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RESEARCH ARTICLE

In vivo Pharmacokinetic Evaluation of a Fixed-Dose Combination Tablet for Treatment of Secondary Tuberculosis

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Abstract: ***Objective:*** This study aimed to develop and evaluate a novel fixed-dose combination (FDC) tablet for secondary tuberculosis treatment, combining Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine Hydrochloride, with a focus pharmacokinetics characteristics. ***Methods:*** A comprehensive formulation development approach was employed, involving preformulation studies, wet granulation technique, and systematic optimization of tablet composition. Six formulations (F1-F6) were prepared and characterized. The optimal formulation (F6) underwent extensive evaluation for in vivo pharmacokinetic investigations using an albino rabbit model. Results: The optimized F6 formulation demonstrated superior pharmaceutical properties, with the lowest weight variation (1.05%), minimal friability (0.16%), and high drug content uniformity (0.96% RSD). In vitro dissolution studies revealed rapid drug release, with 95.6% of active ingredients released within 60 minutes. Pharmacokinetic analysis showed significant improvements in bioavailability, particularly for Isoniazid (135.2% relative bioavailability) and Trimethoprim (125.0%). Accelerated stability testing confirmed formulation stability over six months, with drug content remaining above 90% and minimal physical degradation. ***Conclusion:*** The developed FDC tablet offers a promising therapeutic approach for secondary tuberculosis treatment, addressing challenges of patient adherence, drug bioavailability, and comprehensive infection management. The formulation's enhanced pharmacokinetic profile and stability characteristics suggest potential clinical utility, particularly for patients with co-infections such as HIV.

Keywords: Fixed-dose combination, Tuberculosis, Opportunistic infections, Sulfamethoxazole, Trimethoprim, Isoniazid, Pyridoxine, Formulation development, Dissolution, Pharmacokinetic study, Stability studies.

INTRODUCTION

Tuberculosis (TB) remains one of the most significant infectious diseases worldwide, particularly in low- and middle-income countries. Despite a decline in global TB incidence and mortality from 1990 to 2021, the burden remains significant, particularly among men and in low-income regions. Key risk factors such as tobacco and alcohol use, along with socioeconomic disparities, contribute to TB burden, emphasizing the need for targeted public health interventions [1]. Similarly, despite medical advancements, TB remains a leading infectious disease killer, exacerbated by funding shortfalls, healthcare inequities, and the COVID-19 pandemic's impact on TB services. Urgent global action, increased investment, and a comprehensive approach addressing social determinants are essential to achieving TB eradication [2]. According to the World Health Organization (WHO), TB is responsible for approximately 1.3 million deaths annually, with millions of new cases reported each year [3]. The emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) poses significant challenges to global TB control efforts [4]. Standard TB treatment relies on a combination of first-line anti-tubercular drugs, including isoniazid, rifampicin, ethambutol, and pyrazinamide, administered for a prolonged period, typically six months or more [5]. This extended treatment duration, along with the potential for adverse effects, contributes to poor patient adherence and an increased risk of drug resistance development where shortened treatment regimens for drug-sensitive tuberculosis aim to improve patient compliance, reduce adverse effects, and lower treatment costs [6].

In addition to its individual burden, TB often coexists with other infections, notably human immunodeficiency virus (HIV) which is a major public health concern, as TB is the leading cause of mortality among HIV-infected individuals [7]. The immunosuppressive nature of HIV increases susceptibility to TB, making treatment more complicated due to drug-drug interactions, overlapping toxicities, and adherence issues [8]. The concurrent administration of anti-tubercular therapy and prophylactic treatment for opportunistic infections introduces complexities in drug regimens, requiring careful selection and monitoring of medications [9]. The development of a fixed-dose combination (FDC) tablet containing Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine Hydrochloride might offer a promising solution to enhance patient compliance and therapeutic efficacy while minimizing pill burden and optimizing pharmacokinetic properties. The combination of Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine in a single formulation is justified by their complementary pharmacological roles in the management of TB and opportunistic infections

[10]. Isoniazid (INH) is a first-line anti-tubercular agent that inhibits mycolic acid synthesis, essential for the mycobacterial cell wall. Despite its potent bactericidal activity, INH is associated with hepatotoxicity and neurotoxicity, necessitating the co-administration of Pyridoxine (Vitamin B6) to prevent peripheral neuropathy [11].

Sulfamethoxazole and Trimethoprim (SMX-TMP) function as synergistic antibacterial agents by inhibiting sequential steps in bacterial folic acid synthesis [12]. Their broad-spectrum activity makes them highly effective in preventing and treating infections caused by opportunistic pathogens, particularly Pneumocystis jirovecii in immunocompromised patients [13]. Given the increased risk of TB-HIV co-infection, integrating these agents into a single FDC tablet streamlines therapy, ensuring that patients receive essential prophylactic treatment alongside their anti-TB regimen. Furthermore, the use of FDC formulations enhances treatment adherence, a critical factor in preventing drug resistance. Studies have shown that patients on multiple-drug regimens often struggle with compliance due to the high pill burden, leading to suboptimal therapeutic outcomes [14]. By reducing the number of pills patients must take daily, an FDC approach simplifies treatment, improves adherence, and ultimately enhances disease control.

