Sakuramil S2 mock

This mock intends to illustrate the contents to be included in CTD 2.3.S.2.6 “Manufacturing Process Development” regarding drug substance (drug substance manufactured by chemical synthesis) developed using the Quality by Design methodology (QbD) presented in ICH Q8, Q9 and Q10. It takes into consideration the description into CTD Module 2 (Quality of Summary). In addition, in order to help the readers’ understanding, part of the contents corresponding to 2.3.S.2.2-2.5 and 2.3.S.4.1, 4.5 are also included in this mock. In preparing this mock, we tried to reflect the contents of ICH Q11 Guideline (draft) regarding development and manufacture of drug substance.
The purpose of this mock is to envision development of drug substance using the Enhanced Approach methodology (definition is the same as advanced methodology and QbD approach), not to propose new regulatory requirements or delete any existing regulatory requirement. Also, it does not cover all the items.
Though detailed numbering is not used in CTD Guideline for Module 2, numbering such as 2.3.S.●● is used in this mock for the sake of convenience. Medicinal development through QbD was not considered when CTD guideline was developed. There is a rule of maximum 40 pages for QOS (June/21th, 2009, Iyakushin # 899, appendix 3). The product of this mock was developed through QbD approach, therefore it is necessary to show not only data but depth of understanding of the product and processes to regulators. Therefore, this QOS was prepared without taking account of page restriction.

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2.3.S.2.2 Description of Manufacturing Process and Process Controls

1) Synthetic Routes

1)-1 Synthetic flows of Sakuramil drug substance

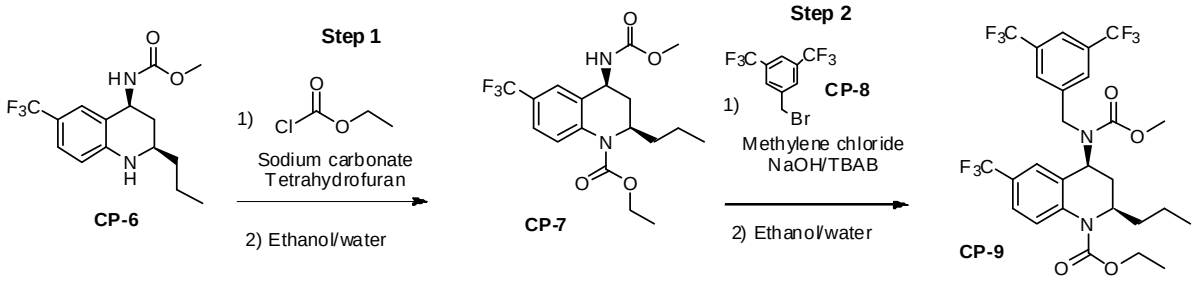


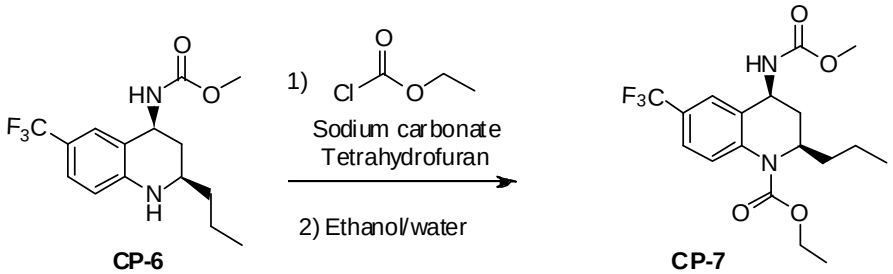
Figure 2.3.S.2.2-1 Sakuramil Manufacturing Scheme

The synthesis of Sakuramil drug substance consists of two steps. CP-6 is reacted with ethyl chloroformate to give CP-7, which is reacted with CP-8 and subsequently crystallized from an ethanol-water solvent mixture to give Sakuramil (CP-9).

