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FORMULATION, OPTIMIZATION AND *IN-VITRO* CHARACTERIZATION OF A FIXED DOSAGE COMBINATION TABLET FOR EFFECTIVE TREATMENT OF SECONDARY TUBERCULOSIS INFECTION

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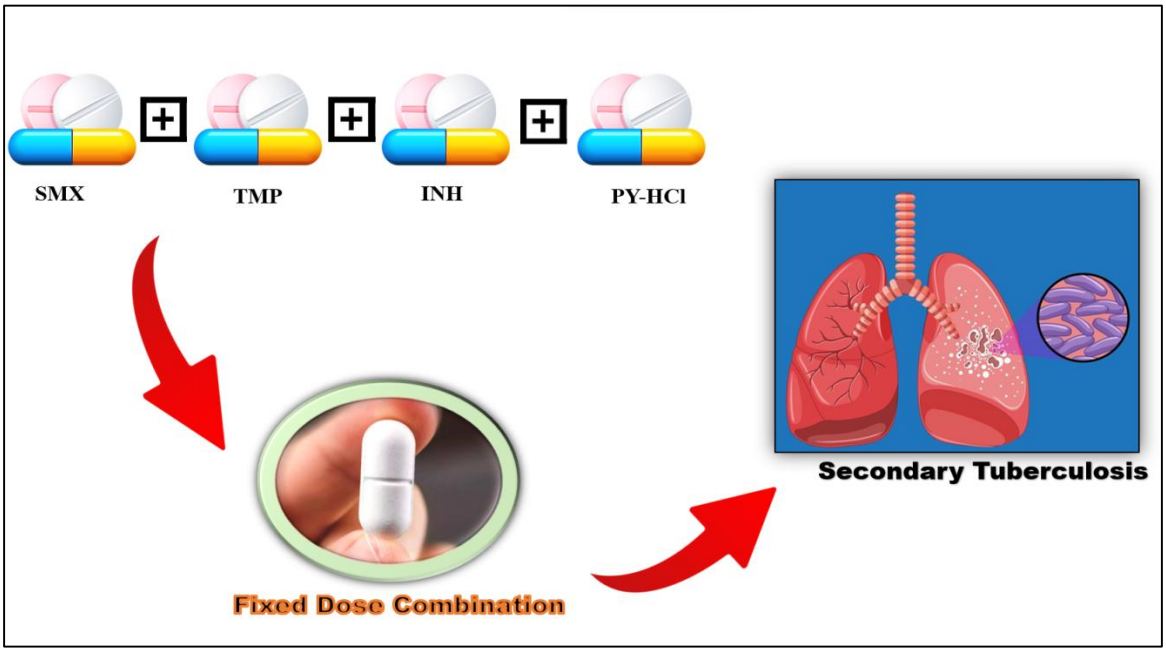
ABSTRACT
Background- Patients with Human Immunodeficiency Virus (HIV) have higher risk of developing tuberculosis (TB) compared to uninfected individuals. The World Health Organisation (WHO) recommends fixed dose combinations (FDC) of antibiotics, anti-tubercular, and vitamins to treat latent TB. Multiple drugs for minimum 6 months are needed to effectively treat active TB. Incorrect mono-therapy and non-adherence increases disease relapse, treatment failure, and drug resistance. FDCs containing 2+ anti-TB drugs improve adherence by reducing tablet count, preventing mono-therapy, aiding dose calculation, increasing acceptance, minimizing errors, and reducing pill burden.
Methodology- A combination of Sulfamethoxazole and Trimethoprim (SMX-TMP) at concentrations of 8.3 µg/ml of SMX and ≥1.6 µg/ml of TMP has been effectively used in patients with extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis.* Given the efficacy of SMX-TMP against XDR M. tuberculosis, this combination was preferred in the current study. Additionally, Isoniazid (INH) is effective against *M. tuberculosis*. However, treatment with INH can lead to a deficiency of the vitamin B6 called Pyridoxine (PY). To address this issue, 10 mg of Pyridoxine hydrochloride (PY-HCl) was added with each 100 mg dose of INH. The aim of this study is to formulate, optimize, and evaluate in vitro a new FDC using SMX, TMP and INH, PY HCl to treat co-infection of HIV and TB. Tablets were prepared with maize starch, povidone K30 (PVP K30), sodium lauryl sulphate (SLS), Starch1500, croscarmellose sodium (CCS), sodium starch glycolate (SSG), colloidal silicon dioxide (Aerosil), and magnesium stearate using the wet granulation method. Dissolution testing was performed in 0.1 N HCl for each tablet separately

and also for the mixture of the three drugs, with quantification done using HPLC.
Results - The optimized batch F8 showed satisfactory dissolution results for the FDC compared to the other formulation batches. Therefore, the FDC tablet containing 800mg SMX, 160mg TMP, 300mg INH and 25mg PY-HCl was successfully designed as compressed tablets meeting the final product specifications.
Keywords – Fixed Dose Combination, Secondary tuberculosis,Sulfamethoxazole, Trimethoprim, Isoniazid, Pyridoxine, sodium starch glycolate.



