# *IN-VIVO* PHARMACOKINETIC STUDIES OF SULFASALAZINE SOLID LIPID NANOPARTICLES

**Hema Devi Tumma, Praveena Chinthala***

*Department of Pharmacy, Chaitanya Deemed to be University, Hanamkonda, Telangana -506001, India.*

**Corresponding Author:** Praveena Chinthala
Email: praveenamr18@gmail.com

**ABSTRACT**

Nanoparticles, particularly Solid Lipid Nanoparticles (SLNs), are increasingly utilized as efficient drug delivery systems due to their ability to enhance bioavailability, provide controlled release, and improve drug stability. SLNs offer significant advantages over traditional drug delivery systems, especially in targeting specific sites, such as the colon, for drugs like sulfasalazine used in the treatment of inflammatory bowel diseases. Sulfasalazine, although effective, suffers from poor solubility and variable bioavailability, limiting its therapeutic potential. In this study, sulfasalazine-loaded SLNs were developed and their in vivo pharmacokinetics were evaluated using a randomized animal model. The pharmacokinetic parameters, including absorption (Tmax), maximum plasma concentration (Cmax), elimination half-life (t1/2), and systemic exposure (AUC), were compared between the SLN formulation and pure sulfasalazine. The results demonstrated that SLNs significantly improved the absorption and bioavailability of sulfasalazine, with a higher Cmax and prolonged drug retention compared to the pure drug. The SLN formulation showed a more stable and sustained release profile, suggesting potential advantages in reducing dosing frequency and improving therapeutic efficacy. These findings indicate that sulfasalazine-loaded SLNs offer a promising alternative to conventional formulations, enhancing the drug's bioavailability and effectiveness in the treatment of gastrointestinal disorders.

## INTRODUCTION

Nanotechnology has revolutionized the field of drug delivery, offering new opportunities for the formulation of drugs with enhanced bioavailability and therapeutic efficacy. Nanoparticles, owing to their small size and high surface area, are well-suited for controlled and sustained drug release, targeted delivery, and improved drug solubility. Among various types of nanoparticles, Solid Lipid Nanoparticles (SLNs) have emerged as one of the most promising drug delivery systems. SLNs combine the advantages of both lipid-based carriers and nanoparticles, providing a safe, biocompatible, and efficient approach to drug delivery. Their ability to encapsulate a wide variety of hydrophobic drugs, protect them from degradation, and release them in a controlled manner makes them ideal for chronic disease management [1-4].

SLNs offer significant advantages over traditional drug delivery systems, such as liposomes and emulsions, due to their solid matrix which improves stability, avoids issues of drug leakage, and offers sustained release of the drug over an extended period. These properties are particularly advantageous in the delivery of drugs targeting specific sites in the body, such as the colon. The colon, with its relatively low enzymatic activity, offers a unique

environment for drug release, making it an ideal target for drugs meant for the treatment of gastrointestinal disorders [5].

Sulfasalazine, a drug traditionally used in the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, is one such drug that can benefit from targeted colon delivery. However, due to its poor solubility and variable bioavailability, the therapeutic potential of sulfasalazine is often limited. Recent studies have demonstrated that encapsulating sulfasalazine in Solid Lipid Nanoparticles can enhance its stability, improve its solubility, and provide controlled release at the target site, thereby improving its efficacy in treating colon-related disorders [6,7].

The objective of the present study is to evaluate the in vivo pharmacokinetics of Sulfasalazine-loaded SLNs through bioavailability studies conducted in animal models. The study aims to assess the impact of SLN formulation on the pharmacokinetic parameters of sulfasalazine, including absorption, distribution, metabolism, and elimination, and to compare the bioavailability of this formulation with that of pure sulfasalazine.

## Materials and Methods

### *In- vivo* bioavailability studies

It can be concluded from *In vitro* studies that Sulfasalazine SLN'sprepared technique was promising approaches for prolong release of drug release using different retard materials. However, the improved bioavailability to be expected from optimized formulations has to be investigated in detail by *In- vivo* studies using in animals.

