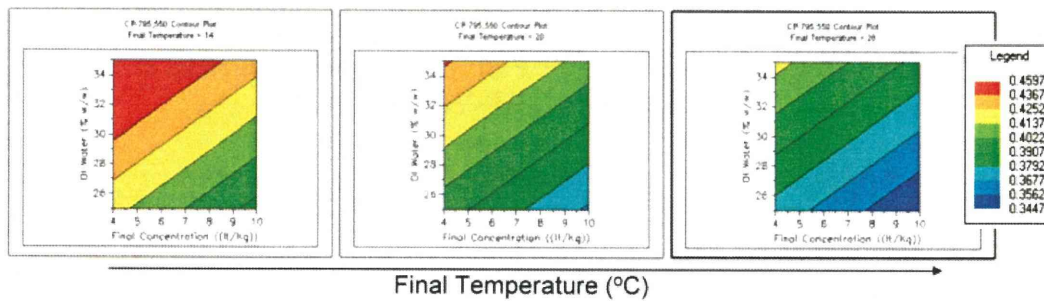


Table 2.3.S.2.6-10 Step 1 Crystallization DoE

Parameter	Low	Standard	High
Cooling Rate (°C/min)	0.15	0.36	0.5
Final Temperature	14	20	26
Final Concentration (L/kg, volume of ethanol relative to CP-6)	4	7.22	10
Addition Time (min)	15	30	60
Volume of Water (% w/w, volume of water relative to ethanol)	10	30	50
Agitation Rate (rpm)	150	test	350
Hold Time prior to Water Addition (hr)	2	test	4
THF (%v/v)	1	test	6

The design space limiting examples from the crystallization experimentation data are presented in Figure 2.3.S.2.6-20 and Figure 2.3.S.2.6-21

**Figure 2.3.S.2.6-20 Ethyl Homolog Levels in Step 1 Crystallization**

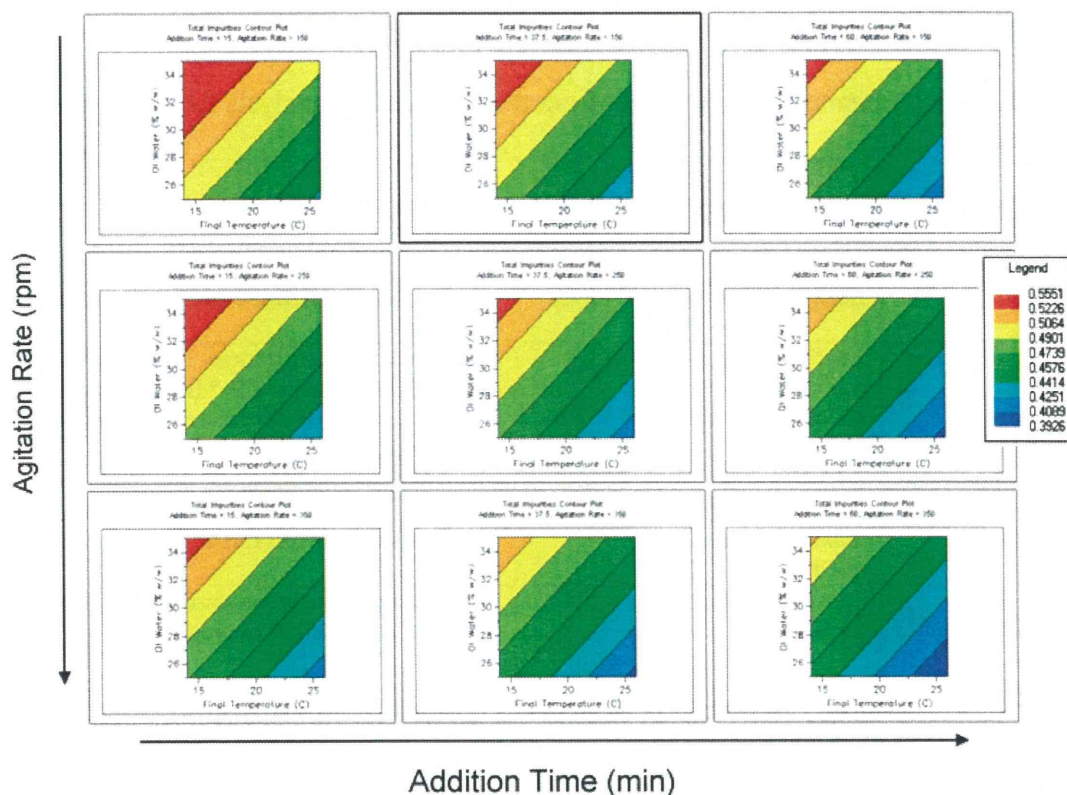


Figure 2.3.S.2.6-21 Total Impurities (%)

Conclusions from the Crystallization DoE

- Ethyl Homolog slightly purges throughout the entire crystallization proposed design space. There is no change of this impurity due to preferential crystallization.
- For all experiments the total impurities ranged from 0.3 to 0.5% taken as area % by HPLC in the isolated product. Scale-up and development experience have shown that these impurities purge well in the Step 1 crystallization below the quality specification for CP-7 and Sakuramil drug substance.
- It is important to note that no new impurities were observed within the proposed design space and levels of existing peaks were below the standard conditions.

Scale and equipment:

- Stressing experiments where cooling rates were varied with prolonged exposure before isolation did not result in any deviation from the impurity profile.

5)-1-1-3 Initial criticality risk assessment from Step 1 reaction and crystallization (including the starting material attributes)

