

Unique Solid Dispersions by Microwave Fusion Technique with Etoricoxib and Thiocolchicoside: *In vivo* Evaluation and Comparison with Marketed Formulation

Hemanth Annepogu¹, Hindustan Abdul Ahad^{2,*}

¹Department of Pharmaceutics, St. Johns College of Pharmaceutical Sciences, Yerrakota, Yemmiganur-518360, AP, India

²Department of Industrial Pharmacy, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, Ananthapuramu-515001, AP, India

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Abstract Aim: The primary objective of the study is to evaluate solid dispersion (SDs) made into tablets containing Etoricoxib (ECB) and Thiocolchicoside (TCS) for different *in vivo* parameters and *in vitro* drug release as an extension of the work described by Annepogu *et al.*, in 2018. Methods: The *in vitro* study was constructed on various evaluation parameters of SDs of ECB and TCS. From earlier study results, we have selected formula-5 SDs of ECB and TCS for *in-vivo* study and comparison with marketed ones. Results: Plasma drug availability was performed on 6 healthy rabbits of both sexes (weighing 2.0-2.5 kg). They were aimlessly alienated into 2 groups of alike size by parallel design. Later, the study was HPLC analysis of the drug in the plasma, which involved preparation of the spiked plasma sample, and an analytical technique was established (simple and cost-effective method for ECB and TCS simultaneous estimation). The plasma samples were assessed for pharmacokinetic parameters. Finally, the F-5 formulation was assessed for *in vitro* drug release by comparison with the marketed preparation (Recox tablets-Hochest Biotech India). Conclusion: The study summarizes that TCS and ECB reached systemic circulation better than the pure drugs, and the kinetic values were appreciable. The drug discharge from the SDs was identical to the marketed one.

Keywords Solid Dispersions, Microwave Fusion, *In vivo*, Bioavailability, Rabbits

1. Introduction

Formulation experts in industries do plenty of trials to improve poorly soluble drugs economically [1]. Among the innumerable tactics for augmenting solubility, the solid dispersion (SDs) methodology [2] is ahead of all as it is meeker and entails fewer struggles than the other tactics [3].

Etoricoxib (ECB) is a Non-Steroidal Anti Inflammatory Drug (NSAID) used to tackle all the diversities of pain and arthritic tenderness [4]. ECB is poorly water-soluble with 92% binding to protein [5] and poor bioavailability [6]. Thiocolchicoside (TCS) is a colchicoside derivative of *Gloriosa superba* and *Colchicum autumnale* [7]. TCS is a muscle relaxant used to challenge sore muscle reductions [8], acute and arthritic glitches [9], and pains [10], and it is devoid of sedative issues [11] compared with other muscle relaxants. It can be co-administered with many NSAIDs. TCS is a yellow crystalline powder that is merely soluble in

ethanol and poorly in water [12].

The old style of making SDs is by fusion [13], in which the polymer carriers used for SDs are not open to uniform heat from the heat source [14]. To overwhelm this, an innovative microwave (MW) melting procedure was adopted as described by Annepogu *et al.*, [17]. Electromagnetic irradiation was pragmatic in an MW oven with 0.3 to 300 GHz of infrared and radio frequencies [15] at wavelengths of 1 mm to 1 m. This practice can be assumed for procurement of fast and incessant heating even in materials bestowing low heat conductivity (e.g., polymers) because the movement of energy is not contingent on heat diffusion [16]. Thus, this innovative MW melting process was assumed in the planning of SDs. Much research has been accomplished on augmenting the solubility of drugs using PEG 8000, PVP K-30 and P188.

Therefore, it is important to enhance the solubility of ECB and TCS, with faster discharge, absorption, and exploitation to relieve acute patients. It also helps to find out what proportions are effective in augmenting the solubility of drugs. The ratios of drugs and excipients were as per table 1.

Table 1. Drug poly blend ratios in solid dispersions

| Drug: Carrier | Drug: Carrier ratio | Formulation code |
|--------------------------|---------------------|------------------|
| ECB+ TCS: carrier blend* | 1:1 | F-1 |
| | 1:2 | F-2 |
| | 1:3 | F-3 |
| | 1:4 | F-4 |
| | 1:5 | F-5 |
| | 1:6 | F-6 |

ECB- Etoricoxib; TCS- Thiocolchicoside;

Carrier blend* contains an equal mixture of PEG 8000+PVP K-30+P188

Among all the above formulations, F-5 possesses all the characteristics of an ideal solid dispersion like improved solubility, good physicochemical/ flow properties, *in vitro* drug discharge, and uniformity in their contents as described by Annepogu *et al.*, [17]. So, F-5 was subjected to *in vivo* trials.

