EXPLORING THE ROLE OF ANTI-SOLVENT EFFECTS DURING WASHING ON ACTIVE PHARMACEUTICAL INGREDIENT PURITY

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ABSTRACT

Washing is a key step in pharmaceutical isolation to remove unwanted crystallisation solvent, rich in impurities, (mother liquor) from the Active Pharmaceutical Ingredient (API) filter cake. This study looks at strategies for optimal wash solvent selection, minimising dissolution of API product crystals while preventing precipitation of product or impurities. Selection of wash solvent to avoid both these phenomena can be challenging but is essential to maintain yield, purity, and particle characteristics throughout the isolation process.

An anti-solvent screening methodology has been developed to quantitatively evaluate the propensity for precipitation of APIs and their impurities of synthesis during washing. This is illustrated using paracetamol and two typical impurities of synthesis during the washing process. The solubility of paracetamol in different binary wash solutions was measured to provide a basis for wash solvent selection. A map of wash solution composition boundaries for precipitation for the systems investigated was developed to depict where anti-solvent phenomena will take place.

For some crystallisation and wash solvent combinations investigated, as much as 90% of the dissolved paracetamol and over 10% of impurities present in the paracetamol saturated mother liquor was shown to precipitate out. Such levels of uncontrolled crystallisation during washing in a pharmaceutical isolation process can have drastic effect on the final product purity.

Whilst precipitation of both product and impurities from the mother liquor can be avoided by using a solvent in which the API has a solubility similar to that in the mother liquor, for example use of acetonitrile as a wash solvent does not result in any precipitation of the paracetamol API or its impurities. However, the high solubility of paracetamol in acetonitrile, would result in noticeable dissolution of API during washing and would lead to agglomeration during the subsequent drying step. Conversely, use of n-heptane as wash solvent for a paracetamol crystal slurry resulted in the highest amount of precipitation amongst the solvent pairings evaluated. This can be mitigated by designing a multi-stage washing strategy where wash solutions of differing wash solvent concentration are used to minimise step changes in solubility when mother liquor and wash solvent come into contact.

1. INTRODUCTION

In the pharmaceutical industry, crystallisation is a widely used purification technique employed to obtain active pharmaceutical ingredient particles of the required size, purity and crystal habit.[0,0]

Following crystallisation, filtration, washing and drying are the isolation steps required to separate the API crystals from the unwanted, impure mother liquor.[3] Ideally, the complete isolation process should be achieved without any changes to the crystals produced during crystallisation. Any breakage or granulation of crystals, or precipitation of dissolved product or impurities from the mother liquor onto the crystal surface should be avoided.[4]

Washing in pharmaceutical manufacturing is still relatively unexplored with very few academic publications[6-8] even though it plays a vital role in isolation since it is pivotal in the removal of impurities and mother liquor from the API filter cake. Washing displaces the mother liquor containing dissolved raw material and impurities from the API crystal product using a wash solvent. Tien (2012), proposes that washing of a filter cake can involve three main mechanisms: (1) displacement of mother liquor from the cake, (2) reslurrying of filter cake, (3) consecutive dilution.[5,7] During conventional washing without resuspension, the wash solvent first displaces the mother liquor from the large pores in the cake; then the mother liquor from the adjacent narrower pores in the cake diffuses into the wash solvent. The resulting solute transport is regarded as axial dispersion. During subsequent washing steps both diffusion and dispersion processes occur in combination.

To be effective the wash solvent should ideally have the following properties:[9]

- The unwanted impurities should have sufficient solubility to ensure they remain in solution or dissolve;
- The API product's solubility should be low to minimise product loss during the washing process;
- The wash solvent should be miscible with the mother liquor to allow diffusion and dilution mechanisms;
- The viscosity of the wash solvent should be similar to the crystallisation solvent to allow for appropriately long contact with the crystals to facilitate impurity removal from the cake without excessive filtration cycle time;[6]
- The API product should have thermal stability in the wash solvent under the drying process conditions needed to remove the wash solvent(s);
- The volatility of the wash solvent should be kept appropriately high to assist with the drying process.

However, some of these wash characteristics can be mutually exclusive. Introduction of wash solvent into the mother liquor wet API filter cake can result in several undesirable outcomes. The anti-solvent effect is one of the problems commonly encountered during washing because of the requirement for the API product to have a low solubility in the wash solvent. As the wash solvent contacts the slightly supersaturated mother liquor present within the filter cake, nucleation may take place initiating an anti-solvent crystallisation. In deed washing with an anti-solvent may lead to uncontrolled anti-solvent crystallisation. Product precipitation occurring in the packed bed of API crystals in the filter cake provides ideal conditions for the formation of solid bridges between crystals leading to severe agglomeration.[9] If the impurity content of the mother liquor is high and the wash acts as an anti-solvent this can have

a drastic effect on the purity of the final product if the impurities are also potentially subject to anti-solvent crystallisation.

This suggests that poorly designed washing processes can modify particle properties, and this impact may be enhanced in the subsequent drying step, which is very likely to further strengthen the agglomerates formed during washing, especially if the solubility in the residual wash solvent is high. It is important therefore to prevent agglomeration during washing and limit the amount of product in solution at the start of drying by optimising the washing process. This research investigates strategies to follow for optimal selection of the wash solvent. The approach involves minimising dissolution of API crystals, whilst preventing any precipitation of dissolved API and impurities. Selecting a wash solvent to avoid these phenomena, when the wash solvent contacts the retained crystallisation solvent in the saturated filter cake, can be challenging but is essential to maintain yield, purity, and particle characteristics throughout the isolation process.

The aim of this work is to develop a quick and simple screening methodology to both qualitatively and quantitatively analyse the propensity of different wash solvents to cause precipitation to occur during the washing process. Paracetamol was selected as a representative API for the experimental work as it is a widely researched compound with a significant body of published data that can be drawn upon to facilitate the experimental work.[10 -13] The approach developed in this study allows quantification of both the amount of paracetamol API precipitating out during washing and the quantity of dissolved impurities that could precipitate out and adversely affect the purity of the final product.

