ($AUC_{0\rightarrow 24h}$) was calculated by trapezoidal method.

RESULTS AND DISCUSSION

***In-Vivo* Pharmacokinetic Study**

Preparation of Calibration Curve of Ibuprofen with Internal Standard Ketoprofen in Rat Plasma (Spiked CC Standards)

Peak of Ibuprofen with internal standard Ketoprofen in rat plasma by HPLC method is illustrated in **Figure 1** and calibration curve of Ibuprofen in rat plasma by HPLC method is illustrated in **Figure 2**.

Comparison of Mean Plasma Concentration - Time Profile of Ibuprofen Drug with its Treated Crystals in Rat Plasma

In-vivo absorption study was carried out to assess the solubility and dissolution enhancement of Ibuprofen in treated crystal formulation. Enhancement of *In-vitro* dissolution of drug from treated crystals could increase the GI absorption of

drug after oral administration. The plasma concentration - time profile of Ibuprofen in *albino* rats (*Wistar* strain) following oral administration to standard and test groups is shown in **Table 4** and **Figure 3**.

By examining the results obtained from the above individual analysis, the plasma concentration-time profile as depicted in **Figure 3** revealed that the test group gave higher plasma concentration (2.95 folds) compared to the standard group. It was observed that Ibuprofen loaded crystals increased the rate of dissolution by way of hydrogen bonding with Saccharin sodium excipient. Hence, there was a drastic increment in the absorption of Ibuprofen from its crystal formulation which also enhanced the pharmacokinetic parameters and bioavailability of the drug.

Table 1. Spiked CC standards in rat plasma for ibuprofen.

Spiking Solution ID	Spiking Solution Conc. (µg/mL)	Spiking Volume (mL)	Plasma Volume (mL)	Total Volume (mL)	Spiked Conc. (µg/mL)	STD ID
Methanol	0	0.5	9.5	10	0	STD BL
SS STD1	2000	0.5	9.5	10	100	STD 1
SS STD2	1000	0.5	9.5	10	50	STD 2
SS STD3	500	0.5	9.5	10	25	STD 3
SS STD4	200	0.5	9.5	10	10	STD 4
SS STD5	100	0.5	9.5	10	5	STD 5
SS STD6	40	0.5	9.5	10	2	STD 6
SS STD7	20	0.5	9.5	10	1	STD 7
SS STD8	10	0.5	9.5	10	0.5	STD8

Table 2. (QC) Spiking Solutions for Ibuprofen

Stock Dil. Conc. (µg/mL)	Volume Taken (mL)	Volume of Methanol (mL)	Total Volume (mL)	Spiking Solution Conc. (µg/mL)	Spiking Solution ID
5000	1.8	3.2	5	1800	SS HQC
1800	1.25	3.25	4.5	500	SS MQC
500	0.3	4.7	5	30	SS LQC

Table 3. Chromatographic conditions for ibuprofen.

Parameters	Used
Column	Gemini C 18, 150 mm x 4.6 mm, 5 µm
Mobile Phase	Formic acid in water, 0.1 %v/v : Methanol, 25 : 75 %v/v
Flow rate	1.00 mL/min
Column oven temperature	40 ± 0.3 °C
Auto sampler temperature	15 ± 3 °C
Volume of injection	10.0 µL
Detector	PDA detector
Retention time	Analyte at about 5.80 minutes ISTD at about 3.15 minutes
Run time	8.0 minutes

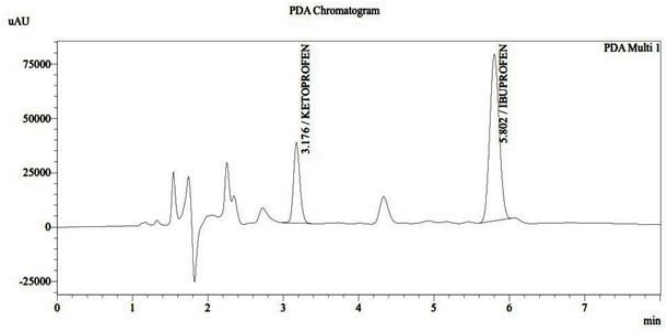

Figure 1 Peak of Ibuprofen with internal standard Ketoprofen in rat plasma by HPLC method.

