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MATRIX ASSISTED CO CRYSTALLIZATION OF ACETAZOLAMIDE FOR ENHANCING AQUEOUS AND LIPIDIC SOLUBILITY

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ABSTRACT

Pharmaceutical Science nowadays is an emerging field to serve mankind and consists of challenges to produce efficient and optimized medicines. The concept of enhancing a drug's efficacy depends upon many factors such as pharmacokinetics, pharmacodynamics of the drug, which again depend upon physicochemical properties of that drug. The therapeutic knowhow of the drug is not enough to prove the reliability of the drug. Many parameters are taken into consideration to formulate market ready drug. Solubility, Permeability are among them who play an important role in Bioavailability of the drug. New approach available for the enhancement of drug solubility is through the application of the co-crystals, it is also referred as molecular complexes. A novel method for the simultaneous production and formulation of pharmaceutical cocrystals, matrix-assisted cocrystallization (MAC), is presented considering Acetazolamide, a BCS Class IV drug with utmost difficulty in formulation and has greater solubility issues. This article focuses on the study of cocrystallization of Acetazolamide using suitable coformer and a matrix for stability commonly referred as MAC product which will be prepared by the ball milling of the three components. The main aim is to produce scalable, amenable method of production of cocrystals and enhancing solubility.

KEYWORDS: Acetazolamide, BCS class, Cocrystallization, matrix, mechanochemical synthesis, insilico predictions.

1. INTRODUCTION

1.1 Concept of Crystallization

A crystalline state is of a drug is considered to be highly pure state. Any drug can be converted into crystal state so as to improve its physicochemical properties that are necessary for its formulation like aqueous solubility and permeability. Co-crystallization is a concept that arises from incorporation of a drug or API(Active Pharmaceutical ingredient) into a crystal lattice using a suitable coformer. Cocrystals of any drug are proven to have improved physicochemical properties and affect Bioavailability, hygroscopicity, mfg process, compressibility, purification, stability, flowability, and many such other performances of a drug without affecting pharmacological activity.[1] Co crystals are defined as crystalline complexes of two or more neutral molecular constituents bound together in the crystal lattice through non covalent interactions. Coformer is an important component that forms crystal lattice with the drug under study. Cocrystals are formed through noncovalent interactions such as hydrogen bonding, ionic bonding, vanderwaals interactions etc.

1.2 BCS Class of drugs

Biopharmaceutics classifies the drugs into 4 classes, among which BCS class II and BCS class IV drugs report poor aqueous solubility, permeability and formulation difficulties.[2] Aiming to enhance solubility of such drugs Crystal Engineering is a promising approach. Features of BCS Class of drugs are explained in table below:

Table 1: BCS class of drugs.

Class	Features	Examples
I	High permeability. High solubility	Paracetamol, metoprolol, theophylline
II	High permeability. Low solubility	Atovaquone, carbamazepine, danazol, glibenclamide, griseofulvin, ketoconazole, troglitazone
III	Low permeability. High solubility	Acyclovir, atenolol, cimetidine, ranitidine
IV	Low permeability. Low solubility	Chlorothiazide, furosemide

1.3 Acetazolamide and its solubility

Acetazolamide is a sulfonamide derivative with diuretic, antiglaucoma, and anticonvulsant properties.

Acetazolamide is a non-competitive inhibitor of carbonic anhydrase, an enzyme found in cells in the proximal tube of the kidney, the eye, and glial cells. Inhibition of this enzyme in the kidney prevents excretion of hydrogen, leading to increased bicarbonate and cation excretion and increased urinary volume, which results in an alkaline diuresis. Acetazolamide reduces the concentration of bicarbonate, resulting in a decreased synthesis of aqueous humor in the eye, thereby lowering intraocular pressure. Although its mechanism of action is unknown, acetazolamide has anti-convulsant properties resulting from indirect effects secondary to metabolic acidosis or direct effects on neuronal transmission. Acetazolamide also produces respiratory stimulant effects in response to changes to both carbon dioxide and oxygen tension levels within the lungs.

