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The possibility of preparing solid Co-crystals forms of sulfamethoxazole-trimethoprim (SMZ-TMP)

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Abstract

This study explores the preparation and characterization of solid co-crystals of sulfamethoxazole (SMZ) and trimethoprim (TMP) to enhance their pharmaceutical properties, particularly solubility and stability. SMZ-TMP co-crystals were successfully obtained using methanol, while a salt form emerged in tetrahydrofuran (THF), demonstrating solvent-dependent behavior. Various characterization techniques, including Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR), and Powder X-ray Diffraction (PXRD), were employed to confirm the formation and properties of the co-crystals. The SMZ-TMP co-crystal exhibited improved thermal stability and distinct molecular interactions, as evidenced by changes in FTIR hydrogen-bonding regions and thermal analysis.

The findings highlight the potential of co-crystallization as a strategy to overcome challenges associated with polymorphism and solubility mismatches in pharmaceutical compounds. Solvent selection proved to be critical in determining the final crystalline or amorphous phase, suggesting a need for systematic solvent screening in future studies. This work underscores the importance of SMZ-TMP co-crystals in improving antibiotic formulations, with broader implications for enhanced drug solubility, stability, and therapeutic performance.

I) Introduction:

A) Mechanism of action of Sulfamethoxazole.

Sulfamethoxazole exhibits five distinct polymorphic forms, hence demonstrating a favorable propensity for co-crystallization. When a chemical compound is found to exist in multiple polymorphic forms, it exhibits structural flexibility and is not constrained to a singular crystalline structure or packing mode. Consequently, the probability of introducing a molecule into an alternative configuration of packing, thereby coexisting with another molecule, is enhanced (Aakeröy, 1997).

Erizal *et al.* (2017) indicated that milling process of sulfamethoxazole and trimethoprim in equimolar yielded the co-crystal. Intact co-crystal was obtained by solution co-crystallization with methanol. Prolongation of milling time accelerated the formation of co-crystal. Physical characterization showed that sulfamethoxazole and trimethoprim co-crystal demonstrated a unique powder X-ray diffraction, thermal and spectroscopic properties.

Sulfamethoxazole (SMZ) is a sulfonamide medication with intermediate duration of action that functions by inhibiting the bacterial production of dihydrofolic acid. This inhibition occurs by competitive binding with para-aminobenzoic acid (PABA) to the enzyme dihydrofolate synthetase (dihydropteroate synthetase). Sulfamethoxazole exhibits bacteriostatic properties. The production of bacterial nucleotides and DNA is reduced by inhibiting the synthesis of dihydrofolic acid.

In terms of chemical structure and antibacterial action, SMZ is similar to sulfisoxazole; however, it is absorbed and eliminated more slowly and has a higher propensity to produce crystalluria. Furthermore, this medication is quite potent and has been in use for a long time. Due to its rather sluggish clearance (half-life of 11 hours), it may be especially helpful in the treatment of pyelonephritis. It is good for urinary tract infections (Rudy and Senkowski, 1973; Kagan, 1974].
The combination of sulfamethoxazole and trimethoprim is classified as an antibiotic. The mechanism of action involves the eradication of germs responsible for many types of illnesses. Currently, the prevailing practice is the utilization of sulfamethoxazole in conjunction with trimethoprim, commonly abbreviated as SMX-TMP (Kemnic and Coleman, 2022). The WHO Model List of Essential Medicines includes the SMX-TMP combination as a first-line therapy for urinary tract infections. Alternative designations encompass sulfisomezole and sulfamethalazole. (NIH 2012).

B) Sulfamethoxazole description

Sulfamethoxazole (SMZ), is a common sulfonamide [N1-(5-methyl-3-isoxazolyl) sulphanilamide]. Its chemical formula is C10H11N3O3S, and its melting point is between 167 and 170 °C (Table 1.4). Sulfamethoxazole is an odorless, tasteless powder that ranges in color from white to off-white. It is a crystalline powder with a molecular weight of 253.28 g/mol and the following structural formula, and it has a plasma half-life of 11 hours (Figure 1) (Rudy and Senkowski, 1973; Riviere and Papich, 2009).

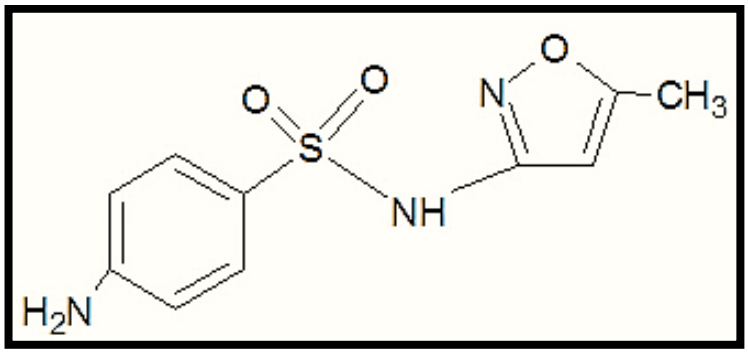


Figure 1. Sulfamethoxazole chemical structure

C) Solubility of sulfamethoxazole in different solvent systems

As indicated in Table 1, sulfamethoxazole is soluble in particular solvent systems. Sulfamethoxazole is more soluble in polar solvents, according to the table.

Table 1. Sulfamethoxazole solubility in various solvent systems at 25 °C.

Solvent	Solubility as g/l
Benzene	0.5
Ethanol	30.6
Petroleum ether	0.2
Chloroform	2.3
Isopropanol	8.8
Methanol	90.3
Ethyl ether	2.7
95% ethanol	37.8
0.1 N NaOH	16.0

D) Sulfasalazine solubility

According to **McDonnell (1976)**, sulfasalazine is essentially soluble in aqueous solutions of alkali hydroxides insoluble in ether, water, benzene and chloroform and hardly soluble in alcohol.

E) Trimethoprim(TMP)

According to Brumfitt *et al.* (1993), the enzyme dihydrofolate reductase is reversibly inhibited by trimethoprim (TMP), which binds to it and prevents the formation of tetrahydrofolic acid from dihydrofolic acid. The concurrent administration of sulfamethoxazole (SMZ) and trimethoprim (TMP) effectively inhibits two sequential enzymatic reactions involved in the manufacture of nucleic acids and proteins, which are vital for the survival of numerous bacterial species.