1. INTRODUCTION

In 2020, there were over 10 million cases of TB worldwide, among which 10% are co-morbid withHIV. TB ranks 13th in terms of death attributes, with 81 per cent of fatalities happening mainly in the African region. TB is the second extremely contagious disease after COVID-19. Nearly 8.8 million (8.5-9.2 million) new cases of TB were reported in 2010, with 1.1 million (0.9-1.2 million) HIV-deleterious TB patients dying from the disease and 0.35 million (0.32-0.39 million) HIV-associated TB patients dyingWHO, 2021. Globally, 1.1 million children have TB infection in 2020. Nearly 86% of new cases in 2020 are from the 30 high TB burden nations (WHO Global, 2020). Globally, the measure of new TB cases is decreasing by about 2% annually. In total, the reduction from 2015 to 2020 was close to 11%. An estimated 66 million lives will be saved with the treatment of TB through diagnosis between 2000 and 2020[1].

The presence of HIV, extensively drug-resistant (XDR) TB, and multidrug-resistant (MDR) TB has significantly undermined global efforts aimed at controlling TB, as indicated by the WHO's report in 2021[2]. Around 6.5 million instances of MDR-TB, or 5% of all newly diagnosed cases of TB, were described in 2010, and > 1.5 million MDR-TB fatalities are anticipated to ensue worldwide every year, representing a mortality level of around 30%. MDR-TB cases varied from 0 to 28.3% and 0 to 61.6%, respectively, among newly diagnosed and previously treated TB cases[3]. The majority of sub-Saharan African nations lack adequate laboratory infrastructure, making it impossible to discover *M. tuberculosis* strains resistant to the two first-in-line treatment medications.

HIV patients are more likely to acquire MDR- and XDR-associated TB, which has shorter survival times and greater fatality rates. MDR-TB and HIV are both balanced fatality combos. Although the influence of HIV infection on MDR-TB patients has received more medical attention, the association between MDR-TB infections is not well known. Contradictory findings have been found in investigations on the relationship between medication resistance and HIV co-infection in TB patients. While some studies in the literature suggested no increased risk, others revealed higher risks for MDR-TB among patients who have a co-infection of both TB and HIV. It is still entirely unknown whether HIV co-infection increases the likelihood of developing medication resistance[4].Multiple drugs must be used for a minimum of six months as part of an effective treatment regimen for individuals with active TB. Incorrect usage of mono-therapeutic regimens and non-adherence to proper treatment are significant risk issues for disease relapse, treatment failure, and the appearance of drug-resistant TB[5].

FDC anti-TB preparations consist of two or more anti-TB medications in each formulation. The WHO has recommended two-drug FDC formulations since 1994, following their introduction in the 1980s. These formulations aim to enhance patient adherence by minimizing the number of tablets and discouraging mono-therapeutic approaches.FDC anti-TB drugs were suggested by the WHO and the Global Union against Tuberculosis and Lung Disease (IUATLD) in 1994.In addition, the FDC is used to aid dosage calculation, increase patients' acceptance, prevent prescribing errors, and reduce pill burden.[6]Presently, the WHO model compendium of FDCs comprises two anti-tuberculae drug combinations (2FDC), typically [rifampin (RIF) + INH (INH)], three-drug combinations (3FDC, RIF + INH + pyrazinamide (PZA)), and four-drug combinations (4FDC, RIF + INH + PZA + ethambutol (EMB))[7].INH, an antibiotic, targets *M. tuberculosis*, the bacterium responsible for TB. INH treatment can result in a deficiency of the vitamin B6. PY combines with INH to form a hydrazone, which is subsequently excreted through urine. To mitigate this issue, 10 mg of PY-HCl was introduced for every 100 mg of INH[8]. For over four decades, TMP and SMX have been utilized together in clinical settings for the treatment of TB. Sold under different brand names such as Septra and Bactrim, this combination effectively addresses infections affecting the urinary, respiratory, and gastrointestinal tracts. The TMP and SMX combination targets the sequential biochemical steps of the folate biosynthesis pathway.

SMX hinders the activity of the enzyme dihydropteroate synthase (FolP), a process facilitated by SMX itself. This inhibition prevents the merging of dihydropterin diphosphate with aminobenzoic acid, which shares structural similarities with SMX. This sequence culminates in the creation of 7,8-dihydropteroate. In the subsequent stage, this compound interacts with glutamate, resulting in the formation of dihydrofolate. This enzymatic progression is followed by the conversion of dihydrofolate into tetrahydrofolate, a process targeted by TMP[9]. This transformation is facilitated by the enzyme dihydrofolate reductase (Dhfr). Mammals absence dihydropteroate synthase and cannot generate folate, but fungi, bacteria, and plants can do so. For the biosynthesis of pyrimidine and purine, as well as the catabolism and biosynthesis of certain amino acids, tetrahydrofolate is a crucial co-factor involved in transferring one-carbon units. The mixture of TMP and SMX inhibits the appearance of drug resistance and has shown synergistic effects against various bacteria. It also acts against different bacteria and viruses, including those that can cause significant infections in patients with HIV[10].

