Synthesis and Characterization of Multicomponent Drug-Drug Co-Crystals

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Introduction:

A critical issue within the pharmaceutical industry concerns the resolution of poor physicochemical characteristics associated with a drug or pharmaceutical compound [1]. Many new high-value drug candidates are largely constrained by low aqueous solubility and chemical instability. This problem is of particular relevance to the biopharmaceutical industry. Biotechnology-derived therapeutics are of growing importance in areas such as medicine and immuno-oncology. Many biotherapeutics are macromolecular in nature – their size and complexity can vastly exceed that of the many low molecular weight pharmaceutical compounds on the market. This correlates with a reduced oral bioavailability. The recurrence of sub-optimal molecular properties has been a key driver in the emergence of supramolecular chemistry and crystal engineering in industry. Physicochemical modulation techniques (such as co-crystallization) have been viewed as viable and effective methods of optimizing the molecular characteristics of formulated active pharmaceutical ingredients [2]. Though very few co-crystals are commercially available, a growing interest across both industry and academia has led to the introduction of new legislation on co-crystal classification, as of February 2018 [3]. It is anticipated that advancements in the area of co-crystallization will have a profound impact on drug development, and ultimately facilitate the design and commercialization of new therapeutics.

In this research project, liquid assisted grinding was used to synthesize binary co-crystals of acetazolamide and theophylline anhydrous in various stoichiometric ratios. The morphologies and physicochemical characteristics of the resulting co-crystals were investigated via microscopy, spectroscopy and powder X-ray diffraction. A multicomponent co-crystal of acetazolamide, theophylline anhydrous and microcrystalline cellulose was synthesized mechanochemically, and its solubility in methanol compared against its binary counterpart at 20℃.

Results:

The following stoichiometric ratios of acetazolamide (A), theophylline anhydrous (T) and microcrystalline cellulose (M) were investigated. With respect to sample nomenclature, A:T 1:1 refers to a mechanochemically synthesised co-crystal created using 0.25 mmol of acetazolamide and 0.25 mmol of theophylline anhydrous in excess methanol at a temperature of 30℃. A similar basis is taken for all other sample ratios:

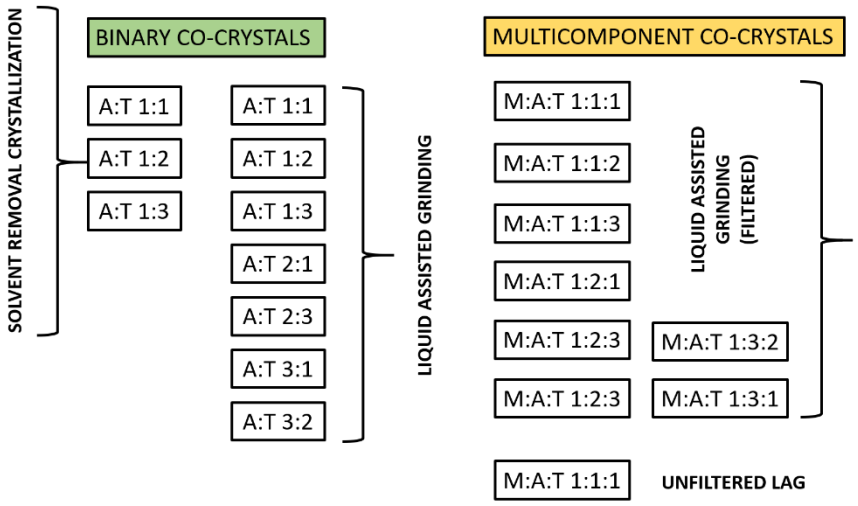
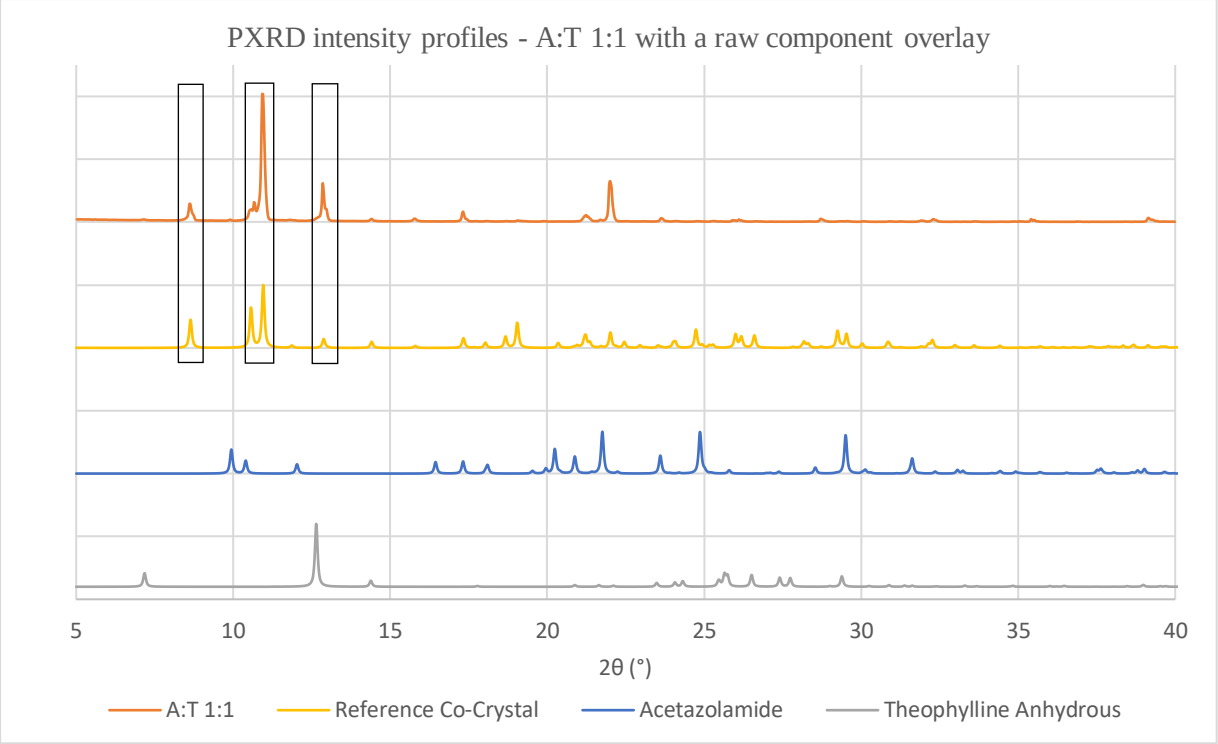