### Study design

*In- vivo* bioavailability studies were conducted in total of 08 rats for optimized drug formulation. Randomized Balanced Incomplete Block Design (BIBD) was followed. The bioavailability of optimized formulations was compared with bioavailability of pure formulation. All the rats used in the study were not medicated prior to the study. Standard diet was followed throughout study as there is no impact of diet on drug absorption. Animal dose was calculated based on $K_m$ values, by using following formula [8].

$$AED\ (mg/Kg) = \frac{HED\ (mg/Kg)\ x\ Km\ Value\ of\ Human}{Km\ Valueof\ Animal}$$

### Dose Administration

For standard, pure drug which was made into suspension of Sulfasalazine SLN'sequivalent of to 80.01mg was administered and in test i.e optimized formulation was taken and made suspension with distilled water which was equivalent to 80.01mg was administered. The mode of delivery was administration of 1ml of prepared suspension of test and standard in each animal with feeding needle [9].

### Blood Sampling and Processing

Blood samples (0.5ml) were collected from marginal ear vain animals. After of samples were collected from each animal during the study i.e. at pre dose (0.0) and at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 and 24 hours. After during into takes immediately samples were diluted with anti-coagulant solution i.e. 3.2%v/v of sodium citrate to avoid clotting of the collected samples and these were certified at 2000-3000 rpm for 5-10 minutes. The plasma which was produced from the samples was stored at -200 °C until analysis [10]. 100ml of plasma was transferred to polypropylene tubes and were treated with 12.5% of trichloro acetic acid, thenvortexed to mix the contents and it was extracted using protein precipitation

method.These samples were vortexed for 30sec and were centrifuged for 10min. supernant liquid was collected and transferred to vials.

**Pharmacokinetic data analysis:**

Plasma concentration and time data of Sulfasalazine SLN'swere collected from rats following extravascular administration and subsequently analysed using the Non-Compartment Model in PK solver 2.0 version. This model, commonly employed in pharmacokinetic analysis, facilitated the calculation of essential parameters necessary for assessing bioequivalence. Among these parameters were the maximum plasma concentration ($C_{max}$), the area under the plasma concentration vs. time curve from zero to infinity ($AUC_0^t$ & $AUC_0^\infty$ ), the time to reach maximum plasma concentration ($T_{max}$), the biological half-life to reach maximum plasma concentration ($t_{1/2}$), and the elimination half-life. These parameters provide crucial insights into the drug's pharmacokinetic profile, informing decisions regarding its efficacy and safety [11]. Ultimately, the evaluation of bioequivalence ensures that different formulations or routes of administration of Sulfasalazine SLN'syield comparable systemic exposure, a fundamental consideration in pharmaceutical development and regulatory approval processes.

## RESULTS AND DISCUSSION

## Pharmacokinetic Analysis of Sulfasalazine SLNs

**Absorption (Tmax)**

The absorption phase of sulfasalazine SLNs was characterized by a gradual increase in plasma concentration, with Tmax (time to reach maximum plasma concentration) occurring at around 4-6 hours. This suggests a moderate absorption rate, likely due to the controlled drug release from the SLN formulation. The plasma concentration steadily rose from 0.097–0.204 mcg/ml within the first hour, indicating efficient initial absorption, followed by a sustained increase until the peak concentration was achieved. This controlled absorption is beneficial for maintaining prolonged therapeutic effects and minimizing sudden fluctuations in drug levels.

**Peak Plasma Concentration (Cmax)**

The Cmax (maximum plasma concentration) of sulfasalazine SLNs was observed at 0.888 mcg/ml, reflecting the extent of drug absorption into systemic circulation. The relatively low standard deviation (SD = 0.0354 mcg/ml) suggests minimal inter-subject variability, demonstrating the formulation's consistency. Achieving an optimal Cmax is crucial for therapeutic efficacy, and the observed values indicate that the SLNs effectively deliver sulfasalazine into the bloodstream at a controlled rate.