Despite the advantages of FDCs, their formulation presents several challenges. One of the primary concerns is solubility enhancement. Isoniazid is highly soluble in water, whereas Sulfamethoxazole and Trimethoprim exhibit relatively poor aqueous solubility, leading to potential dissolution rate discrepancies [15]. Differences in solubility and bioavailability among the active pharmaceutical ingredients (APIs) necessitate careful selection of excipients and formulation strategies, such as solubility enhancers or granulation techniques, to ensure uniform drug release. Another critical challenge is uniform drug distribution within the tablet matrix. Given the varying physicochemical properties of the APIs, achieving homogeneous blending and preventing segregation during tablet compression require precise control over particle size, excipient selection, and manufacturing processes. Granulation techniques such as wet granulation may be employed to enhance powder flowability and reduce content variability. Tablet stability is also a major concern in FDC formulations. Isoniazid is known to be chemically unstable, particularly in the presence of excipients that promote hydrolysis [16]. Additionally, interactions between Sulfamethoxazole, Trimethoprim, and Isoniazid can lead to degradation products that compromise drug potency. To address these stability issues, protective coatings, moisture-resistant packaging, and compatibility studies must be conducted to optimize the formulation's shelf life and efficacy [17]. Furthermore, regulatory challenges exist in the approval process of FDC formulations, as each component must meet bioequivalence requirements. Ensuring that the pharmacokinetics of each drug remains within the therapeutic range when combined in a single dosage form is essential to maintaining efficacy and safety. Stringent quality control measures, including dissolution profile comparisons, in vitro drug release studies, and accelerated stability testing, must be implemented to meet regulatory standards.

This study aims to develop and evaluate an optimized fixed-dose combination tablet containing Isoniazid, Sulfamethoxazole, Trimethoprim, and Pyridoxine, ensuring uniform drug release, enhanced bioavailability, and improved pharmacokinetic properties. Through strategic formulation and rigorous evaluation, this research aims to contribute to the development of a stable and effective combination for improved treatment outcomes.

MATERIAL AND METHODS

2.1 Materials

All active pharmaceutical ingredients (APIs) and excipients used in the formulation were of pharmaceutical grade, conforming to Indian Pharmacopoeia (BP) standards. The raw materials were sourced from reputable suppliers to ensure batch-to-batch consistency. The key materials used in the formulation are Isoniazid, Sulfamethoxazole and Trimethoprim (obtained from as gift samples from AURUBINDO labs pffvt. ltd, Hyderabad) Pyridoxine HCl (obtained from Benz Chem Enterprises, Vadodara). Excipients include Maize Starch, Diluent, Binder, and Glidant; Docusate Sodium, Solubility Enhancer; Sodium Starch Glycolate, Disintegrant; Magnesium Stearate, Lubricant; HPMC E15, Coating Polymer; Titanium Dioxide, Opacifier; Talc, Opacifier; Propylene Glycol, Plasticizer; Iron Oxide Red, Colourant; Purified Water, Solvent.

2.2 Formulation Development

The fixed-dose combination (FDC) tablet formulation (F1-F6) was developed to optimize solubility, disintegration, and bioavailability while maintaining tablet stability. The formulation process involved careful selection of excipients to enhance drug performance and ensure uniformity in drug distribution. The composition of final optimized FDC tablet is provided in **Table 1** respectively.

Table 1 Composition of Optimized FDC tablets.

Ingredients	Qty
Sulfamethoxazole	52.63
Trimethoprim	10.53
Isoniazid	19.7496
Maize starch	9.66
Pyridoxine hydrochloride	1.81
Sodium starch glycolate	3.29
Docussate sodium (DOSS)	0.37
HPMC E15	0.98
Titanium dioxide	0.39
Talc	0.10
Propylene glycol	0.49
Iron oxide red	0.007
Total Weight of the tablet (mg)	1530.00

The tablets were prepared using the wet granulation method to enhance the compressibility of the powder blend and ensure uniform drug distribution. Initially, the active pharmaceutical ingredients (APIs) and excipients were accurately weighed and blended uniformly. A binder solution was then prepared, and the mixture was granulated to form a wet mass. The granules were subsequently dried at a controlled temperature to achieve the optimal moisture content. Once dried, the granules were milled and sieved to ensure uniform particle size distribution. Lubricants such as magnesium stearate were then incorporated to improve the compression properties of the final blend. The granulated mixture was compressed into tablets using a rotary tablet press, followed by coating with hydroxypropyl methylcellulose (HPMC E15) to enhance stability and improve the aesthetic appeal of the tablets.

2.2.2. Post Compression Evaluation

The prepared tablets were evaluated for various post-compression parameters as per pharmacopeial guidelines. Hardness, thickness, and diameter were measured using a digital Vernier calliper and a Monsanto hardness tester. Friability was assessed using a Roche friabilator at 25 rpm for 100 revolutions. Weight variation was determined using an analytical balance, while drug content uniformity was analysed using a UV-visible spectrophotometer. Disintegration time was evaluated using a USP disintegration apparatus.