Figure 1: Identifying stoichiometric ratios for co-crystal samples

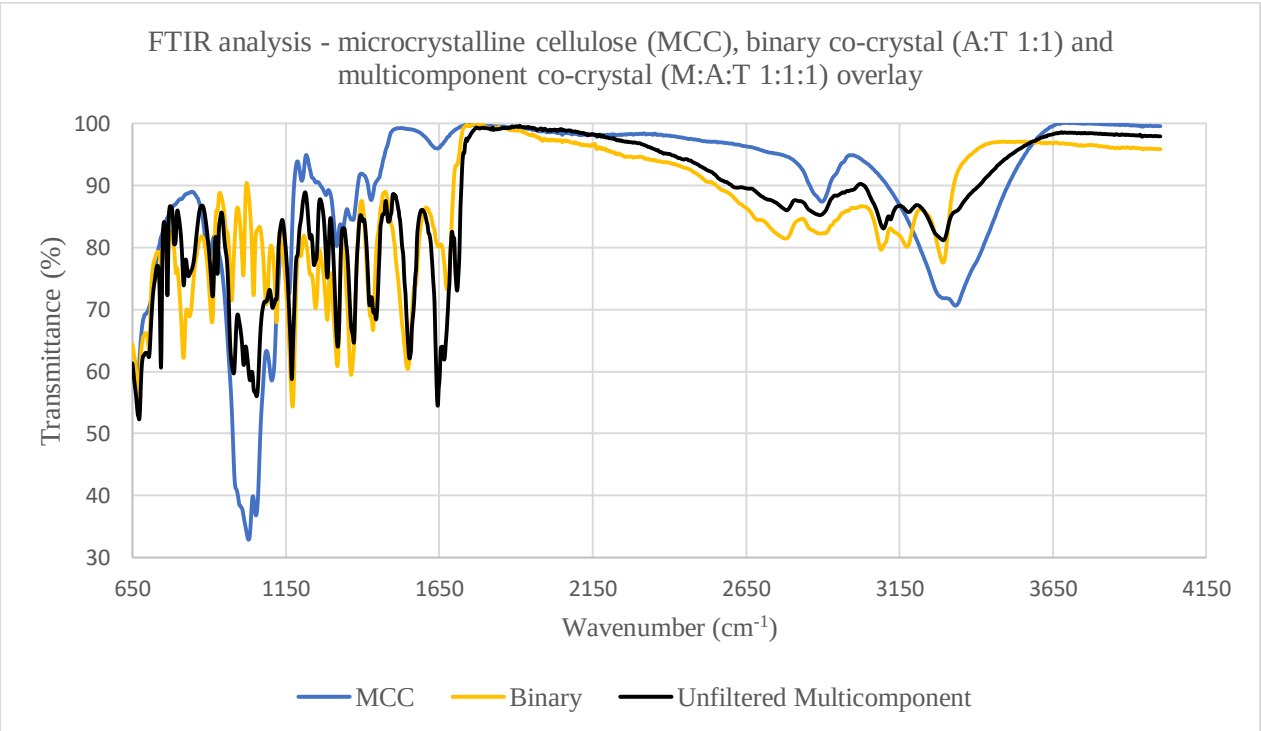
Brightfield microscopy and scanning electron microscopy (SEM) were used to assess the impact of the stoichiometric ratio on co-crystal morphology. Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy and powder X-ray diffraction (PXRD) were utilised to characterize the binding within the co-crystals. A gravimetric method was employed to investigate changes in the solubility of the co-crystals in methanol at different temperatures.