2. Materials and Methods

Materials

Acetonitrile and o-Phosphoric acid and triple glass distilled water (HPLC grade) were delivered by Merck chemicals and other chemicals were of analytical grade. The New Zealand Wistar rabbits for the *in vivo* study were from Krishna Rabbit Farms, Bengaluru. The *in vivo* study was steered in harmony with animal ethical guidelines for investigations in the laboratory.

An approval was taken from the Institutional Animal

Ethics Committee (SJC/2017/COL-16R) ratified by CPCSEA (1561/PO/RE/S/11/CPCSEA) for performing animal experiments.

In vitro Study

The dissolution conditions of SDs of ECB and TCS were performed using a USP-II dissolution apparatus (Lab India Instruments, India) with a paddle speed of 100 rpm using 0.1M HCl as a dissolution medium at 37 ± 0.5 °C. At frequent intervals of every 10 minutes till 1h, a sample of 5 mL was withdrawn (sink conditions were maintained), filtered (0.45 µ nylon disc filter) and absorbance was measured at 25.2 nm (isobestic point for ECCB and TCS) using a UV-Visible spectrophotometer and the amount of drug released was read from calibration curves.

In vivo Study

The *in vivo* studies on various evaluation parameters were achieved as designated by Sharma *et al.*, [18]. We have selected formula-5 SDs (F-5) for *in vivo* study.

In vivo Bioavailability Study

6 white New Zealand rabbits of any gender (weighing 2.0-2.5 kg) were haphazardly alienated into 2 groups of identical size by parallel design. The work was agreed to match the pharmacokinetics of ECB and TCS from selected SDs (F-5) containing 90 mg of ECB and 4 mg of TCS. Food was taken off 10 ± 1 h prior to the *in vivo* study, with water ad libitum. Group A of rabbits orally received SDs (F-5), whereas group B received pure drug suspension in water [19]. Blood samples (2 mL) were collected into heparinized tubes at 0, 0.5, 1, 2, 4, 6, and 8 h after administration of the treatment. The plasma was detached from the heparinized whole blood by centrifugation at 4,000 rpm for 15 min [20]. After parting, plasma samples were immediately transferred to Eppendorf tubes and stored at -20 °C until analysis.

Plasma concentration of drug was dogged by HPLC analysis [21] and the following steps were used for HPLC analysis.

Estimation of ECB and TCS in serum samples

ECB and TCS in serum samples were estimated according to the High-Performance Liquid Chromatographic (HPLC) process [22, 23].

Instrument Conditions

The instrument (HPLC) was used for the study. The conditions were submitted as supplementary files.

Preparation of standard solution and plotting of correction curves

The procedure adopted was as established by Pushparaj

et al., 2017 with little modification [24]. Weighed accurately 90 mg of ECB (for stock solution A) and 4 mg of TCS (for stock solution B) and transferred to a 100 mL volumetric flask, dissolved using the mobile phase with the aid of sonication, and final volume was made with the mobile phase. From these stock solutions, working standard solutions were prepared with suitable dilution with mobile phase to get concentrations of 5-40 µg of ECB and 0.5 to 4 µg of TCS.

Preparation of the spiked plasma sample

This was performed by using the procedure explained by Bhothra *et al.*, 2018 [25]. 250 µL of rabbit plasma, 50 µL of internal standard, 10 µL of ECB and 10 µL of TCS were pipetted into a 10 mL centrifuge tube and to this 2 mL of Acetonitrile was added. A 10 µL of the supernatant layer was collected (after centrifugation at 3200 rpm for 10min) and injected into HPLC. A typical chromatogram is achieved from a sample solution.

Process development

The mobile phase used was a 70:30 (v/v) mixture of freshly prepared buffer 0.1% o-Phosphoric acid and Acetonitrile, which indicated an effective mixture used for the parting. Then, the flow rate tested was 0.4, 0.8, 1.0, 1.2, and 1.5 mL/min. 1.0 mL/min was selected for the determination of ECB and TCS as it has better resolution of the peaks. The stated chromatographic situations were the best to deliver resolution between ECB and TCS at a reasonable time of 4.912 and 2.805 min respectively at the optimum wavelength for detection was 220 nm and no native noise composites eluted at the retention times of ECB and TCS.

