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Purification of amoxicillin trihydrate by impurity-coformer complexation in solution†

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In this work, we demonstrated the purification of amoxicillin trihydrate (AMCT) by the formation of 4-hydroxyphenylglycine (4HPG)-coformer complex in solution. Without advanced knowledge of cocrystal formation of 4HPG, a workflow was established to choose the optimal coformer and the amount of coformer to add for a specific target/impurity system. Forty-seven compounds were chosen and screened due to their functional groups having the potential to form heterosynthons with the functional groups on 4HPG. Using solid-state grinding, eleven compounds were selected as coformers for separation experiments because they can form cocrystals with 4HPG but not AMCT. Four compounds, 2-picolinic acid, L-lysine, L-leucine, and L-isoleucine were shown to enhance AMCT purity the most. The purities of the AMCT crystals crystallized from solutions with a 1 : 10 4HPG : AMCT ratio and with these four compounds were significantly greater than that without the addition of coformer and greater than that obtained from a second crystallization from fresh solvent. By varying the coformer-to-4HPG ratio, we can correlate the purification results to the level of complexation in the solution. In addition, the proposed separation method has practical uses and can be applied to expensive products where low yield is unacceptable.

Introduction

Separation processes are employed in many industries to recover and purify intermediates and final products. In industries that manufacture strictly regulated products with high quality, such as the pharmaceutical and food industries, it is essential to develop efficient and economic separation processes to meet their needs. To do so, it is necessary for these processes to have high selectivity (purity), capacity (maximum applicable amount of material) and recovery (yield). In our previous work with two model systems (benzamide/benzoic acid and cinnamamide/cinnamic acid), we investigated the possibility of purifying structurally similar compounds using selective impurity complex formation in solution followed by crystallization of the target compound.[1] We postulated that coformers that can form cocrystals with the impurity are likely to form complexes with the impurity in solution. In addition, we postulated that the impurity complex is too bulky to fit into the crystal lattice of the target. Therefore, by adding coformers that can form cocrystals with the impurity but not the target to the mixture, we can prevent impurities from substituting into the target crystal lattice. For the two systems we studied, we knew in advance that the impurities can form cocrystal with particular coformers and were able to demonstrate the possibility of purifying mixtures of structurally similar compounds by adding these coformers to the mixtures. The correlation between the level of complexation and the purification result, however, requires additional experimental verification. In this work, a real drug/impurity system, amoxicillin trihydrate/4-hydroxyphenylglycine (AMCT/4HPG; the structures of both molecules are shown in Fig. 1) was chosen to further evaluate the practical use of the proposed method and its mechanism.

Amoxicillin trihydrate (AMCT) is one of the major β-lactam antibiotics which are widely used against broad spectrum of bacteria. It was discovered to have high solubility, high absorption rate and high stability under acidic conditions.[2–4] It is also known for the fact that at the same dosage, the blood level of AMCT is twice as high as that of Ampicillin.[5]

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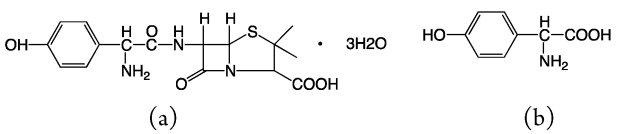


Fig. 1 Structures of (a) amoxicillin trihydrate and (b) 4-hydroxyphenylglycine.

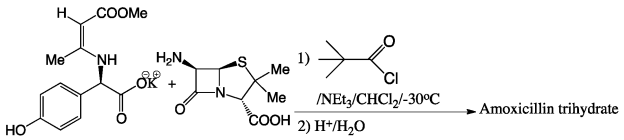


Fig. 2 The industrial process for AMCT (the Dane salt route).

Like other β-lactam antibiotics, AMCT is originally produced using a semisynthetic route. The first generation of AMCT industrial process is based on the Dane salt route (Fig. 2). The yield of this process can go above 90%. However, this reaction demands low temperature (−30 °C), protection, deprotection and activation steps and the usage of several undesirable reagents and solvents (*e.g.* CH_2Cl_2). As a consequence, the amount of waste generated from this process is substantial.[5]