1)-2 Drug Substance Synthesis of Sakuramil

Synthetic process of Sakuramil drug substance is shown below.
Typical batch Size: 350 kg

Step 1: Synthesis of CP-7



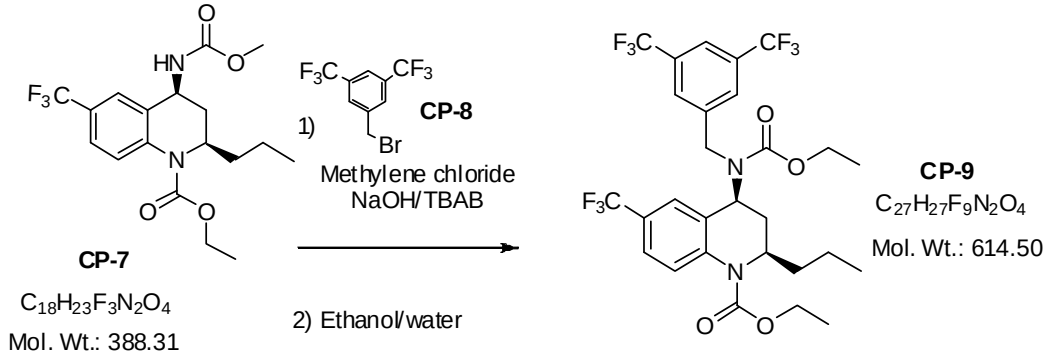
CP-6
$C_{15}H_{19}F_3N_2O_2$
Mol. Wt.: 316.31

CP-7
$C_{18}H_{23}F_3N_2O_4$
Mol. Wt.: 388.31

CP-6, tetrahydrofuran (3 to 15 liters per kilogram of CP-6), sodium carbonate (0.75 to 4.0 molar equivalents per equivalent of CP-6) are combined. Ethyl chloroformate (2.0 to 7.5 molar equivalents per equivalent of CP-6) is added and the mixture is heated at temperature up to reflux. Upon reaction completion, the mixture is filtered, and the filtrate is quenched with a sodium hydroxide solution while maintaining a temperature below 30°C. To the mixture, *n*-hexane is added and stirred, and then the layers are settled and separated. The organic layer is concentrated by distillation with

ethanol for the solvents exchane (final concentration 4 to 10 liters per kilogram of CP-7). Water (25 to 35% weight per weight of ethanol) is added and the mixture is stirred at 14 to 26°C. The resulting crystalline precipitates are filtered, rinsed with ethanol, and dried at temperatures up to 50°C to yield CP-7.

Step 2: Synthetic process for Sakuramil



CP-7 and CP-8 (1.0 -1.1 molar equivalents per molar equivalent of CP-7) are combined in methylene chloride (2 to 4 liters per kilogram of CP-7). Tetra-*n*-butylammonium bromide (0.1 to1.0 kilograms per kilogram of CP-7) and aqueous sodium hydroxide solution (47- 50% solution at 2 to 4 liters per kilogram of CP-7) are added while maintaining the mixture at temperatures between 12-25°C. Upon reaction completion, methylene chloride and water are added, the mixture is separated, and the organic layer is washed with diluted hydrochloric acid. The organic layer is concentrated and displaced by distillation with ethanol (final concentration to 4.5 liters per kilogram of CP-9). Water (25 to 35% weight per weight of ethanol) is added to the mixture. After cooling, the mixture is stirred at 14 to 26°C. The resulting crystalline precipitates are filtered, rinsed with ethanol, and dried at temperatures up to 50°C to yield CP-9 (Sakuramil).

Alternative manufacturing process

In Step 1, trisodium phosphate, dodecahydrate (0.75 to 4.0 molar equivalents per equivalent of CP-6) can be used instead of sodium carbonate as alternative base.

Manufacturing Scale & Yields

Typical batch Size: 350 kg
Typical yields: 80% (Calculated from CP-6 base)

2) Manufacturing Process and Process Controls

2)-1 Synthetic flows

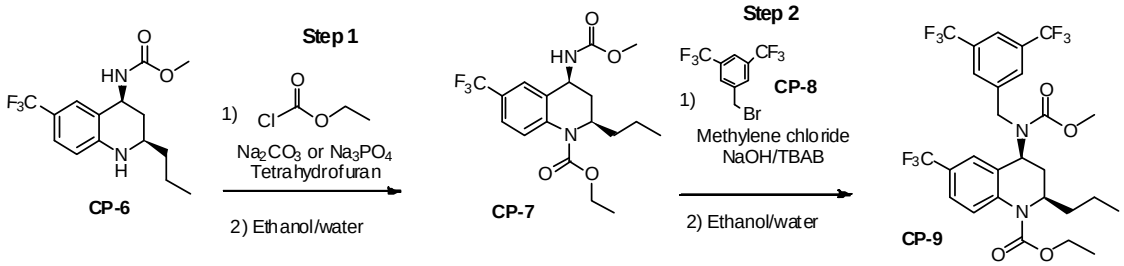


Figure 2.3.S.2.2-2 Sakuramil Manufacturing Scheme

2)-2 Manufacturing processes

Commercial manufacturing processes of Sakuramil drug substance are shown below.