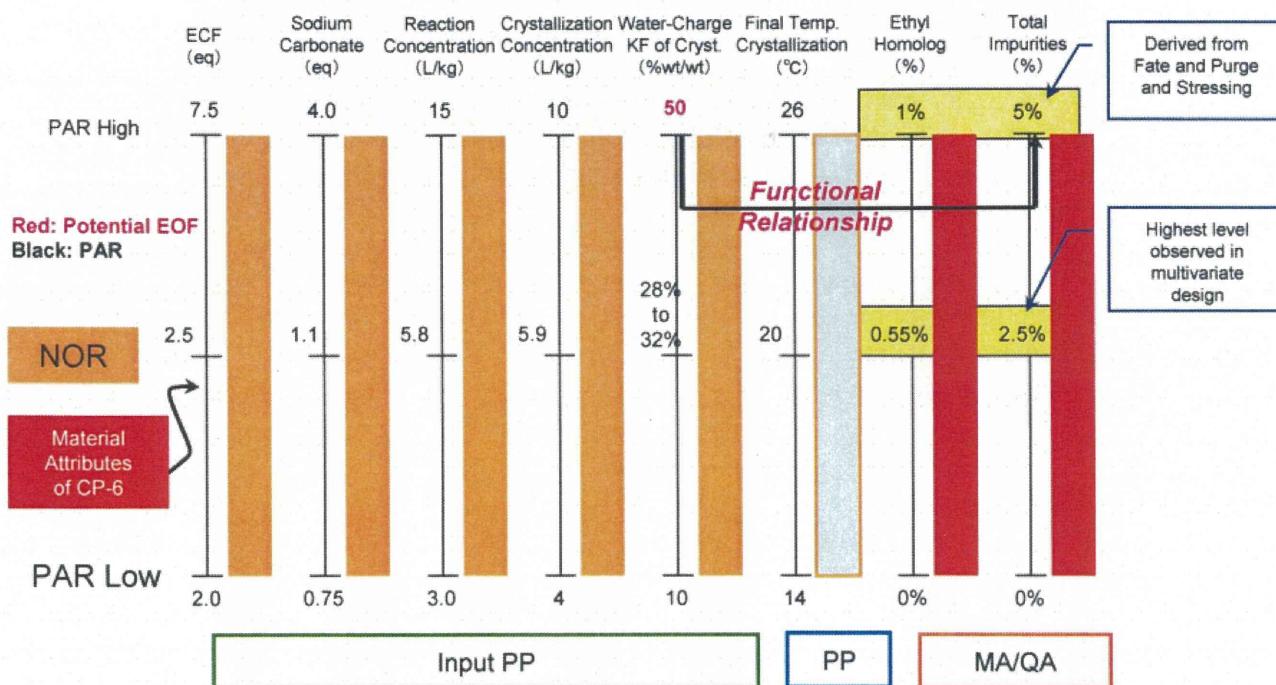
Table 2.3.S.2.6-11 summarizes the output from the multivariate analysis for Step 1 reaction and crystallization.

Table 2.3.S.2.6-11 Summary of Multivariate Analysis Results for Step 1

Parameter	Design Space	NOR	Criticality of Attribute or Parameter with Justification
Ethyl Chloroformate Quantity	2 to 7.5 molar equivalents per molar equivalent of CP-6	2.5	Non-critical: Justification- The unrealistic level of 7.5 eq does not increase the CP-7 (ethyl analog) to $\geq 0.3\%$. Specification is 1% in this reaction and in the final drug substance.
Sodium Carbonate or Trisodium Phosphate Stoichiometry	0.75 to 4 molar equivalents per molar equivalent of CP-6	1.1	Non-critical: Justification- Both have no impact on quality. Both are related to reaction rate but the process quality is not sensitive to differences of bases.
Reaction Concentration (volume of THF relative to CP-6)	3 to 15 liters/kg of CP-6	5.8	Non-Critical: Justification-Both ranges demonstrate low risk to CP-7-1 (ethyl .analog)
Reaction Temperature	reflux	N/A	Non-Critical: Justification-Impact reaction rate, not quality.
Crystallization Ethanol Volume	4 to 10 liters/kg of CP-6	5.9	Minor impact related to total impurities. Requires criticality assessment with Step 2: will be evaluated in holistic design space.
Crystallization Water Volume	10% to 50% wt/wt of water to ethanol	28% to 32%	Statistically relevant and functionally related to total impurities. Requires criticality assessment with Step 2: will be evaluated in holistic design space.
Final Temperature	14 to 26°C	20	Minor impact related to total impurities. Requires criticality assessment with Step 2: will be evaluated in holistic design space.
Drying Temperature	Up to 50°C	42.5°C	Non-Critical: Justification- Higher temperatures and longer exposure demonstrated no degradation.
Scale and Equipment			Non Critical: This homogeneous reaction is not dependent on scale or equipment. Stressing experiments to demonstrate prolonged exposure has no impact on quality.

5)-1-1-4 Multivariate summary for Step 1

Figure 2.3.S.2.6-22 shows the summary of multivariate results for Step 1.



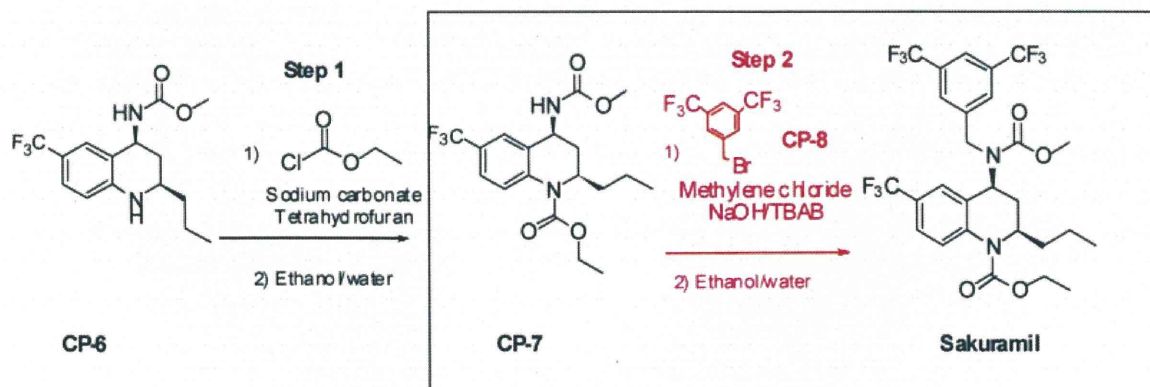
The black arrow shows the functional relationship between QA and PP.

Figure 2.3.S.2.6-22 Combination of Unit Operation Variables for Step 1

5)-1-2 Step 2:

5)-1-2-1 Step 2 reaction

In Step 2, CP-7 is reacted with CP-8 in dichloromethane to give crude Sakuramil drug substance. The crude reaction mixture is quenched, extracted, and exchanged with ethanol; and Sakuramil drug substance is crystallized from an ethanol and water mixture.



5)-1-2-1-1 Impurity quality attributes strategy for Step 2:

Fate and purge data combined with reaction development indicate the level of CP-8 in the crude reaction mixture at levels of 1.2 % risk meeting the 0.10% specification in drug substance. Therefore, monitoring the reaction completion and using CP-8 as the limiting reagent must be seriously evaluated for criticality and control options. The risk assessment combined with prior development knowledge believes this is high risk to uncover multiple edges of failure and be a limiting factor of the design space.

MA of CP-8: A batch was chosen with the “high levels” of impurities in CP-8 for multivariate experimentation to highlight the ability of the design space to control impurities from this starting material. These data complement actual fate and purge data to help evaluate risk and determine acceptable levels and criticality.