Determination of various pharmacokinetic parameters:

From the time versus serum concentration facts, numerous pharmacokinetic parameters such as peak concentration (C_{max}), time at which drug peaks (t_{max}), area under the curve (AUC), elimination rate constant K_{el} , biological half-life ($t_{1/2}$), %absorbed at various times, and absorption rate constant (K_a) [26] were calculated in each case. The peak serum concentration (C_{max}) and time at peak attained (t_{max}) were strong minded [27,28] with the help of correction curves. The serum concentration and time values were plotted on a semi-logarithmic graph paper. The K_{el} was calculated from the slope of the linear line in the elimination phase (the best fit linear regression line for the points in the elimination phase was haggard by the process of fewest squares) [29]. The consistent $t_{1/2}$ was premeditated with the equation $t^{1/2} = 0.693/K_{el}$. %absorbed at various times and K_a was calculated [30] from serum concentration facts by the Wagner and Nelson equation. The AUC was resolute by using the trapezoidal rule. The remaining area from 8 h to ∞ time was calculated [31] using the following eq. 1 and 2.

$$[AUC]_{8-\infty} = \text{Concentration at } 8^{\text{th}} \text{ h} / K_{el} \quad (1)$$

$$\text{Then } [AUC]_{0-\infty} = [AUC]_{0-8 \text{ h}} + [AUC]_{8-\infty \text{ h}} \quad (2)$$

In Vivo investigation protocol

Calculation of Animal Equivalent Dose from Human Dose

To calculate animal equivalent dose (AED) using human dose [32-34] by eq.3 was employed.

$$AED = \frac{\text{Human Dose (mg/kg)}}{\text{Animal weight (kg)}} \text{Human weight (kg)}^{0.33} \quad (3)$$

Using the above equation, considering the average human weight of 70 kg, animal equivalent dose calculations were carried out. Human dose of Drugs in mg: ECB (90 mg) and TCS (4 mg). Calculated Animal Equivalent Dose (AED): ECB (3.98 mg) and TCS (0.18 mg) per kg body weight of the animal.

Treatment of Animals

Healthy rabbits of either sex were fasted overnight. ECB and its SDs were administered at a dose equivalent to 3.98 mg/kg of ECB. TCS and its SDs were administered at a dose equivalent to 0.18 mg/kg of TCS. Each product was repeated 4 times ($n = 4$). The *in vivo* experiments were accompanied by a crossover investigation. The treatment and sampling intervals are revealed in fig. 1.

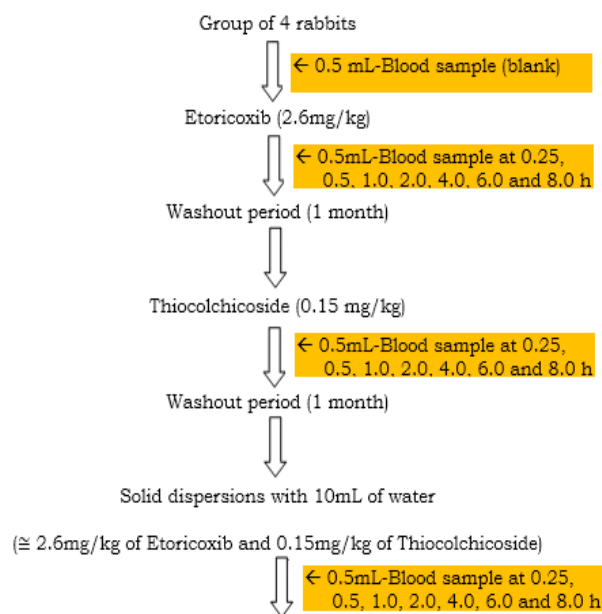


Figure 1. Treatment and sampling intervals for *in vivo* studies

The blood samples (0.5mL) were taken from the marginal ear veins of rabbits. The blood samples were indorsed to clot and centrifuged at 5000 rpm and the serum disjointed was unruffled into dry tubes. All the samples were stored under refrigerated conditions before the assay. The serum concentration of the drugs (ECB & TCS) was resolute by the HPLC process. Various pharmacokinetic parameters were calculated in each case based on the time vs. serum concentration facts [35].

3. Results and Discussion

Results of calibration curves and isobestic point

The standard correction curve of the ECB was revealed in fig. 2. The standard correction curve of TCS is revealed in fig. 3.