Spectroscopy and X-Ray Diffraction: Confirmation of Co-Crystal Formation and Excipient Binding

The veracity of the mechanochemical co-crystal synthesis method used in this investigation was confirmed using Powder X-Ray Diffraction (PXRD). An overlay of PXRD data for the raw components, a binary acetazolamide-theophylline anhydrous co-crystal (A:T 1:1), and a reference co-crystal is given below. Characteristic peaks consistent with the formation of a binary co-crystal are highlighted in black:



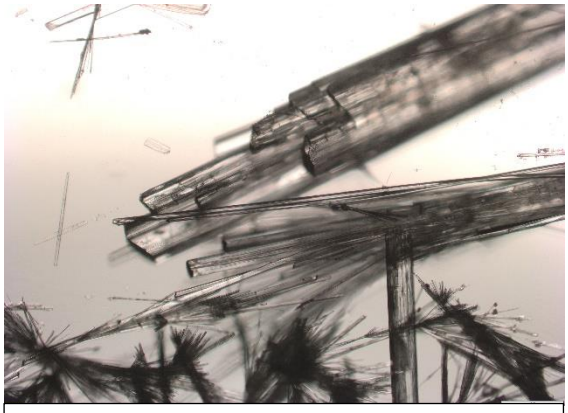
Evidence of binary co-crystal formation via PXRD gave justification for the use of liquid-assisted grinding in other co-crystal syntheses. This method was therefore extended to the synthesis of a multicomponent co-crystal of acetazolamide (ACZ), theophylline anhydrous (THP) and microcrystalline cellulose (MCC). FTIR analysis provided compelling evidence of multi-component co-crystal formation, specifically the binding of MCC to the binary co-crystal:



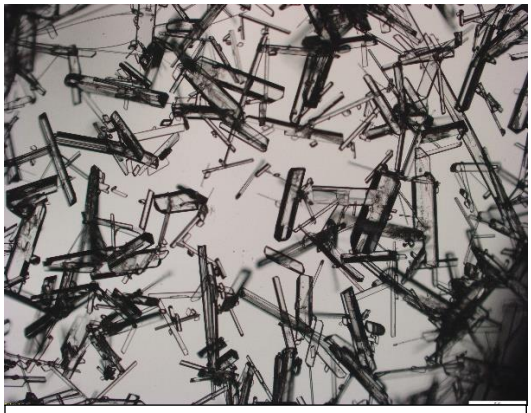
Comparing the multicomponent co-crystal with MCC, there are significant changes in wavenumber regions associated with S=O and C-O stretching (950-1150 cm^{-1}) and C=C stretching (1638-1648 cm^{-1}). Variations in wavenumbers associated with C-O and C-N binding regions (3150-3650 cm^{-1}) were also recorded. The changes in these values are consistent with intramolecular bonding and supramolecular synthon interactions between the APIs. The upward shift in the transmittance of the multicomponent co-crystal at 3300 cm^{-1} is indicative of binding. Comparing the binary co-crystal with the multicomponent co-crystal, the primary regions of difference (with exception, the fluctuations between 3150 and 3650 cm^{-1}) are from 950-1150 cm^{-1} and 1638-1648 cm^{-1}. From 950-1150 cm^{-1}, the binary co-crystal admits a higher transmittance. This likely owes to the favourability of supramolecular synthon interactions between acetazolamide and theophylline over hydrogen bonding in MCC. This may account for the discrepancy in these values, and explain why beyond these regions, the binary and multicomponent co-crystal transmittance is quite similar [4].