With the increasingly tight environmental regulations, the need to replace the synthesis above with an enzymatic synthesis has increased. The enzymatic process takes fewer steps than the chemical synthetic route and can be completed in aqueous solution, at neutral pH, and at ambient temperature. Two approaches to making β-lactam antibiotics were studied: the thermodynamically controlled approach and the kinetically controlled approach. As of today, the kinetically controlled approach is the primary replacement for the chemical synthetic route. In the kinetically controlled approach, the side chain derivative reacts with 6-aminopenicianic acid (6-APA) to form the antibiotic with Penicillin G Acylase (PGA) as catalyst. However, since PGA can be both transferase and hydrolase, it hydrolyzes the side chain derivative and the product, the antibiotic, while it catalyzes the main reaction. Many studies have been done to optimize the reaction condition for maximum yield, selectivity (synthesis-to-hydrolysis ratio), and productivity.[5,6]

The kinetically controlled synthesis of AMCT is presented in Fig. 3. This synthesis has become commercially feasible as the cost of suitable enzymes in robust and immobilized form decreased.[5] In this synthesis, 4-hydroxyphenylglycine methyl ester (HPGM) reacts with 6-APA to produce AMCT. Two hydrolysis reactions occur at the same time. In the first hydrolysis, the reactant, HPGM is hydrolyzed by PGA with the presence of water to form 4-hydroxyphenylglycine (4HPG) and methanol. In the second hydrolysis, the product, AMCT, is hydrolyzed by PGA with the presence of water to form 4HPG and 6-APA. Many studies have been done on the enzymatic synthesis of AMCT to maximize the yield, selectivity, and productivity. Various studies have been done on the effects of reactant concentrations, enzyme concentration, enzyme inhibitor and temperature.[6–8] Despite all the efforts, it is inevitable to have 4HPG present as an impurity.

The importance of separating AMCT from its degradation product has been addressed in the literature.[9] It was shown that the degradation products can inhibit the nucleation of AMCT, which is similar to the negative influence of impurities on the nucleation and growth rate in ampicillin crystallization.[10,9] Factors that affect the amount of impurities

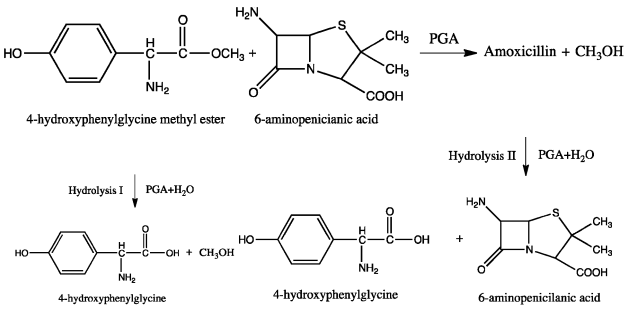


Fig. 3 Enzymatic synthesis of amoxicillin using PGA as the catalyst.

incorporated into AMCT crystal lattice were also studied. The pH of AMCT crystallization process can change the amount of 4HPG incorporated into AMCT crystal lattice. As the process pH increases, the solubility of 4HPG increases. Therefore, the amount of 4HPG in AMCT decreases as the process pH increases.[4] The other factor studied is the presence of degradation products in the AMCT crystallization process. It was shown that the purities of AMCT crystallized with the presence of degradation products, whether at high or low concentration, were at least as pure as the standard material.[9] It is known that USP grade AMCT should contain no more than 1% of D-hydroxyphenylglycine. When AMCT was synthesized through the Dane salt route, crystallization was used to purify and obtain the final products. As the interest in enzymatic synthesis grows, it is necessary to develop other purification methods since different amounts of 4HPG can be incorporated into AMCT in different synthetic route. Many chromatographic methods were developed to separate AMCT from 4HPG.[7,9,11–13] However, in industrial processes, chromatography is often not desirable due to its high cost. Repeated crystallization is often used to enhance the purity of the product but the yield can be sacrificed in the process. It is important to develop a separation method to separate AMCT from 4HPG without sacrificing the yield.

The goal of this work is to demonstrate a method of reducing the amount of lattice substituting impurities in target compound in crystallization by employing coformers which will complex with the impurity in solution and not the target molecule. Using 4HPG as the impurity and AMCT as the target compound, we were able to identify coformers which we have no advanced knowledge of and utilized these coformers to substantially reduce the amount of 4HPG in AMCT crystals. The purities of the resulting AMCT crystals were higher than that obtained by two crystallizations from the initial solutions. A clear correlation between the purification results and the level of complexation was also observed.