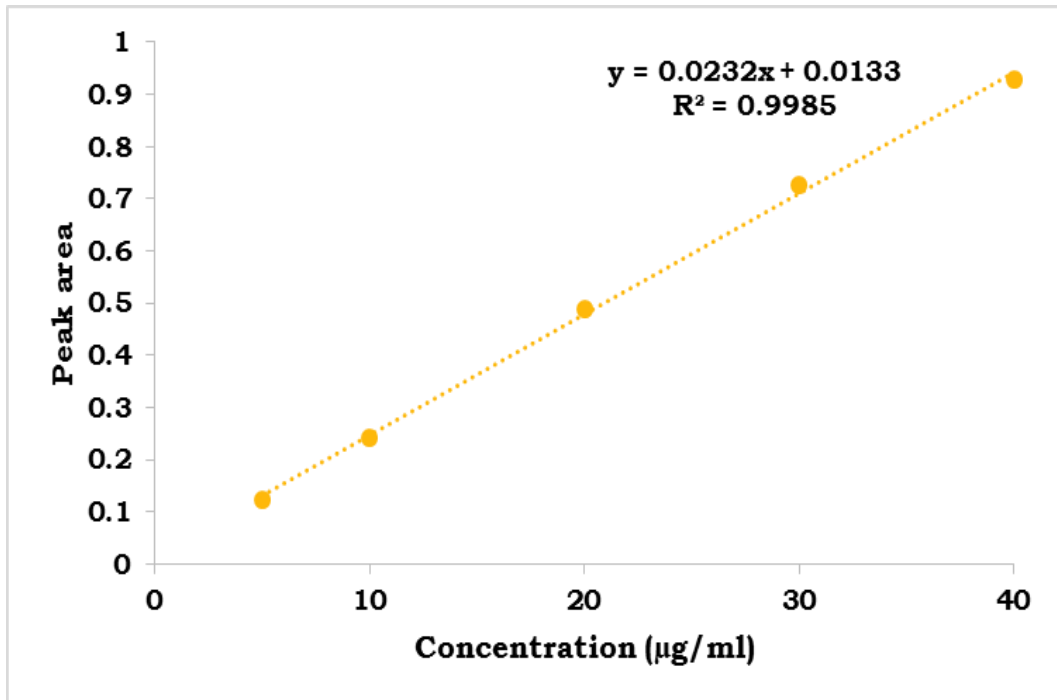


Figure 2. Correction curve for the estimation of ECB in serum by HPLC

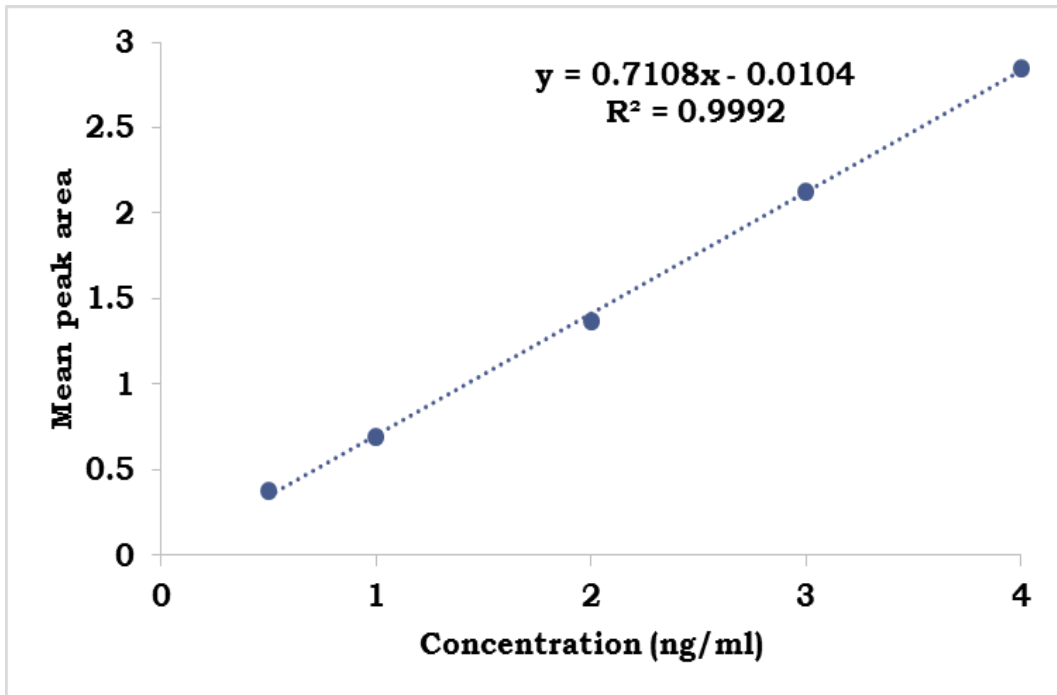


Figure 3. Correction curve for the estimation of TCS in serum by HPLC

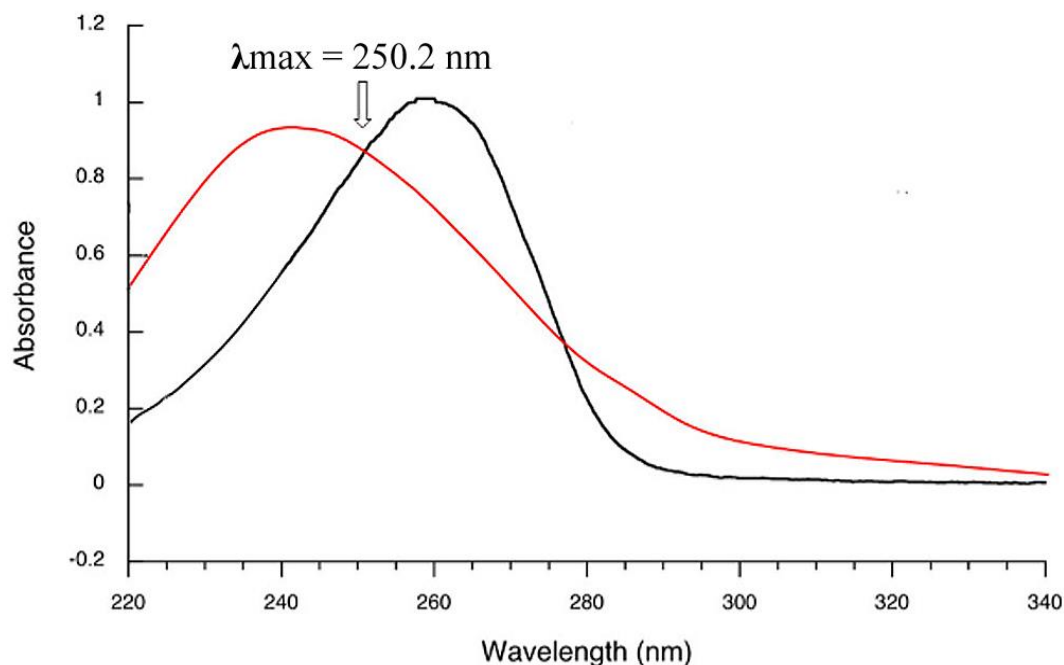


Figure 4. A UV-Overlay spectrum of ECB and TCS

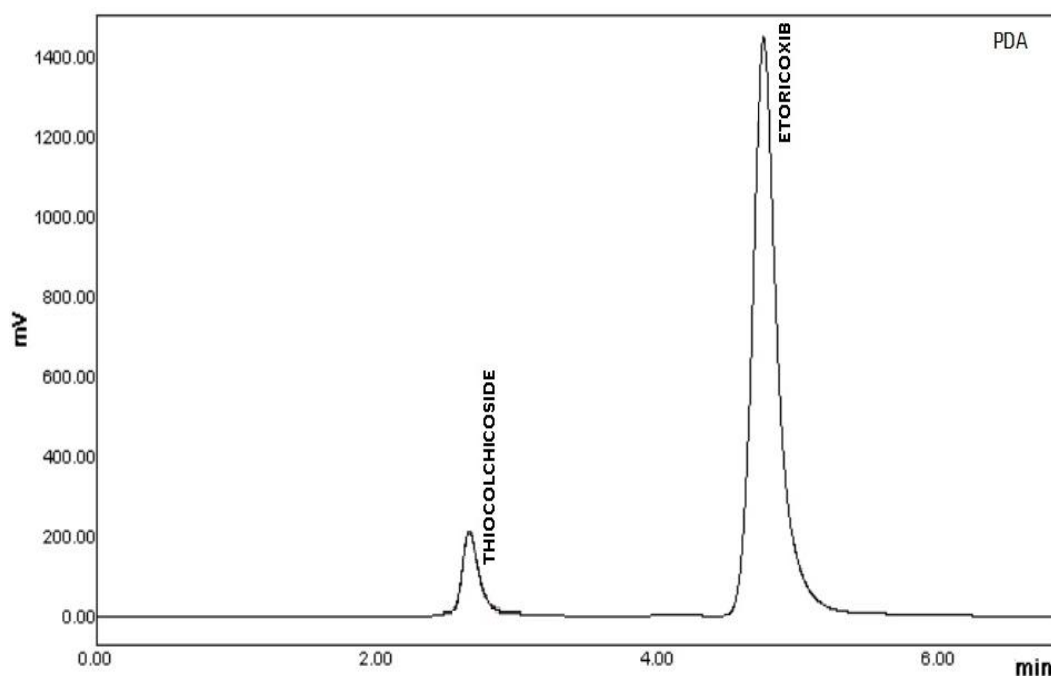


Figure 5. HPLC chromatogram of serum sample (F-5)

An overlay spectrum of ECB and TCS indicated λ_{\max} at 250.2 nm. The Simultaneous approximation of ECB and TCS was done by RP-HPLC. The retention time of ECB was found to be 4.912 min and 2.805 min for TCS and the asymmetric factor was within limits. The UV-Overlay spectrum of ECB and TCS was revealed in fig. 4.

Simultaneous estimation of ECB and TCS by RP-HPLC

The HPLC conditions for a simultaneous guesstimate of ECB and TCS were revealed in table 2. The chromatogram of the serum sample was revealed in fig.5. The regression equation was found to be $24841x+13613$ for ECB and $9219.x+377.8$ for TCS with a correlation of 0.999 for both. The asymmetric values for ECB and TCS were observed to be 1.62 and 1.35 respectively. The description of the HPLC graph and statistical facts about the HPLC chromatogram were revealed in tables 3 and 4. The concentration of ECB

in serum with pure ECB and SDs (F-5) after oral administration was revealed in fig.6. The concentration of TCS in serum with pure TCS and SDs (F-5) after oral administration was represented in fig. 7. The pharmacokinetic parameters estimated with ECB/TCS and its SDs (F-5) in serum after oral administration were revealed in table 5.