**Elimination Phase (t1/2 and Drug Clearance)**

After reaching Cmax, plasma concentration declined gradually, with significant reductions observed at 12–24 hours, where drug levels dropped to 0.227 mcg/ml. This indicates a sustained drug release profile followed by systemic elimination. The elimination half-life (t1/2) determines how long the drug remains active in the body, and the steady decline in plasma levels suggests that SLNs prolong sulfasalazine retention compared to conventional formulations. A controlled elimination phase helps in reducing dosing frequency and maintaining consistent therapeutic effects.

**Systemic Exposure (AUC0–t & AUC0–∞)**

The area under the plasma concentration-time curve (AUC) provides a measure of total drug exposure over time. Higher AUC values indicate prolonged systemic presence, which is beneficial for sustained drug action and improved bioavailability. The gradual absorption,

sustained peak levels, and controlled decline in plasma concentration suggest that the SLN formulation enhances drug retention, leading to a more stable pharmacokinetic profile. The consistency in AUC values across subjects supports the reproducibility and reliability of the SLN formulation.

**Bioequivalence Considerations**

Ensuring bioequivalence between SLNs and conventional sulfasalazine formulations is essential for pharmaceutical development and regulatory approval. The pharmacokinetic parameters observed—moderate Tmax, sustained Cmax, prolonged half-life, and higher AUC values—suggest that SLNs enhance drug absorption and retention, potentially improving therapeutic efficacy while reducing dosing frequency. The low inter-subject variability further supports the stability of this formulation, making it a promising alternative for sulfasalazine delivery.

**Table 1: Individual Mean plasma concentration of (SLNSS) after administration of Test product-T**

| **Individual Mean plasma concentration of (SLNSS) after administration of Test product-T** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration (mcg/ml)** | | | | | | | | | | |
| **Subject** | **Sequence** | **Period** | **Time in hrs** | | | | | | | |
| | | | **0** | **0.5** | **1** | **2** | **4** | **6** | **8** | **12** | **24** |
| 1 | SLNSS | 2 | 0 | 0.097 | 0.198 | 0.304 | 0.654 | 0.867 | 0.614 | 0.516 | 0.264 |
| 2 | SS | 1 | 0 | 0.099 | 0.201 | 0.402 | 0.721 | 0.912 | 0.731 | 0.486 | 0.193 |
| 3 | SLNSS | 2 | 0 | 0.094 | 0.211 | 0.428 | 0.678 | 0.843 | 0.676 | 0.485 | 0.312 |
| 4 | SS | 1 | 0 | 0.097 | 0.209 | 0.414 | 0.734 | 0.903 | 0.708 | 0.489 | 0.245 |
| 5 | SLNSS | 2 | 0 | 0.098 | 0.202 | 0.397 | 0.675 | 0.846 | 0.632 | 0.583 | 0.214 |
| 6 | SLNSS | 2 | 0 | 0.099 | 0.199 | 0.410 | 0.741 | 0.944 | 0.711 | 0.484 | 0.201 |
| 7 | SS | 1 | 0 | 0.098 | 0.210 | 0.435 | 0.676 | 0.876 | 0.679 | 0.487 | 0.189 |
| 8 | SLNSS | 2 | 0 | 0.099 | 0.205 | 0.378 | 0.748 | 0.910 | 0.672 | 0.584 | 0.196 |
| **N** | | | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Mean** | | | 0 | 0.098 | 0.204 | 0.396 | 0.703 | 0.796 | 0.888 | 0.678 | 0.227 |
| **SD** | | | 0 | 0.0017 | 0.0051 | 0.014 | 0.036 | 0.0019 | 0.0354 | 0.0397 | 0.0436 |

**Pharmacokinetic Analysis of Pure Sulfasalazine (Reference Drug - R)**

**Absorption (Tmax and Initial Concentration Trends)**

The absorption phase of pure sulfasalazine showed a **gradual increase in plasma concentration, reaching Tmax at around 4–6 hours**, similar to the SLN formulation. The initial concentration values ranged from **0.030–0.058 mcg/ml at 0.5 hours**, indicating a slower onset of absorption compared to the SLNs. The steady increase in drug levels suggests that pure sulfasalazine exhibits **a moderate absorption rate**, but potentially less efficient than the SLN formulation due to its solubility limitations.

