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An Integrated High-Throughput Screening Approach for Purification of Solid Organic Compounds by Trituration and Crystallization in Solvents

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Abstract:

Trituration and crystallization are essential techniques for purification of intermediates and drug substances in pharmaceutical process development. Traditionally, identifying the optimal solvent conditions for impurity rejection adopts an empirical approach that can be a time and material consuming process. On the basis of thermodynamic considerations for optimizing the purity and the yield of the crystal product, it is necessary to conduct screening experiments with the crude product in different solvents including mixtures of solvents. In this report, we describe the development and the implementation of a high-throughput screening workflow in a 96-well array format for identifying the optimal purification conditions. The impure samples were first triturated at room temperature and subsequently subjected to a thermal cycle in 96 unique solvents or solvent mixtures at a volume of 0.6 mL per well. The compound solubility and the impurity profiles of both supernatant and recovered solid were analyzed by HPLC. Using a mixture of acetylsalicylic acid containing salicylic acid as the impurity, we investigated the effects of material loading per sample and impurity level on product purity/solubility under both screening and scale-up conditions and evaluated the thermodynamic behavior of product−impurity−solvent interactions based on an isothermal ternary phase diagram. During a further case study, a binary solvent system was identified, and the synergistic effect of binary components was demonstrated for purification of an Amgen compound containing three impurities. This high-throughput screening approach is valuable as an integrated part of process development to identify the thermodynamically favored solvent conditions for purification of pharmaceutical compounds.

Introduction

Most organic solids exist in crystalline form. Crystallization is an essential technique for purification and isolation of a solid organic compound. By means of crystallization, the impurities are preferentially dissolved in the solvent of choice and the desired product crystallizes to a high purity solid. The ability to obtain high purity product by crystallization is due to the ordered lattice of a crystal, and factors affecting impurity incorporation in the crystal lattice through equilibrium and nonequilibrium separation have been well studied.[1–4] Small amount of impurities can have dramatic effects on nucleation, crystal growth, crystal size, and morphology.[5,6] In many cases, as an alternative to crystallization, trituration or slurry purification is often used because of its simplicity of operation and effectiveness by eliminating the steps of nucleation and crystal growth.

Fundamentally, solvents play a key role in crystallization or trituration because they can influence crystal product quality through their effects on solution thermodynamics, crystallization kinetics, and crystal interface structure.[7,8] The thermodynamic impacts of solvents on product characteristics (e.g., purity, solubility, yield) are usually given early consideration in process development efforts. Traditionally, identifying the optimal conditions for purification adopts an empirical approach by selecting several different solvents on the basis of the solubility properties of the product and the impurities.[9] To obtain the desired solution properties that can not be obtained with the pure solvents, mixed solvents are often used. In many cases, the solute solubility can be greater in the mixture of solvents than in either of the pure solvents, and crystallization in the mixture of solvents can improve the separation efficiency and the product yield.[10] In crystallization operations, the equilibrium phase diagram often shifts for an impure compound relative to that for the pure compound as a result of product−impurity−solvent interactions.[11] In particular, solubility properties and equilibrium separation can be difficult to predict for a crude product at the early stage of process development when the impurities are often unknown in structure or unavailable as the isolated solids. Therefore, it is necessary to conduct screening experiments with the crude product (e.g., with as many

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representative impurities as possible) and with different solvents including mixtures of solvents, in order to optimize the purity and the yield.

In recent years high-throughput experimentation, by means of automation and miniaturization, has become a fast-growing approach in many areas of pharmaceutical development. Some examples are aqueous solubility determination enabling discovery lead optimization,[12] reaction screening at the Discovery–Development interface,[13] and salt selection and polymorph detection for preformulation.[14] To our knowledge, there has been no report utilizing high-throughput screening techniques for purification of pharmaceutical compounds by solvent trituration and crystallization.

In this report, we have developed and implemented a systematic high-throughput purification screening workflow in a 96-well array format. A user-selected library of 96 solvent compositions was screened under defined trituration and crystallization conditions, and the compound solubility and the impurity profiles were analyzed by HPLC. To reduce the risk of a poor initial choice of solvent that may thermodynamically limit the purification effectiveness, this high-throughput screening workflow evaluates a broad range of solvents or solvent mixtures and at the same time meets the requirements of screening data repeatability and scalability. With an emphasis on solution thermodynamics, we report the screening data to demonstrate the thermodynamic effects of impurity, solvent, and temperature on solubility and purity of the crystal product. However, the kinetic parameters of crystallization, i.e., nucleation, crystal growth, as well as the effect of equilibration duration on solubility and purity, were not explored nor optimized under the defined high-throughput screening conditions. It is also understood that size, shape, and polymorphic form are important features of the crystal product; however, solid-state characterization is beyond the scope of this study. After the hit solvent conditions are identified, it is appropriate to characterize the solid-state properties while optimizing the crystallization conditions during process scale-up.