Materials and methods

Materials

Amoxicillin trihydrate (AMCT) was purchased from Alfa Aesar and used as received. 4-Hydroxyphenylglycine (4HPG, ⩾98%),

and all coformers (see ESI†) were purchased from Sigma-Aldrich and used as received. Hydrochloric acid concentrate (for 1 L of 1.0 M HCl) was purchased from Sigma-Aldrich and measured into a 1 L volumetric flask, which was then filled with HPLC grade water to the mark. Sodium hydroxide was purchased from Sigma-Aldrich and used to prepare a 5.0 M solution. Potassium phosphate monobasic (for HPLC, ⩾99.5%) and dibasic (for HPLC, ⩾99.0%) were purchased from Sigma-Aldrich. Water (H_2O, CHROMASOLV® Plus for HPLC), methanol (MeOH, CHROMASOLV® for HPLC ⩾99%) and acetonitrile (ACN, CHROMASOLV® for HPLC ⩾99%) were purchased from Sigma-Aldrich and used as received.

Coformer selection

To find compounds that have the potential to form cocrystals with 4HPG, we need to find heterosynthons where one functional group is the function group on 4HPG (either the carboxylic acid group and/or the amine group). Forty-seven compounds were chosen for screening based on this criterion. The entire list can be found in ESI. These compounds can be categorized as compounds with functional groups encouraging the formation of heterosynthon with the carboxylic acid group on 4HPG (Group I, with amide, primary, secondary, and tertiary amine group), the amine group on 4HPG (Group II, with carboxylic acid group), and both groups on 4HPG (Group III, with both carboxylic acid and amine group). Solid-state grinding was performed to select coformers that can form cocrystal with 4HPG but not AMCT. We ground 1 : 1 4HPG : coformer mixture using a motor and pestle with few drops of water and measured the powder pattern of the resulting solid using X-ray powder diffraction (XRPD). The powder pattern was then compared to the powder patterns of individual components. If new peaks were observed, the coformer was then selected to the next step. Powder patterns of the thirteen resulting solids that show new peaks can be found in the ESI.† In the following step, the coformer was ground with AMCT in a 1 : 1 molar ratio. Eleven compounds were found to have the potential to form cocrystals with 4HPG but not AMCT (Table 1).

Separation experiments

Three sets of experiments were performed to determine if the coformer could be used to purify AMCT. In the first experiment, AMCT was crystallized with 4HPG, to examine how much 4HPG was incorporated into the AMCT crystal lattice (product 1) and then this product was recrystallized again from fresh solvent to determine the level of purification with two crystallizations (product 2). In the third experiment, AMCT was crystallized with both the impurity and the coformer to examine the amount of 4HPG incorporated after the addition of coformer (product 3). In the initial solution, 1.67 g of AMCT and 4HPG at 1 : 10 and 1 : 5 4HPG : AMCT

Table 1 Compounds that form cocrystal with 4HPG but not AMCT

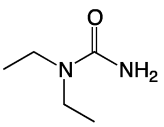
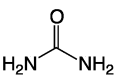
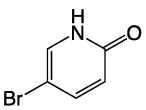
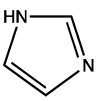
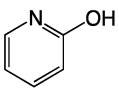
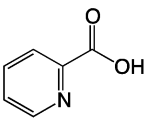
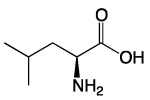
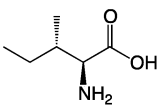
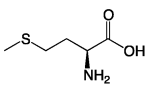
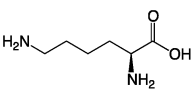
Name	Structure	Name	Structure
1,1-Diethylurea		2-Imidazolidone	
Urea		5-Bromo-2(1*H*)-pyridone	
Imidazole		2-Hydroxypyridine	
2-Picolinic acid		L-Leucine	
L-Isoleucine		L-Methionine	
L-Lysine			

Table 2 Gradient elution program used[a]

Time (min)	0	5	25	30	35
% B/(A + B)	0	0	40	40	0

[a] A = phosphate buffer solution (0.05 M, pH *ca.*5.9); B = 3 : 1 methanol : acetonitrile.

weight ratios (0.167 g and 0.334 g, respectively) were dissolved in 100 ml of 1 M HCl. Coformers were added at a 1 : 1 4HPG : coformer molar ratio. A 0.45 μm PTFE syringe filter was used to remove undissolved solids. The pH of the solution was then adjusted to 4.7 using 5 M NaOH followed by an hour wait. The crystallization was controlled at 4 °C using a 70 : 30 ethylene glycol : water bath. The solids obtained from the crystallization were collected and washed using 2 ml of 15 : 85 water–isopropanol mixture. The solids were examined using X-ray powder diffraction and confirmed to be AMCT. The amount of 4HPG in resulting products was determined using high performance liquid chromatography (HPLC).