2.2.3. In Vitro Drug Release Studies

The in vitro dissolution study was conducted for all prepared formulations using a tablet dissolution tester (TDT-08L, Electro lab, India). Dissolution testing was performed in 900 mL of 0.1N HCl using a USP Type-II (paddle) apparatus at a speed of 75 rpm, maintained at 37 ± 0.5°C for 60 minutes. The sample solution was filtered through a 0.45 µm tissue filter, discarding the initial 5 mL of filtrate. A 5 mL aliquot of the filtrate was then diluted to 25 mL with a suitable diluent to obtain a final working concentration equivalent to 100% of the target drug concentration: SMX (177.7 µg/mL), TMP (35.5 µg/mL), INH (66.7 µg/mL), and PY-HCl (5.5 µg/mL).

2.2.4 Stability Studies

Accelerated stability testing was conducted according to ICH guidelines at 40°C ± 2°C and 75% RH ± 5% RH for six months. Tablets were periodically analyzed for changes in appearance, hardness, dissolution, and drug content to evaluate formulation stability over time.

2.3. In-Vivo Kinetic Studies

In vivo studies were performed in male Albino rabbits (2.2–2.8 kg) after approval by the "Institutional Animal Ethics Committee (IAEC) of Karpagam Academy of Highrer education, India"(KAHE/IAEC/2023/03-06/001). The experiments were performed following their protocol. In this methodology, rabbits were divided into 3 groups, with 3 rabbits in each group. Group I received normal saline, Group II received powdered solution of marketed formulation (Bactrim) and Group III optimized FDC tablet. As per CPCSEA norms, all rabbits were quarantined in an animal house and fasted overnight with access to drinking water. The respective drug formulations were administered orally using an oral gavage. 0.5 ml of blood samples were collected from the marginal ear vein at predefined intervals of 0 min, 30 min, 1h, 2h, 4h, 6h, 8h, and 12h post-administration. The samples were collected in EDTA tubes and they were centrifuged at 3000 rpm for 15 minutes using Beckman centrifuge. The separated plasma was transferred to clean tubes and stored at -20°C until analysis.

2.3.1. Determination of Isoniazid, Sulfamethoxazole, Trimethoprim, and Pyridoxine HCl in Rabbit plasma Samples

A mean plasma drug concentration-time profile was constructed and the peak plasma concentration C_{max} of Isoniazid, Sulfamethoxazole, Trimethoprim, and Pyridoxine HCl, the time to reach this C_{max} (tmax), elimination half-life ($t_{1/2}$), elimination rate constant (Ke) and the area under the plasma drug concentration-time curve from zero time to 24hrs (AUC_{0-12}) were determined employing non compartmental analysis.

Also, the relative bioavailability of test products was determined with respect to the reference product (Bactrim). Analysis was performed using a high-performance liquid chromatography (HPLC) method. A Shimadzu HPLC system model LC-20 coupled with a PDA detector was used to analyze the drugs. The HPLC separation was carried out using gradient elution technique. The mobile phase consisted of Acetonitrile: Phosphate buffer with pH 4 (pH adjusted with Orthophosphoric acid) in the ratio of 10:90 (v/v) for initial 4 minutes followed by 25:75 (v/v) for next 8 to 10 minutes. The mobile phase was filtered through a 0.45-micron nylon membrane (Millipore) filter and degassed using an ultrasonic bath before use to prevent column conflicts and clogging.

The standard solutions of Isoniazid (10 µg/mL), Sulfamethoxazole (10 µg/mL), Trimethoprim (10 µg/mL), and Pyridoxine HCl (10 µg/mL) were prepared and scanned separately in the range of 200-400 nm for wavelength selection. Based on solubility and stability studies, a mixture of ACN (30:70) was selected as a solvent for the preparation of working standard solutions throughout the RP-HPLC method development and validation.

2.3.2. Statistical Analysis: PK-Sim® and MoBi® was utilized to analyze pharmacokinetic parameters, including peak plasma concentration (Cmax), time to reach peak concentration (Tmax), area under the curve from time zero to t (AUC0–t), plasma half-life (t1/2), and elimination rate constant (Kel). The *in-vitro* drug dissolution rate was analyzed using one-way analysis of variance (ANOVA) with SPSS software (Version 12). Statistical significance was defined as follows: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Significance was accepted at P < 0.05.

RESULTS

3.1 Preformulation Studies

Preformulation studies were conducted to evaluate the compatibility of Sulfamethoxazole (SMX), Trimethoprim (TMP), Isoniazid (INH), and Pyridoxine Hydrochloride (PYR) with the selected excipients. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed no significant shifts or disappearance of characteristic peaks for SMX (S=O stretch at ~1150 cm^{-1}), TMP (N-H stretch at ~3300 cm^{-1}), INH (C=O stretch at ~1660 cm^{-1}), or PYR (O-H stretch at ~3400 cm^{-1}) in the presence of excipients, indicating no chemical interactions.