2. APPROACH

2.1 RAW MATERIAL

Paracetamol (PCM) has characteristics typical of APIs and is commercially available, as are its impurities of synthesis. In this research a typical crystalline grade of paracetamol was used (Mallinckrodt Inc., USA). Acetanilide (Sigma-Aldrich, UK, purity 99%) and Metacetamol (Sigma-Aldrich, UK, purity 97%), two structurally related compounds to paracetamol, were used as representative impurities which could be present in the mother liquor during the crystallisation step.[14]

Three commonly used crystallisation solvents appropriate for isolating paracetamol were used: ethanol (purity ≥ 99.8% (GC)), propan-2-ol (IPA) (purity ≥ 99.5% (GC)) and 3-methylbutan-1-ol, (isoamyl alcohol) (purity ≥ 99.5% (GC)) were purchased by Sigma Aldrich.[15] The wash solvents were purchased from Alfa Aeasar: acetonitrile (purity 99+%), isopropyl acetate (purity 99+%) and n-heptane (purity 99%). Acetonitrile was chosen because the API solubility is at the high end of those typically selected as wash solvents and because it is a widely used solvent in industry. n-heptane was selected because the solubility of PCM and the selected impurities is very low, almost negligible. Isopropyl acetate is another commonly used wash solvent in industry and the solubility of API in the isopropyl acetate is in the middle of the two extremes represented by acetonitrile and heptane. A further criterion is that all three wash solvents were chosen to be miscible with the three crystallisation solvents.

To determine the purity of the precipitated material at the end of each experiment, high pressure liquid chromatography (HPLC) was used. The eluents contained water (Water, ultrapure, HPLC Grade) and methanol (Methanol, ultrapure, HPLC Grade,

99.8+%), purchased by Alfa Aesar. Methanol was also used as diluent for some samples.

2.2 SAMPLE PREPARATION

Saturated PCM solution, with impurities included where selected, was prepared in two stages based on previously measured solubility of PCM in the selected crystallisation solvents.[16] Firstly 2% by mass relative to the known PCM solubility of each impurity was added and dissolved in the crystallisation solvent. To ensure complete dissolution of impurities a sonic water bath was used (Elmasonic P300H Ultrasonic, Cole-Parmer Instruments Ltd.). The amount of paracetamol required to saturate the solution was then added and dissolved in similar manner, to prevent any undissolved impurity crystals remaining in the final saturated solution. The saturated solution was filtered before anti-solvent screening experiments to prevent potential seeding effects.

Wash solutions were prepared using a mixture of the selected wash solvent and crystallisation solvent. The different ratios, by volume, of wash solution used in each solvent system is reported in **Error! Not a valid bookmark self-reference.**.

Table 1: The wash solutions of different ratios of wash solvent and crystallization solvent that were tested for each solvent system.

Wash solvent solution identity	Percentage of crystallisation solvent by volume	Percentage of wash solvent by volume
1	90%	10%
2	75%	25%
3	50%	50%
4	40%	60%
5	30%	70%
6	20%	80%
7	10%	90%
8	0%	100%

2.3 ANTI-SOLVENT SCREENING PROCEDURE

Two anti-solvent screening approaches were developed and evaluated, one based on portion-wise addition of the wash solvent to a saturated solution and monitored by visual observation, the other used centrifugation to separate and recover any precipitated particles.

2.3.1 ANTI-SOLVENT SCREENING PROCEDURE – GLASS VIAL METHOD

Figure 1: Glass vial precipitation detection method.

Figure 1 gives a schematic representation of the glass vial method. This method uses a standard 1.8 mL glass HPLC vials. 300 µL of saturated crystallisation solution is first added to the vial using an Eppendorf pipette. Then wash solution is added 2 drops at a time using a 1 mL disposable pipette. After each addition, the vial was shaken and checked for any precipitation of crystals that might have taken place. Wash solvent addition was continued until the total amount of wash solvent added corresponded to

700 µL. Given a typical saturated filter cake contains very approximately 50% by volume API crystals and 50% by volume mother liquor, then a one cake volume wash would broadly match the 2:1 ratio achieved here (the cake void volume depending on particle aspect ratio and packing). The amount of wash solvent used is better expressed as a two-cake void volume wash.[17] The glass vial was then visually inspected at the end of the drop wise addition to check for any precipitation of crystals. If no crystals were formed, the vials were re-inspected the following day (approximately 24 hours later) to determine whether precipitation was possible but a very slow process under the conditions investigated. Whilst precipitation taking significantly longer than the normal duration of the washing step may not be of practical significance it is considered to be useful to know whether precipitation is possible under each of the conditions investigated.

2.3.2 ANTI-SOLVENT SCREENING PROCEDURE – CENTRIFUGE VIAL METHOD

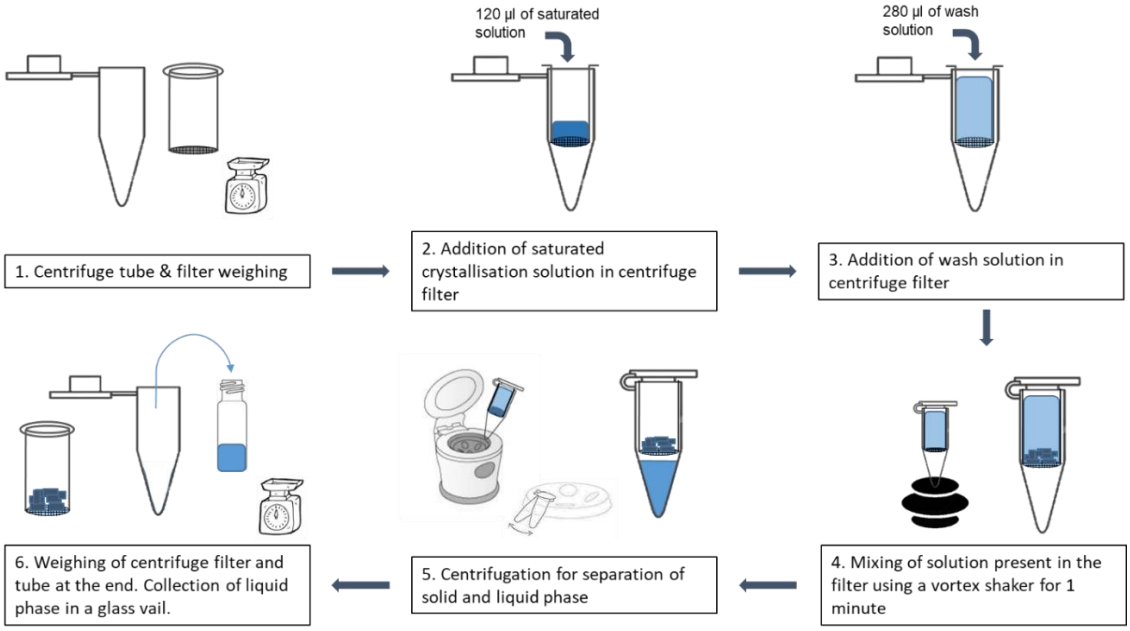

Figure 2: Centrifuge vial precipitation detection method.