High-performance liquid chromatography

The HPLC instrument (Agilent 1260 Infinity) was equipped with a UV diode array detector (Agilent Technologies G1315D). The column used was a Agilent ZORBAX Bonuss-RP 150 × 4.6 mm I.D. column packed with 5 μm particles (Agilent). The maximum wavelength for absorbance was set at 230 nm. The concentrations were analyzed using a 35 min gradient elution program (Table 2).[11]

A liter of 0.05 M phosphate buffer solution (pH *ca.* 5.9) was prepared by measuring 10 ml of 0.5 M K_2PO4 solution and 90 ml of 0.5 KH_2PO4 solution into a 100 ml volumetric flask, which was then filled with H_2O to the mark. The solution was then thoroughly mixed, degased, and filtered through 0.2 μm membrane before used.

X-ray powder diffraction

X-ray powder diffraction patterns were obtained using a PANalytical X'Pert PRO Theta/Theta powder X-ray diffraction system using a monochromatic Cu Kα radiation source with nickel filter λ = 1.5418 Å) generated at 45 kV and 40 mA, using an X'Celerator high-speed detector. The intensities were measured at 2-theta values from 5° to 40° at a continuous scan rate of 5° min^{-1}. Aluminum sample holders with a zero background silicon plate were used to carry out the measurements.

Results

Of the forty-seven compounds we screened, thirteen can form cocrystal with 4HPG. Carbamazepine and 1,2-bis(4-pyridyl)ethane were able to form cocrystals with AMCT and were eliminated from the final list of eleven coformers (Table 1). Of the forty-seven compounds, thirty-one of them are in Group I, six of them are in Group II, and ten of them are in Group III. Several factors were examined to see their effects on the cocrystal formation: molecular weight of the compound, the functional group(s) on the compound, and whether the compound has a phenyl ring or not. For the molecular weight, it was found that 28 compounds have a molecular weight lower than 150. Ten of these compounds can form cocrystal with 4HPG. Out of the 19 compounds with molecular weight higher than 150, only 3 can form cocrystals with 4HPG. That is, if the compound has a molecular weight lower than 150, it has a higher chance to form cocrystal with the 4HPG (35.71% *versus* 15.79%). When we examined the functional group(s), it was observed that no compound in Group II can form cocrystals with 4HPG. The possibility for a compound in Group I and III to form cocrystal with 4HPG was 25.9% (8 out of 31 compounds) and 50% (5 out of 10 molecules), respectively. That is, a compound with functional groups that can form hydrogen bonding with both the carboxylic acid and amine group on 4HPG has a better chance to form cocrystal with 4HPG. Finally, we examined the correlation between having a phenyl ring in the molecule and its possibility of forming cocrystal with 4HPG. The possibility of having a phenyl ring and forming cocrystal with 4HPG is 35.29% (6 out of 17 compounds); the possibility of not having a phenyl ring but forming cocrystal with 4HPG is 23.33% (7 out of 30). These two possibilities are close to each other and no clear correlation was observed.

Separation experiments were performed at a 1 : 10 4HPG : AMCT weight ratio. If coformers were added, a 1 : 1 4HPG : coformer molar ratio was used. The amounts of 4HPG incorporated into AMCT obtained from the crystallization experiments are presented in Table 3. The mass percentage of 4HPG in AMCT crystal after the initial crystallization was 0.98(±0.07) wt%. After a second crystallization (using fresh solvent), the amount of 4HPG decreased to 0.42(±0.05) wt%. With the addition of 2-imizazolidone, urea, and L-methionine, the amount of 4HPG incorporated into AMCT crystal lattice after the initial crystallization increased to 2.45(±0.04) wt%, 1.52(±0.09) wt%, and 1.28(±0.05) wt%, respectively. The addition of 1,1-diethylurea, imidazole, 2-hydroxypyridine, and

Table 3 Amount of 4HPG in AMCT from crystallization experiments with a 1 : 10 ratio of 4HPG : AMCT