Table 4. Comparison of pharmacokinetic parameters of pure ibuprofen drug (STANDARD) with treated crystals (TEST)

Pharmacokinetic Parameters	Ibuprofen Pure Drug (STANDARD)	Treated Crystals (TEST)
C_{max} (ng/mL) ± SD*	16181.12 ng/mL ± 1435.46	29585.68 ng/mL ± 2132.84
T_{max} (h) ± SD*	1.5 h ± 0.04	0.5 h ± 0.03
$AUC_{0\rightarrow24}$ (ng*h/mL) ± SD*	44816.86 ng*h/mL ± 59978.30	132080.30 ng*h/mL ± 55966.76
	* Results are expressed as mean ± SD (n = 4)	
	[a] Significantly different from Pure drug, P < 0.05	

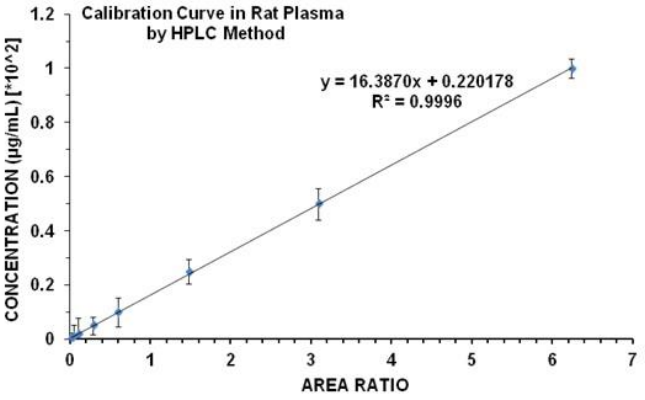

Figure 2 Calibration curve of Ibuprofen in rat plasma by HPLC method.

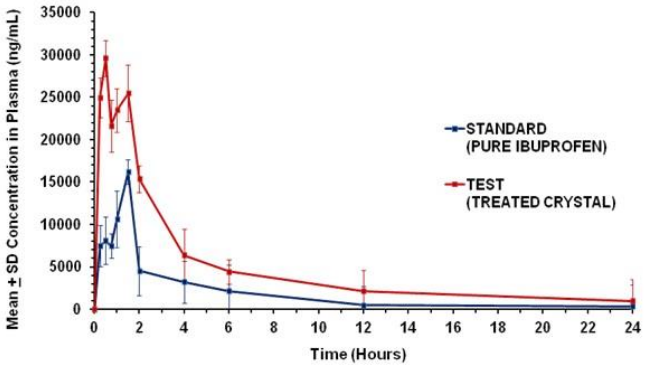

Figure 3 Comparison of mean plasma concentration - time profile following oral dose of pure Ibuprofen (Standard) and Ibuprofen loaded crystal (Test) in rat plasma

Consequently, the pharmacokinetic parameters illustrated in **Table 4** explained that Ibuprofen loaded crystal formulation exhibited better dissolution of drug in stomach. Hence, better absorption was achieved compared to pure drug. As shown in Table 4, T_{max} for standard group was achieved in 1.5 hours while for test group, it was only 0.5 hour. The plasma drug concentration of treated crystals was 29585.68 ng/mL which was again much higher compared to the plasma drug concentration of pure drug i.e., 16181.12 ng/mL.

CONCLUSION

The aim of present work was to study the improvement in the pharmacokinetic parameters of Ibuprofen treated crystals compared to the pure drug as in the previous study it was revealed that the improvement in aqueous solubility, drug release rate and mechanical properties which lead to the improvement in physicochemical, mechanical and pharmacotechnical parameters of Ibuprofen, BCS Class II drug having poor flow property. In the present study, it was found that the test group could drastic increase the pharmacokinetic parameters like C_{max}, T_{max} and AUC compared to the standard group.

CONFLICT OF INTEREST

None

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REFERENCES

1. Nurfazreen, A., Tommy, J.B.E., Khuriah, A.H. Determination of a poorly soluble drugs, Ibuprofen in rat plasma by a simple HPLC analysis and its application in pharmacokinetic study. Int. J. of Pharma. Sci. and Res., 2015; 6(1): 96-102.
2. Shing, F.C., Miles, C., Limin, S., Albert, H.L.C., Changquan, C.S. Simultaneously improving the mechanical properties, dissolution performance, and hygroscopicity of Ibuprofen and

Flurbiprofen by cocrystallization with Nicotinamide. Pharm. Res., 2012; 29(7): 1854-1865.