To evaluate the anti-solvent effect during washing using a centrifuge tube setup, centrifuge filter tubes incorporating a basket with 0.2 µm pore size were used. The small pore size allowed for mixing of the sample solution and the wash solvents to be performed in the filter basket without any solvent leakage into the filter tube.

The procedure was divided into six steps, with a mass balance maintained across each step to take into account any material loss. In a pre-weighed centrifuge filter basket and centrifuge tube, the saturated crystallisation solvent was added and the mass of the filled tube was recorded (Figure 2). The centrifuge filter basket had a capacity of 500 µL, thus 120 µL of saturated crystallisation solvent was added using an Eppendorf pipette, this was followed by the addition of 280 µL of the wash solvent. The choice of solvent volumes allowed a small space to remain at the top of the filter basket to prevent any solvent spillage while mixing the sample using a vortex shaker. After addition of wash solution (step 4 in Figure 2) the solvent was kept in the centrifuge tube basket for 2 hours and then the anti-solvent effect was checked. Longer contact times between mother liquor and wash solvent, e.g., 24 hours, was not investigated as the selected centrifuge vials were found not to seal well enough to completely prevent solvent evaporation occurring if vials were left overnight. Also, the

filter medium in the baskets eventually allowed solvent to drain onto the centrifuge vial, due to gravity, if left over a long period of time. The compromise of two hours was selected as an appropriate amount of time to represent the practical maximum time for which wash solvent would be present in contact with the saturated crystallisation solution in the API cake. The separation of any precipitated solid from the mixture of saturated solution and wash solvent takes place in step 5 of Figure 2. Centrifugation was carried out for 2 minutes at 6000 rpm. The basis of selection of these conditions is reported in the supporting information. The chosen conditions were found to be effective in separating the mixed crystallisation and wash solvent from any precipitated solid particles retained in the centrifuge filter basket.

2.4 ANTI-SOLVENT PROCEDURE ANALYSIS

The solubility of paracetamol in the binary solvent mixtures (crystallisation and wash solvents) was determined experimentally by equilibration and gravimetric analysis.

Liquid and solid phases (HPLC): Calibration curves for pure paracetamol, metacetamol and acetanilide were prepared using a multilevel calibration method reported in the supporting information. An Agilent 1260 Infinity II system was used. The column was an Agilent Poroshell 120 EC-C18 4.6 x 100mm 4µm operated at 40°C, with a flow rate of 1 mL/min. The injection volume was 5µL, data was collected at 243 nm wavelength, and the mobile phase was 80% water and 20% methanol.

Solid phase X-ray powder diffraction (XRPD): The analysis was performed using a D8 (multi-well) powder X-ray diffractometer – Flat plate instrument, Bruker AXS GmbH. The detector rotation (2θ) was set at 2θmin at 4° and 2θmax at 35°. A step size of 0.017° was used and the sec/step duration was set at 1 second.

Solid phase differential scanning calorimetry (DSC): The analysis was performed using a DSC 214 Polyma, NETZSCH-Gerätebau GmbH. Standard aluminium pans were used. The mass of sample added to the pans was maintained around 2-3 mg. The DSC214 Polyma employed a helium purge (inline pressure set at 0.5 bar) and as a protective gas during analysis, and flowing through a chiller unit for sample cooling. The initial temperature was set at ambient, 25 °C, and the final temperature was set at 200 °C. The heating rate used was 10 °C /min. A sample was also run with heating rate of 2 °C /min to check the sensitivity looking for peak separation that might be missed at a high heating rate.

3. RESULTS, INTERPRETATIONS AND DISCUSSIONS

3.1 ANTI-SOLVENT EFFECT – GLASS VIAL METHOD

Figure 3: Ethanol - n-heptane glass vial precipitation qualitative test.

Figure 3 shows the results from the anti-solvent screening carried out for the ethanol - n-heptane solvent system using the glass vial method. The 50:50 solvent ratio (first picture on the left in Figure 3) represents wash solution made up of 50% by volume

ethanol (the crystallisation solvent) with 50% by volume of n-heptane (the wash solvent), respectively. Precipitation was first observed in the ethanol - n-heptane experiments when a wash solution ratio of 40:60 was used (40% ethanol, 60% n-heptane by volume). In this condition of 40:60 wash solution, local and rapid precipitation of crystals was observed as the first few drops of wash solution was added to the saturated crystallisation solvent. These crystals subsequently dissolved back into the mixed liquid phase after a few seconds, once all the wash solution was added to the saturated crystallisation solvent. Therefore, the initial precipitation observed was due to the local supersaturation in a non-mixed environment. As soon as mixing occurred the bulk composition remained undersaturated consequently the crystals dissolved back in solution. However, after leaving the vials for 24 hours, there were three or four small crystals were seen at the bottom of the glass vial, by the naked eye. This delayed precipitation indicates relatively slow kinetics of the system at this composition.

For the samples with compositions of 30:70 to 0:100 ethanol : n-heptane in Figure 3, crystal precipitation occurred as soon as the wash solution was added and mixed with the saturated crystallisation solvent. There is an increase in crystal concentration in the vials going from compositions of 40:60 to 0:100 ethanol : n-heptane as seen in Figure 3. This increase is due to the higher supersaturation achieved in the solvent mixture as the concentration of n-heptane in the wash solution increases, this can be seen in Table 3. Higher supersaturation results in a more thermodynamically unstable solution, which then results in increased precipitation of crystals occurring to allow the solution to return to thermodynamic equilibrium.[18]

This anti-solvent effect (crystal formation due to anti-solvent addition) was seen for 5 different cases, for the combination of crystallisation solvent and wash solvents used in this study. The results for this can be seen in **Error! Not a valid bookmark self-reference.**. For each solvent combination case, if precipitation of crystals was observed then the solvent composition or the solvent proportions of the wash solution at the point where precipitation is first observed is given in **Error! Not a valid bookmark self-reference.**.

In the case of using n-heptane as wash solvent, precipitation was detected in all three different crystallisation solvent systems. The almost negligible solubility of paracetamol in n-heptane combined with its much higher solubility in the crystallisation solvents, results in a supersaturated solution being formed as the wash solution is added to the saturated crystallisation solvent. Table 3 provides the change in saturation (ΔC) in the final solution obtained at the end of washing after all the wash solution is added, for all the different wash ratios used.