Step 1 (Critical Step) (Reaction, Extraction, Purification, Phase Separation, and Drying)

Methyl (2*R*,4*S*)-2- propyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinoline-4-ylcarbamate (CP-6) [1] (230 kg), tetrahydrofuran (1300 L), sodium carbonate (42.4 kg) are combined. Ethyl chloroformate (158~592 kg) is added and the mixture is heated at temperature up to reflux. The mixture is filtered, and the filtrate is combined with a 50% sodium hydroxide solution. To the mixture, *n*-hexane is added and stirred, and the layers are settled and separated. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration (1400 L)). Water (25 to 35% weight per weight of ethanol) is added and the mixture is stirred at 20°C. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 42.5°C to yield Ethyl (2*R*,4*S*)-2-propyl-4-(methoxycarbonylamino)-6-(trifluoromethyl)-3,4-dihydroxyquinoline-1(2*H*)-Carboxylate (CP-7) [2] (product 253 kg, yield 89%).

Step 2 (Critical Step) (Reaction, Extraction, Purification, Phase Separation, and Drying)

CP-7 [2] (250 kg) from Step 1 and 3,5-bistrifluoromethylbenzyl bromide (CP-8) (215 kg) are combined in methylene chloride (750 L). Tetra-*n*-butylammonium bromide (50 kg) and 50% aqueous sodium hydroxide solution (750 L) are added and stirred, and then methylene chloride and water are added and stirred. The mixture obtained is settled and the layers are separated. The organic layer is washed with diluted hydrochloric acid. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration (1800 L)). Water (20 to 35% weight per weight of ethanol) is added, and then the mixture is cooled at the rate of 0.15 to 0.5°C per minute, followed by stirring at 18°C. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 42.5°C to yield Ethyl (2*R*,4*S*)-4-{[3,5-bis(trifluoromethyl)benzyl](methoxycarbonyl)amino}-2-propyl-6-(trifluoromethoxy)-3,4-dihydroquinoline-1(2*H*)-carboxylate [3] (Sakuramil) (product 360 kg, yield 90%).

Alternative manufacturing process

In Step 1, trisodium phosphate, dodecahydrate (101.4 kg) can be used instead of sodium carbonate (42.4 kg) as alternative base.

In-process analysis

Step 2: Reaction is monitored by HPLC under the same conditions as the test method for related substances established for drug substance.
Reaction end-point: Residual CP-8 is not more than 1.2% (area percent)
Step 2: Drying end-point for Sakuramil is confirmed.
Drying end-point: Loss on drying is not more than 0.4%

2.3.S.2.3 Control of Materials

1) Control of Starting Materials

CP-6 and CP-8 are selected as the starting materials for the commercial manufacture of Sakuramil drug substance. Their chemical properties and structures are confirmed and characterized, and the impurities that may affect the impurity profile of Sakuramil drug substance have been specified individually including appropriate control items and limits.

1)-1 Control of CP-6

Table 2.3.S.2.3-1 Specifications for CP-6

Tests		Control value/Acceptance criteria
Description		White to pale yellow crystals or crystalline powder (visual observation)
Identification		The IR spectrum of the sample is comparable to that of the reference standard (IR)
Related Substances	CP-4	≤ 0.3%
	Other (Individual)	≤ 0.1%
	Other Impurities Total	≤ 0.5% (HPLC, area%)
Assay		98-102% (HPLC, absolute calibration curve method)
Residual Solvents	●●	▲▲
Pd Content	Pd	≤ 10 ppm (ICP-MS)