	Amount of 4HPG (%)	Decreased (%)
One crystallization	0.98 ± 0.07	
Second crystallization	0.42 ± 0.05	57
2-Imidazolidone	2.45 ± 0.04	−150
Urea	1.52 ± 0.09	−55
L-Methionine	1.28 ± 0.05	−31
1,1-Diethylurea	0.94 ± 0.07	4
Imidazole	0.72 ± 0.01	27
2-Hydroxypyrdine	0.6 ± 0.05	39
5-Bromo-2(1*H*)-pyridone	0.36 ± 0.04	63
2-Picolinic acid	0.17 ± 0.01	83
L-Lysine	0.17 ± 0.01	83
L-Isoleucine	0.15 ± 0.01	85
L-Leucine	0.12 ± 0.01	88

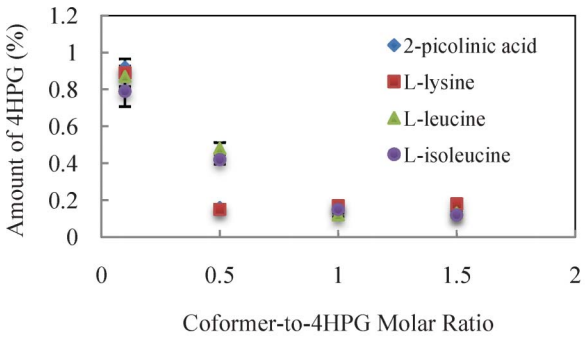


Fig. 4 Effect of coformer amount added on the amount of 4HPG incorporated into AMCT crystal lattice.

5-bromo-2(1*H*)-pyridone decreased the amount of 4HPG after an initial crystallization to 0.94(±0.07) wt%, 0.72(±0.01) wt%, 0.6(±0.05) wt%, and 0.36(±0.04) wt%, respectively. The best separation was obtained from the compounds: 2-picolinic acid, L-lysine, L-isoleucine, and L-leucine where the amount of 4HPG in the AMCT crystal after one crystallization decreased to 0.17(±0.01) wt%, 0.17(±0.01) wt%, 0.15(±0.01) wt%, 0.12(±0.01) wt%. The yield with the addition of coformer was not significantly different from the yield without the addition of coformer.

To investigate the effect of initial impurity concentration on the amount of impurity incorporated into the crystal lattice of the target compound, 4HPG was added at a weight ratio of 1 : 5 to AMCT. The amount of 4HPG in AMCT crystal was 1.13(±0.08) wt%. Compared to the result where the initial molar ratio was 1 : 10, the amount of 4HPG did not increase significantly.

We also investigated the effect of varying the amount of coformer added on the purification results using the four compounds that purify AMCT the most: 2-picolinic acid, L-lysine, L-isoleucine, and L-leucine. For each coformer, four coformer-to-4HPG molar ratios (r) were studied: r = 0.1, 0.5, 1, and 1.5. Purification results are shown in Fig. 4 (a table showing the comparative purification results can be found in ESI). Two trends were observed. In the 2-picolinic acid and L-lysine systems, no significant differences were observed when r = 0.1 (0.92(±0.01) wt% and 0.89(±0.07) wt%, respectively.) The amount of 4HPG decreased dramatically when r = 0.5 (0.16(±0.01) wt% and 0.15(±0.01) wt%, respectively) and no further decrease was observed with increasing amount of coformer added (for r = 1, 0.17(±0.01) wt% and 0.17(±0.01) wt%, respectively; for r = 1.5, 0.15(±0.01) wt% and 0.18(±0.01) wt%, respectively). A different trend was observed in the L-leucine and L-isoleucine systems. In the L-leucine system, the amount of 4HPG decreased from 0.87(±0.06) wt% to 0.12±(0.01) wt% when r increased from 0.1 to 1. Similarly the amount of 4HPG decreased from 0.79±(0.08) wt% to 0.15(±0.01) wt% with increasing r (from 0.1 to 1) in the L-isoleucine system. Additional coformer (r = 1.5) did not further decrease the amount of 4HPG in AMCT crystal (0.13(±0.01) wt% for the L-leucine system and 0.12(±0.01) wt% for the L-isoleucine system).