Although TMP and/or SMX are commonly used to treat bacterial illnesses like Salmonella, Nocardia, Shigella, streptococci, and staphylococci, there has been relatively little research on their effects on *M. tuberculosis.* TMP's least inhibitory concentration against *M.*

tuberculosis strain H37Ra was greater than 128 g/ml. The SMX-TMP combination has demonstrated susceptibility to both drug-resistant and drug-susceptible *M. tuberculosis* strains, with bactericidal activity seen at doses of around 38 g/ml for SMX and 2 g/ml for TMP stated that an XDR-TB strain was susceptible to the combination of TMP-SMX ranging concentrations of 8.3 μg/ml for SMX and ≥1.6 μg/ml for TMP. Therefore, the SMX-TMP mixture was preferred in that study. Consequently, a combination known as 4FDC (SMX/TMP/INH/PY-HCl) has been developed and evaluated to prevent resistance during antibiotic treatment[11]. This 4FDC combination improves patient compliance as per WHO requirements and increases therapeutic efficacy through synergistic effects, providing a broader spectrum of activity. Moreover, it aids in reducing the risk of MDR-TB.

2. MATERIALS AND METHODS

SMX, TMP, INH and PY-HCl were sourced from Virchow laboratories. Maize starch (M.Starch), Sodium starch glycolate, (Dioctyl Sodium Sulfosuccinate) DOSS granular and Magnesium stearate were sourced from Maize products, Amishi drugs, Solvay and Nikita chemicals, respectively.All the analytical chemicals and reagents used in this study were obtained from Rankem gift samples.

2.2.1 Pre-formulation studies[12]

2.2.1.1 Physical Characterization of APIs: The physical characterization of dried granules like the particle size distribution, and bulk & tapped density was characterized by sieve analysis.

2.2.1.2 Determination of particle size distribution: Particle size scattering was done by sieve analysis using an electromagnetic sieve shaker at vibratory torque of 10 for 15 minutes. 16, 20, 30, 40, 60,80 and 100 were arranged from top to bottom respectively. 50 gm of samples subjected to the study. The result of retention on each sieve was noted and expressed in %. [13]

2.2.1.3 Bulk Density: It was calculated by adding 100 g of powder to a 250 ml graduated cylinder without compacting, and recording the volume. Utilizing the following formula, bulk density is determined and represented in grammes per milliliter.

$$Bulk\ density = \frac{Mass\ of\ \ powder\ blend}{Volume\ of\ \ powder\ blend}$$

2.2.1.4 Tapped density: The powder's tapped volume was determined using the tap density apparatus, with 10, 500, and 1250 taps applied. The blend underwent 500 taps, and the percentage volume variation was calculated. Subsequently, an additional 1250 taps were performed, and the percentage variation was recalculated. [14]

$$Tapped\ density = \frac{Mass\ of\ \ powder\ blend}{Tapped\ volume\ of\ \ powder\ blend}$$

2.2.2 Solubility study: The study was conducted by the shake-flask technique in altered media like water, 0.1N HCl, Gastric buffer pH 1.20, Acetate buffer pH 4.50, Phosphate buffer pH 6.80 and buffer pH 7.5. Sampling was done both as individual API and as composite.

2.2.3 Drug-Excipients Interaction Study: Drug substances viz SMX, TMP, PY-HCl and INH and excipients were mixed in a 1:1 ratio and filled in glass vials. The vials were kept in both closed and opened circumstances at 40°C/ 75%RH, 60 °C and UV chamber. Samples exposed to the different conditions were evaluated on the 30th day using HPLC.

2.2.4 Preparation of uncoated tablet formulation: Ingredients and configurations of altered FDC uncoated tablet preparations are given in Table 1.

Table 1. Compositions of different FDC uncoated tablet formulations

S.	Ingredients	Quantity (mg/ tab)

No.		Prototypes							
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Dry mixing									
1	SMX	800.00	800.00	800.00	800.00	800.00	800.00	800.00	800.00
2	TMP	160.00	160.00	160.00	160.00	160.00	160.00	160.00	160.00
3	INH	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
4	M.Starch	93.00	53.00	74.30	81.75	77.50	77.15	87.15	73.45
5	DOSS	×	×	1.20	1.00	1.00	1.25	1.10	1.30
Binder									
6	M. Starch	35.00	40.00	40.00	45.00	40.00	40.00	40.00	50.00
7	PVP K30	10.00	10.00	×	×	×	×	×	×
8	SLS	2.00	2.00	×	×	×	×	×	×
9	Purified water	Q.S.	Q.S.	Q.S.	Q.S	Q.S	Q.S.	Q.S.	Q.S.
Lubrication									
10	M.Starch	50.00	50.00	25.00	20.25	30.00	30.00	20.25	23.25
11	PY-HCl	27.50	27.50	27.50	27.50	27.50	27.50	27.50	27.50
12	Starch 1500	30.00	30.00	×	×	×	×	×	×
13	SSG	40.00	25.00	50.00	50.00	50.00	50.00	50.00	50.00
14	DOSS	×	×	4.50	4.50	4.00	4.10	4.00	4.50
15	SLS	2.50	2.50	2.50	×	×	×	×	×
16	Aerosil	10.00	10.00	×	×	×	×	×	×
17	CCS	25.00	25.00	×	×	×	×	×	×
18	Mg stearate	15.00	15.00	15.00	10.00	10.00	10.00	10.00	10.00
	Total wt.	**1500.0**	**1550.00**	**1500.00**	**1500.00**	**1500.00**	**1500.00**	**1500.00**	**1500.00**

2.2.4.1 Manufacturing Process[15]**:**

Sifting: All the raw materials were sifted through sieve 20.