Experimental Section

(i) Compounds. Acetylsalicylic acid (ASA, purity ≥99.0% purity) and salicylic acid (SA, purity ≥99.0%), purchased from Sigma-Aldrich, were selected as the test compounds. A mixture of 90% (w/w) ASA and 10% (w/w) SA was prepared. To ensure mixing homogeneity, the mixture was dissolved in methanol and the solvent was then removed completely in vacuo. Determined by HPLC peak area at 220 nm, the ASA purity was 91.3% and the SA impurity was 8.7% in the mixture. An Amgen test compound containing three impurities by HPLC at 310 nm, 0.4% of impurity A, 1.7% of impurity B, and 1.1% of impurity C, was received for purification screening.

(ii) Solvents. A representative solvent library for primary screening is shown in Table 1, consisting of 96 solvent compositions. Pure solvents commonly used in pharmaceutical processes were chosen. All of the solvents were subjected to a thermal cycle from 50 to 5 °C under the crystallization screening conditions; therefore, volatile solvents with boiling points lower than 50 °C were not selected. Because pH has a significant effect on solubility, 0.1 N HCl and 0.1 N NaOH aqueous solutions were included as appropriate for the compounds or the impurities having groups capable of ionization. To further diversify the solvent properties, binary solvents were selected and mixed at appropriate ratios, i.e., acetonitrile/water 90/10, 50/50, and 20/80 (v/v). The binary components chosen possess different dielectric constants, functional groups, and side chains and were miscible with each other. The solvent screening library could be modified as appropriate for the compound or the impurities of interest. If a binary solvent was a hit from the primary screening, a binary gradient from 10/90 to 90/10 (v/v) with an increment of 10% could be evaluated in the follow-up screening.

(iii) Purity and Solubility by HPLC. An Agilent 1200 HPLC rapid resolution system was used for determination of purity and solubility of the compound under screening. A reverse-phase HPLC method with gradient elution was developed for separating the product and the impurities of interest from any UV-absorbing solvents, i.e., toluene. The compound purity and the impurity levels were reported as peak area percentages at an appropriate UV detection wavelength. It is important to monitor the compound stability (e.g., thermal, acidic/basic) under the crystallization screening conditions. The compound solubility (mg/mL) was measured by HPLC based on a calibration curve of the compound concentrations against the peak areas, where the compound concentrations were accounted for using the initial compound purity by peak area percent. The initial peak area of the compound at a calibration concentration, together with the peak areas of individual impurities of interest, was recorded for mass balance calculation.

(iv) Screening Protocol. The screening protocol included two consecutive steps of purification using the same sample: first, trituration at room temperature, and second, crystallization by heating–cooling. In both steps, a supernatant sample was taken and its concentration was quantified by HPLC as the compound solubility. The difference between the two steps is that in the first step the solid purity was calculated on the basis of the mass balance between solid and supernatant in a closed compound–solvent system, whereas in the second step the solid was recovered and its purity was directly determined by HPLC. With an aim to identify the hit solvents under the defined trituration and crystallization conditions, one plate of 96 solvent compositions can be screened, and the entire experiment set can be completed in 3 days. The total sample amount required is determined by the material loading level, the solvent volume, and the number of solvents to be screened. A representative screening protocol is outlined below, and a flowchart is shown in Figure 1, in which a solvent volume of 0.6 mL was set and a loading level of 25 mg/mL was defined.

(a) Sample Preparation. A solid dispenser (Symyx Technologies) was used to dispense the desired sample amount (15 mg) into each vial on a 96-well plate with a tolerance of ±0.5 mg. The actual dispensed weight was recorded to the nearest 0.1 mg for mass balance calculation in each vial. A magnetic stir bar was added to each vial using a 96-well stir bar dispenser,

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Table 1. **Representative screening library of 28 pure solvents and 68 mixed solvents**

pure solvent	mixed solvent (v/v)					
water	0.1 N HCl	0.1 N NaOH				
	20% MeCN	20% MeOH	20% EtOH	20% IPA	20% acetone	20% THF
	50% MeCN	50% MeOH	50% EtOH	50% IPA	50% acetone	50% THF
	90% MeCN	90% MeOH	90% EtOH	90% IPA	90% acetone	90% THF
acetonitrile (MeCN)	10% IPAc	10% toluene				
	50% IPAc	50% toluene				
	80% IPAc	80% toluene				
N,*N*-dimethylformamide						
N-methyl pyrrolidone						
methanol (MeoH)						
ethanol (EtOH)	10% IPAc	10% THF	10% toluene	10% heptane		
	50% IPAc	50% THF	50% toluene	50% heptane		
	80% IPAc	80% THF	80% toluene	80% heptane		
n-propanol						
2-propanol (IPA)	10% THF	10% toluene	10% heptane			
	50% THF	50% toluene	50% heptane			
	80% THF	80% toluene	80% heptane			
n-butanol						
isobutanol						
2-methoxyethanol						
acetone						
methyl ethyl ketone	10% THF	10% toluene	10% heptane			
	50% THF	50% toluene	50% heptane			
	80% THF	80% toluene	80% heptane			
methyl isopropyl ketone						
methyl isobutyl ketone						
ethyl acetate						
isopropyl acetate (IPAc)	10% toluene	10% heptane				
	50% toluene	50% heptane				
	80% toluene	80% heptane				
dichloroethane						
1,1,1-trichloroethane						
tetrahydrofuran (THF)	10% toluene	10% heptane				
	50% toluene	50% heptane				
	80% toluene	80% heptane				
2-methyl tetrahydrofuran						
1,4-dioxane						
1,2-dimethoxyethane						
methyl *tert*-butyl ether						
isopropyl ether						
toluene						
cyclohexane						
n-heptane						

and the vials were sealed with a 96-well cap mat. Following the solvent library design, a liquid handler (Symyx Technologies) was used to dispense 600 μL of pure solvent or the appropriate volumes of binary components into each vial to give a loading level of ca. 25 mg/mL.