Microscopy: Influence of Stoichiometric Ratio on Co-Crystal Morphology

The stoichiometric ratio of the individual components was found to profoundly influence the morphology of the resulting co-crystal. Co-crystals with a higher ratio of acetazolamide were smaller in size with a bar-like crystal habit. Increasing additions of theophylline led to larger crystals. They were observed to be more brittle, with elements of hexagonal plate stacking seen within the co-crystal. This is consistent with literature, as theophylline anhydrous is polymorphic, and presents as Form IV at elevated methanol temperatures [5]:



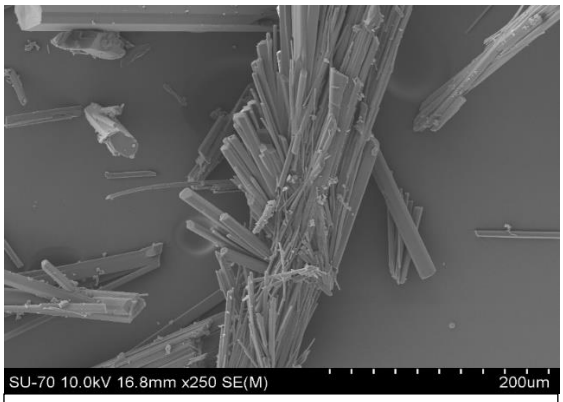
A:T 1:3 – LAG – 500 µm



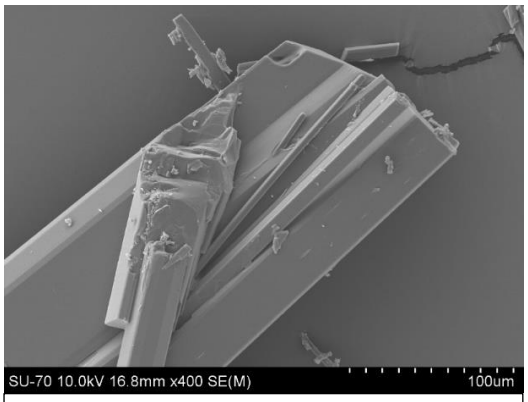
A:T 3:1 – LAG – 500 µm

Observations of stoichiometry mediated changes in morphology and crystal habit were further supported by SEM analysis. Scanning electron microscopy images comparing binary

theophylline-enriched (A:T 2:3) and theophylline-deficient (A:T 2:1) co-crystals are given below:



A:T 2:3 – LAG – 200 µm



A:T 2:1 – LAG – 100 µm

These morphological differences likely derive from an imbalance of heteromeric interactions between the individual co-crystal components during the formation of an equilibrium crystal structure. Mechanistically, acetazolamide and theophylline first dimerize through the self-association of their respective homosynthons. Theophylline dimers form through N-H…O non-covalent bonds, whereas acetazolamide molecules dimerize via N-H…N bonding. Acetazolamide further associates with itself via N-H…O bonds to form a tape. N-H…O bonding of THP-dimers to the ACZ-tape yields a corrugated sheet. Non-covalent C-H…N interactions facilitate the stacking of the sheets, thus creating an interlocking 3D structure [6].