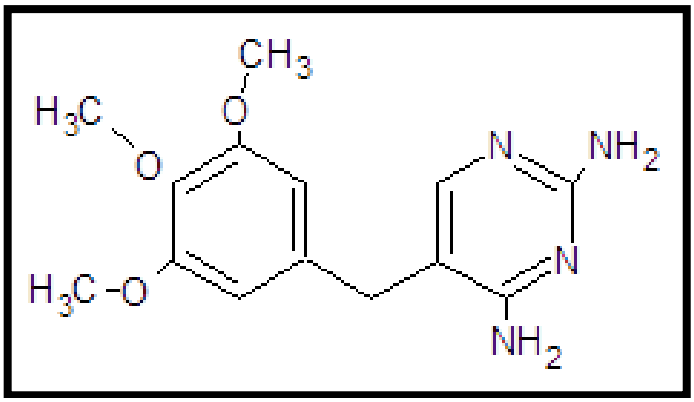


Figure 2 Chemical structure of trimethoprim

F) Mechanism of trimethoprim action

By reversibly blocking the necessary enzyme, dihydrofolate reductase, trimethoprim (TMP) prevents the synthesis of tetrahydrofolic acid from dihydrofolic acid (Brumfitt, 1993). The concurrent administration of sulfamethoxazole (SMZ) and trimethoprim (TMP) effectively inhibits two sequential enzymatic reactions involved in the manufacture of nucleic acids and proteins, which are crucial for the survival and growth of several bacterial species (see Figure 2).

G) Description of trimethoprim

The chemical name for trimethoprim (TMP) is 2,4-diamino-5-(3,4,5 trimethoxybenzyl)pyrimidine. Its melting point ranges from 199 to 203 °C and its molecular formula is C14H18N4O3 (Table 2). It has the following structural formula (Figure 3) and is a cream-colored or white crystalline or crystal powder with a molecular weight (290.3 g/mol) (Manius, 1978).

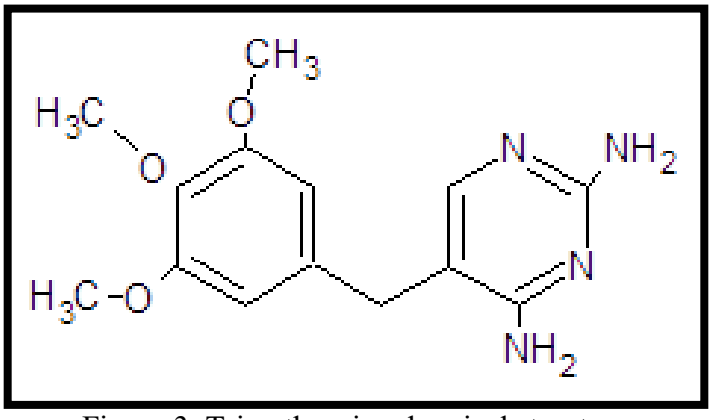


Figure 3. Trimethoprim chemical structure

H) Trimethoprim solubility

The solubility data for trimethoprim in various solvents at 25 °C are presented in Table 2.

Table 2. Trimethoprim solubility in various solvents at 25 °C.

Solvent	Solubility as g/100 ml
Methanol	1.21
Isopropanol	0.12
Chloroform	1.82
95% ethanol	0.81
Benzene	0.002
Acetone	0.35
Ethyl ether	0.003
Water	0.04

I) Synergistic combination of sulfamethoxazole with trimethoprim:

Trimethoprim, a dihydrofolate reductase inhibitor, which prevents the conversion of dihydrofolate to tetrahydrofolate, is typically used together with sulfamethoxazole.

Most frequently, sulfamethoxazole is used in the formulation known as co-trimoxazole, which is sold under the trade name Bactrim, in a 5:1 molar ratio with trimethoprim (Gilman *et al.* 1990).

Bacterial resistance appears to develop more gradually when sulfamethoxazole and trimethoprim are used together than when either drug is used alone, according to *In vitro* research (Rudoy *et al*., 1974). The co-crystallization of medicinal molecules in trimethoprim with co-forming chemicals will be attempted in this work. The ultimate goal is to enhance SMZ's solubility, antibacterial activity, and/or other physical and chemical characteristics.

The purpose of this study was to determine whether it is feasible to combine trimethoprim with the sulfa antibiotic medication sulfamethoxazole to produce its common solid crystalline forms with GRAS status.

II) Materials and methods:

The active pharmaceutical ingredients (APIs) used in this study were trimethoprim and sulfamethoxazole (monoclinic form), both obtained from Sigma-Aldrich, India. The co-crystallization experiments utilized a range of co-formers, including nicotinamide, saccharin, L-tartaric acid, salicylic acid, benzoic anhydride, L-aspartic acid, D-tartaric acid, maleic acid, L-glutamic acid, oxalic acid dihydrate, citric acid, benzoic acid, succinic acid, and 4-hydroxybenzoic acid (monohydrate), all procured from the same supplier.

The solvents employed included ethanol (96%), methanol, benzyl alcohol, propan-2-ol, 1,4-dioxane, acetone, chloroform, diethyl ether, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), ethyl acetate, butanone, and butanol.

A) Compound identification

Each chemical was verified using the provided Certificate of Analysis (CFA) reports. For initial identification and characterization, melting points were determined, followed by thermal analysis using Differential Scanning Calorimetry (DSC)**.**

B) Experimental methods

1) Prepare methods co-crystals:

There are several ways to create co-crystals, such as gradual solvent-free grinding, solvent evaporation (crystallization from solution), sublimation, vapour diffusion, slow cooling (flask method), crystallization from melt, solvent drop grinding, and slurry technique (Schultheiss and Newman 2009; Shekunov and York 2000)**.**Solvent evaporation method (crystallization from solution) In order to prepare the co-crystals from the solution, the compounds' solubility was crucial (Blagden *et al*. 2007**)**.