There was no anti-solvent effect observed where acetonitrile and isopropyl acetate were used as the wash solvents. In acetonitrile, PCM has the highest solubility of all the wash solvents used. The binary solvent solubility for the acetonitrile cases shows how the operating dilution line is below the solubility curve.

The calculated ΔC values for all acetonitrile cases, Table 3, shows that supersaturation is not achieved and so no precipitation should be observed. In fact, any paracetamol crystals present would be subject to dissolution in these unsaturated conditions. Isopropyl acetate was also found to have ΔC values < 1 for all the wash solution ratios, hence no precipitation should have been detected.

Table 2: Precipitation caused for different solvent combinations - glass vial method. The crystallisation solvent used is listed on the left side of the table with the corresponding wash solvent across the top of

the table. The ratio of the wash solution at which precipitation is first observed in the solvents system for paracetamol API case is given here. (The bold numbers correspond to the volume ratio of crystallisation solvent in the wash solution, while the italic number corresponds to the volume ratio of wash solvent in the wash solution.)

		Wash solvent		
		n-heptane	*Acetonitrile*	*Isopropyl acetate*
Crystallisation Solvent	**Ethanol**	**40** – *60*% (v/v)	No nucleation	**10** – *90*% (v/v)
	Isopropanol	**40** – *60*% (v/v)	No nucleation	**0** – *100*% (v/v)
	Isoamyl alcohol	**20** – *80*% (v/v)	No nucleation	No nucleation

Table 3: ΔC achieved for the solvent combinations used. Blue cells represent scenarios where nucleation and crystallisation was observed. Orange cells represent scenarios where local supersaturation resulted in immediate nucleation and then dissolution of crystals as bulk under saturation is reached on mixing.

ΔC values		**Ratio of the wash solution sample**								
		100:0	**90:10**	**75:25**	**50:50**	**40:60**	**30:70**	**20:80**	**10:90**	**0:100**
Solvent Combinations	**Ethanol - Acetonitrile**	1.00	0.79	0.63	0.51	0.49	0.49	0.49	0.50	0.52
	Ethanol - Isopropyl Acetate	1.00	0.89	0.79	0.71	0.70	0.70	0.71	0.73	0.76
	Ethanol - Heptane	1.00	1.06	1.17	1.43	1.58	1.77	2.01	2.34	2.82
	Isopropanol - Acetonitrile	1.00	0.71	0.53	0.42	0.41	0.41	0.42	0.44	0.48
	Isopropanol - Isopropyl Acetate	1.00	0.90	0.81	0.72	0.71	0.70	0.70	0.71	0.73
	Isopropanol - Heptane	1.00	1.08	1.24	1.63	1.86	2.17	2.60	3.23	4.26
	Isoamyl Alcohol - Acetonitrile	1.00	0.65	0.46	0.36	0.36	0.36	0.37	0.39	0.43
	Isoamyl Alcohol - Isopropyl Acetate	1.00	0.85	0.46	0.36	0.63	0.63	0.64	0.67	0.71
	Isoamyl Alcohol - Heptane	1.00	1.09	1.25	1.63	1.83	2.08	2.38	2.77	3.28

The glass vial method used for anti-solvent effect screening was found to be effective for qualitative analysis of the wash solvent effect. The precipitation of crystals formed due to interaction between wash solution and the mother liquor is observable and this method can be used as a “quick” first approach to assess wash solvent compatibility.

However, quantitative analysis, to determine the amount and identity of solute precipitating out of the solution required a different methodology, where separation of liquid and solid phase is achievable. To get a better quantitative result, an improved wash screening analysis was devised to overcome these separation issues. Hence the centrifuge vial method was developed.

3.2 ANTI-SOLVENT EFFECT – CENTRIFUGE VIAL METHOD

Table 4: Precipitation caused by different solvent combinations - centrifuge vial method. The crystallisation solvent used is reported on the left side of the table whilst the wash solvent across the top of the table. The ratio of wash solution at which precipitation is first observed in these solvent systems for paracetamol as a representative API is reported here. (The bold numbers correspond to the volume ratio of crystallisation solvent in the wash solution, while the italic number corresponds to the volume ratio of wash solvent in the wash solution.)

		Wash solvent		
		n-heptane	*Acetonitrile*	*Isopropyl acetate*
Crystallisation Solvent	**Ethanol**	**30** – *70*% (v/v)	No nucleation	No nucleation
	Isopropanol	**30** – *70*% (v/v)	No nucleation	No nucleation
	Isoamyl alcohol	**10** – *90*% (v/v)	No nucleation	No nucleation

Table 4 shows the anti-solvent effect observed using the centrifuge vial method. This is similar to the glass vial method described in Figure 3 shows the results from the anti-solvent screening carried out for the ethanol - n-heptane solvent system using the

glass vial method. The 50:50 solvent ratio (first picture on the left in Figure 3) represents wash solution made up of 50% by volume ethanol (the crystallisation solvent) with 50% by volume of n-heptane (the wash solvent), respectively. Precipitation was first observed in the ethanol - n-heptane experiments when a wash solution ratio of 40:60 was used (40% ethanol, 60% n-heptane by volume). In this condition of 40:60 wash solution, local and rapid precipitation of crystals was observed as the first few drops of wash solution was added to the saturated crystallisation solvent. These crystals subsequently dissolved back into the mixed liquid phase after a few seconds, once all the wash solution was added to the saturated crystallisation solvent. Therefore, the initial precipitation observed was due to the local supersaturation in a non-mixed environment. As soon as mixing occurred the bulk composition remained undersaturated consequently the crystals dissolved back in solution. However, after leaving the vials for 24 hours, there were three or four small crystals were seen at the bottom of the glass vial, by the naked eye. This delayed precipitation indicates relatively slow kinetics of the system at this composition.

For the samples with compositions of 30:70 to 0:100 ethanol : n-heptane in Figure 3, crystal precipitation occurred as soon as the wash solution was added and mixed with the saturated crystallisation solvent. There is an increase in crystal concentration in the vials going from compositions of 40:60 to 0:100 ethanol : n-heptane as seen in Figure 3. This increase is due to the higher supersaturation achieved in the solvent mixture as the concentration of n-heptane in the wash solution increases, this can be seen in Table 3. Higher supersaturation results in a more thermodynamically unstable solution, which then results in increased precipitation of crystals occurring to allow the solution to return to thermodynamic equilibrium.[18]

This anti-solvent effect (crystal formation due to anti-solvent addition) was seen for 5 different cases, for the combination of crystallisation solvent and wash solvents used in this study. The results for this can be seen in **Error! Not a valid bookmark self-reference.**. For each solvent combination case, if precipitation of crystals was observed then the solvent composition or the solvent proportions of the wash solution at the point where precipitation is first observed is given in **Error! Not a valid bookmark self-reference.**.