(b) Screening under Trituration Conditions. The sealed 96-well samples were stirred at 400 rpm for 2 h at room temperature and subsequently left standing without stirring for an additional 1 h at room temperature. The sealed plate was centrifuged at 1650 rpm for 10 min to allow separation of the compound supernatant from undissolved solid. After centrifuging, an aliquot of supernatant (50 μL) from each vial on the 96-well plate was taken, accurately diluted, and examined by HPLC for solubility and impurity profiling. The dilution factor was obtained based on the difference from the compound loading level to the HPLC calibration concentration, i.e., 25× dilution from 25 to 1 mg/mL. Based on the mass balance in a closed compound−solvent system for each sealed vial, the purity of the compound or the impurity of interest in the residual solid after trituration was calculated and expressed as the peak area percent shown below. An Excel macro program was written to automate these calculations.

$$A(i)\% = \frac{A_{(i,0)} \times W_0/(V \times C) - A_{(i,1)} \times D}{\sum_{i=1}^{n} A_{(i,0)} \times W_0/(V \times C) - A_{(i,1)} \times D} \quad (1)$$

where $A_{(i,0)}$ is the initial area of peak i at a HPLC calibration concentration of the compound, W_0 is the compound weight to the nearest 0.1 mg, V is the solvent volume (mL), C is the HPLC calibration concentration (mg/mL), D is the dilution factor from the loading level to the HPLC calibration concentration, $A_{(i,1)}$ is the area of corresponding peak i in the supernatant sample after dilution, and n is the number of peaks of interest.

(c) Screening under Heating–Cooling Conditions. After triturated at room temperature, the sealed samples on the 96-well plate were heated to 50 °C, stirred at 400 rpm for 1 h, cooled linearly from 50 to 5 °C in 8 h, and held at 5 °C for 8 h. The samples were then centrifuged at 1650 rpm for 10 min at 5 °C, and an aliquot of the supernatant (50 μL) in each vial on