2) Dry grinding

In the process of preparing co-crystals, the resultant product acquired through grinding typically exhibits a similar composition to that achieved through solution-based methods. The grinding was done for a set amount of time (Blagden *et al*., 2007 and Etter *et al.*, 1993). A few co-

crystal materials can only be synthesized through the process of neat or dry grinding, while a significant portion of these materials can be synthesized using both solvent drop grinding and solution growth methods (Yadav *et al*., 2009 and Lynch *et al*., 1991).Solvent drop grinding.Another method for creating co-crystals is solvent assisted grinding. (Blagden *et al.* 2007 and Shan *et al.* 2002) and Trask *et al*.(2005).

3) Sublimation

In this procedure, a watch glass-covered petri dish was filled with a modest amount of the respective co-formers in stoichiometric proportions. To see if co-crystals were developing on the watch glass, the petri dish was gently heated on a hot plate (Guillory 1999).

4) Slow cooling (Flask method)

The solvent and flask that will be used both need to be dust-free. Over the course of many days, the warm solution-filled flask is allowed to cool in warm water (Jones 1981).

5) Vapour diffusion:

Typically, this method (Figure 4) is the most effective for obtaining single crystals. A solution at small amount (the component to be crystallized is in the excellent solvent) is placed in an open tube and stored in a desiccator. this method is also appropriate when there is only a little amount of material available (Jones 1981).

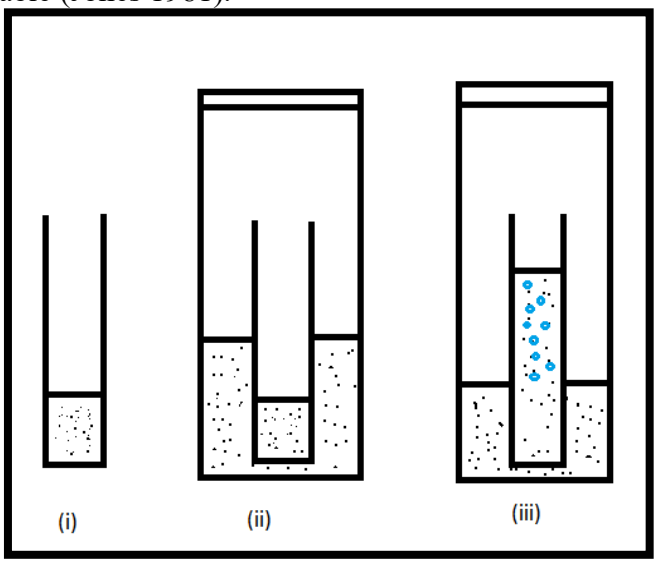


Figure 4. The process of vapour diffusion. I. Compound-in-solution that will eventually crystallize. (ii) Be sure to firmly insert the precipitant and the stopper. After a period of time, crystals begin to form.

This approach has the advantages that multiple tubes can be set up in the same container, as opposed to the sealed desiccator that is frequently utilized, and that very small amounts can be utilized on a spot plate. The process of crystallization is a little slower and can take many days**)**.The process of crystallization is a little slower and can take many days Lu and Afr (2012).

6) Slurry technique

To promote co-crystallization, equimolar amounts are dissolved in a small solvent amount at room temperature, the slurry is agitated for an extended period of time (24 hours or more), the temperature is maintained, and the solution is filtered (Tiekink 2006).

7) Crystallization from the melt:

It is possible to create co-crystals by simply melting two co-crystal formers and then cooling them. If a melt does not produce co-crystals, a melt seed may be utilized in a crystallization solution to produce co-crystals (Braga et al., 2009).

8) Identification of a quality crystal:

The crystal examination process requires the use of a microscope since the quality of the crystals cannot be determined instantly (Jones, 1981).

9) Analytical methods.

Various analytical techniques were utilized to analyze and characterize the distinct forms of the active pharmaceutical ingredient (API), including hot stage microscopy, thermogravimetric analysis, differential scanning calorimetry, elemental analysis, powder X-ray diffraction, single X-ray diffraction, Fourier transform infrared spectroscopy, ultraviolet spectrophotometry and dissolution.

10) Hot stage microscopy (HSM).

A limited quantity of the specimen was utilized. The samples underwent treatment with silicon oil while being observed under the microscope, a crucial step in detecting solvents within the sample.

11) Differential scanning calorimetry (DSC)

Differential scanning calorimetry is used for thermal analysis to determine the melting point and rate of decomposition of the sample.

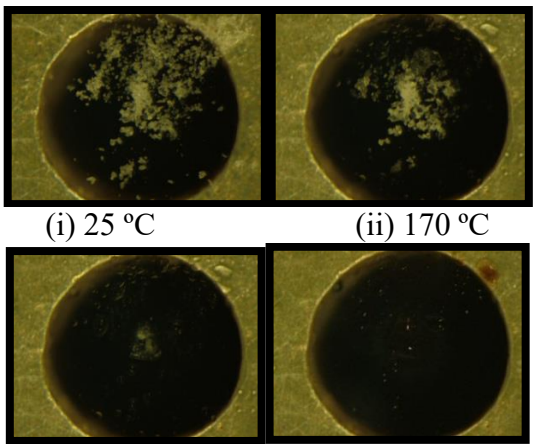
III) Results and discussions

Sulfamethoxazole (SMZ) characterization:

SMZ identification:

Microscopy hot Stage (MHS):

The photographs of SMZ were taken at the following times: (i) the beginning of analysis at temperature of room; (ii) 1st indication of melting; (iii) the completion of the melt; (v) and (vi) the beginning of breakdown with a brown discoloration of the powder at 255 ºC (Figure 5).



(i) 25 ºC (ii) 170 ºC

(iii) 173 ºC (v) 255ºC

Figure 5. HSM for SMZ at various temperatures

Differential Scanning Calorimetry (DSC)

The DSC trace showed that SMZ had a melting point that peaked at 170.7 ºC with a single endotherm between 168 ºC and 172 ºC (Figure 6). The deterioration of SMZ was seen between 255 and 260 ºC, and the DSC results agree with the HSM findings.