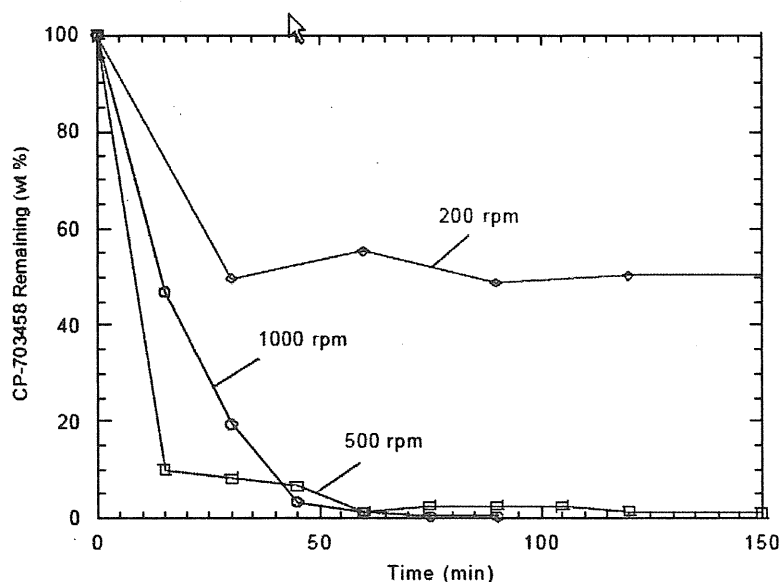
Reaction:

In the reaction between CP-8 and CP-7, CP-8 is used as the limited reagent. A PAT method (REACTR) will be used in the reaction to evaluate the disappearance of CP-8. In addition, total “other” impurities were monitored while probing the ranges in the experimental design to identify if 1. Any new impurities are being generated, and 2. Any levels of known trace impurities already controlled in the standard process increase (< 0.1%).

From the risk assessment, parameters that could potentially influence the levels of CP-8 (and total impurities) in drug substance were identified. This assessment identified that the stoichiometry of CP-8, phase ratio, and reaction concentration were the process parameters that had the highest risk of impacting the potential quality attributes of drug substance. An experimental strategy was designed to determine the effects of this process parameter on controlling these quality attributes, improve process understanding and robustness, and establish the design space for the Step 1 manufacturing process. NaOH concentration and TBAB catalyst eq. are non critical. Development range demonstrated no impact on quality or rate. Historical data / prior knowledge around phase transfer reactions are supports.

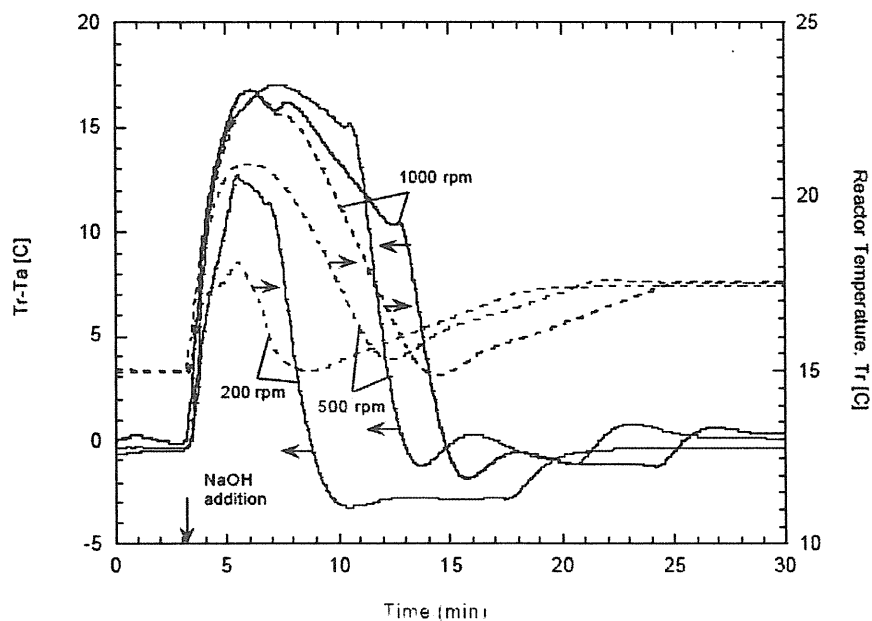
Scale and equipment consideration: (Prior to multivariate design to establish the design space)

As with many phase transfer reactions, the reaction rate will be severely impacted if the aqueous and organic phases are not adequately dispersed; however, product purity does not appear to suffer even after 24 hours under poor mixing conditions, indicating that the reaction is robust enough to withstand any mixing issues that may be encountered on scale (e.g. temporary loss of agitation).



Shows remaining CP-7 (wt%) HPLC results for experiments using agitation rates of 1000 rpm (red circles), 500 rpm (blue squares), and 200 rpm (green diamonds).

Figure 2.3.S.2.6-23 Relation between Agitation Rates and Remaining CP-7 (CP-703458)



Uncorrected heat flow (left axis, solid lines, represented by $Tr-Ta$) and reactor temperature profile (right axis, dotted lines) for experiments at 1000 rpm (black), 500 rpm (blue), and 200 rpm (red). After returning to baseline (15°C), the reaction temperature was increased to 17.5°C over 10 min.

Figure 2.3.S.2.6-24 Relation between Agitation Rates and Reactor Temperature Profile

As expected, mixing has a very strong influence upon the rate of the phase transfer reaction, which proceeds faster at higher agitation speeds. If adequate dispersion of the aqueous and organic phases is not achieved, as was the case at 200 rpm in this series of experiments, the reaction may not be complete even after 24 hours. Even though only 70% conversion was reached after 24 hours at 200 rpm, reaction completion was achieved in 1.5 hours after increasing the agitation rate to disperse the phases.

Despite these results, the reaction appears robust enough such that in all of the experiments, product purity profiles were typical for all of the complete reactions, and issues with purity related to mixing are not anticipated upon scale-up.

Continued stressing experiments were executed to understand if there is any impact on prolonged time difference between laboratory and manufacturing times on the quality attributes. This will be confirmed in the design space of the reaction and crystallization. In addition, the multivariate experiments mimicked the “worst case” scenario of the proposed commercial equipment and its limitations (example; the heating and cooling profile was executed over hours with the same ability as the manufacturing facility).

Parameters and ranges explored in the DoE:

Design: An efficient 3-parameter hybrid response surface design for coupling reaction. Because the level of CP-8 can be controlled within spec ($\leq 0.1\%$) in the downstream crystallization, the whole study ranges for coupling reaction is the design space.