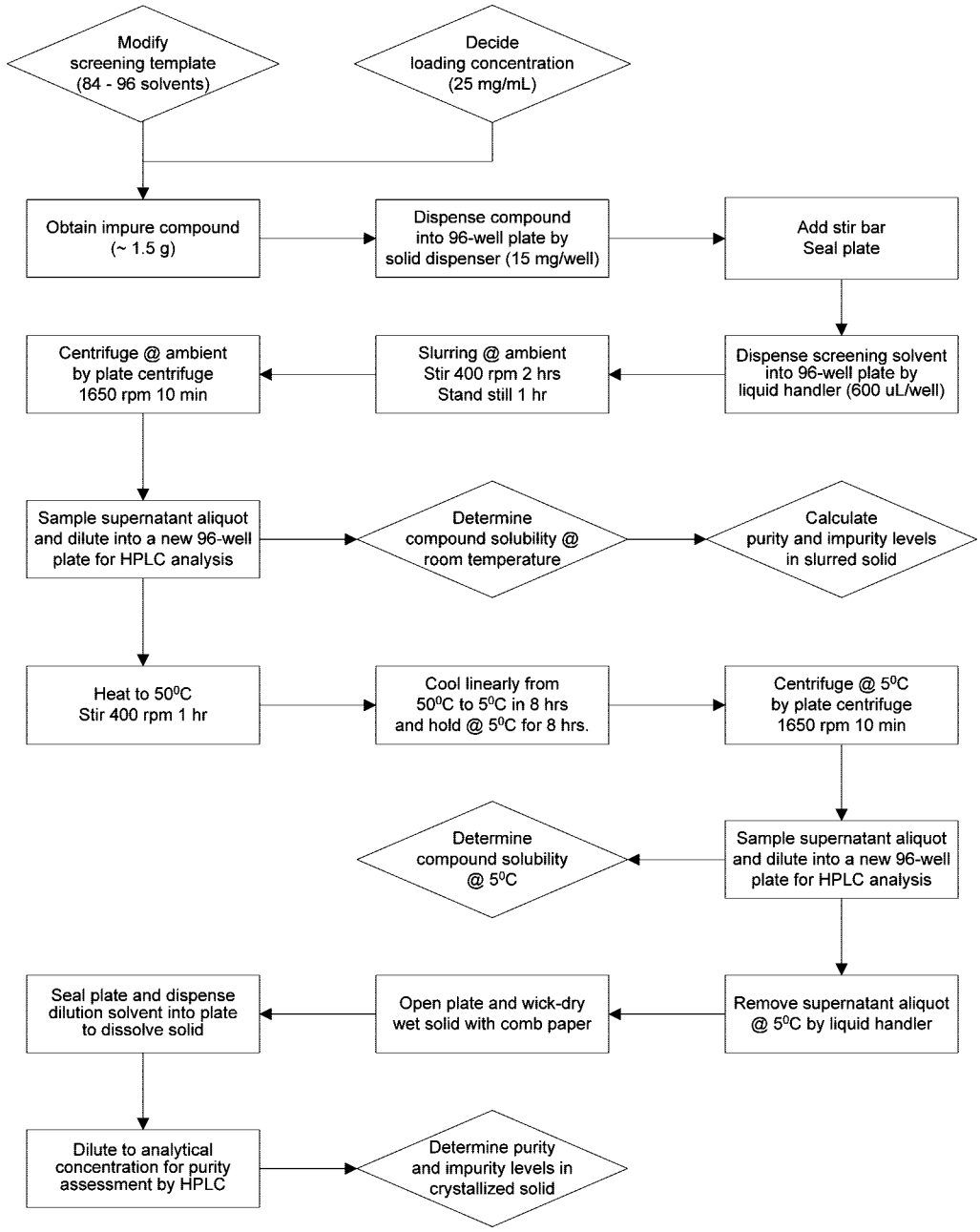

Figure 1. **Flowchart of high-throughput screening for purification by solvent trituration and crystallization.**

the 96-well plate was taken and diluted accurately for HPLC quantification of solubility at 5 °C. The remaining supernatant at 5 °C was subsequently removed using the liquid handler. The 96-well plate was opened, and a precut slice of porous paper was dipped to dry the solid in the vial. In order to fully dissolve the solid in each vial at a loading level of ca. 25 mg/mL, a solvent with the highest solubility at room temperature identified from the trituration screening results, i.e., methanol, was chosen and the solvent volume necessary for dissolution was estimated and added to each vial. Each vial solution was then diluted and analyzed by HPLC for purity and impurity profiling of the solid recovered under crystallization conditions.

(v) Follow-Up Screening and Scale-Up. By screening the solvent library, often several good solvent systems, single or binary, could be identified in which the purity of the triturated or crystallized solid was higher than the starting purity and the recovery yield was acceptable on the basis of solubility measurement. For binary solvent hits, a follow-up screening was warranted to investigate the gradient effect of binary components from 10/90 to 90/10 (v/v) with an increment of 10%. As an integrated part of process development, the hit solvent conditions identified from this screening workflow were scaled-up appropriately to verify the screening hits and to fine-tune the purification process parameters.

Results and Discussion

(i) Workflow Validation. The ASA/SA mixture (ASA purity 91.3%, SA impurity 8.7%) was selected to confirm the utility of this workflow, using a screening library of 96 solvent compositions at a loading level of 25 mg/mL. The mixture was first triturated at room temperature and then heated to 50 °C

Table 2. **Purification screening and scale-up data for a mixture of ASA/SA in toluene**

	trituration			heating–cooling		
	ASA purity (%)	ASA solubility at 25 °C (mg/mL)	ASA yield (%)	ASA purity (%)	ASA solubility at 5 °C (mg/mL)	ASA yield (%)
screening at 25 mg/mL ($n = 6$)	99.4 (±0.1)	3.9 (±0.1)	NA	99.6 (±0.0)	1.9 (±0.0)	NA
scale-up at 25 mg/mL ($n = 1$)	99.0	3.8	72.8	99.9	2.2	75.6
screening at 100 mg/mL ($n = 6$)	99.1 (±0.1)	6.5 (±0.3)	NA	99.4 (±0.1)	2.1 (±0.1)	NA
scale-up at 100 mg/mL ($n = 1$)	99.0	6.2	89.5	99.9	2.6	91.7

The ASA/SA mixture initially contained 91.3% ASA and 8.7% SA by HPLC peak area at 220 nm. The mean is reported with the standard deviation in parenthesis for the screening results.

and cooled to 5 °C in order to identify the optimal solvent conditions for purification and recovery of ASA as the desired product. The ASA solubility in each of the 96 solvents at room temperature and at 5 °C was determined by HPLC. The ASA purity of the solid remaining after trituration was calculated on the basis of mass balance. At the end of heating–cooling cycle, the solid was recovered, dried but unwashed, and the ASA purity was determined by HPLC.

The screening criteria for a hit solvent were defined to be (1) ASA purity greater than 99% and (2) ASA solubility lower than 5 mg/mL under either trituration or heating–cooling conditions. The ASA solubility criterion of 5 mg/mL was selected to ensure that a product recovery of 80% could be achieved with a loading level of 25 mg/mL in this validation screening. From the screening results, toluene was identified as the only solvent to meet the above criteria. The toluene-containing binary solvents did not exert a higher purification power than toluene itself despite the fact that the ASA solubility was affected by the polarity of the binary solvent mixtures; for example, the ASA solubility became lower in heptane/toluene but higher in methyl *tert*-butyl ether/toluene than in toluene.