In the case of using n-heptane as wash solvent, precipitation was detected in all three different crystallisation solvent systems. The almost negligible solubility of paracetamol in n-heptane combined with its much higher solubility in the crystallisation solvents, results in a supersaturated solution being formed as the wash solution is added to the saturated crystallisation solvent. Table 3 provides the change in saturation (ΔC) in the final solution obtained at the end of washing after all the wash solution is added, for all the different wash ratios used.

There was no anti-solvent effect observed where acetonitrile and isopropyl acetate were used as the wash solvents. In acetonitrile, PCM has the highest solubility of all the wash solvents used. The binary solvent solubility for the acetonitrile cases shows how the operating dilution line is below the solubility curve.

The calculated ΔC values for all acetonitrile cases, Table 3, shows that supersaturation is not achieved and so no precipitation should be observed. In fact, any paracetamol crystals present would be subject to dissolution in these unsaturated conditions. Isopropyl acetate was also found to have ΔC values < 1 for all the wash solution ratios, hence no precipitation should have been detected.

Table 2. Due to the opaque character of the polypropylene centrifuge vials, nucleation and crystallisation phenomena were much harder to observe compared to using the clear glass vials.

Comparing the results shown in Table 4 with those obtained using the glass vial method, (see Figure 3 shows the results from the anti-solvent screening carried out for the ethanol - n-heptane solvent system using the glass vial method. The 50:50 solvent ratio (first picture on the left in Figure 3) represents wash solution made up of 50% by volume ethanol (the crystallisation solvent) with 50% by volume of n-heptane (the wash solvent), respectively. Precipitation was first observed in the ethanol - n-heptane experiments when a wash solution ratio of 40:60 was used (40% ethanol, 60% n-heptane by volume). In this condition of 40:60 wash solution, local and rapid precipitation of crystals was observed as the first few drops of wash solution was added to the saturated crystallisation solvent. These crystals subsequently dissolved back into the mixed liquid phase after a few seconds, once all the wash solution was added to the saturated crystallisation solvent. Therefore, the initial precipitation observed was due to the local supersaturation in a non-mixed environment. As soon as mixing occurred the bulk composition remained undersaturated consequently the crystals dissolved back in solution. However, after leaving the vials for 24 hours, there were three or four small crystals were seen at the bottom of the glass vial, by the naked eye. This delayed precipitation indicates relatively slow kinetics of the system at this composition.

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Table 2), reveals some differences. Due to the opaque nature of the centrifuge vials, the nucleation observed due to local supersaturation effects for isopropyl acetate which were seen in glass vial method, could not be discerned in the centrifuge vial experiment (orange cells in Table 3). In all the cases of n-heptane as wash solvent, nucleation was observed for wash solution ratios with higher n-heptane concentrations. This offset in observation of the anti-solvent effect can again be attributed to the opaque nature of the centrifuge vials giving difficulties in visualisation of precipitation of few, small crystals. Also, the slow crystallisation kinetics noticed in the 40:60 ethanol : n-heptane case in the glass vial experiments is not noticed in the centrifuge method as the solution is only left for 2 hours compared to 24 hours in the glass vial method.

However, the quantitative analysis achieved using the centrifuge vial method was found to be much more successful as almost complete separation of solid crystals from liquid solution was achieved. After stage 6 in Figure 1, HPLC is performed on both the separated solid and liquid samples. Quantitative results obtained from two different scenarios, ethanol – acetonitrile, and ethanol – n-heptane and are given below. The two scenarios presented illustrate the results which would be obtained for most cases depending on whether precipitation is observed or not.

3.2.1 CENTRIFUGE VIAL METHOD – QUANTITATIVE ANALYSIS: ETHANOL – ACETONITRILE (NO NUCLEATION)

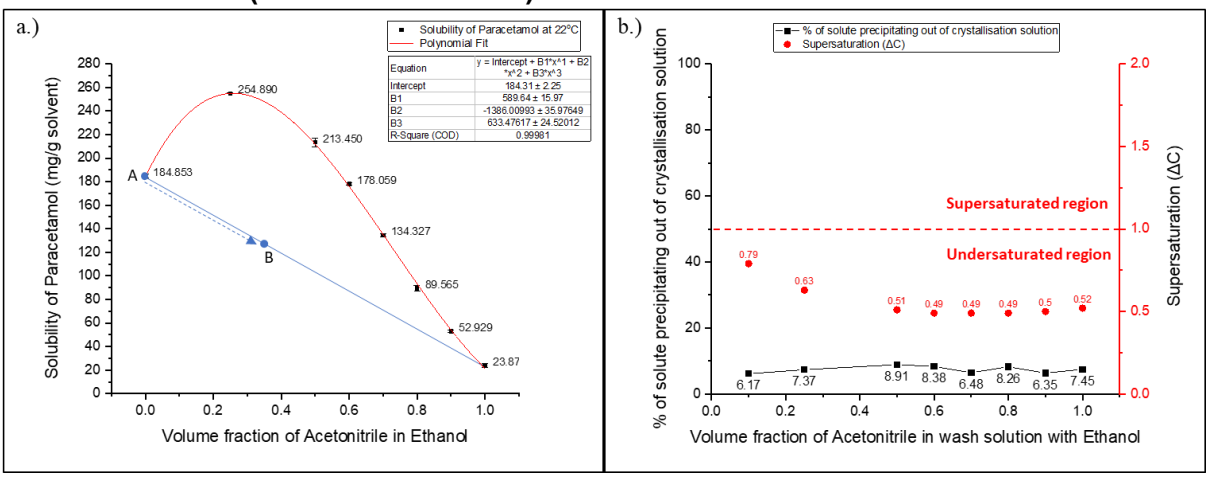

Figure 4: Quantitative analysis of the ethanol-acetonitrile case. a.) Solubility of paracetamol in ethanol-acetonitrile binary solvent mixture at 22 ºC. b.) Percentage of solute precipitating out of solution for different wash solution compositions is shown in the Y axis on the left hand side of the graph, with the supersaturation achieved in the solution when different ratio of wash solution is added to the saturated crystallisation solvent shown on the Y axis on the right hand side of the graph.