Table 2.3.S.2.6-12 Step 2 Reaction DoE

Parameters Reaction	Low	Standard	High
CP-8 quantity (stoichiometry of CP-8 relative to CP-7)	0.9	1.05	2
Phase ratio for water/organic phase (Ratio NaOH aq volume/ DCM volume)	0.25	1	1.25
Reaction concentration (L/kg, Volumes of DCM relative to CP-7)	0.25	3	5

DCM: Dichloromethane

Conclusion for reaction

- In all experiments the highest level of CP-8 was less than 1%. (1.2% is the specification for CP-8 that purges to 0.1% in the crystallization step.)
- Fate and purge data demonstrate that 5% of unreacted CP-7 purges well below the 0.1% for unspecified impurities. In addition, an experiment containing 5% of CP-7 was run in the design space with high levels of water to confirm that it meets the 0.10% unspecified level.

- There was no deviation from acceptance criteria and no Edges of Failure (EoF) were observed in the parameter multivariate experiments and/or the stressing experiments. At the high end of the three parameters studied in a multivariate analysis, the level of CP-8 was at 1%. The acceptance criteria for CP-8 is 1.2%. The combination of these three parameters all at high levels is unrealistic for manufacturing; therefore warrants non critical designations.

Design-Expert® Software

Impurity CP-8

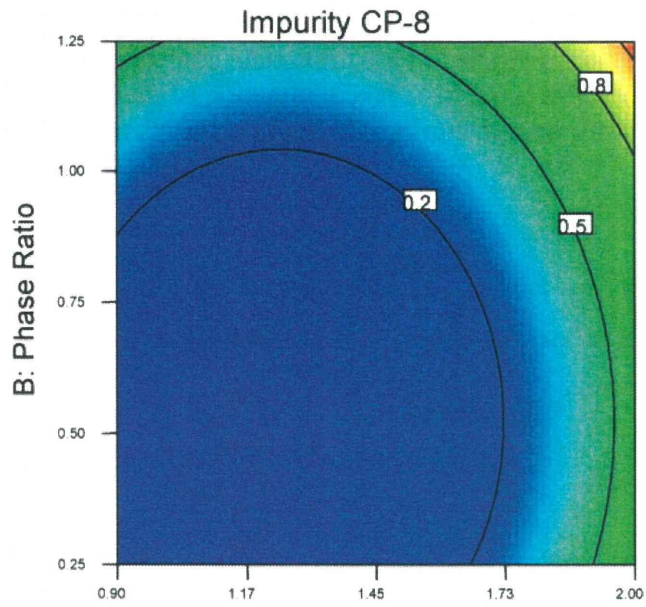


X1 = A: CP-8

X2 = B: Phase Ratio

Actual Factor

C: Reaction Conc = 0.25



A: CP-8

Design-Expert® Software

Impurity CP-8

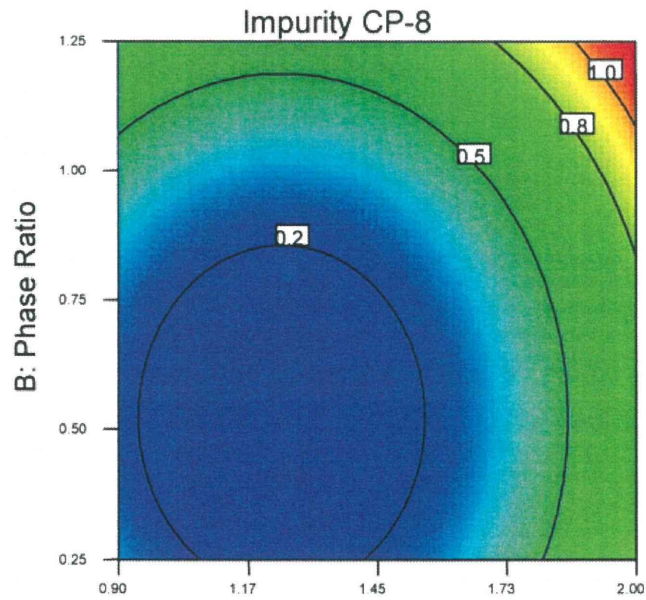


X1 = A: CP-8

X2 = B: Phase Ratio

Actual Factor

C: Reaction Conc = 3.00



A: CP-8

Figure 2.3.S.2.6-25 Relation between Remaining CP-8 and Process Parameters

5)-1-2-2 Crystallization:

From the risk assessment, parameters that could potentially influence the levels of CP-8 (and total impurities) in drug substance were identified and listed in the table below. An experimental strategy was designed to determine the effects of this process parameter on controlling these quality attributes, improve process understanding and robustness, and establish the design space for the Step 2 crystallization manufacturing process.

Scale and equipment consideration: Crystallizations are known to be scale and equipment dependent. However, for this product, physical characteristics were determined to be non-critical (see TPP above). The design of this crystallization will be focused on the impurity potential CQA. Stressing experiments are executed to understand if there is any impact on prolonged time difference between laboratory and manufacturing times on the quality attributes. This will be confirmed in the design space of the reaction and crystallization.

Data were collected on the 4 genotoxic impurities throughout the multivariate design to confirm our proposed control strategy.

Stressing experiments were executed to understand if there is any impact on prolonged time difference between laboratory and manufacturing times on the quality attributes. In addition, the multivariate experiments mimicked the “worst case” scenario of the proposed commercial equipment and its limitations (example; the heating and cooling profile was executed over hours with the same ability as the manufacturing facility).

Parameters and ranges explored in the DoE:

A $2^{(7-3)}$ fractional factorial design was used for crystallization. The maximum amount of CP-8 from reaction DoE1 is 1.2%. Assuming spiking in 3% CP-8 (2 or 3 times the maximum amount in DoE1) in crystallization study, cooling rate and DI water are identified to be critical process parameters (CPP) to control CP-8.

Table 2.3.S.2.6-13 Step 2 Crystallization DoE

Parameters Crystallization	Low	Standard	High
Cooling Rate (°C/min)	0.15	0.36	0.5
Final Temperature (°C)	14	18	24
Final Concentration (L/kg, volume of ethanol relative to CP-9))	3	4.5	8
Addition Time (min)	15	30	60
Water quantity (% w/w, ethanol relative to water quantity)	20	28-32	35
Agitation Rate (rpm)	150	test	350
Hold Time prior to Water Addition (hr)	2	test	stress

Conclusions from the Crystallization DoE

- As shown in Figure 2.3.S.2.6-26, the impurity level increases with the combination of high level of DI water and a high cooling rate. Therefore, the level of DI water and the cooling rate are determined to be CPP.
- For all experiments there were not any observed impurities from CP-8, the reaction, and CP-7 that exceeded 0.1%
- It is important to note that no new impurities were observed within the proposed design space and levels of existing peaks were below the standard conditions.

Scale and equipment:

- Stressing experiments where cooling rates were varied with prolonged exposure before isolation did demonstrate potential for failure of CP-8. Therefore, the design space includes an evaluation of any new equipments temperature controls and demonstration that it can control the cooling rate such that the specification of CP-8 is maintained.