Acetazolamide is a potent carbonic anhydrase inhibitor, effective in the control of fluid secretion, in the treatment of certain convulsive disorders and in the promotion of diuresis in instances of abnormal fluid retention. Acetazolamide is not a mercurial diuretic. Rather, it is a nonbacteriostatic sulfonamide possessing a chemical structure and pharmacological activity distinctly different from the bacteriostatic sulfonamides.[3]

Solubility of Acetazolamide

Acetazolamide is very slightly soluble in water; sparingly soluble in boiling water. At 250C, an aqueous solubility of 0.72 mg/mL was reported. Between pH 1.68 and pH 8.17, solubilities of 0.8–2.8 mg/mL were reported. Another source reports the solubility between pH 4 and pH 7 at 250C to be approximately the same (0.8–1 mg/ mL). At 378C, equilibrium solubilities of acetazolamide in pH 1.2 and pH 7.4 were reported to be 1.23 and 2.43 mg/mL, respectively. At 250C, between pH 1.68 and pH 8.17, solubilities of 1.26– 2.79 mg/mL were reported. Also, an aqueous solubility of 0.70 mg/mL was reported for acetazolamide.[4]

1.4 Solubility Enhancement

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the solvent used as well as on temperature and pressure. The extent of solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase its concentration in the solution.[5]

Solubility measurement is a valuable activity throughout drug discovery. It is the guidance towards the properties of drug that are important for its formulation. Solubility is important because information of solubility assists diverse research activities which are mentioned as follows:

1. Pharmacokinetic screening
2. Optimization of structures
3. Biological activity assessment
4. Animal dosage form selection for efficacy
5. Pharmacokinetics and toxicity

40% marketed drugs have low solubility and 80-90 % drug candidates fail to reach market because of solubility issues. Many methods are available but some methods undergo amorphization so less effective, Solvent containing methods need location of system in phase diagram, concentration of solvent matters and have chemical and physical stability issues. Thus control of stoichiometry and high quality of co crystals can be achieved by matrix assistance. Matrix during ball milling, acts as a catalytic solvent, promotes mixing and reduces shear stress and eventually crystal damage.

1.5 Matrix and coformer

Matrix is any polymer that has low melting point, high stability and affinity towards drugs but inert in nature. It is important that matrix should act as catalyst in formation of cocrystal, and also impart the cocrystal a stable form. Hot melt extrusion is latest technology where matrix is useful in the formation of cocrystals. The cocrystal particles formed are embedded in the matrix material, which solidifies upon exiting the extruder. The end product of the MAC process, termed MAC product, is a matrix-embedded cocrystal with the level of controlled stoichiometry offered by both thermal and mechanical cocrystal production methods.[6] The crystal quality and ease of production makes this process popular one.

Co formers are any chemicals, acids, amides or any ones who have strong hydrogen bonding capacity. They are responsible for formation of the new crystalline form of the drug that will be better in solubility and permeability than the old drug. Coformers need to be efficient enough to form crystal with the drug and should also be compatible with the drug under study.[7] Coformer can be selected on the basis of trial and error and by supramolecular synthons, (Cambridge Structural Database), Hansen solubility parameter and knowledge of Hydrogen bonding between Drug and coformer. Acetazolamide Co-crystals by MAC method using Tartaric acid as coformer and with Poloxamer 407 as matrix agent is the novel concept presented here.

2. MATERIALS AND METHODOLOGY

2.1 Materials

Acetazolamide was received from Ctx Life Sciences, Mumbai as a gift sample.

Tartaric acid received from Shree Ambabai Talim Sanstha's Diploma In Pharmacy College, Miraj Poloxamer 407 procured from Rajesh chemicals, Mumbai.

2.2 Methodology

2.2.1 In-Silico Approach for Selection of Co-formers-CAD Studies

3D structure of the Acetazolamide and selected

coformers were developed using **V life engine** and optimized via application of **Merck molecular force field** to get an optimized structures. The optimized structures are utilized to analyze the property and Non covalent interactions.

2.2.2 Preparation of Reference standard by solvent evaporation method

The reference Standard cocrystals were prepared by using **Ethanol** solvent . Acetazolamide and Tartaric acid were taken in 1:1 molar ratio, i.e in stoichiometric quantities and mixed with 5 times greater amount of solvent ethanol. The solution was heated upto 60^{o} C until clear solution is obtained. The solution was kept in a porcelain dish for 24 hrs in open air until all the solvent evaporated and pure crystals were obtained. The crystals were collected, weighed and analysed.