Table 5: Ratio of wash solvent in the final solution mixture.

Ratio of wash solution used (v/v) (crystallisation: wash)	90:10	75:25	50:50	40:60	30:70	20:80	10:90	0:100

Volume fraction of wash solvent in final solution (end point of the final solution)	0.07	0.175	0.35	0.42	0.49	0.56	0.63	0.7

As reported in the binary solvent mixture solubility data reported in Table 5 and in Figure 4, the ethanol and acetonitrile system does not show any anti-solvent effect. The blue line in Figure 4, graph a, represents the change in concentration of the API in the resultant solution mixture as the wash solution is added to the saturated crystallisation solvent. Point A in the graph is the starting API concentration of the saturated ethanoic solution. As the wash solution is added to the saturated crystallisation solvent, the concentration of API in the solution will change and move down following the path of the blue line. The calculated end point of the overall solution is dependent on the wash solution that is used, given in Table 5. Hence, the end point of the API concentration in the new system (mother liquor and wash solvent) depends on the composition and quantity of wash solution being used (in this study the total volume ratio of the system is fixed at 700 µL wash solution to 300 µL mother liquor). For example, if an experiment with wash solution comprising equal volumes of the wash solvent and the crystallisation solvent is used, then looking at Table 5, the final composition of the overall solution (containing saturated crystallisation solution and wash solution) would be 35% by volume acetonitrile in ethanol. The value of paracetamol concentration at 35% by volume wash solvent can then be determined from the blue line in graph a, of Figure 4, which would correspond to around 127 mg paracetamol/g solvent, point B.

The difference between the diluting line and the solubility curve dictates whether precipitation could take place. For the case of ethanol and acetonitrile (Figure 4), since the diluting line is below the solubility curve, the actual concentration of paracetamol in system is below the solubility limit. Therefore, the solution would be undersaturated and no precipitation would occur.

In Figure 5b, the red dots represent the corresponding supersaturation across the solvent composition investigated, showing no precipitation of the API or the impurity as the system is in the undersaturated region. Table 6 shows the solubility data of the API and the selected impurities in the pure solvents used in this study. Since the solubility of the impurities, metacetamol and acetanilide, in pure solvent is similar or greater than that of paracetamol, and only 2% by mass of impurity is present in each crystallisation solution, any impurity present in the precipitated material would be due to incorporation in API crystals rather than independent crystallisation of the impurities as separate crystalline species.

Table 6: Experimental solubility determined of Metacetamol and Acetanilide in the selected solvent at 25 ^{O}C.

	Solvent	**Solubility (g paracetamol/g solvent) (at 25 ^{o}C)**	**Solubility (g metacetamol/g solvent) (at 25 ^{o}C)**	**Solubility (g acetanilide/g solvent) (at 25 ^{o}C)**
Crystallisation Solvent	Ethanol	0.2057	0.2944	0.3322
	Isopropanol	0.1243	0.1948	0.1957
	Isoamyl Alcohol	0.0549	0.1049	0.1656
Wash Solvent	Acetonitrile	0.0294	0.0776	0.2060

	Isopropyl Acetate	0.0076	0.0246	0.0896
	N-heptane	0.0003	0.0003	0.0004

Even though there is no precipitation observed in the ethanol - acetonitrile case, the measured percentage precipitation value remains constant at around 7 ± 1% as indicated by the black squares in graph b of Figure 4. This consistent amount of precipitation along the varying wash solution composition used can be explained by the presence of crystallised material formed from solution left on the porous media of the centrifuge vial basket. This crystallisation is therefore occurring during the solvent evaporation.

Since no precipitation takes place in ethanol - acetonitrile solvent combination, this does not automatically make acetonitrile a good candidate as the wash solvent for paracetamol in ethanol crystallisation solvent. Selecting a wash solvent with a modest solubility of paracetamol API can reduce the isolation yield by dissolution of the particles forming the API cake. Figure 4, graph a, shows that the operating dilution line is below the solubility curve and so the acetonitrile wash solution would tend to dissolve some of the paracetamol crystals present in the filter cake. Also, and probably of greater importance, the residual acetonitrile wash solution left in the deliquored cake would be likely to result in particle agglomeration during the drying process. Evaporation of the residual wash solution in the API cake would cause crystallisation of the dissolved solute on the crystal surfaces forming crystal bridges in the API cake (as seen in **Error! Reference source not found.**).