1)-2 Control of CP-8

Table 2.3.S.2.3-2 Specifications for CP-8

Tests		Control value/Acceptance criteria
Description		White to pale yellow crystal or crystalline powder (visual observation)
Identification		The IR spectrum of the sample is comparable to that of the reference standard (IR)
Related Substances	CP-8-25I	≤ 0.05%
	CP-8-24I	≤ 0.05%
	Other (Individual)	≤ 0.1%
	Other Impurities Total	≤ 1.0% (HPLC, area%)
Assay		≥ 97% (HPLC, absolute calibration curve method)

1)-3 Control of starting materials through life cycle

Control of Starting Material Suppliers for CP-6 and CP-8

In addition to the high degree of process and analytical control of CP-6, IROHA-corp and all commercial manufacturers (current and future) are obligated to comply with IROHA-corp's *Management of Change* policy that stipulates all major changes to the synthesis of CP-6 must be evaluated to substantiate that the impurity profile of CP-6 has not been adversely affected.

Representatives of procurement, quality, manufacturing, and technical development groups will participate in the evaluation and review of proposed new suppliers of starting materials (SM) or process changes by existing suppliers.

A qualification protocol will be prepared which may include the following activities:

- Overview of the new synthesis/ scheme or process modifications
- Receive sample(s) of SM from prospective supplier or from modified process accompanied by documentation showing method of synthesis.
- Test sample(s) versus current SM specification. Analytical test results must meet all acceptance criteria. Perform other orthogonal analyses as deemed necessary and appropriate. Based upon method of synthesis and structures of potential impurities, make determination that analytical methods are sufficient.
- Laboratory performance testing and preparation of 3 pilot batches to a downstream intermediate or drug substance will generally be required. The resulting intermediate and/or API will be tested versus current specifications. All established acceptance criteria must be met. The number of batches evaluated may be decreased to 1 depending on reliability of the supplier or risk of minor modifications, etc.
- Information generated is reviewed by the responsible personnel.

After execution of the qualification protocol, the following actions will be taken.

- If the evaluation determines that SM produced by the new site or modified process contains no new impurities and is equivalent in quality to SM produced by the current site and / or process, the new supplier and / or process modifications will be commercially qualified and approved in accordance with internal change control procedures.

- If the evaluation determines there is a need to revise the specifications or analytical methods for the SM, intermediates or drug substance, an appropriate post-approval variation will be filed.

This mock describes manufacturer's control strategy/policy for starting material suppliers through life cycle. The topics relating to life cycle management described in this mock are those relating to operation of manufacturer under quality system. Therefore, most of those might be covered under GMP, however, those are not considered an item submitted to regulatory authority at J-NDA. However, the starting material CP-6 described in this mock is a custom compound developed by IROHA Corp, and it is expected that manufacturer can acquire manufacturing method from starting material supplier. Therefore, items relating to lifecycle management are included in this mock from the justification view of starting material.

2) Control of Raw Materials

The raw materials, solvents and reagents used in the proposed manufacturing process of Sakuramil drug substance, and their test items, acceptance criteria, and processes where each material is used are provided in Table 2.3.S.2.3-3. No raw materials which have potential BSE/TSE risk are used in the manufacturing process of Sakuramil drug substance.