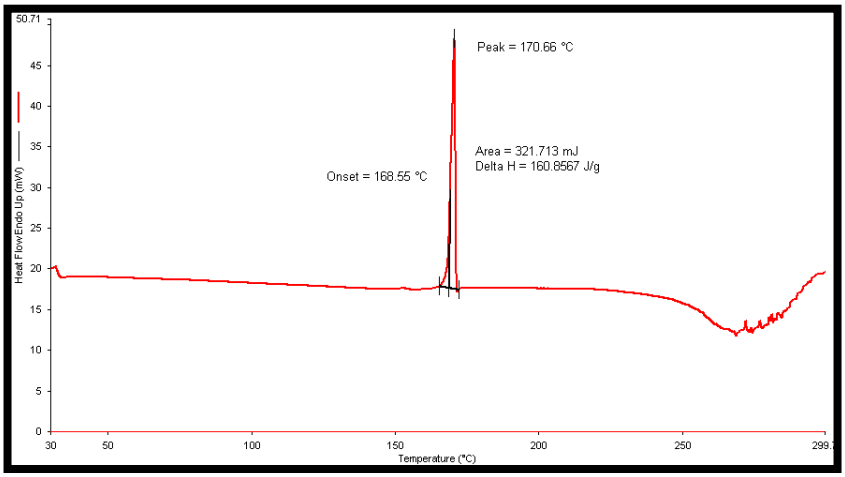


Figure 6. DSC trace for SMZ

Infrared spectroscopy (FTIR)

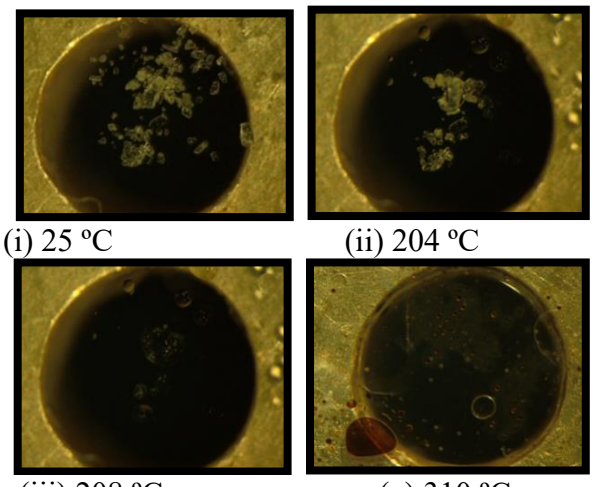
The utilization of Fourier transform infrared spectroscopy was employed to investigate the spectrum characteristics of SMZ.

Trimethoprim (TMP) characterization

TMP identification

Microscopy Hot Stage (MHS)

The photographs of TMP were captured at different temperature conditions: (i) at ambient temperature of 25 ºC, (ii) when the early signs of melting were observed at 204 ºC, (iii) when complete melting of TMP occurred at 208 ºC, and (v) at the onset of decomposition at 310 ºC (Figure 7).



(i) 25 ºC (ii) 204 ºC

(iii) 208 ºC (v) 310 ºC

Figure 7. HSM of TMP

Differential Scanning Calorimetry (DSC)

According to the TMP DSC trace (Figure 8), the melting point was 202.5 ºC, and the endotherm appeared between 199 ºC as well as 203 ºC. The HSM observations and the DSC data showed good correlation. In addition, breakdown started around 310 ºC.

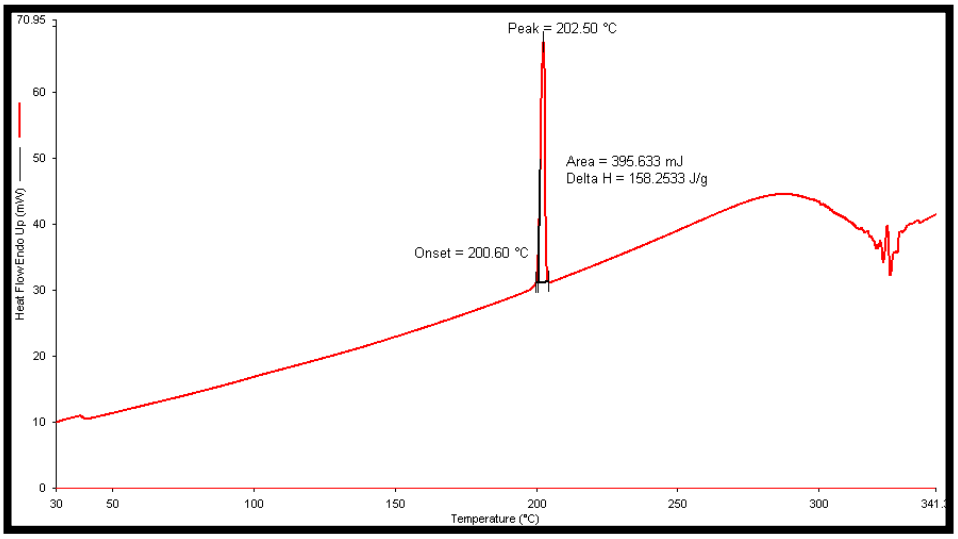


Figure 8. DSC trace for TMP

Infrared spectroscopy (FTIR)

The assignments of the noticed fundamental bands have been presented in Table 3.

Table 3. TMP infrared spectroscopy absorption bands

Compound	Standard frequency bands (cm^{-1}) [6]	Experimental frequency bands (cm^{-1})	Associated of functional group(s)
TMP	3414	3316	Symmetric NH_2 stretching
	3516	3468	Asymmetric NH_2 stretching
	3010	3106	Aromatic CH stretching
	2966, 2940, 2840	3015, 2929, 2834	Aliphatic CH stretching
	1506	1506	Aromatic ring
	1236, 1129	1234, 1123	Aromatic methoxy

The standard spectrum was achieved using spectroscopy infra-red from a TMP solution in chloroform (14.8 mg/ml, path length 0.1 mm NaCl), whereas the experimental spectrum was listed utilizing the ATR method, which accounts for the difference between the standard and experimental frequencies.

Characterization of co-formers:

HSM, DSC, TGA, PXRD, and FTIR were used to characterize each co-former. Table 4 lists the major functional groups for each co-former that the FTIR spectra revealed.