2.2.3 Optimization and Preparation of Cocrystals by ball milling

The ratio of drug to coformer was taken as 1:1, considering the molecular masses, they were initially weighed. Molecular mass of Acetazolamide is 222.3g/mol and that of Tartaric acid is 150.08g/mol. Thus, 1:1 Stoichiometric quantities of Acetazolamide and Tartaric acid were mixed initially in a mortar pestle.Percentage of matrix was an important parameter to be optimized and that was done using melting point similarity between pure Acetazolamide and MAC batches. The three different % of matrix decided were 10,15,20% .The matrix were then added to the mixtures of Acetazolamide and Tartaric acid and 3 batches were ball milled for the specific period of time evaluated. The time of ball milling was optimized by considering quality of cocrystals obtained The mechano chemical synthesis is done by ball milling technique. The details of the method is explained in the table.

Table 2: Ball milling Technique.

Method	Ball milling Technique
Ball mill used	Shreeji chemicals, SATS Pharmacy college, Miraj
Milling time	10, 15 ,20 minutes
Speed of Ball mill	30rpm
% of matrix	10 , 15,20 %
Amount of sample	2gm per batch

The amount of sample taken was 2gm as per the capacity of ball mill used and the speed of rotation was kept 30 rpm. All the batches were collected and weighed after milling and analyzed for further studies.

2.2.4 Evaluation of cocrystals by FTIR and THERMAL ANALYSIS.

Melting point determination of each sample was performed by capillary method and noted. The % of matrix was selected with help of similarity in melting point of Drug and formulated batches. Melting point was also useful in authentication of the drug, coformer and matrix as well.

FTIR studies were performed at Quadrrant Lab, (FDA approved) Madhavnagar with BRUKER. The drug , Reference standard and MAC product with selected % matrix was analyzed.

3. RESULTS AND DISCUSSION

3.1 In-Silico Approach for Selection of Co-formers-CAD Studies

2D QSAR module of the **Vlife MDS 4.6** was utilized for the property calculation, Acetazolamide and selected coformers showed number of H-Acceptor and H-Donor which might be playing vital role in the intermolecular interactions. Calculated property of Acetazolamide and selected coformers are summarized in following table.

Table 3: Calculated properties of coformers and Aceatzolamide.

Sr no	Name of coformer	H-Acceptor Count	H-Donor Count	XlogP	Slogp
1	Acetazolamide	6	2	0.363	-0.426
2	Benzoic acid	2	1	1.534	1.385
3	Para amino benzoic acid	3	2	0.786	0.967
4	Succinic acid	4	2	-0.604	-0.064
5	Tartaric acid	6	4	-1.654	-2.123

The above information explains that Tartaric acid has more H-Donor and H- acceptor count than all the other 3 cofomers selected. Tartaric acid is acceptable due its lower Gibb's free energy and thus can prove to enhance solubility of acetazolamide by hydrogen bonding.

Inter molecular interaction studies:

Intermolecular interactions was calculated via **Biopredicta module of V life MDS 4.6,** to analyze possible role of coformers in the crystallization of acetazolamide. All selected coformers showed hydrogen bonding with acetazolamide which might be responsible for the solubility enhancement of acetazolamide.

Table 4: Type of Interactions and distance of Various coformers with Acetazolamide.

Sr. No	Coformer	Type ofInteractions	Distance in A^O
1.	Benzoic acid	Hydrogen bond (Green Color)	1.548
			1.565
2.	Para amino benzoic acid	Hydrogen bond (Green Color)	2.147
			2.208
3.	Succinic acid	Hydrogen bond (Green Color)	1.878
			1.526
		Hydrophobic (Blue Colour)	3.421
			4.707
4.	Tartaric acid	Hydrogen bond	2.108
		Hydrophobic (Blue Colour)	3.558
			3.838

It is clear from the above information that Tartaric acid and Benzoic acid show the shortest distance for hydrogen bonding. But Tartaric acid has also shown more H-donor and H-acceptor count and hence it has proven to be most suitable coformer undergoing hydrogen bonding with Acetazolamide.