Table 2. Instrument (HPLC) Conditions for simultaneous estimation of ECB and TCS

| Parameter | Chromatographic condition |
|-----------------------|--|
| Instrument | Shimadzu |
| Chemstation/ Software | LC Solutions |
| Column | Phenomenex Luna C18 (250x4.6 mm; 5 μ) |
| Mobile phase | Water (0.1% o-Phosphoric acid) and Acetonitrile (70:30 v/v) |
| Wavelength (nm) | 250.2 |
| Flow rate (mL/min) | 9.0 |
| Run time (min) | 7.0 |

Table 3. Description of the HPLC graph

| Name | Retention Time (min) | Peak Area | Asymmetric factor |
|------|----------------------|-----------|-------------------|
| ECB | 4.912 | 1037568 | 1.62 |
| TCS | 2.805 | 37281 | 1.35 |

Table 4. Statistical facts of HPLC chromatogram

| Parameters | ECB | TCS |
|--|----------------------|----------------------|
| Linearity ($\mu\text{g/mL}$) | 10 – 60 | 1 – 6 |
| Regression equation | $y = 24841x + 13613$ | $y = 9219.x + 377.8$ |
| Correlation coefficient | 0.999 | 0.999 |
| Slope | 24841 | 9219 |
| Intercept | 13613 | 377.8 |
| Limit of Detection ($\mu\text{g/mL}$) | 1.76 | 0.24 |
| Limit of Quantification ($\mu\text{g/mL}$) | 5.35 | 0.72 |
| Values in mean \pm SD; trials made (n=3) | | |

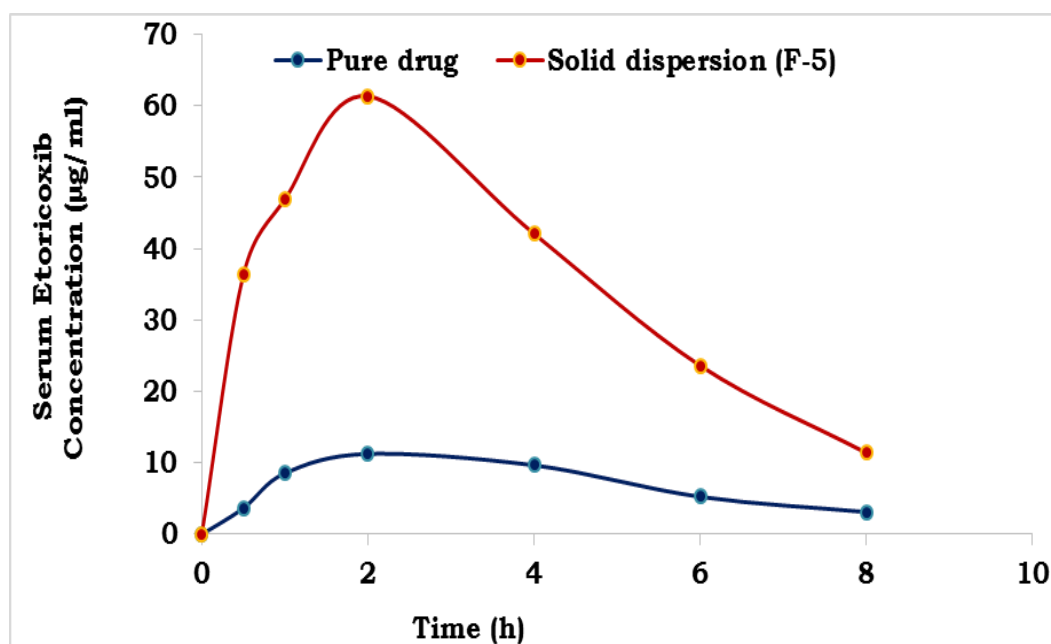


Figure 6. Serum concentration of ECB and its SDs (F-5) in rabbits (p.o)