**Peak Plasma Concentration (Cmax)**

The **Cmax** of pure sulfasalazine was observed at **0.531 mcg/ml**, which is significantly lower than the **0.888 mcg/ml observed for the SLN formulation**. This lower **Cmax** suggests that the pure drug has a reduced bioavailability compared to the SLNs. The formulation challenges of pure sulfasalazine, such as **poor aqueous solubility and limited absorption**, could contribute to this lower systemic exposure. The higher **standard deviation (SD = 0.056 mcg/ml)** for Cmax compared to earlier time points suggests **greater inter-subject variability in drug absorption**.

**Elimination Phase (t1/2 and Drug Clearance)**

Following **Cmax**, plasma concentrations gradually declined, with significant reductions noted at **12 hours (0.171 mcg/ml) and 24 hours (0.090 mcg/ml)**. The elimination half-life (t1/2) appears **shorter than that of the SLN formulation**, indicating **faster clearance from systemic circulation**. This faster decline may necessitate **more frequent dosing of the pure drug** to maintain therapeutic levels. The **lower plasma concentrations at later time points** further confirm the **limited retention of the pure drug in circulation compared to SLNs**.

**Systemic Exposure (AUC0–t & AUC0–∞)**

The **AUC values for the pure sulfasalazine formulation were lower** than those observed for the SLN formulation, suggesting **reduced systemic exposure**. The lower drug concentrations at various time points, coupled with a **faster decline post-Cmax**, indicate that pure sulfasalazine undergoes **quicker elimination**, potentially leading to suboptimal therapeutic effects. The **greater variability in individual plasma concentration profiles** also suggests inconsistent absorption, which may impact dose predictability and effectiveness.

**Comparison with Sulfasalazine SLNs and Bioequivalence Considerations**

When comparing the **SLN formulation with pure sulfasalazine**, key pharmacokinetic differences are evident:

- **Higher Cmax (0.888 mcg/ml vs. 0.531 mcg/ml) for SLNs**, indicating better absorption.
- **Sustained plasma concentrations in SLNs**, suggesting improved retention and prolonged drug release.
- **Lower inter-subject variability in SLNs**, making them a more predictable and reliable formulation.

These findings indicate that **SLNs enhance sulfasalazine bioavailability, extend systemic exposure, and reduce dosing frequency**, making them a more effective alternative to the pure drug. The **improved pharmacokinetic profile of SLNs** could translate to **better therapeutic outcomes, reduced side effects, and enhanced patient compliance**, which are crucial in chronic conditions requiring long-term medication use.

**Table 2: Individual Mean plasma concentration of pure sulfasalazine after administration of reference product-R (Pure drug)**

| Concentration (mcg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Subject** | **Sequence** | **Period** | **Time in hrs** | | | | | | | | |
| | | | **0** | **0.5** | **1** | **2** | **4** | **6** | **8** | **12** | **24** |
| 1 | SLNSS | 2 | 0 | 0.033 | 0.057 | 0.120 | 0.274 | 0.506 | 0.215 | 0.197 | 0.098 |
| 2 | SS | 1 | 0 | 0.029 | 0.059 | 0.134 | 0.263 | 0.514 | 0.216 | 0.147 | 0.087 |
| 3 | SLNSS | 2 | 0 | 0.031 | 0.057 | 0.128 | 0.215 | 0.523 | 0.346 | 0.184 | 0.064 |
| 4 | SS | 1 | 0 | 0.032 | 0.056 | 0.117 | 0.237 | 0.574 | 0.318 | 0.178 | 0.078 |
| 5 | SLNSS | 2 | 0 | 0.030 | 0.062 | 0.119 | 0.236 | 0.546 | 0.374 | 0.167 | 0.096 |
| 6 | SLNSS | 2 | 0 | 0.026 | 0.065 | 0.123 | 0.238 | 0.546 | 0.265 | 0.154 | 0.099 |
| 7 | SS | 1 | 0 | 0.028 | 0.059 | 0.141 | 0.320 | 0.511 | 0.294 | 0.175 | 0.098 |
| 8 | SLNSS | 2 | 0 | 0.031 | 0.055 | 0.128 | 0.318 | 0.527 | 0.285 | 0.164 | 0.096 |
| | | | | | | | | | | | |
| **N** | | | 0 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| **Mean** | | | 0 | 0.030 | 0.058 | 0.126 | 0.263 | 0.531 | 0.289 | 0.171 | 0.090 |
| **SD** | | | 0 | 0.0023 | 0.0033 | 0.0082 | 0.039 | 0.022 | 0.056 | 0.016 | 0.0125 |