The 3 D structures are presented as follows:

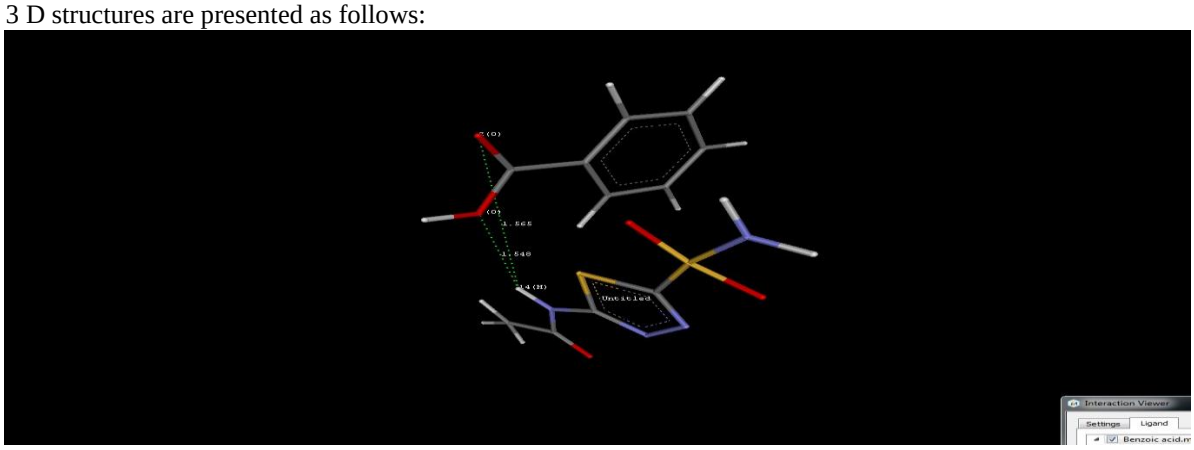

Figure 1: Interaction of Acetazolamide with Benzoic acid.

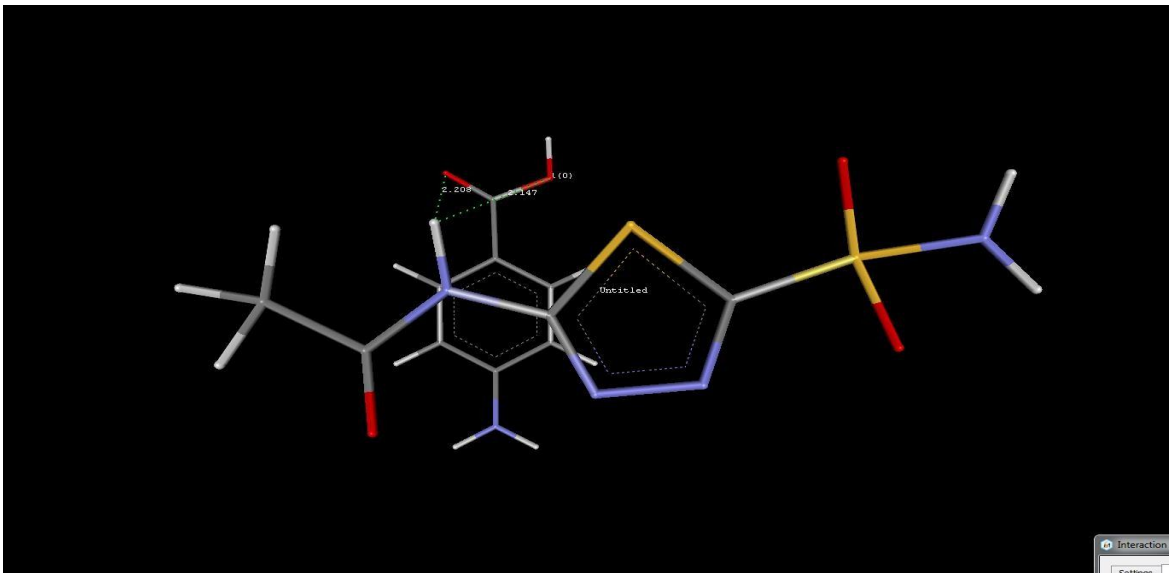

Figure 2: Interaction of Acetazolamide with p-AminoBenzoic acid.