To evaluate the reproducibility of the screening results, the workflow was repeated for the hit solvent toluene in six replicated wells, and the summary data is shown in Table 2. With a loading level of 25 mg/mL containing 91.7% ASA and 8.7% SA by HPLC peak area, the calculated ASA purity of the solid remaining after trituration was 99.4% and its standard deviation was 0.1%. The ASA solubility in toluene at 25 °C was 3.9 mg/mL with a standard deviation of 0.1 mg/mL. The same wells were subsequently heated to 50 °C and then cooled to 5 °C. The ASA solubility in toluene at 5 °C was 1.9 mg/mL with a standard deviation less than 0.1 mg/mL. The ASA purity in the unwashed solid recovered after heating–cooling was 99.6% and its standard deviation was less than 0.1%. It was demonstrated that toluene met the defined screening criteria of ASA purity greater than 99% and ASA solubility less than 5 mg/mL under both trituration and heating–cooling conditions, and the screening results were repeatable. Furthermore, although the solid recovered was unwashed, the ASA purity was higher after heating–cooling than after trituration at room temperature; therefore, heating–cooling was identified by this screening protocol as a more favorable process to purify ASA.

By identifying toluene as the optimal solvent, scale-up experiments were carried out to validate the screening results. With a scale-up factor of 100 while maintaining the loading level at 25 mg/mL, 1.5 g of the ASA/SA mixture in 60 mL of toluene was triturated at room temperature and heated to 50 °C then cooled to 5 °C. At the end of equilibration, an aliquot of the supernatant was sampled and the ASA solubility was determined by HPLC. The triturated or crystallized solid was filtered under vacuum and washed three times with toluene prior to purity assessment by HPLC. As shown in Table 2, the ASA solubility was 3.8 and 2.2 mg/mL, respectively, at 25 and 5 °C from the scale-up experiments, consistent with the screening results. The ASA purity was 99.0% from the trituration scale-up experiment, comparable to but relatively lower than the calculated purity of 99.4% from the screening experiment. The relatively higher impurity incorporation in the scale-up sample could possibly be due to incomplete separation and washing of the triturated crystals that left a small amount of impurity with the solid phase. During the heating–cooling scale-up experiment, it was observed that the solid was completely dissolved at 50 °C and recrystallized upon cooling to 5 °C. The ASA purity of the recovered crystals was 99.9%, higher than the trituration scale-up result. This was consistent with the screening data, indicating that crystallization by heating–cooling was a more efficient process for purification of ASA than trituration at room temperature. As expected, the ASA purity of the recovered and washed crystals from scale-up was higher than that of unwashed crystals from screening.

It was demonstrated that the screening results were reproducible and validated by the scale-up experiments. The advantage of this screening workflow is that it applies trituration and crystallization consecutively on the same sample to identify the optimal conditions. A filtration or wash step was not attempted in this screening workflow due to its complexity of integration with a 96-well plate. Though the calculated purity based on mass balance could be higher and the measured purity from unwashed crystals could be lower, the screening data enabled identification of the optimal solvent conditions for scale-up purification.

(ii) Effect of Loading and Impurity Levels on Purification. Process conditions often favor operation with high concentrations of solute, i.e., a ratio of 10 mL of solvent to 1 g of compound, or 100 mg/mL. To verify the success rate at a loading level of 100 mg/mL, the purification screening workflow was repeated by mixing 60 mg of the same ASA/SA mixture in 0.6 mL of toluene in six replicated wells.

As shown in Table 2, the ASA purity was 99.1% after trituration and 99.4% after crystallization at 100 mg/mL loading level, both with a standard deviation of 0.1%. Although the purity results at 100 mg/mL loading level were slightly lower than those at 25 mg/mL loading level, it indicated that the ASA/SA mixture could be purified at a high loading level to obtain

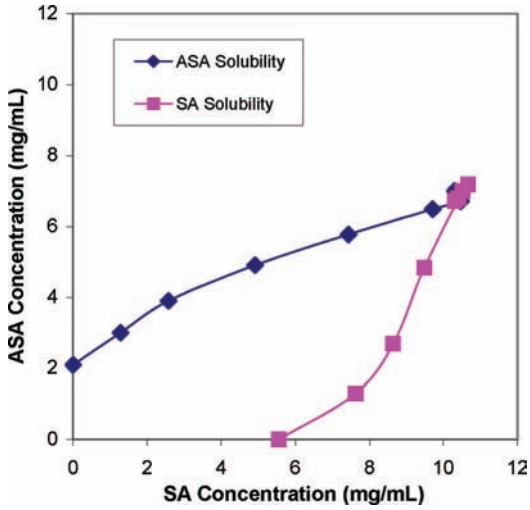

Figure 2. **Solubility of ASA and SA in the mixtures of ASA/SA in toluene at 25 °C. The ASA solubility and the SA concentration were determined by HPLC from each of the ASA/SA mixtures prepared by mixing SA (0–20 mg/mL) with ASA (100 mg/mL) in toluene. The SA solubility and the ASA concentration were determined by HPLC from each of the ASA/SA mixtures prepared by mixing ASA (0–20 mg) with SA (100 mg/mL) in toluene. The mixtures were equilibrated at 25 0C for 24 h.**