Dry mixing: The dispensed quantities of SMX, TMP, INH, maize starch, and DOSS were introduced into a rapid mixer granulator and mixed at slow speed for 15 minutes.

Paste preparation: M. Starchwas suspended in purified water to prepare a slurry. Purified water was boiled and the slurry was added to it. The mixture was stirred until a translucent paste formed and then allowed to cool to room temperature.

Granulation: The above binder paste was added into the dry mixing part with continuous mixing in Rapid Mixer Granulator.

Drying: Further, the granules were transferred into FBD bowl and dried by maintaining the inlet temperature between 50°C and 60°C. Observed LOD Limit is 2.00% - 2.50%.

Sizing: The dry particles were sized through a 20# sieve of the mechanical sifter

Pre-lubrication: The previously sifted PY-HCl, Maize starch, Docusate sodium and Sodium starch glycolate granular were added and varied for 10 minutes at a deliberate speed.

Lubrication: The previously sifted Magnesium stearate was added to an octagonal blender and mixed for 3 minutes slow speed.

Compression: The final granules were compressed using a compression machine with (21.60 X 10.00 mm) caplet shaped, punches having both sides break line.

2.2.5 Evaluation of Core Tablet Compression Parameters[16]

Tablets are evaluated as per Pharmacopeial specification (Pharmacopeial Standards, 2021).

2.2.5.1 Weight of the tablet: Twenty tablets were randomly selected from each batch and individually examined. The average weight of these twenty tablets was then calculated.

2.2.5.2 Tablet Thickness: The tablets' thickness was assessed using a Mitutoyo Vernier caliper (Digital). This involved measuring ten tablets from each batch of the formulation. Similarly, the tablets' length and width were also measured using the same method as the tablet thickness assessment.

2.2.5.3 Tablet hardness: An automated hardness tester was used to gauge the tablets' hardness. Ten tablets from each batch of formulation were confirmed for hardness to make the determination.

2.2.5.4 Friability: To determine the tablets' friability, 10 fully intact tablets were placed into the friability test apparatus from each batch and operating for 4 min (4 × 25 Revolutions = 100 Revolutions). Initial and final tablet weights were expressed as W_1 and W_2 g. The loss of weight is expressed as (W_1-W_2) g. Thus % friability is measured based on the following equation.

$$\% \, Friability = \frac{Loss \ of \ weight}{Initial \ weight} \times 100$$

2.2.5.5 Disintegration Time: The fragmentation time of tablets from each preparation was assessed using a disintegration device produced by Scientific to determine their *in vitro* disintegration time.

2.2.6 Validation of HPLC method: Initially, a novel HPLC method was developed to analyze a new fixed drug dosage in tablet and dissolution samples. This method was designed to be reasonable, economical, and suitable. Subsequently, the technique underwent validation to assess various factors such as system appropriateness, choosiness, linearity, accurateness, precision, and robustness.

2.2.6.1 Instrumentation: HPLC analysis was conducted using a Shimadzu (Japan) HPLC instrument. The setup included a CMB-20 Alite system controller, two LC-20AT pumps, a SIL-20A auto-sampler, and a CTO-10ASVP column oven. Ultraviolet detection was achieved with an SPD-20A UV-VIS detector from Shimadzu (Japan). The data obtained from the drug study were managed using LC solution software (Version 1.2, Shimadzu, Japan) on a Pentium PC running the Windows XP operating system.

2.2.6.2 Chromatographic Conditions for dissolution: Separation was achieved from the C18 column (250 mm × 4.6 mm; 5 µm, Hyperosil BDS is suitable) at 30 ºC temperature. (Mobile phase A) 13.6 g of potassium dihydrogen phosphate in a beaker containing 800 mL of water. 2.2 g of sodium heptane sulfonic adjusted pH to 3.0 with 10% OPA & made up to 1000 ml with water. Mobile phase trials were done resulting in the ratio of 10% OPA and methanol being 5:5 and adjusted the pH 3.0±0.05 using diluted OPA. Peaks were obtained at 280nm in a UV detector. About 50 µL of the sample was injected at a flow rate of 1.0 ml/min. Column and sample temperatures at 25 ºC and 10 ºC respectively. Retention times for SMX, TMP, INH and PY-HCl were 9.9, 17.8, 3.5, and 5.8 respectively using the gradient elution method.

On a Pentium PC running Windows XP, the drug investigation data were collected and treated using LC solution software (Version 1.3, Shimadzu, Japan). The validation of the approach took

into account factors including system appropriateness, choosiness, linearity, accuracy, precision, and robustness.[17]

2.2.6.3 Preparation of standard and sample solution for dissolution: In a 200 mL volumetric flask, the antibiotics consisting of 177.0 mg of SMX, 35.0 mg of TMP, and 66.0 mg of INH were combined. To dissolve the mixture, 20 mL of methanol and 5 mL of PY-HCl stock solution were added, followed by sonication for a duration of 3 to 5 minutes. The resulting solution was then brought to the desired volume with the dissolution medium. To prepare the standard solutions, separate aliquots of SMX, TMP, INH, and PY-HCl were taken in 100 mL volumetric flasks. Each aliquot was diluted with the portable phase to achieve five different concentrations (80%, 90%, 100%, 110%, and 120% of the goal concentration). The final volume of each standard solution was adjusted to the mark in the respective volumetric flask with the mobile phase.