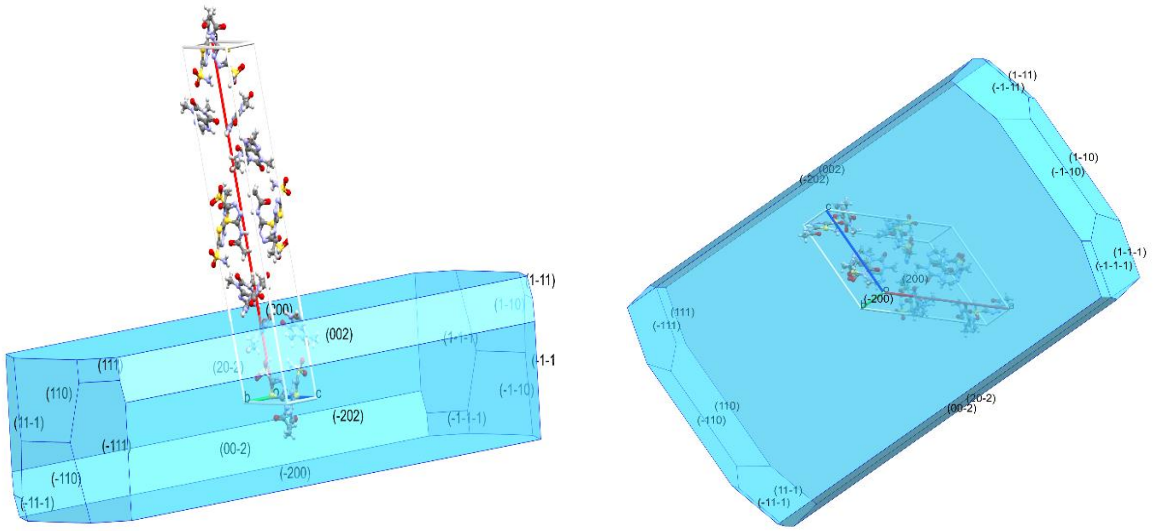


Figure 2: BFDH representation of an equimolar binary acetazolamide-theophylline co-crystal (C2/c, A:T 1:1). The binary co-crystal is characterized as a monoclinic system – with respect to crystal symmetry, it is centrosymmetric.

It is theorized that an inequality in the stoichiometric ratio of the components would interfere with the underlying mechanism of co-crystal formation, in particular the stacking of corrugated sheets.

Solubility in Methanol: Improved Physicochemical Properties of Multicomponent Co-Crystals

The solubility of a binary co-crystal (A:T 1:1) was determined in methanol at 10℃, 20℃ and 30℃. Co-crystal solubility was determined in quintuplicates, and is detailed below in both a graphical and tabular format:

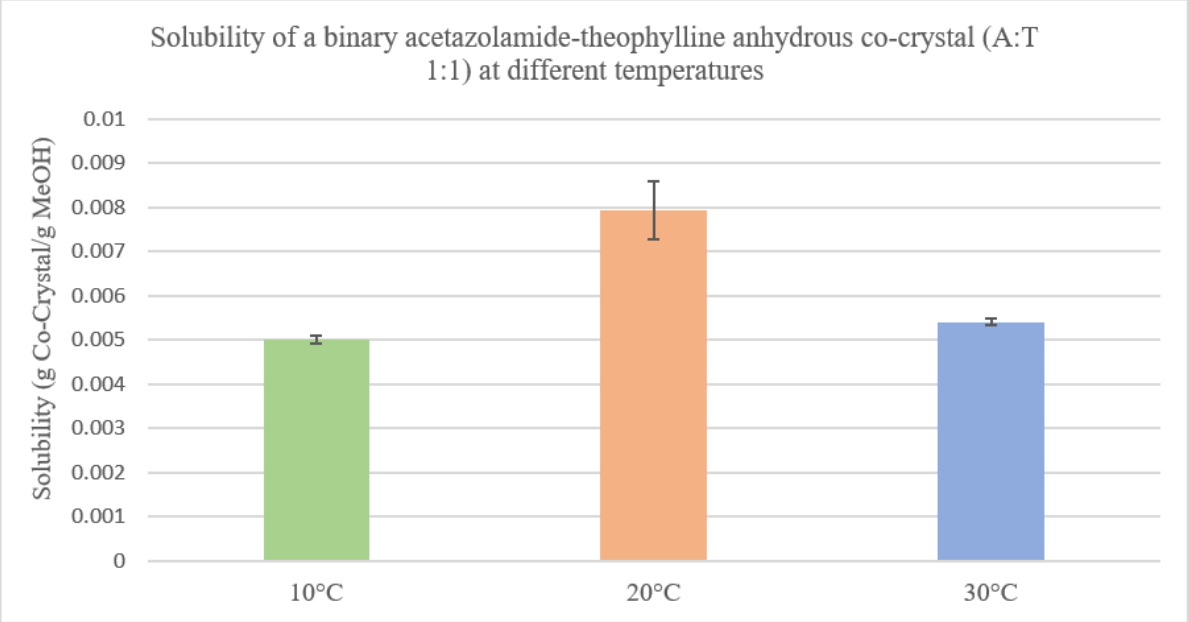


Table 1			
Solubility of a Binary Co-Crystal in Methanol at Different Temperatures			
Temperature (℃)	**Average Solubility (g/g)**	**Standard Deviation (g/g)**	**Replicates**
10	0.00501	0.00008	n = 5
20	0.00792	0.00066	n = 5
30	0.00541	0.00006	n = 5