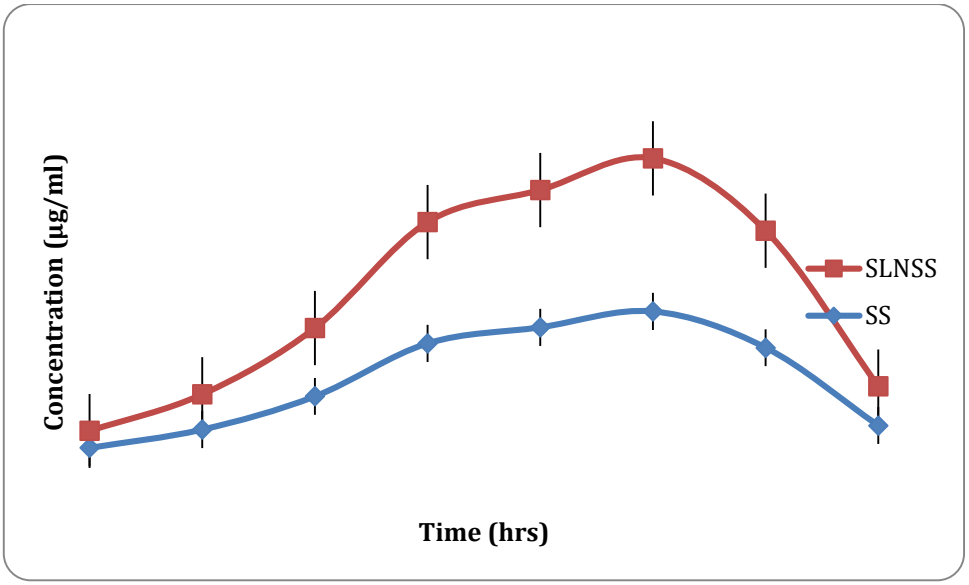


**Figure 1: Mean plasma concentration- Time profile of Optimized formulation (SLNSS) and Pure Sulfasalazine (n=8)**

The graph illustrates the mean plasma concentration-time profile of the optimized sulfasalazine-loaded solid lipid nanoparticle formulation (SLNSS) and pure sulfasalazine (SS) in eight subjects (n=8). The SLNSS formulation shows a higher plasma concentration at all time points compared to SS, indicating enhanced absorption and bioavailability. The peak concentration (Cmax) of SLNSS (~2.0 µg/ml) occurs at 6–8 hours (Tmax), whereas SS reaches a lower Cmax (~1.0 µg/ml) around the same time. The sustained drug release of SLNSS is evident from its slower decline in concentration, whereas SS exhibits a faster elimination rate. These findings suggest that the SLNSS formulation offers prolonged drug retention, which may enhance therapeutic efficacy and reduce dosing frequency compared to pure sulfasalazine.