Table 2.3.S.2.3-3 Specifications for Raw Materials

Raw materials	Process*	Test Item	Acceptance Criteria
Ethyl chloroformate (ECF)	Step 1	Description (visual) Identification (GC) Phosgene (limit test) Assay (GC)	Clear, colorless to pale yellow liquid Principal peak corresponds to that of the reference standard ≤ 5000 ppm ≥ 98.9%
Tetra-*n*-buthylammonium bromide (TBAB)	Step 2	Description (visual) Identification (qualitative test) Assay (titration)	White to pale yellowish white crystals or crystalline powder bromide: positive ≥ 98.0%
Trisodium phosphate, dodecahydrate ($Na_3PO_4 \cdot 12H_2O$)	Step 1	Description (visual) Identification (qualitative test) Arsenate (arsenic limit test) Assay (titration)	White crystals or crystalline powder Phosphate salt: positive Sodium salt: positive ≤ 1 ppm ≥ 99.0%
Sodium carbonate	Step 1	Description (visual) Identification (qualitative reaction) Assay (titration)	White powder Carbonate salt: positive Sodium salt: positive ≥ 99.0%
Sodium hydroxide	Steps 1 & 2	Description (visual) Identification (qualitative reaction) Assay (titration)	White granulated or flaked solid Sodium salt: positive ≥ 93%
Hydrochloric acid (HCl)	Step 2	Description (visual) Identification (qualitative test) Assay (titration)	Clear and colorless liquid Sodium salt: positive 35.0% - 37.0%
Tetrahydrofuran (THF)	Step 1	Description (visual) Identification (IR) Purity (GC)	Clear and colorless liquid Sample exhibits main absorption at about 2970, 2860, 1460, 1380, 1180, 1070, 910 and 650 cm^{-1} ≥ 99.5%
n-Hexane	Step 1	Description (visual) Identification (IR) Purity (GC)	Clear and colorless liquid Sample exhibits main absorption at about 2960, 1470, 1380 and 730 cm^{-1} ≥ 96.0%
Methylene chloride (Dichloromethane; DCM)	Step 2	Description (visual) Identification (GC) Purity (GC)	Clear and colorless liquid Principal peak corresponds to that of the reference standard ≥ 99.5%
Ethanol	Steps 1 & 2	Description (visual) Identification (IR) Water (water determination) Purity (GC)	Clear and colorless liquid Sample exhibits main absorption at about 3330, 2975, 1454, 1090 and 881 cm^{-1} ≤ 0.2% ≥ 99.5%
Water	Steps 1 & 2		Meets JP "purified water"

*Process the Materials is Used

2.3.S.2.4 Control of Critical Steps and Intermediates

1) Control of Critical Steps

Critical process parameters identified from manufacturing process criticality assessment are summerized in Table 2.3.S.2.4-1. As each step containes critical process parameters, all manufacturing steps are designated as critical steps.

Table 2.3.S.2.4-1 Critical Process Parameters (CPP) in the Manufacture of Sakuramil

Parameter	Design Space	Normal Operating Range	Criticality of Parameter and Justification
Step 1			
Water addition in the crystallization (% by weight with respect to ethanol)	25%-35%	28%-32%	Statistically and functionally relates to CQA of drug substance.
Step 2			
Cooling rate (°C/min)	0.15-0.5°C/min	0.36°C/min	Critical: at high limit WITH a high limit of DI water
Water addition in the crystallization (% by weight with respect to ethanol)	20-35%	28-32%	Critical: at high limit WITH a high limit of cooling rate

2) Control of Intermediates

The specification for the intermediate CP-7 is provided in Table 2.3.S.2.4-2.

For CP-7, 25 post-market commercial batches will be tested for the following test items. If all the batches are confirmed to meet the acceptance criteria via design space, subsequent batches will not undergo this test but move into real time release test (RTRt) in a parametric sense. After moving into RTRt, test will be conducted every 25th batch or one batch every year, whichever more frequent.

Table 2.3.S.2.4-2 Control of CP-7

Tests		Control value/Acceptance criteria
Related substances (HPLC)	CP-7-1	≤ 1.0%
	Impurities total	≤ 5% (HPLC, area%)

2.3.S.2.5 Process Validation and/or Evaluation

The commercial process for the synthesis of Sakuramil drug substance does not include aseptic processing or steriliazation. Therefore, this section is not applicable.

2.3.S.2.6 Manufacturing Process Development

Introduction

The development program for Sakuramil has utilized the principles outlined in ICH Q8/Q11, Q9, and, thus, Quality by Design. This Manufacturing Process Development section for Sakuramil is organized as follows:

1. Potential Critical Quality Attributes (CQA) of Sakuramil Drug Substance
2. Development History
3. Starting Material Justification and Commercial Manufacturing Process Selection
4. Risk Assessment for Knowledge Space and Control Strategy Development
5. Unit Operation Design Spaces for Each Step of the Sakuramil Drug Substance
6. Manufacturing Process Criticality Assessment: Summary of Final Design Space and Control Strategy

1) Potential Critical Quality Attributes (CQA) of Sakuramil Drug Substance

1)-1 Quality Target Product Profile for Sakuramil Drug Product

The quality target product profiles (QTPP) for Sakuramil drug product are shown below.

Efficacy of drug product:
- Immediate-release tablet containing 60 mg of drug substance.
- As the drug substance is poorly soluble, tablets are molded by way of spray dried dispersion intermediates.