Table 4. FTIR absorption bands for the 14 co-formers

Compound	Experimental frequency bands (cm^{-1})	Standard frequency bands (cm^{-1})	Associated of functional group(s)
Saccharin (SC)	969	975 [11,12]	S-N-C
	1715	1722[11]	O=C-NH
	3400, 3093	3410, 3100 [11]	CO-NH
	1592	1595[11]	Aromatic –C=C-
Nicotinamide (NC)	1614	1600- 1630 [9]	C=C Stretch
	1673	1700 [9]	C=O
	3357	3500-3300[10]	N-H Stretch; amide
Salicylic acid (SCA)	1609	1615-1580[7]	Aromatic ring
	1654	1650[8]	C=C-

	3231	3220 [8]	C = O (carboxylic) -OH
Oxalic acid (as the dihydrate) (OA)	1611 3415	1690 – 1760 [7,10] 3335-2500[10]	C=O O-H
Tartaric acid	1723 3404	1690 – 1760 [7,10] 3335-2500 [10]	C=O O-H
Benzoic anhydride (BAN)	1829 1773	1800- 1830[10] 1740- 1775[10]	C=O C=O
Succinic acid (SUA)	2980 1680	3335-2500 [10] 1690 – 1760[7,10]	O-H Carboxylic C=O stretch
Citric acid monohydrate (CA)	1671 3067-2542	1690 – 1760 [7,10] 3335-2500 [10]	C=O O-H
Maleic acid (MA)	1703 2951-2507	1725-1700[10] 3335-2500[10]	C=O O-H
L-aspartic acid (L-AA)	2951 1684	3345-3325[7] 1690 – 1760 [7,10]	NH_2 C=O
L- glutamic acid	3012 1637	3345-3325[7] 1690 – 1760 [7,10]	Aliphatic NH_2 C=O
4- hydroxybenzoic acid	3377- 3543 1670	3300- 2500 [13] 1687[13]	Bonded-OH stretch Carbonyl –C=O stretch
Benzoic acid	3071-2552 1679	3300- 2500 [13] 1687[13]	Bonded-OH stretch Carbonyl –C=O stretch

To identify co-crystals of SMZ, several studies were carried out utilizing various methodologies and a variety of solvents or mixed solvent systems (miscible solvents). Table 5 lists a few representative outcomes.

Table 5. Representative experiments utilizing different methods to prepare a SMZ co-crystal.

Drug	Co-former	Method	Solvent	Ratio	Result
SMZ	NC	Dry grinding	-	1:1	SMZ
	NC	Slow evaporation	Methanol	1:3	Mixture of SMZ and NC
	SCA	Dry grinding	-	1:2	SCA
	OA	Vapour diffusion	Ethanol: chloroform	1:1	No crystal
	OA	Solvent assisted grinding	Acetone	1:1	SMZ form I
	SC	Slow evaporation	Ethanol	1:1	Mixture of SMZ and SC
	NC	Slow evaporation	Acetic acid	1:1	SMZ form I
	D-TA	Slow evaporation	Methanol	1:3	SMZ form I
	SUA	Slow evaporation	1,4-dioxane	1:1	Decomposition
	BAN	Slurry method	Methanol	1:1	Co-crystal

SCA	Slow evaporation	Propan-2-ol	1:1	Hydrate form of SMZ
4-HBA	Slow evaporation	Mixed solvent water+ acetone	1:1	Hydrate form of 4-HBA
TMP	Melt	-	1:1	Amorphous form of the co-crystal
MA	Slow evaporation	Distilled water: methanol	1:1	Hydrate form of SMZ
TMP	Slow evaporation	Methanol/ Ethanol/ Propanol	1:1	SMZ-TMP Co-crystal

Sulfamethoxazole complexes preparation:

Sulfamethoxazole benzoic anhydride co-crystal (SMZ-BAN):

Preparation of SMZ-BAN co-crystal utilizing the slurry method:

A total of sulfamethoxazole (0.16 g) and benzoic anhydride (0.142 g), in a 1:1 molar ratio, were accurately measured and combined within a glass vial. The methanol-saturated mixture was subjected to supersaturation and agitated using a magnetic stirrer on a heated plate at a temperature of 55 ºC for a duration of 15 minutes, employing the slurry technique. The sample underwent filtration using a 0.45 µm filter, thereafter, being transferred to a separate vial and sealed with perforated parafilm®. Subsequently, the specimen was placed in a refrigeration unit set at a temperature range of 4 - 6 ºC in order to facilitate the process of crystallization.

The crystal was made utilizing a variety of various techniques, including dry grinding and gradual evaporation with acetone, ethyl acetate, and propan-2-ol as the solvents.

Preparation of amorphous sulfamethoxazole - co-former forms:

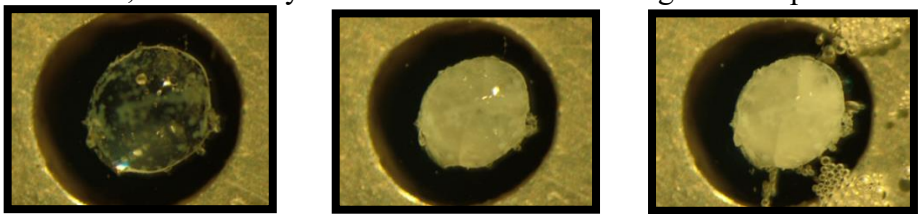
Amorphous Sulfamethoxazole-trimethoprim (SMZ-TMP):

Preparation of amorphous SMZ-TMP:

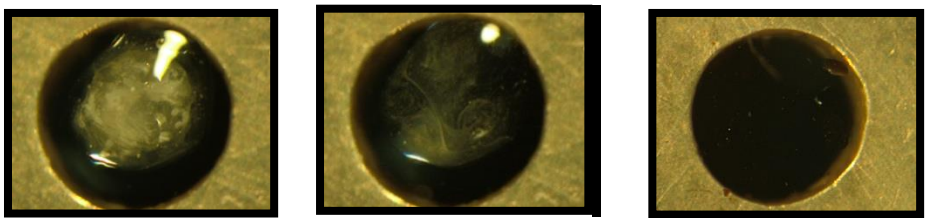
Manual weighing, mixing, and grinding of sulfamethoxazole (0.040 g) and trimethoprim at 0.046 g (1:1 molar ratio) were performed in a small pestle and porcelain mortar. The grinding took place for 30 minutes. The ground product was a tiny amount was put to a DSC pan and set on a hot plate. The sample was made to liquefy at a temperature of 180 ºC. The sample was then taken out and let to cool before setting up. It took on a glassy look, which is the first sign of an amorphous shape.