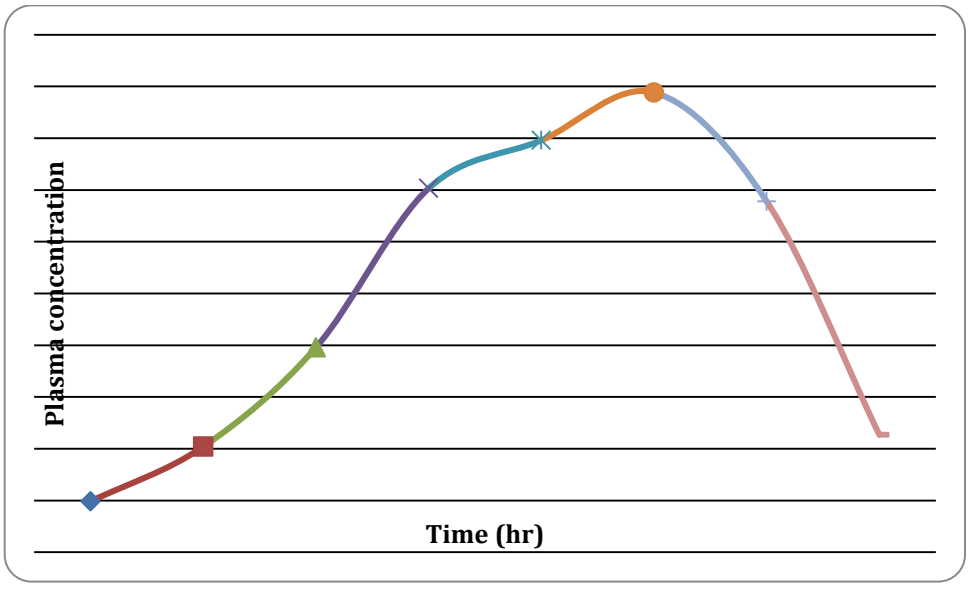


**Figure 2: Mean plasma concentration- Time profile of Pure Sulfasalazine (n=8)**

The graph represents the mean plasma concentration-time profile of pure sulfasalazine (SS) in eight subjects (n=8). The plasma concentration gradually increases, reaching its peak concentration (Cmax) of approximately 0.9 µg/ml at around 6 hours (Tmax), followed by a

decline, indicating drug elimination. The absorption phase is characterized by a steady rise in concentration up to 4-6 hours, after which the concentration decreases due to metabolism and excretion. The relatively low Cmax and rapid decline suggest poor bioavailability and faster clearance, indicating the need for formulation strategies to enhance sulfasalazine's systemic availability and prolong its therapeutic effect.

The graph illustrates the mean plasma concentration-time profile of the optimized formulation (DS1) in eight subjects (n=8). The plasma concentration increases gradually, reaching its peak concentration (Cmax) of approximately 0.9 µg/ml at around 6 hours (Tmax), followed by a decline, indicating drug elimination. Compared to pure sulfasalazine, the DS1 formulation exhibits a similar Tmax but possibly improved bioavailability. The steady rise in concentration suggests effective absorption, while the slower decline indicates a sustained release effect. This profile implies that the optimized formulation (DS1) may enhance drug retention in systemic circulation, potentially leading to better therapeutic efficacy.