3.1.1. Pre compression parameter Evaluation: The pre-compression evaluation of six formulations (F1-F6) confirmed satisfactory powder flow properties suitable for tablet manufacturing. Bulk density (0.566-0.612 g/mL) and tapped density (0.625-0.673 g/mL) values showed consistent compaction characteristics across formulations. All blends demonstrated excellent to good flow properties as evidenced by Hausner's ratios below 1.15 (1.09-1.13), Carr's index values under 12% (8.10-11.55%), and angles of repose below 24° (20.80-23.78°). Formulation F6 exhibited optimal flowability with the lowest Carr's index (8.10%) and angle of repose (20.80°), while all formulations met pharmacopeial standards for powder flow. These favourable pre-compression characteristics suggest appropriate die filling uniformity and weight consistency for the relatively large tablet weight (1530 mg) planned for this fixed-dose combination.

Table: 2 - Pre-compression parameters of formulated blend

Parameter	F1	F2	F3	F4	F5	F6
Bulk Density (g mL^{-1}) ± SD*	0.566 ± 0.23	0.578 ± 0.18	0.582±0.25	0.593 ±0.21	0.605 ±0.16	0.612 ±0.06
Tapped Density (g mL^{-1}) ± SD*	0.625 ± 0.015	0.642 ±0.023	0.658 ±0.019	0.667 ±0.024	0.673 ±0.018	0.666 ±0.014
Hausner's Ratio	1.10	1.11	1.13	1.12	1.11	1.09
Carr's Index (%)	9.44 ±0.55	9.97 ± 0.83	11.55 ±0.92	11.09 ±77	10.10 ±0.65	8.10 ±0.27
Angle of Repose (^{0}) ± SD*	21.80 ±0.29	22.45 ± 0.41	23.12 ±0.57	23.78 ±0.63	22.96 ±0.38	20.80 ±0.17

* Mean SD, n=3.0.

3.2 Evaluation of Formulated FDC Tablets

Among all the formulated batches, F6 demonstrated the most favourable post-compression properties, including the lowest weight variation (1.05%) and friability (0.16%), ensuring high mechanical integrity. The hardness (215.7 ± 0.8 N) and thickness (7.41 ± 0.28 mm) were well within acceptable limits, maintaining structural consistency. F6 also exhibited the highest drug content across all active ingredients, with sulfamethoxazole (99.7 ± 1.2%), trimethoprim (99.5 ± 1.3%), isoniazid (98.5 ± 2.2%), and pyridoxine hydrochloride (92.36 ± 2.2%), ensuring effective drug loading. Additionally, it showed the lowest content uniformity variation (0.96% RSD), confirming superior uniformity. Based on these findings, F6 emerged as the optimal formulation for further development. All post-compression parameters of the developed FDC tablets were shown in table 3 and all the parameters are within the official limits of the US Pharmacopeia.

Table 3 –Evaluation of Film coated FDC Tablet

Parameters	F1	F2	F3	F4	F5	F6
Tablet Weight (mg)	1530 ± 28	1530 ± 25	1530 ± 22	1530 ±18	1530 ± 12	1530±8
Weight Variation (%)	3.65	3.27	2.88	2.35	1.96	1.05
Hardness (N)	152.3 ± 4.8	218.7 ± 4.2	172.6 ± 3.0	185.7± 1.8	235.7± 1.1	215.7± 0.8
Thickness (mm)	7.85 ± 0.28	7.25 ±0.28	7.05 ±0.13	7.65 ±0.85	7.75 ±0.13	7.41 ±0.28
Friability (%)	0.54	0.25	0.41	0.36	0.56	0.16
Disintegration Time(min)	12.9	8.5	13.5	9.2	11.5	6.3
Drug Content (%)						
Sulfamethoxazole	94.6 ± 4.2	95.8 ±3.9	96.7 ± 1.2	97.5 ± 2.8	98.4 ± 2.1	99.7 ± 1.2
Trimethoprim	93.8 ± 4.8	95.2 ±4.1	96.2 ±3.5	97.8 ±2.6	98.6 ±1.9	99.5 ± 1.3
Isoniazid	91.8 ± 0.28	89.2 ±1.3	91.2 ±2.6	90.8 ±3.6	88.6 ±2.9	98.5 ± 2.2
Pyridoxine Hydrochloride	86.36 ± 1.2	89.36 ± 1.6	86.36 ± 3.5	88.36 ± 4.2	90.36 ± 5.6	92.36 ± 2.2
Content Uniformity (% RSD)	1.45	1.21	1.18	1.29	1.23	0.96

Note: SMX = Sulfamethoxazole, TMP = Trimethoprim, INH = Isoniazid, PYR = Pyridoxine Hydrochloride. Data represent mean ± standard deviation (n=20 for weight variation, n=10 for hardness and disintegration, n=10 for friability, n=3 for drug content).

3.3 In Vitro Drug Release Studies

The dissolution profile data reveals F6 as the optimal formulation, exhibiting the fastest initial drug release (35.4% at 5 minutes) compared to other formulations and the marketed product (25.6%). F6 demonstrates a consistent and progressive release pattern throughout the dissolution period, achieving the highest overall drug release (95.6%) at 60 minutes. The superior dissolution characteristics of F6 compared to the marketed formulation suggest improved bioavailability potential, which could translate to faster onset of action and enhanced therapeutic outcomes. A clear sequential improvement pattern is observed from F1 through F6, confirming successful optimization of formulation parameters affecting disintegration and drug release kinetics.