Hot Stage Microscopy (HSM)

SMZ-TMP (Figure 9) pictures were taken at (i) the beginning of the analysis at 25 ºC of the glassy substance, (ii) the sample becoming opaque at 100 ºC, (iii) bubbling on its own at 150 ºC, (v) the first sign of melting at 180 ºC, (vi) the melt's completion at 188 ºC, and (vii) the SMZ-TMP breaking down at 230 ºC, as shown by discoloration around the edges of the picture.



(i) 25 ºC (ii) 100 ºC (iii) 150 ºC



(v) 180 ºC (vi) 188 ºC (vii) 230 ºC

Figure 9. Amorphous SMZ-TMP HSM photographs

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA):

Since no solvent was utilized to manufacture the product, TGA was employed to establish whether or not a solvent was included in the amorphous SMZ-TMP. This implies a 5.8% (n=1.9) mass loss of water and is compatible with the HSM findings shown in figure 10 and the TGA trace in 3.15. The sample melts at 180 ºC after experiencing mass loss with regard to water loss in the region of 100 ºC to 150 ºC.

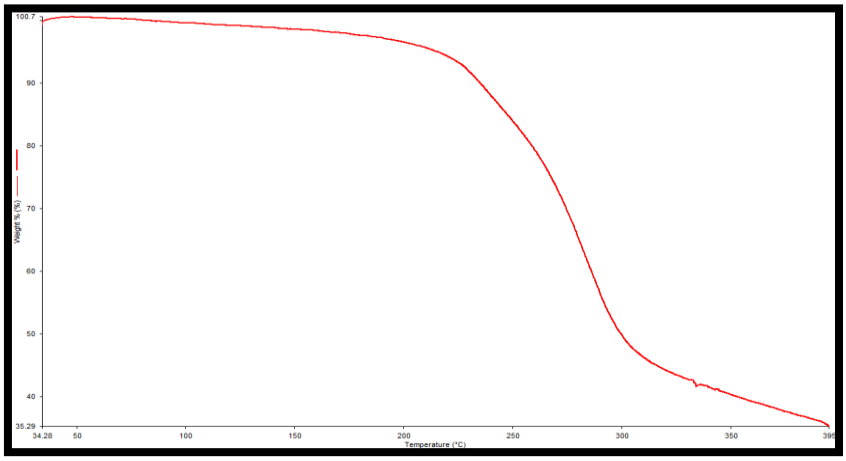


Figure 10. TGA of amorphous SMZ-TMP

The DSC revealed that the amorphous SMZ-TMP had a peak melting point of 180.6 ºC (Figure 11), with a single endotherm between 177 ºC and 183 ºC and an exotherm for the presence of the water molecules between 106 ºC and 120 ºC. The HSM and TGA analyses have verified both of these occurrences, and the thermal events are distinct from those for the parent APIs SMZ (m.p. 167–170 ºC) and TMP (m.p. 200–203 ºC). At around 230 ºC, the amorphous SMZ-TMP started to degrade.

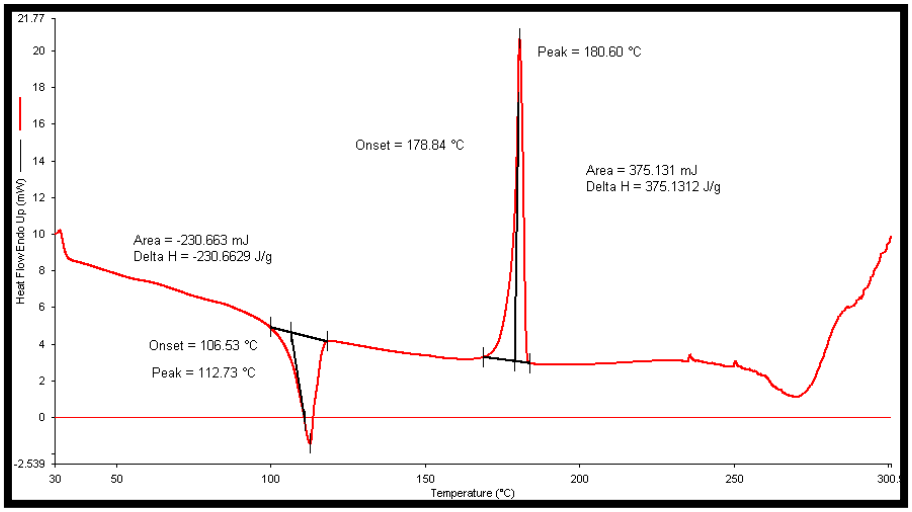


Figure 11. DSC trace of amorphous SMZ-TMP

Infrared spectroscopy (FTIR)

When compared to the SMZ and TMP spectra, Figure 12 reveals a reduction in the strength of the peaks of amorphous SMZ-TMP. Amorphous precipitate is indicated by a change in the peak patterns, with certain peaks vanishing (Table 6). The hydrogen-bonding zone (2000–4000 cm^{-1}), the fingerprint region (<1000 cm^{-1}), and the region between 1350 and 1450 cm^{-1} are all different in the amorphous sample.

The preceding studies (DSC, HSM, and TGA) indicated that the amorphous sample had a broad hydrogen-bonding system that likely contains a sizable number of water molecules bonded in diverse ways. Due to the uneven packing of the molecules in this sample, intensity irregularities often result from an amorphous structure.