CP-6 potential genotoxic impurities (PGI) data:

Highest levels of PGI detected in multivariate designs for Step 1 and Step 2 are shown in Table 2.3.S.2.6-14.

Table 2.3.S.2.6-14 PGI Data Supporting Proposed Control Strategy

	In CP-6 (SM)	In CP-7 (Step1)	In Sakuramil (Step 2)
CP-6	N/A (98%)	< 200 ppm	< 10 ppm
CP-3	0.1%	< 10 ppm	< 1 ppm
CP-4	0.3%	< 10 ppm	< 1 ppm
CP-5	0.1%	< 10 ppm	< 1 ppm

Control Strategy for CP-4, CP-5, CP-3:

In CP-6: Important MA CP-4 ($\leq 0.3\%$) + CP-5 and CP-3 ($\leq 0.1\%$ each) = ≤ 10 ppm total for these three in Sakuramil

Control Strategy for CP-6 (Starting Material):

< 10 ppm in Sakuramil when process through Steps 1&2 design space (specification for CP-6 as a CQA in drug substance: ≤ 10 ppm)

Therefore: Overall Genotoxic Control strategy = the total of these two control points ensure that CP-5, CP-3, CP-4, and CP-6 to be < 25 ppm (25 ppm is the TTC based on the daily dose of Sakuramil drug substance).

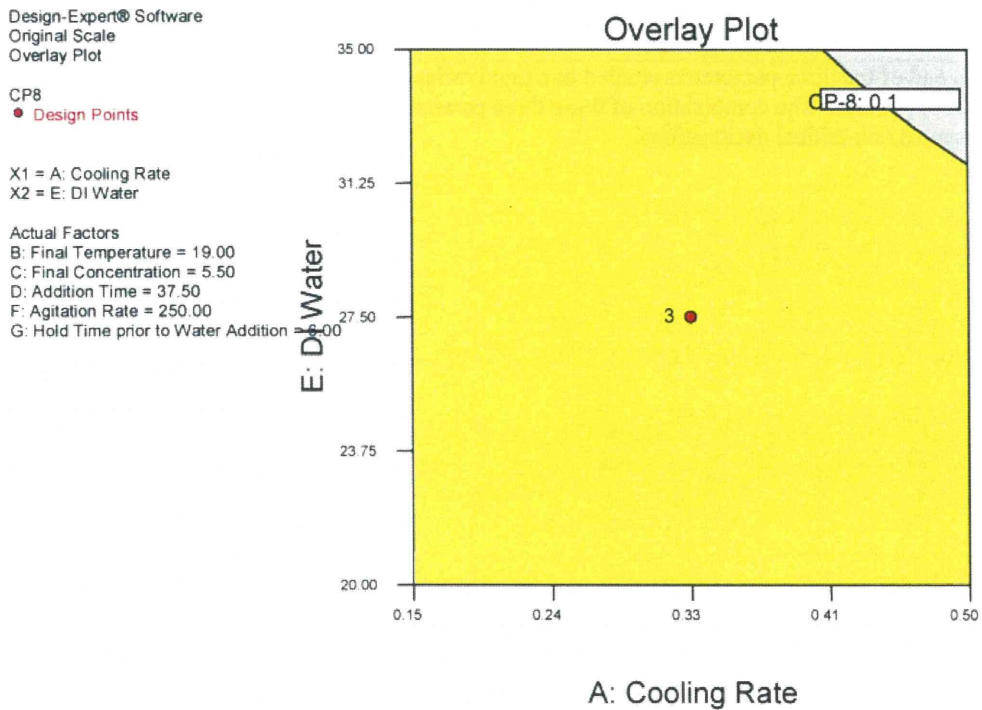
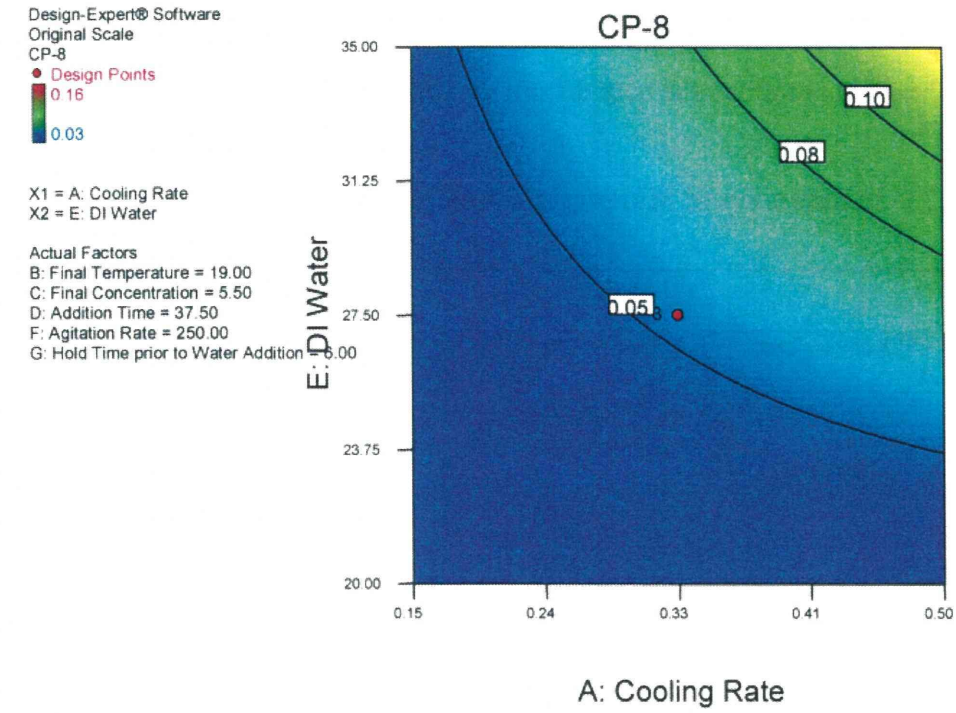


Figure 2.3.S.2.6-26 Relation between Remaining CP-8 and Crystallization Process Parameters

Initial Criticality Risk Assessment from Step 2 Reaction and Crystallization (including the Starting Material Attributes): to identify critical attributes or parameters.

Table 2.3.S.2.6-15 summarizes the output from the multivariate analysis for Step 2 reaction and crystallization.