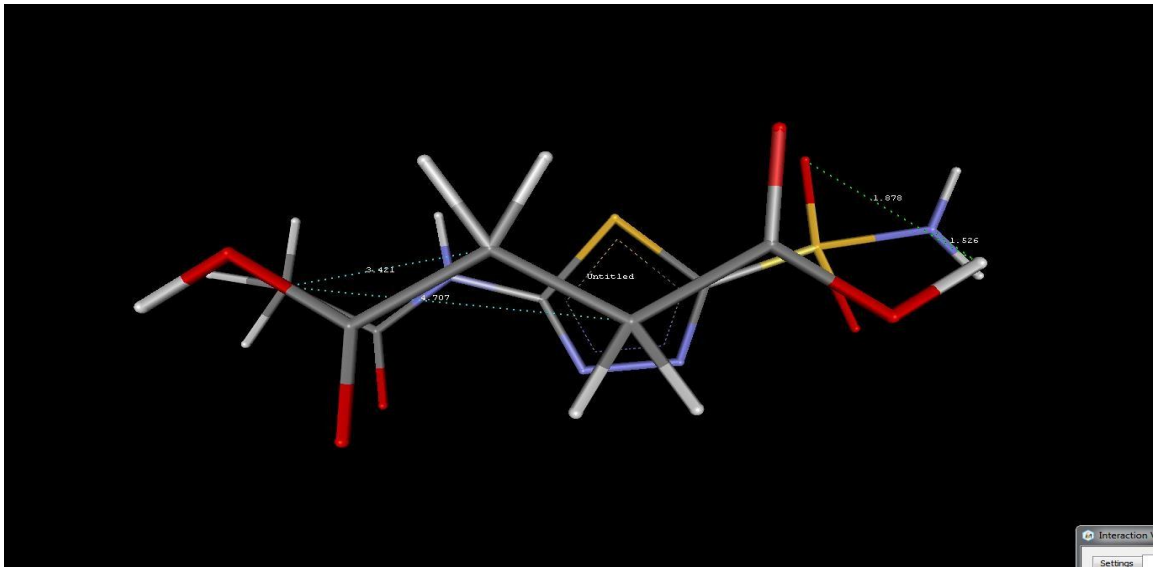

Figure 3: Interaction of Aceatazolamide with Succinic Acid.

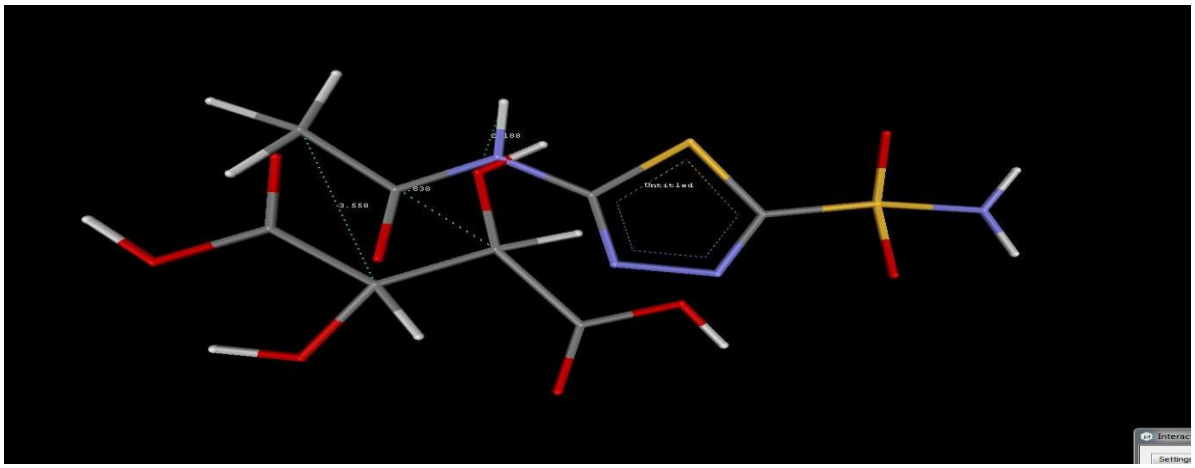

Figure 4: Interaction of Acetazolamide with Tartaric Acid.