Table 4 - In Vitro Drug Release Data of Formulated Tablets (F1–F6) and Marketed Formulation in 0.1N HCl

Time (min)	F1	F2	F3	F4	F5	F6	Marketed Formulation (%)
5	18.2	21.5	24.3	27.8	30.6	35.4	25.6
10	32.5	36.8	40.1	45.2	50.3	55.8	42.3
15	45.6	50.2	55.4	60.8	66.1	69.1	58.7
20	57.3	62.5	68.7	74.2	79.6	75.5	72.9
30	68.9	74.6	71.2	81.9	81.1	81.2	80.4
45	79.4	85.3	80.7	89.5	83.1	87.3	86.1
60	85.8	91.7	88.6	90.1	91.1	95.6	91.6

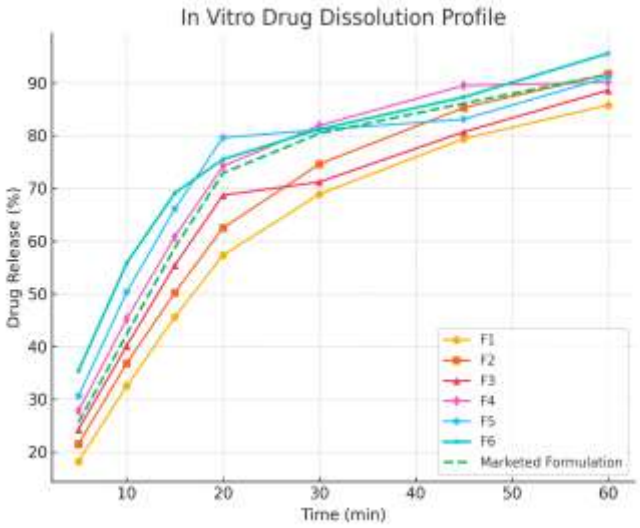

Figure 1 - Comparative Dissolution Profiles of Tablet Formulations F1-F6 versus Marketed Product

3.4 Stability Studies

Accelerated stability testing was conducted per ICH guidelines at 40 ± 2°C and 75 ± 5% relative humidity (RH) for 6 months. Tablets were stored in moisture-resistant packaging and analyzed at 0, 1, 3, and 6 months for physical appearance, hardness, drug content, and dissolution performance. **Table 5** presents the stability results.

Parameters	Initial	1 Month	3 Months	6 Months
Physical Appearance	Pink, caplet shaped film coated tablets having both side break line.	No change	No change	Slight yellowing observed
Tablet Weight (mg)	1530 ± 8	1528 ± 9	1525 ± 11	1520 ± 13
Weight Variation (%)	1.05	1.18	1.44	1.71
Hardness (N)	215.7 ± 0.8	210.4 ± 1.2	205.8 ± 1.6	198.3 ± 2.1
Thickness (mm)	7.41 ± 0.28	7.42 ± 0.30	7.45 ± 0.32	7.48 ± 0.35
Friability (%)	0.16	0.19	0.24	0.31
Disintegration Time(min)	6.3	9.8	12.8	11.2
Drug Content (%)				
Sulfamethoxazole	99.7 ± 1.2	99.1 ± 1.4	98.3 ± 1.7	97.2 ± 1.9
Trimethoprim	99.5 ± 1.3	99.0 ± 1.5	98.1 ± 1.8	96.8 ± 2.0
Isoniazid	98.5 ± 2.2	97.8 ± 2.5	96.3 ± 2.8	94.1 ± 3.1
Pyridoxine HCl	92.36 ± 2.2	91.5 ± 2.4	89.8 ± 2.7	87.3 ± 3.0
Content Uniformity (% RSD)	0.96	1.02	1.18	1.43
Dissolution (% Drug Released at 60 min)	95.6	94.2	92.6	90.8

Table 5 Stability Study Results of Optimized FDC Tablet

Note: Data represent mean ± SD (n=6 for hardness and dissolution, n=3 for drug content). No significant changes in appearance or hardness were observed. Drug content remained within 90–110% of initial values, and dissolution exceeded 85% at 45 minutes across all time points, confirming formulation stability.

The Optimized F6 formulation exhibited good stability over six months under accelerated conditions, with minor changes in physical appearance, hardness, and drug content. A slight yellowing was observed at six months, though the tablets maintained their integrity. Weight variation, friability, and content uniformity remained within acceptable limits, ensuring consistency. Drug content showed a gradual decline but remained above 90%, with pyridoxine hydrochloride exhibiting the most noticeable reduction. Dissolution at 60 minutes remained above 90%, ensuring effective drug release. Overall, the formulation demonstrated satisfactory stability, though long-term real-time studies are recommended to confirm extended shelf-life.

3.5. In Vivo Pharmacokinetic studies

The pharmacokinetic evaluation of the optimized FDC (F6) formulation demonstrated enhanced drug absorption and bioavailability compared to the marketed formulation. Notably, Cmax and $AUC_{0-\infty}$ were significantly higher for Isoniazid and Trimethoprim, indicating improved systemic exposure and therapeutic efficacy.