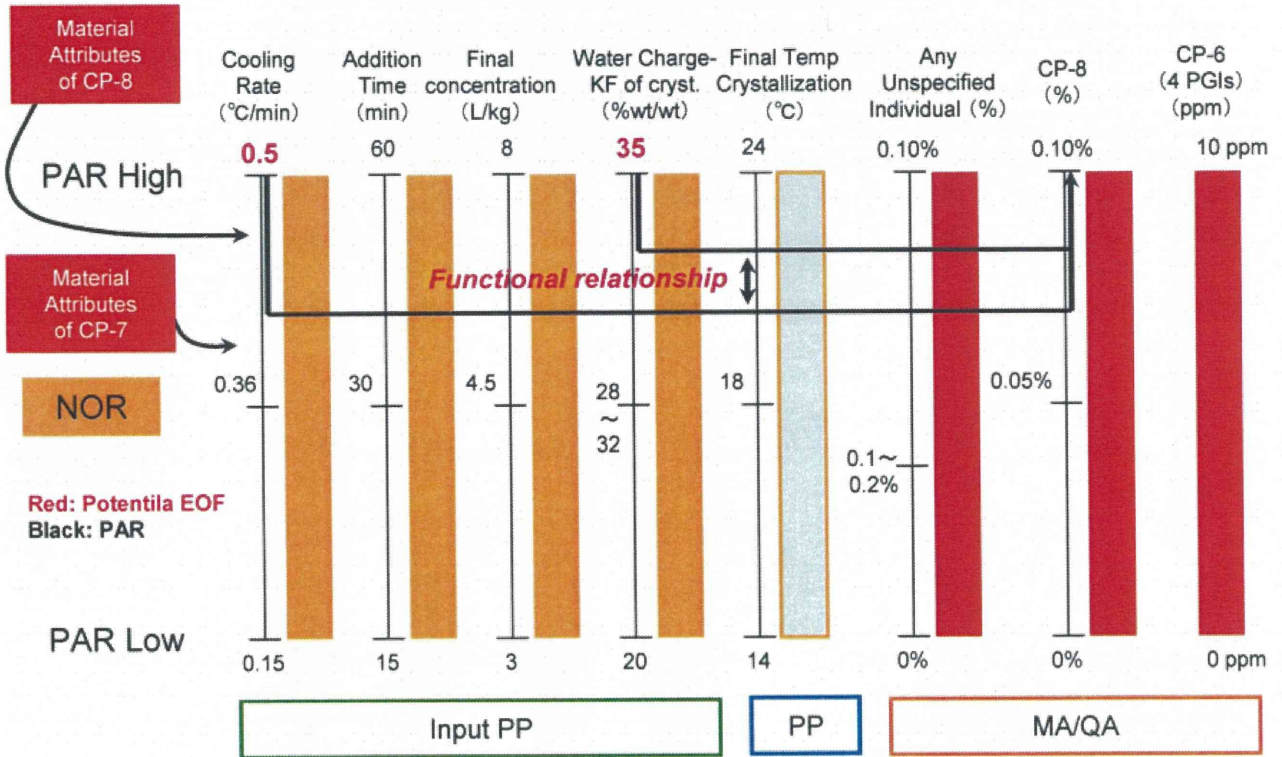
Table 2.3.S.2.6-15 Summary of Multivariate Analysis Results for Step 2

Parameter	Design Space	NOR	Criticality of Attribute or Parameter with Justification
CP-8 quantity (CP-8 equivalents relative to CP-7)	0.9 to 2 eq	1.05	Non-Critical*
Phase Ratio (DCM/NaOH aq)	0.25 to 1.25	1	Non-Critical*
Reaction Concentration (Volumes of DCM relative to CP-7)	0.25 to 5 volumes	3	Non-Critical*
Cooling Rate (°C/min)	0.15 to 0.5°C/min	0.36	Critical: at high limit WITH a high limit of water
Final Temperature (°C)	14 to 24°C	18	Non critical: no impact on residual CP-8
Final Concentration (L/kg, ethanol quantity relative to CP-9)	3 to 8	4.5	Non critical: no impact on residual CP-8
Addition Time (min)	15 to 60	30	Non critical: no impact on residual CP-8
Water (% w/w, water/ethanol)	20 to 35	28 to 32	Critical: at high limit WITH a high limit of cooling rate
Agitation Rate (rpm)	150 to 350	test	Non critical: no impact on residual CP-8
Hold Time prior to Water Addition (hr)	2 and up	test	Non critical: no impurities were isolated in larger quantities upon extended hold times prior to filtration.

* At the high end of the three parameters studied in a multivariate analysis, the level of CP-8 was at 1%. The acceptance criteria for CP-8 is 1.2%. The combination of these three parameters all at high levels is unrealistic for manufacturing; therefore warrants non critical designations.

5)-1-2-3 Multivariate summary for Step 2

Figure 2.3.S.2.6-27 shows the summary of multivariate results for Step 2.



The black arrow shows the functional relationship between QA and PP.
 PGIs: Potentially genotoxic impurities

Figure 2.3.S.2.6-27 Combination of Unit Operation Variables for Step 2

6) Manufacturing Process Criticality Assessment: Summary of Final Design Space and Control Strategy

The following is the final risk assessment resulting in the overall process design space and control strategy for each identified critical process parameter and critical quality attribute.

- High risk (and moderate risk) process parameter ranges will be presented as commitments in the manufacturing process description in S.2.2.
- SM or raw material MA which are functionally related to drug substance CQA will be defined along with acceptance criteria in S.2.3.
- Important material attributes for in situ process controls (PAT) or isolated intermediates will be defined along with acceptance criteria in Intermediate specifications in S.2.4.

Table 2.3.S.2.6-16 Summary of Control Strategy and Design Space for Overall Manufacturing Process

MA/Drug Substance CQA	Control Strategy	Design Space
Ethyl Homolog $\leq 1.0\%$ in drug substance	<ul style="list-style-type: none"> • Design Space for Step 1 (parametric control) • Specification in CP-7 of $\leq 1\%$ to be used when appropriate. <ul style="list-style-type: none"> ○ Test for ethyl homolog CP-7-1 in CP-7 for 25 batches at commercial launch. If demonstrates control via design space, eliminate this test and use RTRt in a parametric sense. 	<p>The design space for Step 1 demonstrated the highest possible amount (even under stressing conditions) of ethyl homolog to be 0.3%. This is well below the 1% specification/qualified level in drug substance.</p> <p>No edges of failure were identified in the Step 1 design space. This is a very robust process.</p> <p>No CPP's identified for the reaction of Step 1.</p>
Total impurities NMT 5% (Step 1, intermediate MA) And unspecified impurities of $\leq 0.10\%$ (Step 2, DS CQA) <i>Intermediate MA & DS CQA's are functionally related</i>	<ul style="list-style-type: none"> • Specification for total impurities in Step 1 of $\leq 5\%$ • Specification for unspecified impurities of $\leq 0.10\%$ each in drug substance. 	<p>It is well recognized that water (the "poor" solvent) can increase the level of impurities. The NOR for this parameter is 28-32% for both Steps 1&2;</p> <ul style="list-style-type: none"> • The % water for the crystallizations in Step 1 and Step 2 at the high level are CPP's. <ul style="list-style-type: none"> ○ 50% water Step 1 CPP ○ 35% water Step 2 CPP
CP-8 of $\leq 0.10\%$ in drug substance	<ul style="list-style-type: none"> • RTRt: Primary control: PAT: $\leq 1.2\%$ of remaining CP-8 at the reaction completion. <ul style="list-style-type: none"> ○ In the event of PAT failure ○ HPLC can be used as well for determining the level of CP-8 of 1.2% as an in-process analysis ○ HPLC in drug substance of $\leq 0.10\%$ 	<p>CP-8: It was demonstrated that the RTRt is acceptable to control this impurity to $< 0.10\%$ via an in-process PAT method in combination with the crystallization design space of Step 2.</p>