a high recovery yield with the desired ASA purity of ≥99%. It was found that the ASA solubility of 6.5 mg/mL at 25 °C at 100 mg/mL loading level was significantly higher than that of 3.9 mg/mL at 25 mg/mL loading level. On the contrary, the ASA solubility of 2.1 mg/mL at 5 °C at 100 mg/mL loading level was comparable to that of 1.9 mg/mL at 25 mg/mL loading level. The increased product solubility at a higher loading level of an impure mixture could be attributed to enhanced interactions between product and impurity molecules in the solution phase where a higher concentration of impurity was present. Temperature could have a profound effect on these interactions, and the impurity effect on the product solubility could be weaker at a lower temperature. To better understand the thermodynamic behavior of product−impurity−solvent interactions, an isothermal ternary phase diagram of the ASA/SA mixtures in toluene was established as shown in Figure 2. Solubility of pure ASA and pure SA in toluene at 25 °C was 2.1 and 5.6 mg/mL, respectively. In a ternary system of ASA/SA in toluene, the maximum ASA solubility was 7.0 mg/mL, and the maximum SA solubility was 10.5 mg/mL, in equilibrium with both solid phases of ASA and SA. It was interestingly noted that the ASA solubility gain (7.0–2.1 = 4.9 mg/mL) was equal to the SA solubility gain (10.5–5.6 = 4.9 mg/mL). From the phase diagram, it was learned that ASA could be thermodynamically separated from the ASA/SA mixture by rejecting the SA impurity concentration up to 10.5 mg/mL at 25 °C.

Scale-up experiments were carried out at a loading level of 100 mg/mL to validate the screening results. Six grams of the ASA/SA mixture (90% ASA and 10% SA by weight, or 91.3% ASA and 8.7% SA by HPLC peak area) in 60 mL of toluene was triturated at room temperature or heated to reflux and then cooled to 5 °C. It was noticed that the ASA/SA mixture was not soluble at 100 mg/mL in toluene at 50 °C; therefore the scale-up conditions deviated from the screening conditions by heating to reflux until the mixture became completely dissolved. The ASA solubility in toluene at 25 and 5 °C, as well as the ASA purity of the recovered, filtered, and washed solid after trituration or crystallization, were determined by HPLC. As shown in Table 2, the scale-up solubility was consistent with the screening solubility at the same loading level. At 100 mg/mL loading level, the purity of the triturated and recrystallized solid was 99.0% and 99.9%, respectively, and both values were the same as those at 25 mg/mL loading level. It was proven that the scaled-up purification of the ASA/SA mixture at 100 mg/mL loading level was successful under the optimal conditions identified from the screening workflow.

It was demonstrated from this study that the thermodynamically favored solvent conditions could effectively minimize impurity incorporation. The impurity concentration could have a dramatic effect on the product solubility, and vice versa. The increased product solubility could reduce the actual recovery yield as compared to the theoretical yield calculated based on the solubility of the pure product. On the other hand, the increased impurity solubility could ease the impurity rejection; therefore, purification can be achieved thermodynamically in optimal solvent systems at high loading levels of the crude product. The extent of solubility change for the product or the impurity could be greatly affected and controlled by temperature, which is an important parameter to be fine-tuned during scale-up purification.

(iii) Effect of Multiple Impurities and Binary Solvents on Purification. Commonly, the compound to be purified contains multiple impurities. A complex molecule with multiple impurities may require trituration or crystallization in mixed solvents, which poses a great challenge to identify the optimal solvent compositions to reject all the impurities efficiently. An Amgen compound was screened using this workflow, and its HPLC chromatogram at a standard concentration of 2.0 mg/mL is shown in Figure 3. The compound had three impurities, 0.4% of impurity A, 1.7% of impurity B, and 1.1% of impurity C, detected under reverse-phase HPLC conditions at 310 nm. At a loading level of 20 mg/mL, the impure compound was triturated at room temperature and then heated to 50 °C and cooled to 5 °C. From the library of 28 pure solvents and 68 mixed solvents screened, 2-propanol/heptane and ethanol/heptane were identified as the hit solvents under the room temperature trituration conditions. The screening results from the heating–cooling conditions were equivalent to but not better than those from the room temperature trituration conditions. As the chromatograms show in Figure 4, the compound solubility at room temperature was higher in 2-propanol/heptane than in 2-propanol or heptane alone. Most importantly, the supernatant liquors became enriched, and all three impurities A, B, and C, were freely soluble in 2-propanol/heptane.

To further understand the effect of mixed solvents on solubility and purification, a follow-up screen was conducted by extending the binary ratio of 2-propanol/heptane and ethanol/heptane from 10/90 to 90/10 (v/v) with an increment of 10%. The impure compound was triturated in the mixed solvents at a loading level of 20 mg/mL at room temperature. Plots of the compound solubility and the individual impurity level against the binary solvent gradient are shown in Figure 5. As can be