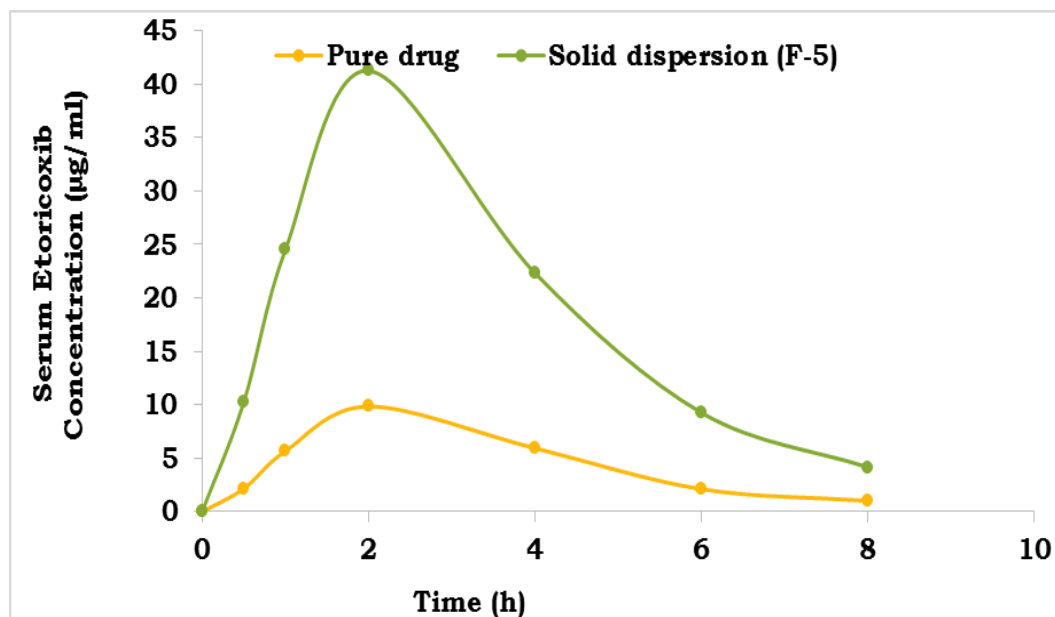


Figure 7. Serum concentration of TCS and its SDs (F-5) in rabbits (p.o)

Table 5. Pharmacokinetic parameters estimated with ECB/ and its SDs (F-5) in serum when administered orally

| Pharmacokinetic parameter | Concentration of ECB in Serum | | Concentration of TCS in Serum | |
|--|-------------------------------|--------------------|-------------------------------|--------------------|
| | ECB | SDs (F-5) | TCS | SDs (F-5) |
| C_{max} ($\mu\text{g/mL}$) | 11.260 \pm 0.62 | 061.370 \pm 1.35 | 09.840 \pm 0.23 | 041.260 \pm 1.28 |
| T_{max} (h) | 02.000 \pm 0.00 | 002.000 \pm 0.00 | 02.000 \pm 0.00 | 002.000 \pm 0.00 |
| K_{el} (h^{-1}) | 00.126 \pm 0.01 | 000.205 \pm 0.01 | 01.223 \pm 0.05 | 000.135 \pm 0.01 |
| $T^{1/2}$ (h) | 02.451 \pm 0.05 | 003.380 \pm 0.09 | 01.573 \pm 0.03 | 001.648 \pm 0.05 |
| $(\text{AUC})^{0-8}$ ($\mu\text{g.h/mL}$) | 57.408 \pm 2.48 | 283.705 \pm 5.65 | 36.433 \pm 1.28 | 148.167 \pm 5.26 |
| $(\text{AUC})^{0-\infty}$ ($\mu\text{g.h/mL}$) | 60.530 \pm 5.95 | 295.085 \pm 6.59 | 37.453 \pm 0.97 | 152.317 \pm 4.95 |
| K_a (h^{-1}) | 00.506 \pm 0.02 | 001.710 \pm 0.03 | 01.938 \pm 0.06 | 001.746 \pm 0.09 |

Values in mean \pm SD; trials made (n=3)

After a single dose of design F-5 (ECB: 3.98 mg/kg and 0.18 mg/kg of TCS), the symmetrical mean C_{max} values of design F-5 (61.37 \pm 1.35 $\mu\text{g/mL}$ for ECB and 41.260 \pm 1.28 $\mu\text{g/mL}$ for TCS), were higher than those of pure drugs {ECB ($P < 0.05$), which was 11.26 \pm 0.62 $\mu\text{g/mL}$ and TCS ($P < 0.05$), which was 09.84 \pm 0.23 $\mu\text{g/mL}$. C_{max} of SDs (F-5) was 5.45 times more than ECB and 4.19 times more than TCS. The T_{max} values of the design F-5 were equivalent to the pure drug. The $\text{AUC}_{(0-8h)}$ values of the design (ECB 283.705 \pm 5.65 $\mu\text{g.h/mL}$ and TCS 148.167 \pm 5.26 $\mu\text{g.h/mL}$) were higher than those of the pure drugs (ECB 57.408 \pm 2.48 $\mu\text{g.h/mL}$ and TCS 36.433 \pm 1.28 $\mu\text{g.h/mL}$). The $\text{AUC}_{(0-8h)}$ of SDs (F-5) was \sim 5 folds more than ECB and \sim 4 times more than TCS. The $\text{AUC}_{(0-\infty)}$ values of the design (ECB 295.085 \pm 6.59 $\mu\text{g.h/mL}$ and TCS 152.317 \pm 4.95 $\mu\text{g.h/mL}$) were obviously higher than those of the pure drugs (ECB 60.530 \pm 5.95 $\mu\text{g.h/mL}$ and TCS 37.453 \pm 0.97 $\mu\text{g.h/mL}$). The $\text{AUC}_{(0-\infty)}$ of SDs (F-5) was \sim 5