	<ul style="list-style-type: none"> Crystallization of Step 2 (parametric RTRt): if CP-8 is $\leq 1.2\%$ at reaction completion, then the crystallization design space demonstrated $<0.1\%$ in drug substance. 	Step 2 is determined to be a critical step. Two CPP's were identified. Cooling rate and % water quantity
Genotoxic impurities (GTI): total of 4 GTI's NMT 25 ppm in drug substance	<ul style="list-style-type: none"> Specification for CP-6 in drug substance: CP-6 of ≤ 10 ppm in drug substance Specification for CP-6 only (no test for these three in drug substance): <ul style="list-style-type: none"> CP-4 (0.3%) CP-5 and CP-3 (0.1% each) 	The data demonstrated that if CP-6 is <10 ppm in drug substance, then the total of the 4 GTI's can not exceed 25ppm IF those 3 met the specification for each in CP-6. Rational: These impurities are extremely lipophilic and have unprecedented purge factors. Fate and purge experiments demonstrated that even at levels of 1% in CP-6, they still were all well below the TTC for the total.
Chirality (enantiomers and diastereomers) NMT 0.10% of any in drug substance	<ul style="list-style-type: none"> Specification in CP-6 <ul style="list-style-type: none"> $\leq 1\%$ of enantiomers $\leq 1\%$ of diastereomers Crystallization design space of Step 2 It should be noted that the methods for drug substance are specific for the stereoisomers and will be inevitably controlled as "unspecified". 	All of the enantiomers and diastereomers were demonstrated in the design space fate and purge program to be well below the 0.10% level in drug substance.

Scale and equipment:

- Step 1 is not scale and/or equipment dependent. Changes in scale and equipment will be managed in the quality systems
- Step 2 is only scale and equipment dependent on cooling rate control. Changes in scale and equipment will require adequate risk analysis, confirmation, and validation that the controls continue to ensure the cooling rate (CPP) can deliver drug substance with acceptable quality.

Starting Materials: CP-6 and CP-8. These tests will be maintained and used when necessary. They will be used when identifying new potential vendors or supply of CP-6.

Important Material Attributes: See justification for moderate and low risk matters above in Starting Material Sections.

Table 2.3.S.2.6-17 Specifications for Important MA with Justification

Important MA	Specification	Justification
SM CP-6:		
CP-4	$\leq 0.3\%$ ¹	This indicating test to ensure the following: CP-6 MA: CP-4 (0.3%) + CP-5 and CP-3 (0.1% each) = <10 ppm total for these three in Sakuramil.
CP-5	$\leq 0.1\%$	
CP-3	$\leq 0.1\%$	
SM CP-8:		
CP-8-25I	$\leq 0.05\%$	Guarantees $\leq 0.10\%$ in drug substance
CP-8-24I	$\leq 0.05\%$	Guarantees $\leq 0.10\%$ in drug substance

¹ Fate and purge supports up to 1%.