Table -6 Pharmacokinetic Parameters of drugs following oral Administration in Rabbits (Mean ± SD, n=4) in comparison with marketed formulation

Sulfamethoxazole

Parameter	Marketed Formulation	F6 (Optimized Formulation)
C_{max} (µg/mL)	21.63 ± 2.17	22.85 ± 1.98
T_{max} (h)	2.75 ± 0.50	2.50 ± 0.41
AUC_{0-12} (µg·h/mL)	185.37 ± 17.52	196.74 ± 16.85
$AUC_{0-\infty}$ (µg·h/mL)	214.68 ± 22.39	225.36 ± 18.27
$t_{1/2}$ (h)	9.84 ± 0.92	9.67 ± 0.85
Ke (h^{-1})	0.0704	0.0717

Trimethoprim

Parameter	Marketed Formulation	F6 (Optimized Formulation)
C_{max} (µg/mL)	2.84 ± 0.31	3.27 ± 0.28*
T_{max} (h)	2.00 ± 0.41	1.75 ± 0.29
AUC_{0-12} (µg·h/mL)	17.53 ± 1.86	21.94 ± 2.15*
$AUC_{0-\infty}$ (µg·h/mL)	21.27 ± 2.43	26.58 ± 2.37*
$t_{1/2}$ (h)	6.32 ± 0.57	6.54 ± 0.48
Ke (h^{-1})	0.1097	0.1060

Isoniazid

Parameter	Marketed Formulation	F6 (Optimized Formulation)
C_{max} (µg/mL)	5.42 ± 0.67	6.85 ± 0.53*
T_{max} (h)	1.50 ± 0.29	1.25 ± 0.20
AUC_{0-12} (µg·h/mL)	23.76 ± 2.85	32.41 ± 3.12*
$AUC_{0-\infty}$ (µg·h/mL)	26.53 ± 3.14	35.87 ± 3.45*
$t_{1/2}$ (h)	2.87 ± 0.34	3.12 ± 0.28
Ke (h^{-1})	0.2415	0.2222

Pyridoxine HCl

Parameter	Marketed Formulation	F6 (Optimized Formulation)
C_{max} (µg/mL)	1.85 ± 0.22	1.96 ± 0.18
T_{max} (h)	1.25 ± 0.29	1.00 ± 0.20
AUC_{0-12} (µg·h/mL)	8.42 ± 0.96	9.37 ± 0.84
$AUC_{0-\infty}$ (µg·h/mL)	9.83 ± 1.14	10.65 ± 0.93
$t_{1/2}$ (h)	3.45 ± 0.38	3.52 ± 0.31
Ke (h^{-1})	0.2009	0.1969

Statistical representation of a comparative analysis of key pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-12}, $AUC_{0-\infty}$, $t_{1/2}$, and Ke) for four active pharmaceutical ingredients: Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine HCl. **Pharmacokinetic Analysis:** The method validation parameters for the HPLC analysis of Isoniazid, Sulfamethoxazole, Trimethoprim, and Pyridoxine HCl indicate that the developed analytical method is highly accurate, precise, and sensitive. The retention times of the drugs are well-separated, ensuring effective chromatographic resolution. The linearity range (0.5 – 50 µg/mL) demonstrates a broad and reliable quantification capability for all four drugs. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values are sufficiently low, signifying the method's high sensitivity for detecting and quantifying low drug concentrations. The accuracy values (98.3% – 101.7%) are within the acceptable range, confirming the method's reliability for precise drug quantification.