3. Zalte, A.G., Darekar, A.B., Gondkar, S.B., Saudagar, R.B. Preparation and Characterization of Ibuprofen cocrystals by using solvent drop grinding method. World J. of Pharma. res., 2014; 3(4): 1392-1402.
4. Castro, S., Bruni, S.S., Lanusse, C., Allemandi, D., Palma, S. Improved Ibuprofen Dissolution Rate in Pluronic 188 Solid Dispersions. AAPS Pharm. Sci. Tech., 2010; 11(4): 1518–1525.
5. Cavalcanti, N.C.T., Giovana, D.S., Maria, A.M.T., Jose, L.S.S., Leila, B.L., Davi, P.S. Assay and physicochemical characterization of the antiparasitic Ibuprofen. Braz. J. of Pharma. Sci., 2012; 48(2): 281–290.
6. Nallasivan, P.K., Saranya, K.C., Kumar, R.S., Jeevanantham, S., Venkatnarayanan, R. Simultaneous determination of Ibuprofen and Tizanidine in bulk drug and its combined dosage form by spectrophotometry. Scholars Res. Lib., 2010; 2(6): 289-295.
7. Healy, A.M., Worku, Z.A., Kumar, D., Madi, A.M. Pharmaceutical solvates, hydrates and amorphous forms: A special emphasis on cocrystals. Adv. Drug Del. Rev., 2017; 117(1): 25-46.
8. Zalte, A.G., Darekar, A.B., Gondkar, S.B., Saudagar, R.B. Preparation and Characterization of Ibuprofen cocrystals by using solvent drop grinding method. World J. of Pharma. res., 2014; 3(4): 1392–1402.
9. Chandira, R.M., Bhowmik, D., Yadav, R., Jayakar, B., Kumar, K.P.S. Formulation and evaluation the oral tablets Ibuprofen. The Pharma. Inno., 2012; 1(9): 32–43.
10. Vaghela, P.D., Tank, H.M., Patel, N.U., Chavda, J.R., Shah, P.I. Improvement of Physicomechanical and Pharmacotechnical Parameters of Ibuprofen by Crystal Engineering. Int. J. Pharm. Sci. Rev. Res., 2016; 39(1): 173-182.
11. Ibuprofen: Pharmacology: Mechanism of Action, Drug Bank, Open Data Drug & Drug Target Database, 2013.
12. Wishart, D.S. Drug bank and its relevance to pharmacogenomics, Pharmacogenomics. 2008; 9(8).
13. Rao, P., Knaus, E.E. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J. of Pharmacy & Pharma. Sci., 2008; 11(2): 81–110.
14. Kakuta, H. et al. Cyclooxygenase-1-selective inhibitors are attractive candidates for analgesics that do not cause gastric damage. Design and in vitro/in-vivo evaluation of a benzamide-type cyclooxygenase-1 selective inhibitor. J. of Med. Chem., 2008; 51(8): 2400–11.
15. Non-steroidal anti-inflammatory drugs, British National Formulary, 2016.
16. Joint Formulary Committee, British National Formulary, 65 ed.; London, UK: Pharmaceutical Press, 2013; 665–671.
17. Rossi, S. Australian Medicines Handbook, Adelaide: The Australian Medicines Handbook Unit Trust, 2013.
18. Ibuprofen, The American Society of Health System Pharmacists, 2011.
19. Rahman, Z., Agarabi, C., Zidan, A.S., Khan, S.R., Khan, M.A. Physico-mechanical and Stability Evaluation of Carbamazepine Cocrystal with Nicotinamide. AAPS Pharm Sci Tech., 2011; 12(2): 693–704.
20. Reis, C.P. et al. Ibuprofen Nanoparticles for Oral Delivery: Proof of Concept. J. Nanomed. Biothera. Discov., 2013; 4(1).
21. Yong, C.S. et al. Enhanced oral bioavailability of Ibuprofen in rats by poloxamer gel using poloxamer 188 and menthol. Drug develop. and ind. pharmacy, 2005; 31: 615– 622.
22. Ghosh, M.N. In: Fundamentals of experimental pharmacology, Ch. 25, 5th ed.; Hilton and Company, Calcutta, 2011; 165–171.

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