Tartaric acid having H-bonding with shortest distance and more H-donor, acceptor count was selected as suitable coformer.

3.2 Thermal analysis

Melting point determination was done by capillary method of each of the sample. Acetazolamide, Tartaric acid, Poloxamer 407, all the 3 batches with different matrix percentages underwent melting point determination. The results are given in the table below.It was clear that the batch having 20% matrix had melting point similar to that of the pure drug which shows that matrix 20% batch yields a MAC product with no change in Acetazolamide moiety.

Table 5: Melting points of different samples.

Sr no	Name of entity	Melting point in ^{o}C
1.	Acetazolamide procured drug	257.5
2.	Tartaric acid	172.60
3.	Poloxamer 407	50.30
4.	ACZ-TA Reference standard	255.2
5.	MAC product with 10% matrix	256.4
6.	MAC product with 15% matrix	256.9
7.	MAC product with 20% matrix	257.1

From the above figures, MAC PRODUCT WITH 20% MATRIX was selected for further studies as it showed less variation in Melting point with respect to pure Acetazolamide drug. It also confirmed formation of MAC product with no any change in the intrinsic property of Acetazolamide moiety.

3.3 Ftir Analysis

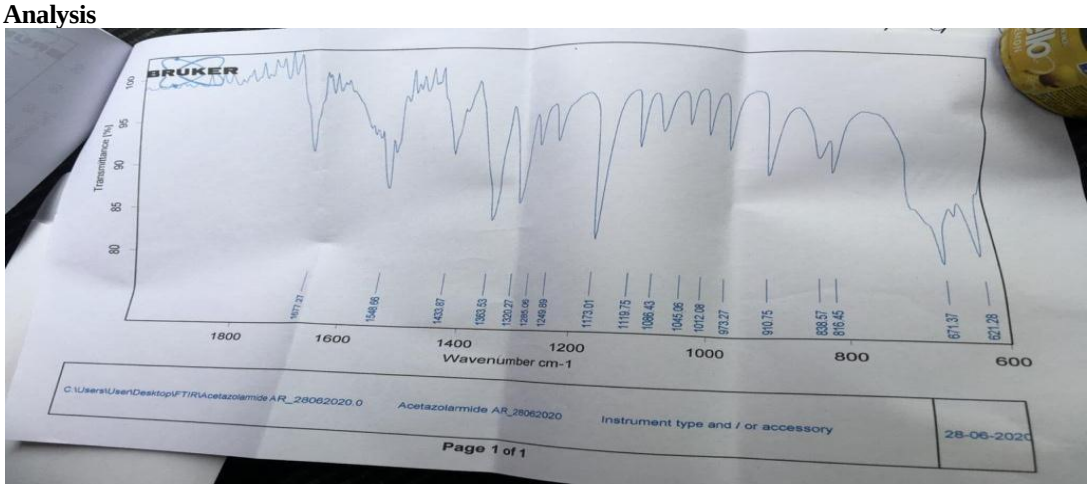

Figure 5: FTIR Spectra of procured Acetazolamide drug.