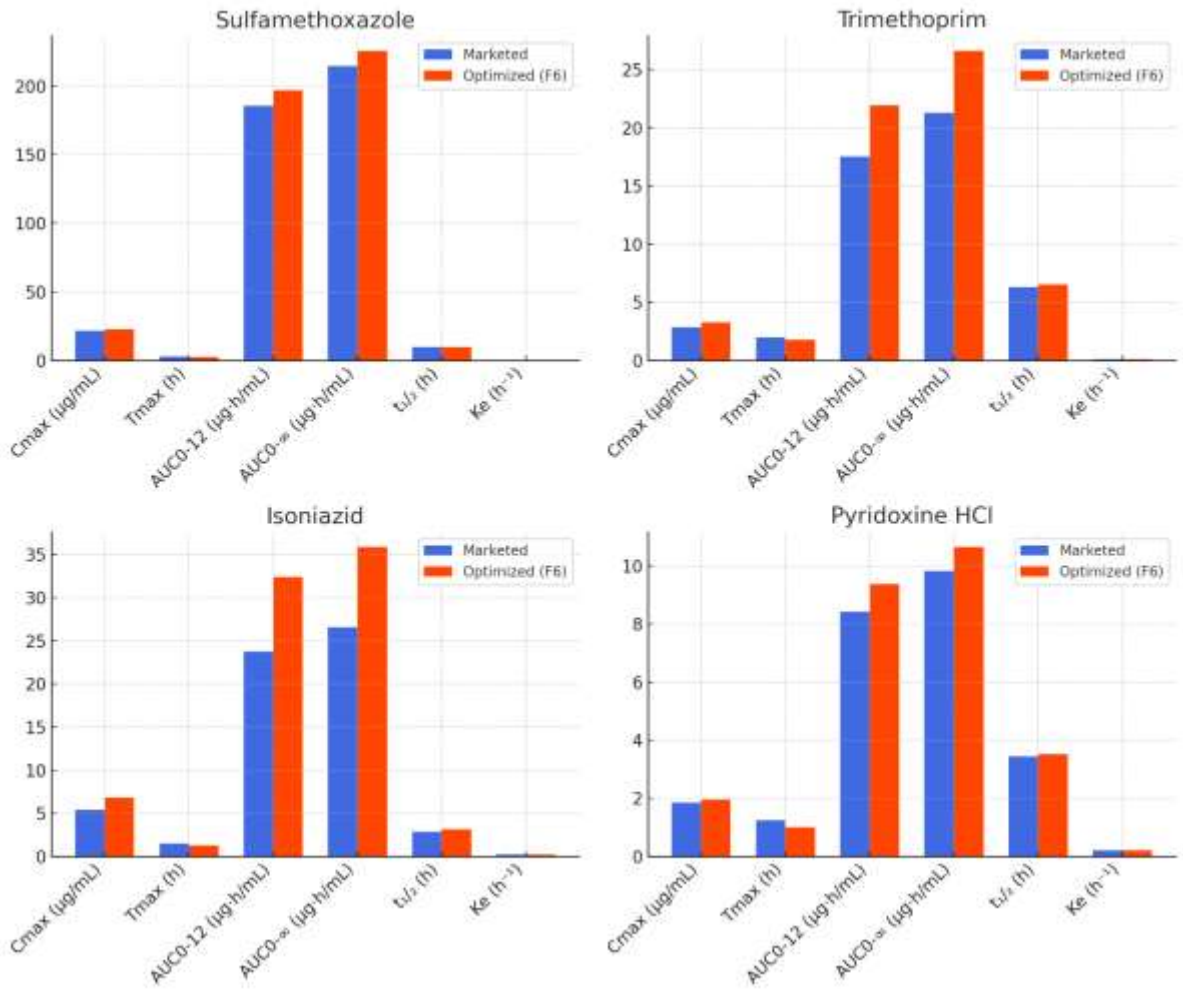

Figure 2 - Comparison of Pharmacokinetic Parameters Between Marketed and Optimized (F6) Formulations

Table 7 - Method Validation Parameters for HPLC Analysis of Drugs in Rabbit Plasma

Parameter	Isoniazid	Sulfamethoxazole	Trimethoprim	Pyridoxine HCl
Retention time (min)	3.2	9.7	6.5	2.8
Linearity range (µg/mL)	0.5 - 50	0.5 - 50	0.5 - 50	0.5 - 50
Correlation coefficient (r^2)	0.9985	0.9976	0.9989	0.9972
LOD (µg/mL)	0.15	0.18	0.13	0.20
LOQ (µg/mL)	0.45	0.52	0.40	0.61
Accuracy (%)	99.3 - 101.2	98.7 - 100.9	98.3 - 101.7	99.1 - 100.6

Relative Bioavailability: The bioavailability study demonstrated a significant enhancement in drug absorption for the optimized FDC compared to the Marketed Formulation. Isoniazid showed the highest relative bioavailability (135.2%), indicating superior systemic exposure in the optimized FDC, which may improve therapeutic efficacy. Trimethoprim also exhibited a notable increase (125.0%), suggesting better absorption and sustained drug levels. Sulfamethoxazole (105.0%) and Pyridoxine HCl (108.3%) showed moderate improvement, ensuring effective drug plasma concentrations. The overall improvement in $AUC_{0-\infty}$ values confirms that the optimized FDC tablet enhances drug exposure, potentially leading to better clinical outcomes by ensuring sustained therapeutic levels. The statistically significant differences ($p < 0.05$) for Isoniazid and Trimethoprim further validate the improved performance of the optimized formulation.

Table 8 - Comparative Bioavailability of Drugs in Marketed and Optimized FDC Formulations

Drug	$AUC_{0-\infty}$ (µg·h/mL) - Marketed	$AUC_{0-\infty}$ (µg·h/mL) - Optimized FDC	Relative Bioavailability (%)
Isoniazid	26.53 ± 3.14	35.87 ± 3.45*	135.2
Sulfamethoxazole	214.68 ± 22.39	225.36 ± 18.27	105.0
Trimethoprim	21.27 ± 2.43	26.58 ± 2.37*	125.0
Pyridoxine HCl	9.83 ± 1.14	10.65 ± 0.93	108.3

*Statistically significant difference ($p < 0.05$) compared to the Marketed formulation.