2.2.6.4 System suitability: On a Pentium PC running Windows XP, the drug study data were collected and processed using LC solution software (Version 1.3, Shimadzu, Japan). The validation of the approach took into account factors including system suitability, choosiness, linearity, correctness, exactness, and strength.

2.2.6.5 Selectivity: To determine selectivity, the existence of excipients commonly used in tablet formulation was considered. Initially, a sample containing 100% of the new fixed-dosage drug was injected. Subsequently, samples containing three placebo formulations, which included the communal excipients, were inserted to assess the fussiness of the method.

2.2.6.6 Linearity: To evaluate the linearity of the process, standardization curves were constructed. Standard solutions with varying concentrations ranging from 50% to 150% of the target concentration were prepared. To ensure reproducibility, each concentration level was measured in six repeats to authenticate the consistency of the sensor response. The peak ranges from the chromatograms were strategized against the corresponding applications to generate the standardization curves. Subsequently, regression analysis was conducted on the data to determine the calibration equation and association factors.

2.2.6.7 Accuracy: Accuracy was assessed using the spike and recovery method. Placebo formulations were supplemented with varying levels of the new fixed-dose combination antibiotic. The accuracy was then calculated by determining the proportion of the solution improved through the assay.

2.2.6.8 Precision: The accuracy of the method was evaluated through an intra-day study focusing on repeatability and an inter-day study to examine ruggedness. For intra-day precision (repeatability), four repeated analyses of three reference solutions (at 90%, 100%, and 110% of the target concentration) were performed on the same day. Inter-day precision (also known as intermediate precision) was assessed by analyzing standard solutions (at 90%, 100%, and 110% of the concentration) over three consecutive days in the same testing facility. The results indicated that the relative standard deviation was consistently below 2.0%.

2.2.6.9 Robustness: To assess the method's robustness, samples were analyzed under various conditions with slight modifications to the mobile stage factor, movement rate (1.2 and 1.4 ml/min), and temperature (28 ºC and 32 ºC)[18]

2.2.7 *In* vitro dissolution test validation through HPLC

In vitro dissolution, work was passed out for all the prepared preparations using a tablet dissolution tester (TDT-08L, Electro lab, India). A dissolution examination was carried out for

the formulation and commercial standard product in 900 mL of 0.1N HCl, Paddle (USP-II), 75 rpm speed, maintained at 37°C ± 0.5°C for 60 min and analyzed[19].

The sample solution was sifted through a 0.45 µm tissue filter. An initial 5 mL of filtrate was discarded. About 5 mL of the filtrate was dilute in 25 mL of diluent to make the ultimate concentration of the working sample equivalent to 100% of the object concentration as SMX of 177.7 µg/mL, TMP of 35.5 µg/mL, INH of 66.7 µg/mL and PY-HCl of 5.5 µg/mL.

2.2.9Assay validated using HPLC

20 tablets were triturate in a mortar pestle. The process powdered tablets having 800.0 mcg/mL of SMX, 160.0 mcg/mL for TMP, 300.0 mcg/mL for INH, and 25.0 mcg/mL for PY-HCl were dissolved in 150 ml of the diluent, sonicate for 25 to 30 minutes with sporadic shaking and then make up the volume with diluent to obtain the resulting solution. Filtered the solution through a 0.45 µm film filter.The HPLC was used to inject a 10 µl solution, and the potency was estimated using the created calibration curve[20].

3. RESULTS AND DISCUSSION

3.1 Pre-formulation studies

3.1.1 Physical characterization of granules

Bulk concentration and tapped density through mesh analysis, elastic guide (%), Hausner's ratio and particle size distribution of dried granules of API like SMX, TMP, and INH were evaluated and tabulated in Table 2.

Table 2. Physical characterization of dried granules of FDC

API	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (%)	Hausner's ratio	Particle size of fine granules
SMX	0.5000	0.7575	34.000	1.5151	21:79
TMP	0.5000	0.7143	30.000	1.4285	64:36
INH	0.7936	0.8602	7.9365	1.0862	36:64

APIs used in this study were establish to have very lowly compressibility and flow properties, henceforth wet granulation technique was preferred to attain better correlation and good run property of the granules.

3.2 Solubility assay of API as an individual and composite form[21]

A solubility study was performed for SMX, TMP, INH and PY-HCL. The study was conducted by a shake-flask process in different media like water, 0.1N HCl, Gastric buffer pH 1.20, Acetate buffer pH 4.50, Phosphate buffer pH 6.80 and buffer pH 7.5. Sampling was done both as an individual API (Table 3) and as a composite (Table 4).