folds more than ECB and \sim 4 times more than TCS. These fallouts suggest that the absorption rate and bioavailability of SD design F-5 are remarkably quicker and greater than that of pure drugs. *In vivo* animal trials in rabbits revealed good levels of ECB and TCS in serum compared to pure ECB/TCS.

Comparison of *in vitro* drug dissolution with the marketed sample

Comparison of *in vitro* drug dissolution of optimized design (F-5) with the marketed sample was revealed in fig. 8. The comparison of *in vitro* drug dissolution of optimized design (F-5) with the marketed sample revealed that the drug discharge from the SDs was on par with the marketed dosage form, i.e., the formulated SDs tablets indicated a complete drug discharge in 60 min, whereas the marketed product was found to discharge only 95% of the drug in 60 min.

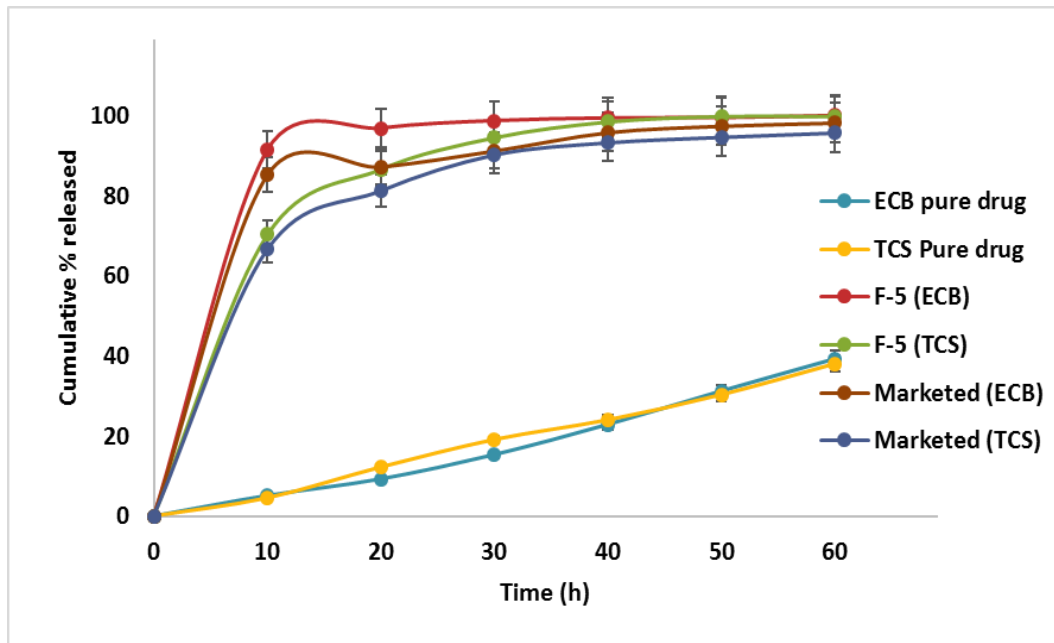


Figure 8. Comparison of *in vitro* drug dissolution of optimized design (F-5) with the marketed sample

4. Conclusions

A combination of Etoricoxib (ECB) and Thiocolchicoside (TCS) was prepared as solid dispersions (SDs). The best design (among F-1 to F-6) i.e., F-5, was subjected to *in vivo* studies in rabbits. The simultaneous appraisal of ECB and TCS was urbanized by RP-HPLC. *In vivo* animal trials in rabbits revealed good levels of ECB and TCS in serum associated with pure ECB and TCS. The pharmacokinetic parameters, viz., C_{max} , AUC were enriched relative to pure ECB/TCS drug. The drug reaching the systemic circulation from the SDs was quicker and appreciable than the pure drugs.

Abbreviations

ECB- Etoricoxib
 TCS- Thiocolchicoside
 NSAID- Non-Steroidal Anti-Inflammatory Drugs
 SDs- Solid Dispersions
 MW- Microwave
 AUC- Area Under Curve
 AED- Animal Equivalent Dose

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Declaration of Competing Interest

The author declared no conflict of interest.

Authors Contribution

Hemanth Annepogu performed the work, and Hindustan Abdul Ahad reviewed, revised, and arranged the work as per the journal format.

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