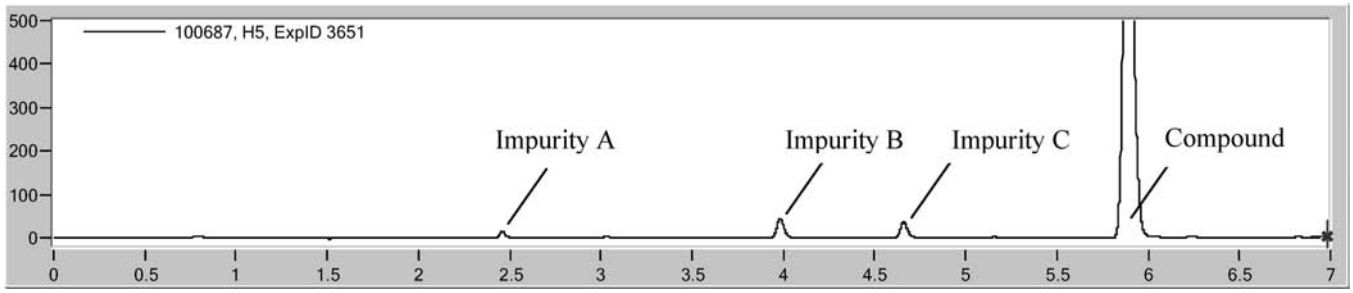

Figure 3. **Standard HPLC chromatogram of an Amgen compound at 2.0 mg/mL in 2-methoxyethanol, containing 0.4% impurity A, 1.7% impurity B, and 1.1% impurity C, respectively, at 310 nm.**

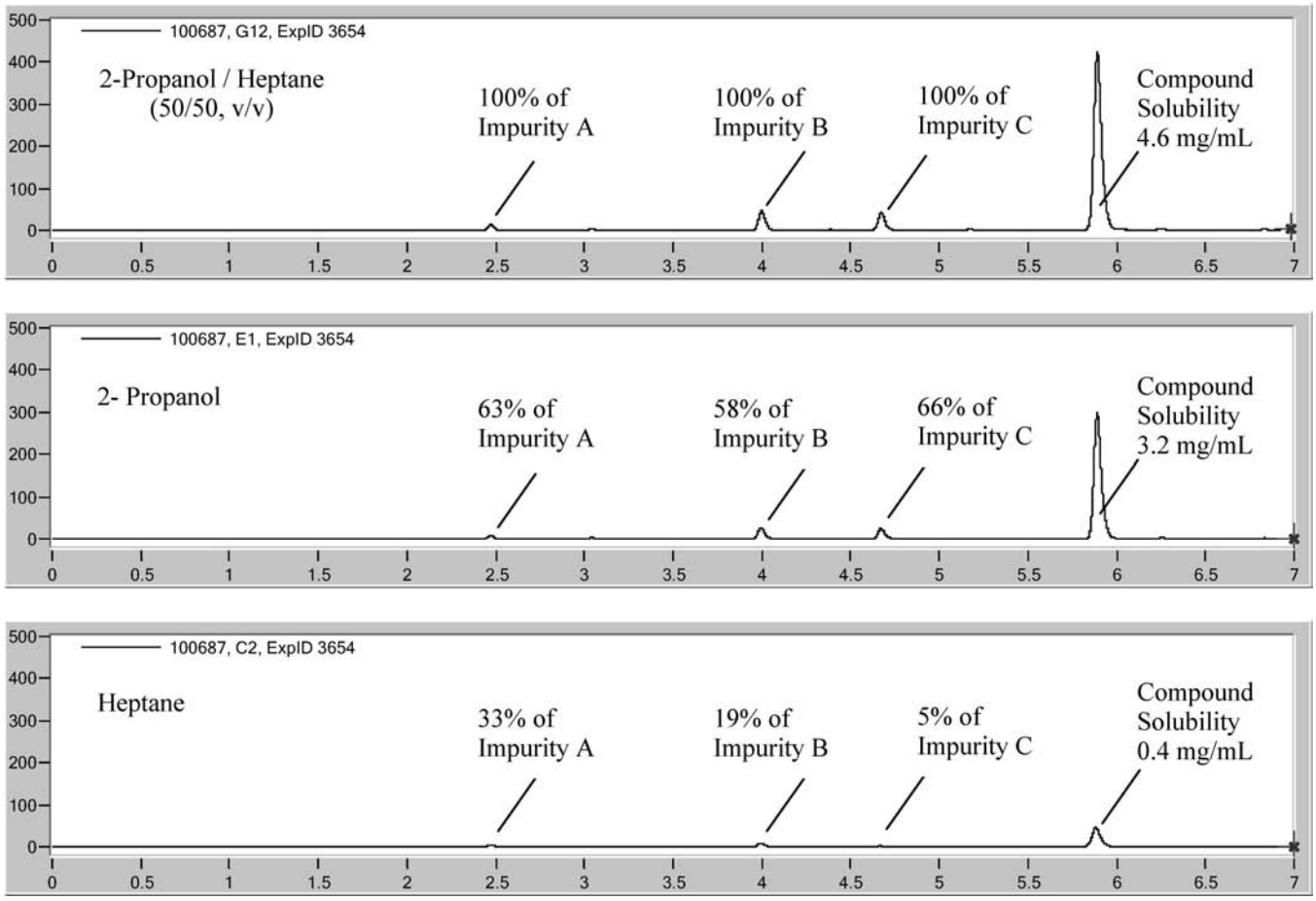

Figure 4. **HPLC chromatogram of the supernatant sample (dilution 10×) from the Amgen compound at a loading level of 20 mg/mL after trituration at room temperature in 2-propanol/heptane (50/50, v/v), 2-propanol, and heptane, respectively. The percent of each impurity enriched in the supernatant liquor was calculated from the impurity peak area detected in the supernatant sample relative to the impurity peak area detected in a standard sample at a concentration of 2.0 mg/mL.**

seen, the optimal ratios of 2-propanol/heptane were from 70/30 to 60/40 (v/v), where the compound solubility ranged from 3.7 mg/mL to 4.2 mg/mL, and the total impurities were less than 0.1%. The synergistic effect observed between 2-propanol and heptane on the solubility and the purification of this compound was observed. Predicting such a binary solvent composition for a successful purification would be almost impossible. High-throughput screening provides a useful approach to identify the optimal solvent compositions in a rapid and material-sparing manner.