3.2.2 CENTRIFUGE VIAL METHOD – QUANTITATIVE ANALYSIS: ETHANOL – n-HEPTANE

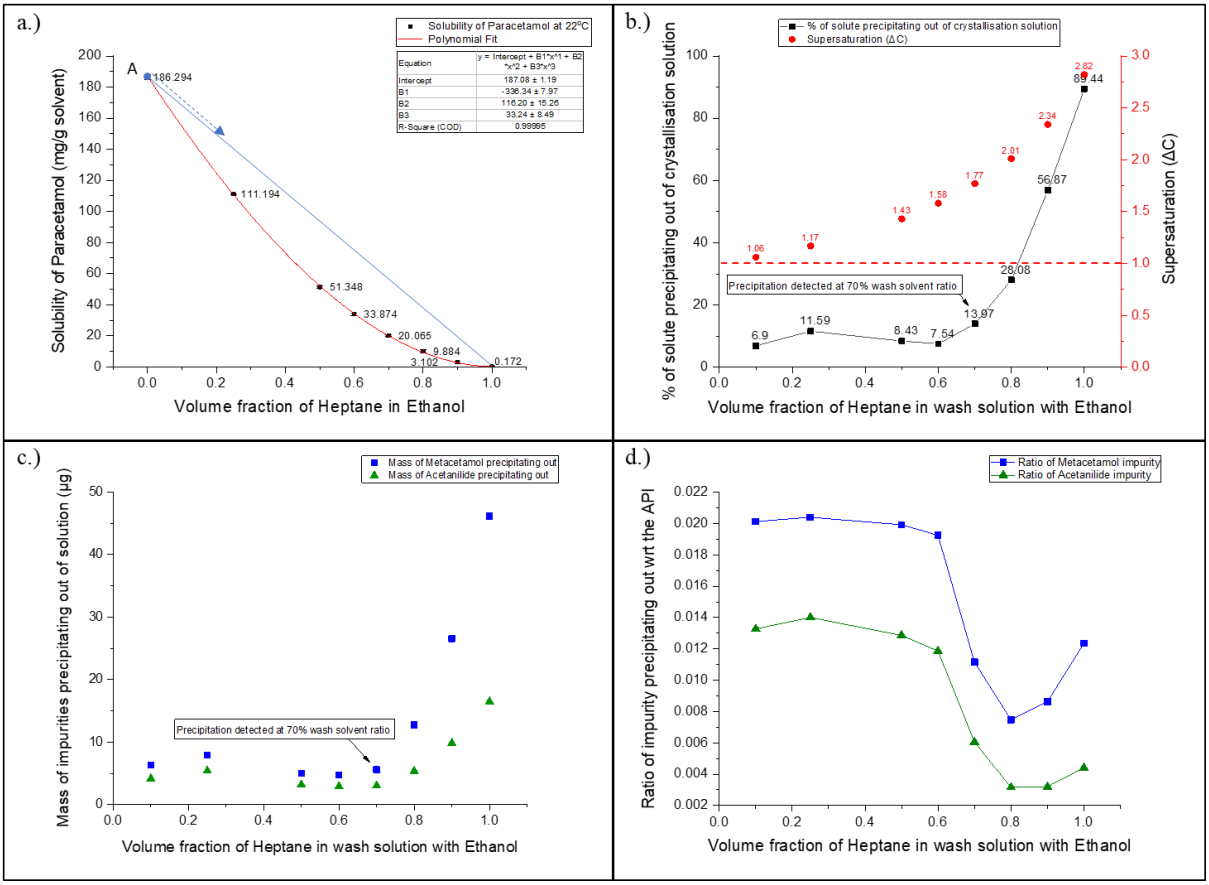

Figure 5: Quantitative analysis of the ethanol - n-heptane case. a.) Solubility of paracetamol in ethanol - n-heptane binary solvent mixture at 22 °C. b.) Percentage of solute precipitating out of solution for different wash solution compositions is shown in the graph together with the supersaturation achieved in the solution when different ratio of wash solution is added to the saturated crystallisation solvent. c.) Mass of impurities precipitating out when using different ratios of wash solution. d.) Ratio of impurities precipitating out with respect to the paracetamol (API) precipitating out for each of the different ratios of wash solutions used.

As reported in Figure 3 and validated by the paracetamol solubility data determined for the ethanol - n-heptane binary solvent mixture (Figure 5, graph a), precipitation was detected. The figure shows paracetamol supersaturation was generated as wash solution is added to the saturated crystallisation solution. The end point composition of the solution on the operating dilution line would be dependent on the ratio of wash solution added (Table 5). Since the API concentration in the system would be higher than the solubility of the API in the solution (blue dilution line above the solubility curve), supersaturation would be generated and precipitation would be likely to be observed.

Graph b in Figure 5 shows the percentage of solute precipitating out of solution (black line with square points) and the supersaturation level reached (red dots) for the different ratios of wash solution used. Precipitation of the solute was detected after a wash solution of 60% n-heptane and 40% ethanol by volume is used. Before that, the percentage of solute shown as precipitating out of the system is due to the retention of solution in the membrane similar to the effect observed in the ethanol - acetonitrile case, section 3.2.1. Any increase in n-heptane above 60% in the wash solution shows a significant increase in the amount of solute precipitating out of solution with around 89% of the dissolved solute precipitating out of the solution when using pure n-heptane as the wash solvent. This increase in the amount of precipitation taking place is consistent with the increase in the supersaturation value as the amount of n-heptane increases in the system, as seen on the Y axis on the right-hand side of graph b.

HPLC of the precipitated crystals was used to determine the composition of the crystals and to quantify the amount of impurities precipitating out of the solution. Graph c in Figure 5 shows the amount of impurities, both metacetamol and acetanilide, that were precipitated in the case of ethanol-n-heptane solvent system. There is a gradual increase in the amount of impurity precipitating out of the system, after 0.7 heptane volume fraction at which point precipitation is first detected. Knowing the initial concentration of API and impurities dissolved in the crystallisation solution (0.01769 g of PCM, 0.00031 g of metacetamol, and 0.00035 g of acetanilide), over 10% of the metacetamol and around 5% of the acetanilide impurities were precipitated out of the solution when the wash solution used was pure n-heptane. Graph d in Figure 5 shows the ratio of impurity precipitating out compared to the API in the solution, where the ratio is the mass of impurity over the mass of API. As the impurities are uniformly dispersed throughout the solution, the ratio of impurity from 0.1 to 0.6 volume fraction of n-heptane in ethanol are relatively constant, graph d, Figure 5. This is because there is no precipitation observed in these samples, the impurities are only present because of the retention of solution in the porous membrane.
After a wash volume fraction of 0.6 n-heptane is exceeded, the precipitation of solute increases, there is a decrease in ratio of impurity precipitating out. Since the amount of impurities in the system is only 2% by mass, at the start of the precipitation process this ratio change is caused by the paracetamol API that is present in the system precipitating out. When the volume fraction of n-heptane in the wash solution reaches

0.8, there is an increase in the ratio of impurity precipitating out with respect to the API. Because the impurity concentrations in the mother liquor are so low, it is unlikely that the impurities are crystallizing as separate crystals. Rather that they are being incorporated in the crystals of paracetamol. When the volume fraction of n-heptane reaches 0.8, around 50% of the dissolved paracetamol solute is precipitated out of solution and the supersaturation level is around 2, under these conditions the paracetamol crystal precipitation is rapid and the impurities are easy incorporated into the API crystals. This effect is also seen in the other two solvent mixture cases where precipitation is observed; isopropanol - n-heptane and isoamyl alcohol - n-heptane.