The solubility of the multicomponent co-crystal (M:A:T 1:1:1) was determined at 20℃. In the preparation of the multicomponent co-crystal solution, filtration of the MCC was recorded. Though this introduced a degree of error, samples with a lower quantity of bound MCC still admitted a two-fold increase in methanol solubility compared with the binary co-crystal at

20℃. A maximal ten-fold increase in methanol solubility was observed when comparing the binary and unfiltered multicomponent co-crystals at 20℃:

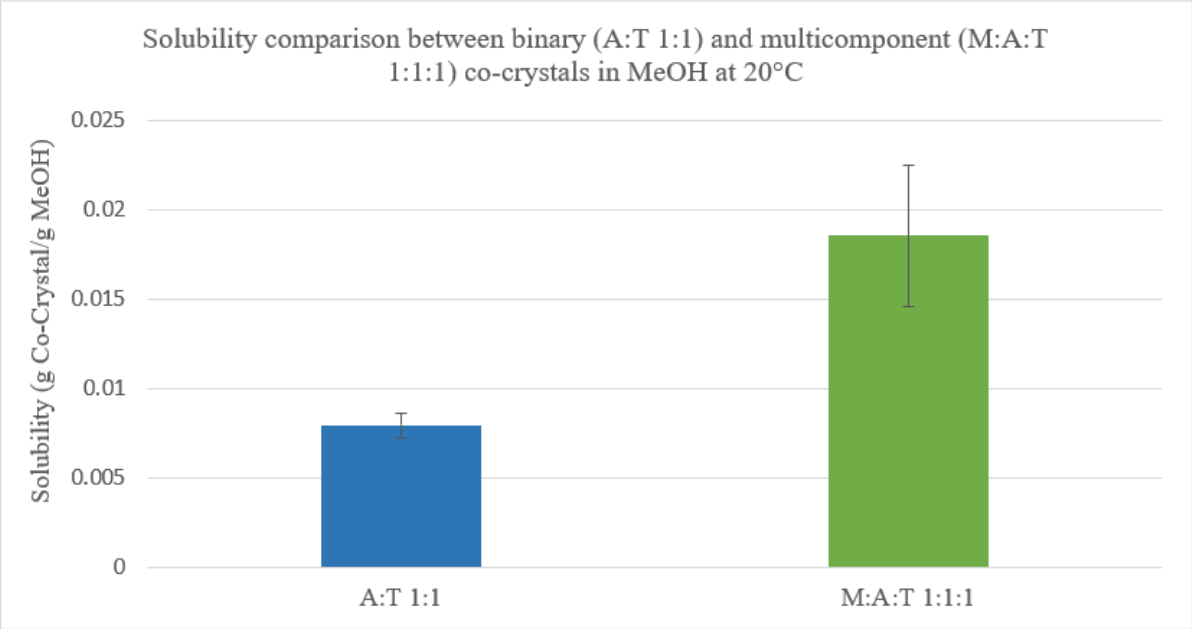


Table 2					
Solubility of a Binary and Filtered Multicomponent Co-Crystal in Methanol at 20℃					
A:T 1:1			**M:A:T 1:1:1**		
Average Solubility (g/g)	**Standard Deviation (g/g)**	**Replicates**	**Average Solubility (g/g)**	**Standard Deviation (g/g)**	**Replicates**
0.00792	0.00066	n = 5	0.01854	0.00395	n = 4

Conclusion:

Liquid assisted grinding was investigated as a method of co-crystal synthesis. The stoichiometric ratio of the individual components was observed to profoundly influence co-crystal morphology. A multicomponent co-crystal was successfully synthesized via liquid assisted grinding, with FTIR data highlighting changes in intramolecular bonding. The solubility of the acetazolamide-theophylline co-crystal was determined at 3 temperatures. There was an observed increase in the solubility of the co-crystal on inclusion of MCC, indicating that multicomponent co-crystal synthesis positively modulates the physicochemical characteristics of poorly soluble drug-drug co-crystals.

Further Work:

The results of this research will be used as the basis for further investigation into the synthesis of multicomponent drug-drug co-crystals. Additional characterization studies are conjectured to yield formulations that both optimize the physicochemical characteristics of the APIs, and provide a greater insight into the underlying supramolecular architecture of multicomponent co-crystals.

References:

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