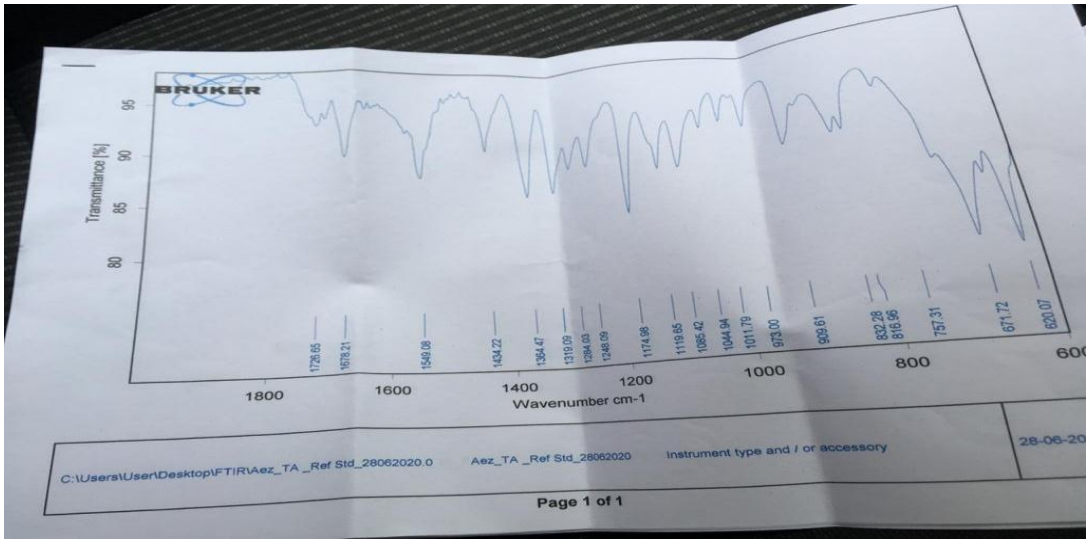

Figure 6: FTIR spectra of Reference std prepared by solvent evaporation method.

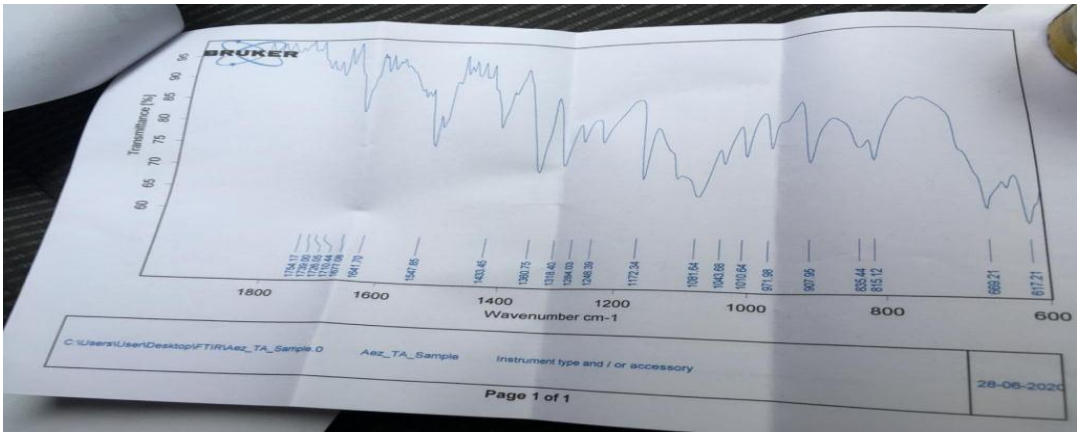

Figure 7: FTIR spectra of MAC product with 20%matrix.

All the 3 FTIR Spectra have similarity in peaks and thus confirm successful formation of MAC product without any alteration in Acetazolamide moiety.

The characteristic peaks of Acetazolamide noted in all the 3 spectras are given in the table below: Table no 6. FTIR Peaks of Acetazolamide.

Sr no	Peak observed cm-1	Peak Reported cm-1	Bond	Functional group
1.	620.07	621	C-S Stretching	-
2.	816.96	818	NH out of plane bending	-
3.	1011.79	1013	S=0 Stretching	-
4.	1434.22	1435	CH_3	CH_3 Asymmetric bending
5.	1678.21	1680	C=O Stretching NH_2 Scissoring	Aromatic Carboxylic Amino

4. CONCLUSION

BCS Class IV drug, Acetazolamide was successfully transformed into its MAC product. The process of ball milling was useful in obtaining cocrystals. Matrix-assisted cocrystallization is an effective method for producing high-quality cocrystals, while simultaneously incorporating them into a functional matrix material with formulation implications. The Acetazolamide- Tartaric acid -Poloxamer 407, MAC product, prepared by the ball milling of the three components, showed qualitatively and quantitatively, to be compositionally equivalent to a reference standard in the same proportions. The FTIR and Thermal Analysis proved the existence of MAC product.This proposed method for the simultaneous production and formulation of pharmaceutical cocrystals is solvent free, scalable, and amenable to continuous manufacturing, giving it great potential for utilization in commercial production of cocrystal based pharmaceutical products. Mechanochemical synthesis of cocrystals by ball milling is an promising approach for supporting concept of Green Chemistry.

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