Table 3. Solubility report for individual API samples in different media

S.No	Media	pH (Drug + Media)	Solubility (mg/ml)			
			SMX	TMP	INH	PY-HCl
01	Water	4.62	0.12	0.69	0.97	1.01
02	0.1N hydrochloric acid	1.32	0.77	1.00	1.00	0.99
03	Gastric buffer pH 1.2	1.74	0.51	0.97	0.97	0.99
04	Acetate buffer pH 4.5	4.57	0.12	1.01	1.01	1.00
05	Phosphate buffer pH 6.8	6.55	0.55	0.98	1.01	0.96
06	Phosphate buffer pH 7.5	6.86	0.56	1.01	1.01	0.96

Table 4. Solubility report for composite API samples in different media

S.No	Media	pH (Drug + Media)	Solubility (mg/ml)			
			SMX	TMP	INH	PY-HCl
01	Water	4.62	0.17	0.48	0.99	0.99
02	0.1N hydrochloric acid	1.32	0.59	1.00	1.00	1.02
03	Gastric buffer pH 1.2	1.74	0.49	0.93	0.99	1.01
04	Acetate buffer pH 4.5	4.57	0.11	1.00	1.00	1.01
05	Phosphate buffer pH 6.8	6.55	0.48	0.13	1.00	1.00
06	Phosphate buffer pH 7.5	6.86	0.48	0.14	1.00	1.01

The results obtained show that the maximum solubility of 0.49 mg/ml for SMX,0.93 mg/ml for TMP,0.99 mg/ml for INH and 1.01 mg/ml for PY HCl in gastric buffer pH 1.2.

3.3 Drug- excipients interface study

Before formulating FDC, drug excipients interactions were studied based on the appearance of the sample at augmented conditions (40°C/75% RH) and with the effect of heat (60°C) after 30 days. Drug- excipients compatibility study based on the appearance of chemicals like SMX, and PY-HCl is white, amorphous fine powder during initial and after thirty days at 40°C/75% RH and at 60°C. Drug- excipients compatibility study based on the appearance for chemicals like TMP, INH is white, slightly granular powder and white crystalline powder, respectively during initial and after 30 days at 40°C/75% RH and at 60°C. Drug- excipients compatibility study based on appearance for chemicals like placebo is pink coloured, amorphous fine powder during initial and after 30 days at 40°C/75% RH and at 60°C.

Drug- excipients compatibility is study based on the appearance of a mixture of chemicals like Placebo + SMX, and Placebo + TMP is pink coloured, slightly granular powder and for the mixtures like Placebo + INH, and Placebo + PY-HCl, the appearance is pink colored, slightly granular powder, and pink coloured, amorphous fine powder, respectively during initial and after 30 days at 40°C/75% RH and at 60°C. Drug - excipients compatibility study based on the appearance of a mixture of chemicals like SMX + TMP + INH + PY-HCl (D.S.), and (D.S.)+ Maize Starch is a white, amorphous fine powder and white, amorphous powder during initial and after 30 days at 40°C/75% RH and at 60°C.

Drug-excipient compatibility was evaluated through the assay method using HPLC. After performing this study, it was concluded that the API and excipients are both compatible because there are no changes that occurred both physically and chemically. Hence it is suitable for the development of formulation.

3.4 Method validation using HPLC

The main aim of authentication of a systematic process is to reveal that it is appropriate for its future usage. The designated HPLC methodology has been significantly authenticated for its identified degradation and unidentified scums as per ICH guidelines.

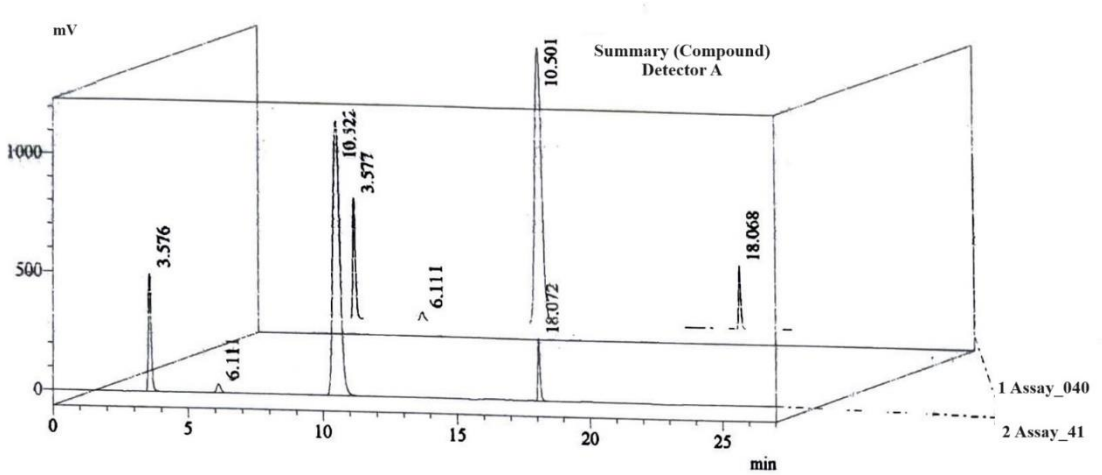


Figure:1 - Chromatogram of each drug (SMX, TMP, INH, and PY-HCl) during the initial stage