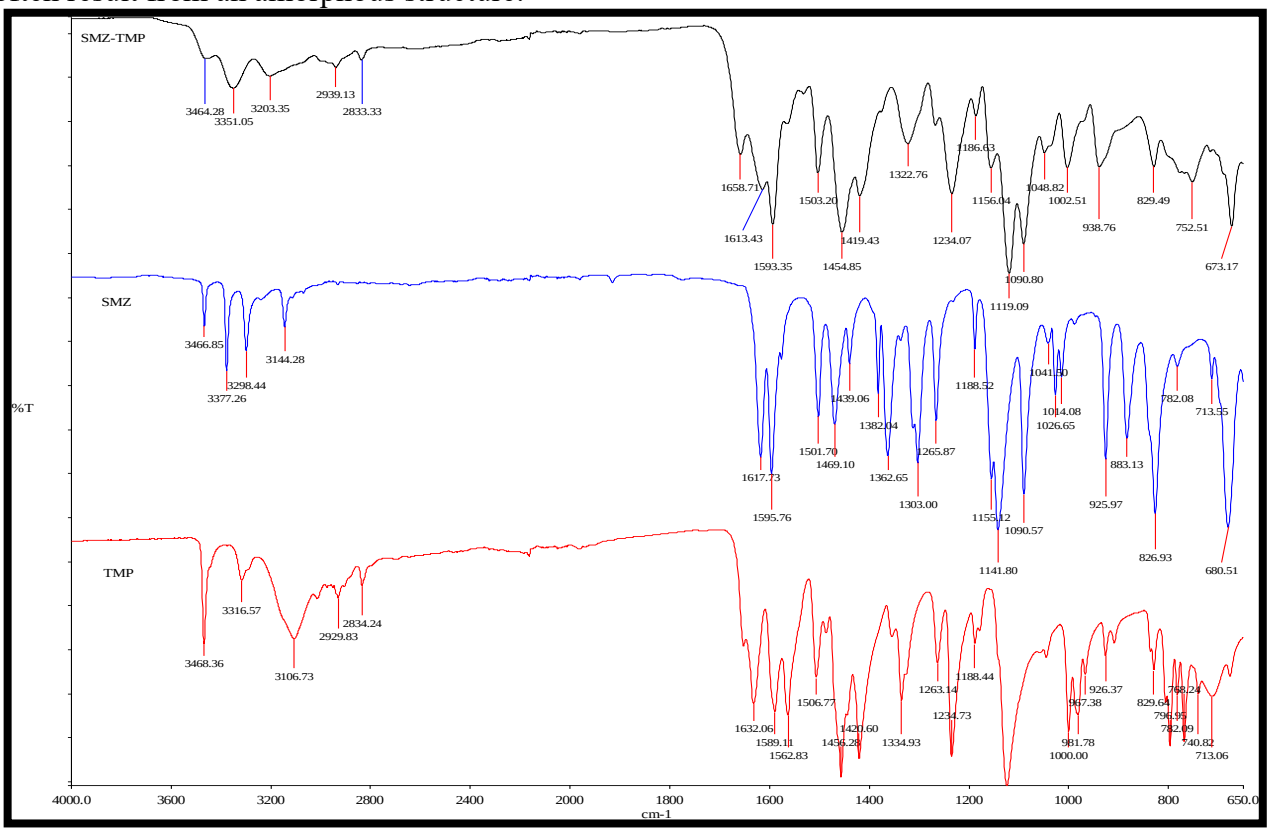


Figure 12. FTIR spectra of amorphous SMZ-TMP, SMZ and TMP

Table 6. Selected infrared data (from FTIR) for the compound SMZ-TMP compared to SMZ and TMP

SMZ standard frequency bands (cm^{-1})	TMP standard frequency bands (cm^{-1})	Amorphous SMZ-TMP frequency bands (cm^{-1})	Interactions and comment
3466	3468	3464	-NH-H•••NH_2
NH_2 Asymmetric	NH_2 Asymmetric	NH_2	Decrease in the intensity.
3298	3468	3203-3464	-HN-H•••NH
NH stretch	NH_2 Asymmetric	NH- NH_2	Decrease in the intensity.
3377	3316	3351	-NH-H•••NH_2
NH_2 symmetric	NH_2 symmetric	NH_2	Decrease in the intensity.
3298	3316	3203-3351	-HN-H•••NH
NH stretch	NH_2 symmetric	NH- NH_2	Decrease in the intensity.
3466	1123	3464-1119	-OCH_2-H•••NH_2

NH_2 Asymmetric	Aromatic methoxy	NH2-OCH_3	Decrease in the intensity.
3298	1123	3203-1119	-OCH_2-H•••NH
NH stretch	Aromatic methoxy	NH-OCH_3	Decrease in the intensity.
3377	1123	3351-1119	-OCH_2-H•••NH_2
NH_2 symmetric	Aromatic methoxy	NH_2-OCH_3	Decrease in the intensity.

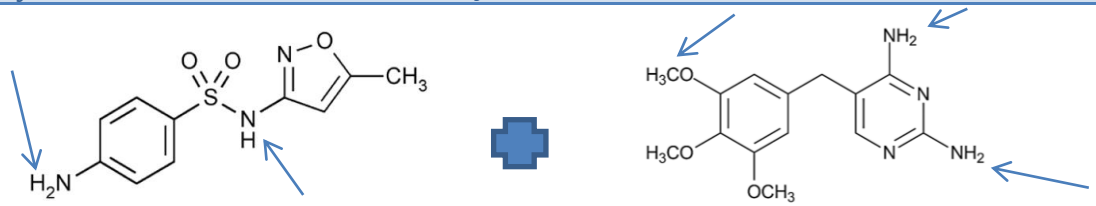


Figure 13. Indication of the functional groups that could interact with the molecules of SMZ and TMP to create SMZ-TMP from FTIR spectra.

According to Table 6, the IR spectra of the SMZ-TMP sample was noticeably various from that of the parent compounds (TMP and SMZ), indicating the formation of a new molecule. When a neutral amino group (-NH_2) displays a strong NH_3 stretch around 1665-158, 1530-1490 cm^{-1} and a high stretching peak at 3466 cm^{-1}, a co-crystal is created. Additionally, a sharp decline in peak intensity points to a co-crystal that is amorphous.