Discussions

Three characteristics of compounds were examined to find their effects on the formation of 4HPG cocrystal: molecular weight, different types of functional groups, and the existence of phenyl ring. It was found that if the compound has a molecular weight smaller than 150, it has a higher chance to form a cocrystal with 4HPG. One possible explanation could be the steric effect. The larger the molecular weight, the larger the molecule. Hence, the steric effect would add difficulty when the functional group on the coformer candidate interacts with the functional group on 4HPG to form a crystalline material. However, further investigation would be required to determine if this alone is accounting for the observation. It was found that compounds with both carboxylic acid and amine groups have a high possibility to form cocrystals with 4HPG. This observation could be explained by the numbers of cyclic hydrogen bonds formed between 4HPG and the coformer. If a compound has both carboxylic acid and amine groups (compounds in Group III), it can potentially form two sets of cyclic hydrogen bonds with the amine and carboxylic acid groups on 4HPG. However, if a compound has only one functional group that can interact with either the carboxylic acid or the amine group on 4HPG (compounds in Group I and II), it only has the potential to form one set of cyclic hydrogen bond. It is reasonable to conclude that the more hydrogen bonds that a compound can form with 4HPG, the more likely the compound can form a cocrystal with 4HPG. As for the phenyl ring, no clear correlation was observed between the existence of phenyl ring and the cocrystal formation. Studies have shown that intra/intermolecular π–π interaction between the two phenyl rings either on the same or different components were found in cocrystals.[14–19] However, compared to hydrogen bonding, π–π interactions are weak interactions and should never used as the main design principle. Sometimes the existence of phenyl rings can have a steric effect and prevent two components from forming cocrystal. We can conclude that the steric effect and functional groups on coformer candidates are the two most important factors when we design cocrystals.

For the eleven coformers that separation experiments were performed with, 2-picolinic acid, L-lysine, L-leucine, and L-isoleucine enhanced AMCT purity the most. Compared to all other coformers whose functional group can only interact with one functional group on 4HPG, these compounds have functional groups that can interact with both functional groups on 4HPG to form two sets of cyclic hydrogen bonds. Based on our assumption where the stronger the coformer interacts with 4HPG in the solid state, the coformer will have a higher possibility of forming complexes with the impurity in the solution. High amount of complexes must form between

these four coformers and 4HPG. High levels of complexation contributed to the enhancement of purification.

The purification results obtained from a single crystallization reduced the impurity concentration to as low as 0.12($\pm$0.01) wt% from 0.98($\pm$0.07) wt% when no coformer was added. In addition, crystals obtained from a second crystallization without the addition of coformer were still significantly less pure than crystals made from a single crystallization with the coformer. This indicates the potential usefulness of the method. The most common way to improve product purity is to recrystallize the compound of interest. However, by doing so, the yield is sacrificed to increase the purity. On the other hand, in our separation method, in addition to the high purity gained, the yield was not sacrificed. Our proposed separation method has the potential to be applied on expensive products when low yield is unacceptable.

We can correlate the level of complexation to the purification results with the addition of various coformer amounts. At r = 0.1, no significant decrease was observed in any system. This can be due to the lack of complex formation. For the 2-picolinic acid and L-lysine systems, best purifications were achieved at r = 0.5 and additional coformers cannot further decrease the amount of 4HPG in AMCT crystal lattice. This fact suggested that the level of complexation increased when r was increased from 0.1 to 0.5 and the maximum level of complexation was achieved at r = 0.5. The optimum coformer-to-4HPG molar ratio for these two systems were at r = 0.5 since we can achieve the best purification without adding excess amount of coformer. For L-leucine and L-isoleucine systems, the decrease of 4HPG amount with increasing r (from 0.1 to 0.5 and 1) indicated that the level of complexation increased with increasing amount of coformer added. The fact that no further purification was observed when additional coformer was added (after r = 0.5 for 2-picolinic and L-lysine systems and after r = 1 for L-leucine and L-isoleucine systems.) implied that the maximum level of complexation was achieved.

With these observations, we verified the practical use of our proposed separation method with AMCT/4HPG as our model system. General rules for cocrystal formation were established. Separation results with various amounts of coformers suggest that the purification was due to the interaction between the coformer and 4HPG and the level of complexation in the solution.

Conclusions

The practical use of the proposed separation method with a real drug/impurity system was evaluated. Forty-seven compounds were selected because they have functional groups that can form heterosynthons with functional groups on 4HPG. Eleven were confirmed to form cocrystal with 4HPG but not AMCT using solid-state grinding. Separation experiments were performed and four compounds were found to decrease the amount of 4HPG incorporated into AMCT crystal lattice the most: 2-picolinic acid, L-leucine, L-isoleucine, and L-lysine. The amounts of 4HPG incorporated in AMCT in these four systems were lower than that in an initial crystallization and a second crystallization. We were able to correlate purification results to the level of complexation by varying the amount of coformer added.

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Notes and references

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