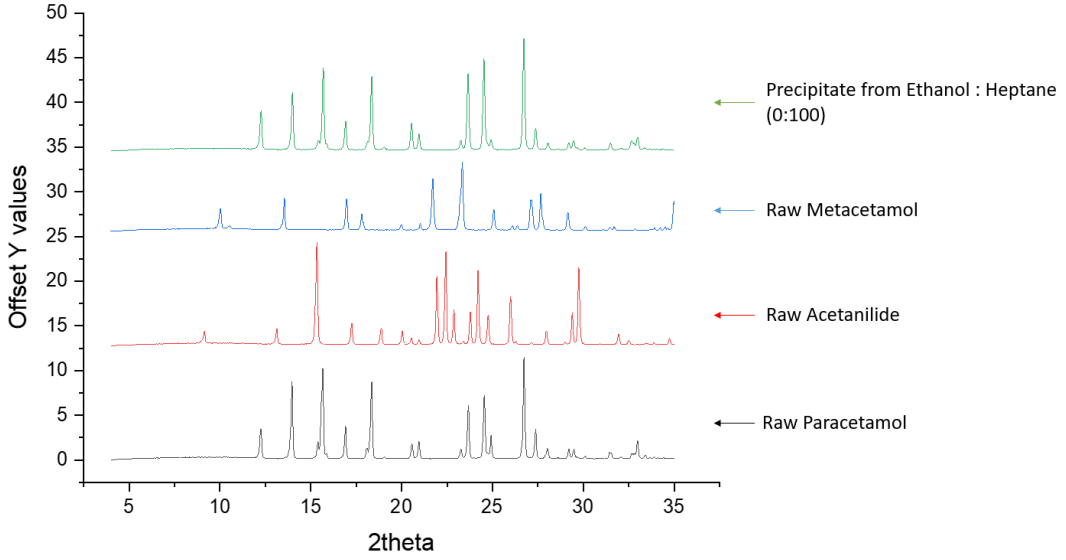

Figure 6: XRPD results for raw paracetamol, metacetamol and acetanilide together with the precipitate sample obtained from ethanol – n-heptane sample.

XRPD analysis was performed on the precipitate obtained from the ethanol - n-heptane experiments to analyse the structure of the crystalline material. The diffraction data in Figure 6 generated from pure paracetamol, metacetamol and acetanilide provide reference XRPDs. From the sample of precipitated material shown in Figure 6, only paracetamol crystals of form 1 are seen to be present, there are no peaks corresponding to metacetamol or acetanilide. DSC analysis was performed to investigate the effect of presence of impurities in the precipitate samples. The amount of impurities in the precipitate samples were found to be smaller than would be needed to be detected because the measured melting temperature of the samples correspond to the melting temperature of pure paracetamol and no other thermal effect related to the impurity species was observed.

The lack of peaks at 2θ values corresponding to impurities in the XRPD and absence of significant melting point reduction in the DSC result is presumed to be due to the small amount of impurities present in the precipitate sample compared to the API, as indicated by the HPLC assays. This low concentration of impurities falls below the detection limit of the two techniques, XRPD and DSC, and hence could not be observed.[19,20]

Use of pure n-heptane as wash solvent in the cases examined would not be an ideal washing strategy due to precipitation of both paracetamol and its impurities of synthesis in the system. Precipitation can be minimised or possibly eliminated by using a two or more-stage washing strategy. In the example case, the first wash can be carried out using a 50:50 ethanol : n-heptane wash solution. This would allow for most

of the saturated ethanoic solution in the API cake to be displaced by the wash solution without causing precipitation. To further improve purity and aid with the drying process, a second wash can then be carried out using pure n-heptane. This washing strategy minimises the risk of precipitation in the first wash by using a wash solution with higher API solubility. Also, the residual n-heptane in the final deliquored API cake, is relatively easily evaporated and because of the low solubility of the API, compared to the 50:50 ethanol : n-heptane wash solution would ensure quicker drying and should also prevent crystalline bridges forming during wash solvent evaporation, minimising agglomeration.

4. CONCLUSIONS

The quality of the crystalline product is primarily dominated and controlled in the crystallisation process but may be influenced by the downstream isolation processes. For overall isolation process optimization, it is important to understand and mitigate the adverse effect caused during the washing process. Lack of knowledge and understanding of the washing process can have a dramatic impact on the final crystal product quality achieved at the end of the drying process. Designing an optimum washing regime is crucial to avoid API product batches that are out of specification.

This study investigates wash solvent selection and introduces a simple and material sparing methodology to help better design washing regimes for API isolation to prevent risk of impurity precipitation during washing. The glass vial anti-solvent methodology was found to be very effective as a qualitative evaluation based on visual detection of precipitation occurring during washing. Effects such as local nucleation can be identified using this method to provide an insight into the kind of process that can be taking place at the washing front inside a saturated API cake during washing.

The centrifuge vial anti-solvent methodology was found to be very efficient at quantitatively determining the amount of precipitation that can take place during a washing process. The composition of the precipitated crystals can be then determined using HPLC technique.

In this work paracetamol was used as the model compound. The solubility of the API was experimentally determined at different crystallisation and wash solvent ratios. The two anti-solvent evaluation methodologies developed in this study are straightforward to conduct and were able to provide a good indication of the effects that would occur within a paracetamol API cake during washing. The glass vial method readily indicates if precipitation is likely to occur due to the solvent interaction in a washing process. If so, then the centrifuge vial method can be used to determine the extent and composition of the precipitation taking place.

Both of the methods developed are quick and easy to perform and allow for prompt wash solvent evaluation. The qualitative results obtained in the 1 mL glass vial method were successfully replicated in 100 mL volumes. The small sample size required for this technique prevents any solvent wastage and is in line with environmental sustainability. The centrifuge vial method could be further improved by using clear, larger 1 mL vials rather than the opaque 500 µL vials used. However, sourcing such vials with membrane basket compatible with the solvents used in this study proved difficult. Furthermore, using centrifuge vials which are fully air tight would have allowed for mimicking of glass vial method, where the solvent system could be allowed to equilibrate over 24 hours. However, 20-minute solvent contact time together with vortex mixing is found to be sufficient and the three replicates of each experiment obtained similar result with very good repeatability.

From the results, acetonitrile wash solvent did not cause any anti-solvent effect in the case of paracetamol crystal washing. Use of heptane wash solvent on the other hand caused anti-solvent effect in the case of all 3 crystallisation solvents used in this study. However, these finding alone does not make acetonitrile a good candidate for wash solvent or heptane a poor wash solvent. Developing the right washing strategy and hence choosing the appropriate wash solvent strategy depends on the aim/objective of the washing procedure within the API isolation processes. If removal of impurity is the main focus, then a wash solvent with high solubility can be used (such as acetonitrile in the case of paracetamol), but the yield would be adversely affected and there is a significant risk of agglomeration on drying. However, if complete removal of mother liquor together with minimal effect on the crystal product is the aim, then a multi-step washing strategy should be devised as exemplified in the ethanol - n-heptane solvent mixture example reported in this study. This would allow for removal of mother liquor with a significantly decreased chance of precipitation occurring and hopefully a corresponding expectation of a reduction in agglomerate formation during drying.

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