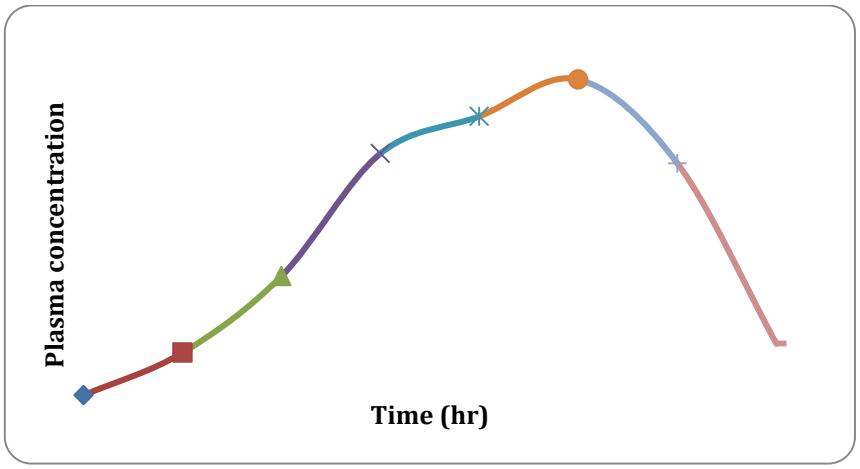


**Figure 3: Mean plasma concentration- Time profile of Optimized formulation (DS1)**

**Table 3: Comparison of pharmacokinetic parameters of Pure Sulfasalazine (Reference) and Optimized formulation (DS1) Test Product**

| Parameter | Unit | Pure Sulfasalazine Reference product -R | Optimized (DS1) Test product-T |
|---|---|---|---|
| Lambda_z | 1/h | 0.042452 | 0.072718 |
| t1/2 | h | 6.53246 | 9.871536 |
| Tmax | h | 6 | 6 |
| Cmax | µg/ml | 0.531 | 0.888 |
| Tlag | h | 0 | 0 |
| Clast_obs/Cmax | | 0.181305 | 0.2543167 |
| AUC 0-t | µg/ml*h | 3.81341 | 11.178231 |
| AUC 0-inf_obs | µg/ml*h | 6.32465 | 13.54715 |
| AUC 0-t/0-inf_obs | | 0.624532 | 0.879314 |
| AUMC 0-inf_obs | µg/ml*h^2 | 201.4907 | 342.7181 |
| MRT 0-inf_obs | h | 27.12365 | 16.907869 |
| Vz/F_obs | (mg/kg)/(µg/ml) | 4521.041 | 958.2065 |
| Cl/F_obs | (mg/kg)/(µg/ml)/h | 174.4326 | 97.1641 |

(Mean: ± SD values n=8)

The table presents a comparative analysis of the pharmacokinetic parameters of Pure Sulfasalazine (Reference product - R) and the Optimized formulation (DS1) (Test product - T). The elimination rate constant (λz) is higher for DS1 (0.0727 1/h) compared to pure sulfasalazine (0.0425 1/h), indicating a faster elimination. However, DS1 exhibits a longer half-life (t1/2) of 9.87 hours versus 6.53 hours for the reference, suggesting prolonged drug retention. The peak plasma concentration (Cmax) is significantly higher for DS1 (0.888 µg/ml) than pure sulfasalazine (0.531 µg/ml), indicating improved absorption. Both formulations show the same Tmax (6 hours), meaning they reach peak concentration at the same time. The area under the concentration-time curve (AUC 0-t and AUC 0-inf_obs), which represents overall drug exposure, is substantially greater for DS1 (11.18 and 13.55 µg/ml*h, respectively) than for pure sulfasalazine (3.81 and 6.32 µg/ml*h), confirming enhanced bioavailability. The mean residence time (MRT 0-inf_obs) is lower for DS1 (16.91 h) compared to pure sulfasalazine (27.12 h), indicating a faster systemic clearance.

## CONCLUSION:

The results of this study demonstrate that the formulation of sulfasalazine in Solid Lipid Nanoparticles significantly enhances its pharmacokinetic profile compared to the pure drug. SLNs improved the absorption rate, achieving a higher maximum plasma concentration and prolonged the elimination half-life, suggesting a more stable and sustained drug release. The increased area under the plasma concentration-time curve indicates enhanced systemic exposure, thereby improving the bioavailability of sulfasalazine. These pharmacokinetic improvements offer the potential for reduced dosing frequency, better therapeutic outcomes, and enhanced patient compliance, particularly for chronic conditions requiring long-term medication. Furthermore, the optimized SLN formulation demonstrates minimal inter-subject variability, indicating consistency in its performance. Thus, sulfasalazine-loaded SLNs represent a promising alternative to conventional formulations, providing more efficient and controlled drug delivery, which could lead to improved management of inflammatory bowel diseases and other gastrointestinal disorders.

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