Conclusions

A systematic high-throughput screening workflow in a 96-well array format has been developed and validated for purification of impure compounds by trituration in solvents at room temperature followed by crystallization under a heating–cooling cycle. With a library of 28 pure solvents and 68 mixed solvents, the screen was carried out to identify the optimal solvent conditions to meet the user-defined criteria for purity and solubility. When the hit was a binary solvent, a follow-up screening was executed to investigate the gradient effect of binary components from 10/90 to 90/10 (v/v). Furthermore, it was demonstrated from this study that the crystallization solvent and the impurity concentration could have dramatic effects on the purification efficiency. Through purification screening of the crude product, it was possible to identify the optimal solvent conditions to effectively minimize impurity incorporation. The deliverables of this screening workflow are (1) to identify an

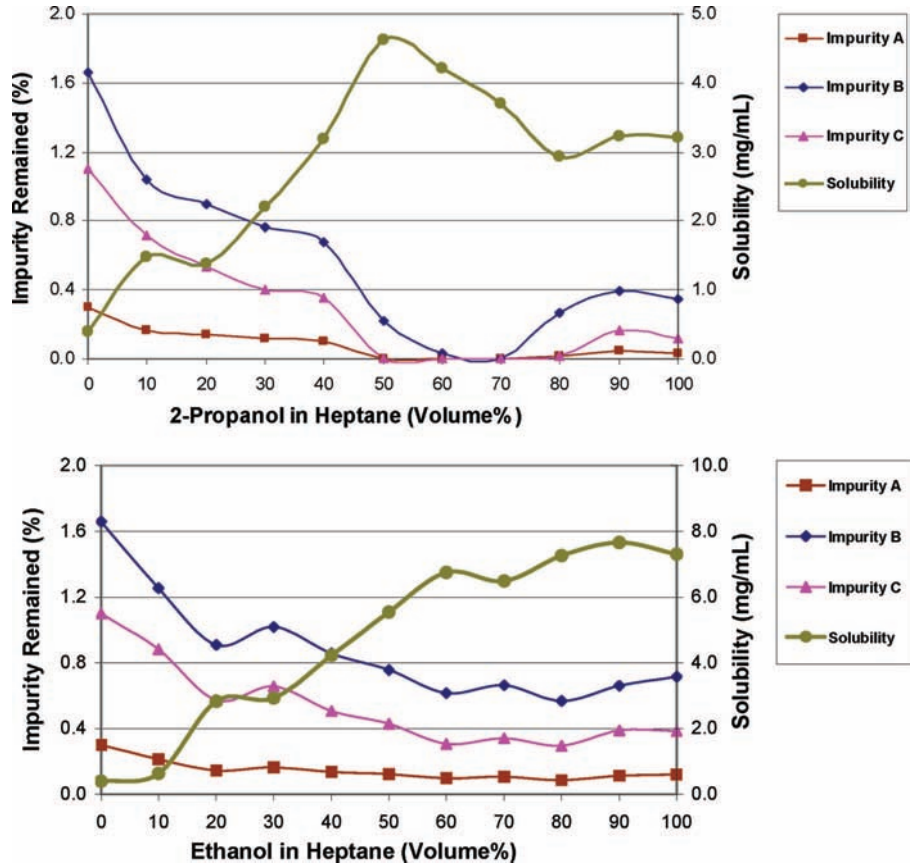

Figure 5. **Compound solubility and impurity level versus binary solvent gradient. The Amgen compound initially contained 0.4% impurity A, 1.7% impurity B, and 1.1% impurity C by HPLC peak area at 310 nm. The binary solvents of 2-propanol/heptane and ethanol/heptane were screened by triturating the impure compound at a loading level of 20 mg/mL at room temperature. The supernatant sample was analyzed by HPLC for solubility measurement. The individual impurity remaining in the triturated solid was calculated on the basis of mass balance.**

optimal solvent to purify the impure compound at a low loading level, i.e., 20 mg/mL, in order to minimize the sample amount required for screening; (2) to identify an efficient pathway, either by room temperature trituration or by heating–cooling crystallization; (3) to scale-up the optimal solvent conditions at a high loading level, i.e., 100 mg/mL, in order to maximize the product yield while maintaining the desired purity. Because temperature and equilibration time are the important factors of purification, it is required to fine-tune these parameters during scale-up following the initial hit solvent identification from screening. This high-throughput screening approach is valuable as an integrated part of process development for purification of intermediates and drug substances.

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