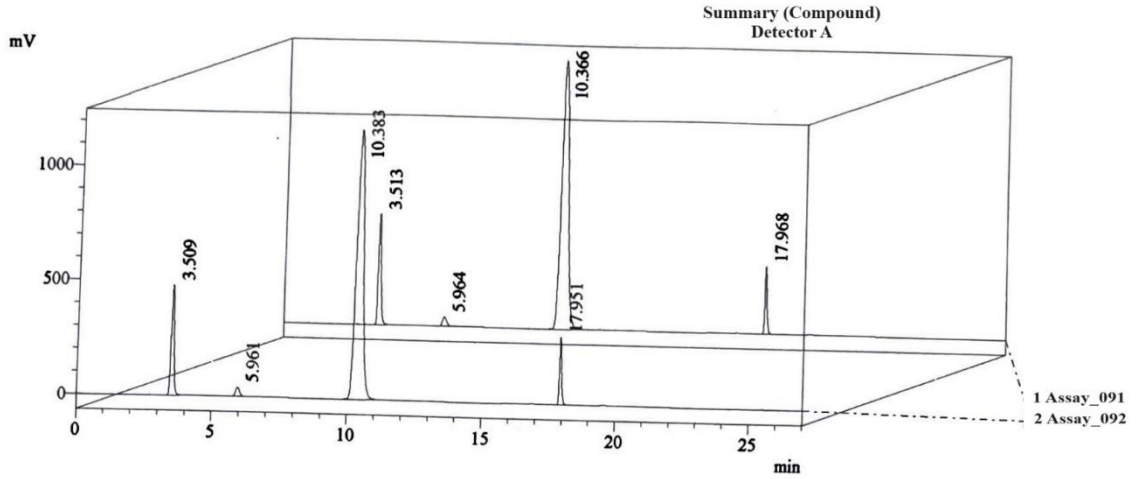


Figure: 2- Chromatogram of each drug (SMX, TMP, INH, PY-HCl) during the 30th day at 40 ºC/75% RH (at final stage)

Figure 1 & 2 represents the chromatogram of each drug at the initial (1st day of preparation) and after 30 days interval at 40ºC/75% RH. By comparing the chromatogram of each drug (SMX, TMP, INH, PY-HCl) at the initial (1st day of preparation) and after 30 days intervals at 40ºC/75% RH, the retention time (t_R) for each drug remains the same (Figure 1 and 2). The retention time for INH remains to be approximately 3.5 min in both chromatograms and was inferred from the drug peak. t_R for SMX remains to be approximately 10.5 min in both chromatograms. $t_R$for TMP and PY remains to be approximately 17 min and 5.8 min in both chromatograms. These HPLC results infer that each of the drugs (SMX, TMP, INH, and PY-HCl) becomes inert even for 30 days at 40°C /75% RH.

3.5 Assay % validation using HPLC

Table 6 shows the HPLC assay % validation of each drug and excipients.

Table 5. HPLC assay validation of each drug and excipient

Mixture		Assay % (Open study)			
		Initial	30 days (40°C /75% RH)	30 days(60°C)	UV
SMX		98.10	98.59	98.25	99.39
TMP		99.37	97.72	99.06	96.61
INH		98.68	98.43	98.84	98.93
PY Hydrochloride		98.49	101.92	101.64	100.43
Placebo + SMX		97.71	98.48	98.32	99.14
Placebo + TMP		97.71	97.72	99.10	96.73
Placebo + INH		100.48	97.49	99.10	99.14
Placebo +PY hydrochloride		106.55	97.30	97.70	98.77
SMX + TMP + INH + PY hydrochloride (D.S.)**	SMX	99.53	98.87	98.15	99.43
	TMP	101.60	98.20	97.84	98.82
	INH	101.69	98.28	97.84	98.75
	Vit. B_6	107.77	108.98	107.29	108.47
(D.S.) + Maize Starch	SMX	101.46	97.59	98.67	98.41
	TMP	101.58	96.74	98.15	97.74
	INH	100.17	96.77	98.16	97.69

	Vit B_6	107.92	107.16	107.60	107.48
(D.S.) + Sodium Starch Glycolate	SMX	99.00	98.79	98.39	98.06
	TMP	97.32	98.18	98.07	97.30
	INH	100.14	98.19	98.08	97.16
	Vit B_6	107.92	108.79	107.62	106.86
(D.S.) + Docusate Sodium granular	SMX	98.42	97.62	98.34	98.78
	TMP	99.25	96.69	98.11	98.12
	INH	102.72	96.77	98.18	98.13
	Vit B_6	107.49	107.12	107.73	108.47
(D.S.) + Magnesium Stearate	SMX	97.82	97.29	97.81	97.40
	TMP	100.68	96.80	98.08	97.13
	INH	102.55	96.92	98.08	97.04
	Vit B_6	108.21	107.32	107.58	106.72
(D.S.) + Titanium dioxide	SMX	98.52	97.80	98.57	97.99
	TMP	102.46	96.95	98.44	97.29
	INH	95.62	97.12	98.45	97.23
	Vit B_6	107.30	107.56	108.02	106.83
(D.S.) + Ferric oxide red	SMX	96.97	99.82	96.70	99.10
	TMP	99.48	99.35	96.14	98.25
	INH	102.79	99.38	96.21	98.40
	Vit B_6	105.72	110.40	105.48	108.23
(D.S.) + Talc	SMX	98.18	99.74	96.55	98.24
	TMP	99.62	99.03	97.44	97.99
	INH	97.42	99.27	95.81	97.37
	Vit B_6	104.66	110.20	105.11	107.08
(D.S.) + Propylene glycol	SMX	97.78	99.70	96.57	98.24
	TMP	99.23	99.40	96.36	97.75
	INH	99.95	99.17	95.99	97.35
	Vit B_6	103.99	110.06	105.24	107.70
(D.S.) + HPMC E 15	SMX	98.58	99.19	96.10	97.62
	TMP	96.77	99.50	96.45	97.75
	INH	99.57	99.16	95.95	97.31
	Vit B_6	104.99	110.06	105.25	107.09
(D.S.) + Placebo	SMX	100.71	98.16	97.19	97.89
	TMP	95.09	97.31	96.75	97.07
	INH	102.06	97.49	96.80	97.02
	Vit B_6	106.34	108.15	106.20	106.72