A peak at 3464 cm^{-1} (-NH_2) shows no protonating is taking place, which supports the idea that SMZ-TMP is an amorphous co-crystal. The term "amorphous co-crystal" will now be used to describe SMZ-TMP.

Powder X-ray diffraction (PXRD)

The amorphous SMZ-TMP PXRD signals lacked any prominent peaks that are indicative of a crystal material. The absence of these peaks (Figure 13), however, points to an amorphous shape (Egami and Billinge 2003).

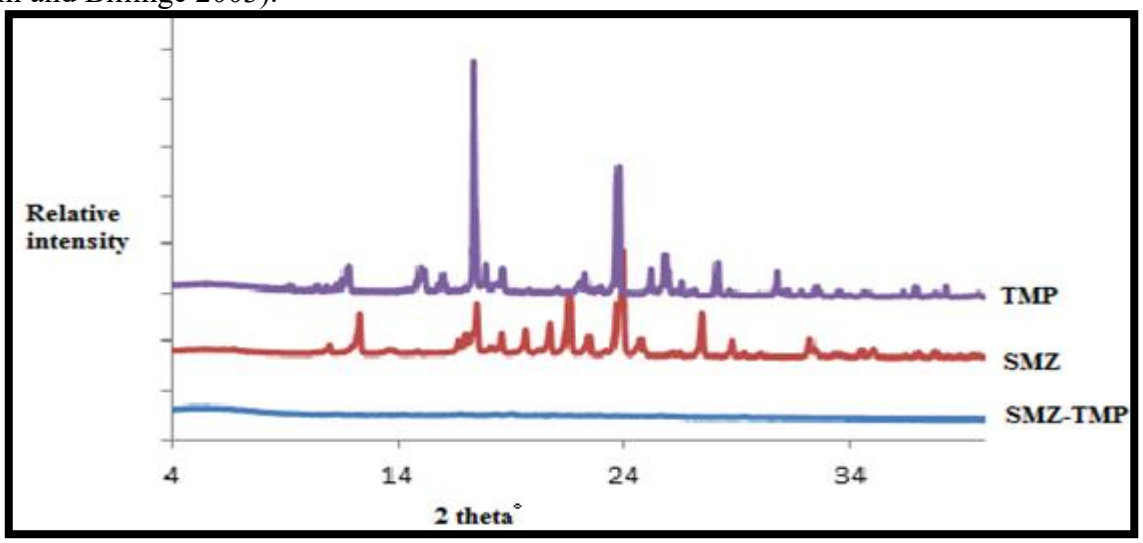


Figure 14. PXRD patterns of amorphous SMZ-TMP, SMZ, TMP

We were able to explain the structural characteristics of the amorphous SMZ-TMP using FTIR thanks to the analytical results of the experimental system, and we were able to connect the end product to the crystalline phase that (Zaini *et al.*, 2009) described in their published research and that they had produced using the slurry approach. In order to establish that the glassy amorphous form is a co-crystal form of SMZ-TMP, it was re-crystallized using several solvent systems into a co-crystal with a melting point of 180 ºC, the same as the amorphous SMZ-TMP.

The SMZ-TMP substance, which lacks a crystalline structure, has an amorphous nature, resulting in the material's inability to undergo X-ray diffraction.

Challenges faced when forming a sulfamethoxazole co-crystal:

Recrystallization problems:

Sulfamethoxazole has 5 polymorphs **(Bettinetti *et al*. 1982 and Deo *et al.* 1980)** and a hemihydrate **(Takasuka** and **Nakai 2001)** form. The hemihydrate form of SMZ recrystallized as a result of co-crystallization with the appropriate co-formers in the chosen solvent system. One of the five identified polymorphs also underwent recrystallization throughout the experiment.

Quality of the crystals:

The most difficult issue in the trials was the quality of crystal. Single crystal X-ray diffraction analysis needs a suitable crystal with specified qualities and a particular size. Because so many crystals were fibrous or opaque in nature, it was challenging to create crystals with these characteristics. For single crystal XRD, other crystals were far too thin.

Long periods of growth are common for crystals of high grade. It took six months to generate a single co-crystal of sufficient quality for a single X-ray diffraction examination, according to Portalone and Colapietro (2004).

Solubility:

Sulfamethoxazole is soluble in a certain system of solvents. Furthermore, the 2 compounds must have the same solubility, or comparable solubilities, in the same solvent in order to make co-crystals of the API and co-former; otherwise, the less soluble molecule would precipitate (Yadav *et al*., 2009). But "similar solubility" by itself won't ensure success. Sometimes we must employ solvents that the API or the co-former does not entirely dissolve in in order to calculate solubility. One of the components may be acting as a surface-active agent, promoting solubility, to explain this behavior.

Stability:

The pharmaceutical sector, for instance, places a premium on a solid therapeutic material's relative humidity stability because of the practical effects that the hydrate form has on formulation, storage, and packing (Byrn *et al*., 1999). Even if the solvent for sulfamethoxazole is not water because of the environment, this issue constantly arises. Additionally light-sensitive is sulfamethoxazole. Sulfamethoxazole Similar compounds are produced via light-catalyzed oxidation .

While, some co-crystal materials can only be produced by solid-state grinding, several co-crystal materials can be made using both solution solid-state grinding and increase (Lynch *et al*., 1991).

IV)Conclusions:

This study successfully explored the feasibility of combining sulfamethoxazole (SMZ) with trimethoprim (TMP) and other co-formers to form solid crystalline and amorphous phases. The SMZ-TMP system crystallized as a co-crystal in methanol and as a salt in tetrahydrofuran (THF), demonstrating solvent-dependent polymorphic behavior similar to other sulfonamide-trimethoprim combinations. Additionally, an amorphous SMZ-TMP co-crystal was obtained through melt solidification, exhibiting distinct thermal and spectral characteristics. The study also achieved the preparation of sulfamethoxazole-oxalic acid (SMZ-OA) and sulfamethoxazole-benzoic anhydride (SMZ-BAN) phases through vapor diffusion and slurry techniques, respectively. These findings highlight the potential of co-crystallization to enhance the physicochemical properties of SMZ, such as solubility and thermal stability, which are crucial for optimizing pharmaceutical formulations.

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