CPP Identified in the Overall Design Space

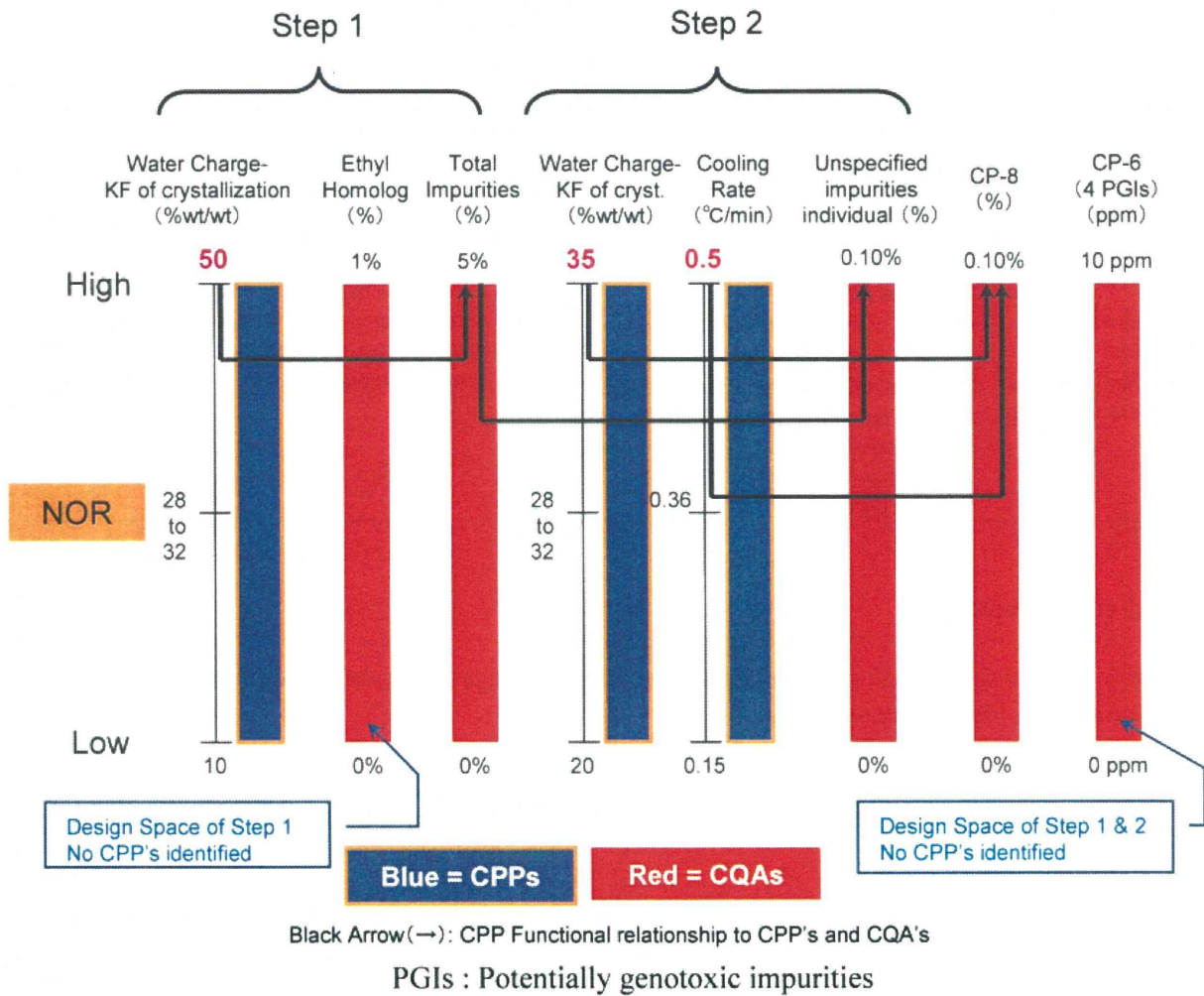


Figure 2.3.S.2.6-28 CPP and CQA (IMA) Identified for Step 1 and Step 2

2.3.S.4 Control of Drug Substance

2.3.S.4.1 Specifications

Table 2.3.S.4.1-1 Specifications for Sakuramil Drug Substance

Test Items		Test Method	Acceptance Criteria
Description	Appearance	Visual observation	White solid
ID	IR	IR	The IR spectrum of the sample is comparable to that of the reference standard
	Chiral HPLC	HPLC	The retention time of the principal peak of the sample corresponds to that of the reference standard
Purity	Heavy metals	Method 2 of the General Test of the current JP	≤ 20 ppm
	Rerated substances (1) CP-9-1 CP-8	HPLC	≤ 1.0% ^a ≤ 0.10% ^a
	Rerated substances (2) Others (individual) Total	HPLC	≤ 0.10% ≤ 0.5%
	Genotoxic impurities CP-6	HPLC	≤ 10 ppm
	Residual solvents CH ₂ Cl ₂	GC	≤ 600 ppm ^b
Loss on drying		General Test of the current JP	≤ 0.5%
Residue on ignition		ICH Q4B harmonized method	≤ 0.2%
Assay		HPLC	98.0 to 102.0% (dried basis)

a) RTRt will be applied.

b) Skip testing will be applied.

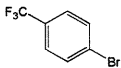
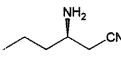
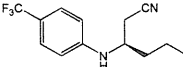
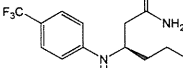
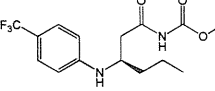
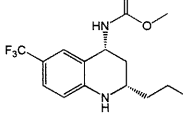
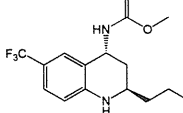
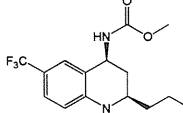
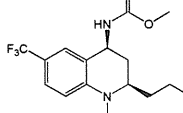
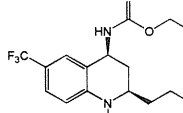
Test will be conducted every 25th batch or one batch every year, whichever more frequent.

2.3.S.4.5 Justification of Specification

Table 2.3.S.4.5-1 Summary of Control Strategy for Sakuramil (Abstract)

Control System API CQA (2.3.S.2.6)/ Limit↓	Process Control (including In-process Tests and Process Parameters)	Control of Material Attributes (Raw Material /SM /Intermediate)	Impact on Manufacturing Process Design	CQA will be Tested in API/ CQA will be Set as Specification for API (2.3.S.4.1)
Related Substances (1)				
- CP-9-1: ≤ 1.0%	DS (Step 1)	CP-7-1: ≤ 1% in intermediate CP-7		No/Yes
- CP-8: ≤ 0.10%	DS (Step 2 Crystallization)	RTRt: 1.2% (Step 2 Reaction)		No/Yes
Related Substances (2)				
- Stereo Isomers: ≤ 0.10%	DS (Step 2 Crystallization)	Enantiomers: ≤ 1% Diastereomers: ≤ 1% in SM CP-6	No racemisation/ No undesired cyclization	Yes/Yes (Controlled with other impurities.)
- Other Impurities: ≤ 0.10%				Yes/Yes
- Impurities Total: ≤ 0.5%		Total: ≤ 5% in CP-7		Yes/Yes
Genotoxic Impurities				
- CP-6: ≤ 10 ppm		- CP-4 : ≤ 0.3% in CP-6		Yes/Yes
- CP-3,4,5,6 Total: ≤ 25 ppm	DS(Step 2 Crystallization)	- CP-6: ≤ 10 ppm in drug substance - CP-4: ≤ 0.3% in CP-6, CP-3&5: ≤ 0.1%	High reactivity. Can be removed in crystallization step due to hydrophobic nature.	No/No
Residual Solvents				
- EtOH: ≤ 5000 ppm	LOD: ≤ 0.40% (Step 2 in-process control in crystallization)			No/Yes
- THF: ≤ 720 ppm	The manufacturing process following Step 1.		Purge more significantly than the concentration limit of ICH Q3C (≤10%) in the manufacturing process following Step 1.	No/No
- <i>n</i> -Hexane: ≤ 290 ppm				No/No
- Dichloromethan: ≤ 600ppm	The solvent replacement and crystallization in Step 2.		Purge more significantly than the concentration limit of ICH Q3C (≤10%) by solvent replacement and crystallization in Step 2.	Yes/Yes
Assay				
Sakuramil : 98 - 102%				Yes/Yes

Appendix-1 Evaluation of Potential Organic Impurities in Sakuramil Drug Substance

Code	Structure	Origin	Genotoxic Evaluation	Classification
CP-1		Raw material for the manufacture of CP-6	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-2		Raw material for the manufacture of CP-6 (Chiral pool material)	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-3		<i>In situ</i> intermediate in the manufacture of CP-6	Aniline functional group based genotoxicity alerting structure.	Control of genotoxic impurities
CP-4		Intermediate in the manufacture of CP-6	Aniline functional group based genotoxicity alerting structure. Positive in the Ames Assay.	Control of genotoxic impurities
CP-5		<i>In situ</i> intermediate in the manufacture of CP-6	Aniline functional group based genotoxicity alerting structure.	Control of genotoxic impurities
CP-6-E		Enantiomer of CP-6	Aniline functional group based genotoxicity alerting structure.	Control of genotoxic impurities (Control as CP-6)
CP-6-D1		Diastereomer 1 of CP-6	Aniline functional group based genotoxicity alerting structure.	Control of genotoxic impurities (Control as CP-6)
CP-6		Starting material	Aniline functional group based genotoxicity alerting structure. Positive in the Ames Assay.	Control of genotoxic impurities
CP-7		Intermediate	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-7-1		Ethyl homolog of CP-7	No genotoxicity alerting structure.	Control in accordance with ICH Q3A