HPLC assay % was carried out for each drugs such as SMX, TMP, INH and PY Hydrochloride during initial, after 30 days at 40°C /75% RH, after 30 days at 60 °C and under UV light conditions. In addition, HPLC assay % was carried out for combinations such as Placebo + SMX, Placebo + TMP, Placebo + INH and Placebo +PY hydrochloride during the initial, after 30 days at 40°C /75% RH, after 30 days at 60 °C and under UV light conditions. Assay % was carried out for 4FDC combination (SMX + TMP + INH + PY hydrochloride (D.S.)) during initial, after 30 days at 40°C /75% RH, after 30 days at 60 °C and under UV light conditions. Assay % for 4FDC combination along with each excipient like (D.S.) + Maize Starch, (D.S.) + Sodium Starch Glycolate, (D.S.) + Docusate Sodium

granular, (D.S.) + Magnesium Stearate, (D.S.) + Titanium dioxide, (D.S.) + reduced Ferric oxide, (D.S.) + Talc, (D.S.) + Propylene glycol, (D.S.) + HPMC E 15, and (D.S.) + Placebo was studied during initial, after 30 days at 40°C /75% RH, after 30 days at 60 °C and under UV light conditions. These HPLC assay % results infer that each of the drugs (SMX, TMP, INH, PY-HCl) remain inert in all studied conditions. Even these four drugs showcase no interactions between them while mixing at all studied conditions which were inferred through the tabular value of D.S. 4FDC (D.S.) along with excipients like maize starch, sodium starch glycolate, docusate sodium granular, magnesium stearate, titanium dioxide, reduced ferric oxide, talc, propylene glycol, and HPMC E 15 showcase no interactions between each of them and hence it is found to be a suitable drug formulation.

The core tablet specification of the new FDC drug is tabulated in Table 7.

3.6 Physical and chemical characterization of Different FDCs

The prepared formulation was physically characterized in Table 5.

Table 6. Physical characterization of different prepared formulations

Physical Parameters	**Developmental Trails**							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Average weight (mg)	1600	1590	1550	1550	1500	1510	1505	1500
Length (mm)	21.58	21.60	21.59	21.59	21.61	21.60	21.60	21.60
Width (mm)	10.02	10.02	10.00	10.01	9.58	10.00	10.00	10.00
Thickness (mm)	8.00	6.50	8.00	7.50	7.70	7.70	7.71	7.70
Hardness (N)	270	265	265	250	250	200	200	150
DT (Min: Sec)	15:30	15:00	12:15	11:43	10:23	05:25	02:12	00:60
Friability (%)	1.2	0.98	0.88	0.87	0.65	0.55	0.34	0.29

Table 7. Chemical characterization of different prepared formulations

Chemical parameters		**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Dissolution (%)	SMX	93.80	96.97	96.17	97.02	96.26	96.72	98.55	99.89
	TMP	100.24	100.39	102.11	101.50	102.05	99.98	101.09	100.39
	INH	102.38	100.7	105.35	103.95	104.56	103.60	102.47	103.53
	PY-HCl	112.73	110.94	116.64	115.91	111.12	108.95	109.25	105.12
Assay (%)	SMX	100.5	98.82	98.82	100.02	98.52	98.81	99.09	99.39
	TMP	100.52	95.87	100.22	101.53	98.61	101.57	100.39	100.63
	INH	100.35	101.40	105.56	103.39	100.76	106.99	104.65	99.76
	PY-HCl	109.81	112.89	115.72	109.39	108.08	114.73	110.96	112.77

4. SUMMARY AND CONCLUSION

In conclusion, comprehensive evaluations of formulation batches containing varying concentrations of SMX, TMP, INHand PY-HCl were conducted, revealing compatibility and absence of interactions between drugs and excipients. These findings, verified through HPLC analysis under different conditions, demonstrate the robustness of the chosen drug combination and excipients. The optimized batch, F8, exhibited superior dissolution results compared to other formulations, leading to the successful design of a FDCtablet comprising

800mg SMX, 160mg TMP, 300mg INH, and 25mg PY-HCl. The study supports the belief that the proposed formulation, along with the specified container/closure system and manufacturing processes, is suitable for ensuring the requisite quality, safety, and stability of the product, making it well-suited for its intended use.

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