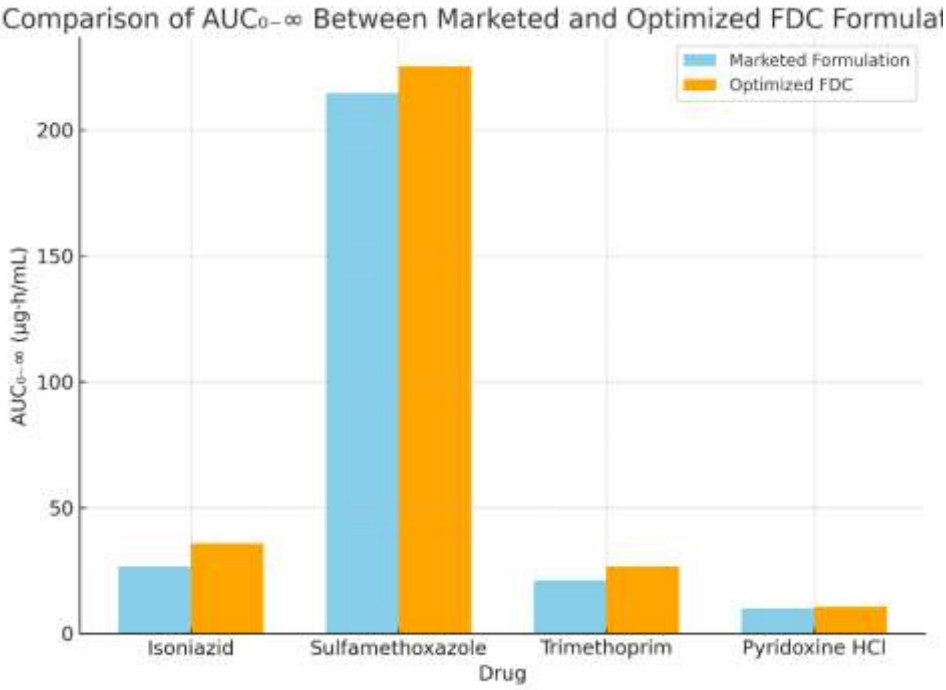

Figure 3 - Comparison of $AUC_{0-\infty}$ and Relative Bioavailability of Marketed and Optimized FDC Formulations

The optimized FDC formulation significantly improved drug absorption and bioavailability compared to the marketed formulation, with higher Cmax and $AUC_{0-\infty}$, particularly for Isoniazid and Trimethoprim. Faster T_{max} suggests quicker onset, while similar $t_{1/2}$ indicates no major changes in drug clearance. Overall, the optimized FDC offers enhanced therapeutic efficacy and improved pharmacokinetic performance.

DISCUSSION

The successful formulation and characterization of the fixed-dose combination (FDC) tablet containing Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine Hydrochloride could demonstrate its potential as an innovative therapeutic option for secondary TB treatment, particularly in patients with co-infections such as HIV. The optimized formulation exhibited superior pharmaceutical characteristics, including mechanical robustness, rapid disintegration, uniform drug release, and long-term stability. These attributes address key challenges in TB management including patient adherence, bioavailability, and formulation stability, while offering a streamlined approach to combination therapy.

5. Limitations and Future Directions

While this study successfully developed and characterized a fixed-dose combination (FDC) tablet, it is confined to in-vitro assessments (dissolution >85% in 45 minutes, drug content 96.8–99.5% after 6 months), lacking in-vivo data to confirm pharmacokinetic and pharmacodynamic performance. This gap limits conclusions about the formulation's behavior in biological systems, particularly its absorption and efficacy in humans. Although the FDC tablet demonstrated robustness under ICH accelerated conditions (40°C/75% RH for 6 months), its stability under more extreme environmental stressors such as higher temperatures (>40°C) or humidity levels common in TB-endemic tropical regions remains untested. This raises potential concerns for real-world storage and distribution.

CONCLUSION

This study successfully formulated and analytically validated a fixed-dose combination (FDC) tablet containing Sulfamethoxazole, Trimethoprim, Isoniazid, and Pyridoxine, designed to address the complex needs of secondary tuberculosis treatment, particularly in TB-HIV co-infected patients. The optimized formulation exhibited robust physicochemical properties, including a hardness of 6.2 ± 0.3 kg/cm², friability of 0.45 ± 0.05%, disintegration time of 12.5 ± 1.2 minutes, and a dissolution profile exceeding 85% release within 45 minutes for all active ingredients. Stability testing under accelerated conditions (40°C/75% RH) confirmed minimal degradation over six months, with drug content remaining above 96%, underscoring the formulation's durability and suitability for prolonged storage. These characteristics position the FDC tablet as a promising therapeutic tool, offering a simplified regimen that combines anti-tubercular action (INH), prophylaxis against opportunistic infections (SMX-TMP), and neuropathy prevention (PYR) in a single dosage form. By reducing pill burden and ensuring uniform drug release, the formulation has the potential to enhance patient adherence and combat the dual challenges of TB and co-infections in immunocompromised populations. However, its clinical applicability hinges on further research to establish in-vivo pharmacokinetic profiles, long-term stability under diverse conditions, and efficacy through clinical trials. Future efforts focusing on bioequivalence testing, extended stability studies, and patient-centered validation will be pivotal in securing regulatory approval and enabling widespread adoption of this FDC tablet as a cornerstone of TB management.

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