Safety of drug product:
- The drug substance is Sakuramil. (Identification)
- Control of potential impurities in the drug substance.

1)-2 Physical Properties of Sakuramil Drug Substance

During development, only one polymorph of Sakuramil drug substance has been observed in the synthetic manufacture and during extensive polymorph screening. In addition, as Sakuramil drug substance is poorly soluble, tablets are molded after manufacturing the Sakuramil Spray Dried Dispersion Intermediate (SDDi) to enhance absorption of the drug. Sakuramil drug substance is dissolved in acetone at 40 mg/mL, which is far less than the solubility limit of Sakuramil drug substance in acetone (>1000 mg/mL). The impact of polymorph and particle size is very little.

Therefore, routine control of polymorph and particle size for Sakuramil drug substance are not critical quality attributes (CQA).

1)-3 Control of Impurities for Sakuramil Drug Substance

As impurities may impact the safety of the drug product, the manufacturing process impact on impurity control was investigated. Impurities vary through the manufacturing process of Sakuramil. Therefore, CQA of Sakuramil can be controlled by understanding and controlling the formation routes and fate and purge of impurities. In addition, as Sakuramil is developed as optically-active substance, investigation on stereoisomers is also necessary.

1)-4 Potential CQA of Sakuramil Drug Substance

Potential CQA of Sakuramil have been identified as shown in Table 2.3.S.2.6-1 based on QTPP for Sakuramil drug product and ICH Q6A.

Table 2.3.S.2.6-1 Potential CQA of Sakuramil

Quality Attribute	Tests	Criticality	Rationale
Description	Description	Not critical	ICH Q6A*, Universal test
Identification	IR, chiral HPLC	Critical	ICH Q6A, Universal test
Potency	Assay	Critical	ICH Q6A, Universal test
Purity	Related substances	Critical	ICH Q6A Flow Chart #1, Universal test
	Genotoxic impurities	Critical	Genotoxic impurities have been identified.
	Residual solvents	Critical	Class 2 solvent are used in the manufacturing process.
	Metal residues	Critical	Pd catalyst is used in the manufacturing process of the starting material CP-6.
	Heavy metals	Not critical	Pharmacopoeial test
	Residue on ignition	Not critical	Pharmacopoeial test
Physicochemical property	Melting point	Not critical	No polymorphs heve been observed.
Particle size	----	Not critical	ICH Q6A Flow Chart #3 In the manufacturing process of the drug product, the drug substance is dissolved and then spray-dried to make dispersion intermediate.
Polymorphism	----	Not critical	ICH Q6A Flow Chart #4 No polymorphs have been observed. And also, in the manufacturing process of the drug product, the drug substance is dissolved and then spray-dried to make dispersion intermediate.
Optical activity	Stereoisomers	Critical	ICH Q6A Flow Chart #5 Sakuramil drug substance is optically-active substance.
Water content	Loss on drying	Not critical	Sakuramil drug substance shows no hygroscopic property.
Microbial limit	Not applicable	Not critical	ICH Q6A Flow Chart #6 Sakuramil drug substance is not capable of supporting microbial growth or viability.

*Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (PFSB/ELD Notification No. 568, May/01/2001)

Interpretation:

A critical quality attribute (CQA) of a drug substance is defined as a drug substance characteristic that has a direct impact on the safety and efficacy of the drug product / QTPP-quality target product profile (for example, the impurity profile of the drug substance). A control strategy will ultimately be established with the appropriate control and point in the overall manufacturing process to ensure consistent quality is met for each CQA. Options for controlling each drug substance CQA will be evaluated in parallel with the design space development to understand the functional relationship between all possible opportunities, such as, material attributes, PAT, design space parameters, engineering controls, scale and equipment, etc.

The potential critical quality attributes of the drug substance are defined as the drug substance tentative specifications. For the purposes of defining each of unit operation design spaces for the Sakuramil drug substance manufacturing process, the impact of the process on impurity control was primarily investigated up to and including the final isolation of the drug substance.

If control of physical properties of the drug substance (e.g., polymorph, particle size, etc.) is necessary, such properties are controlled in the final isolation step (crystallization) and subsequent steps.

1)-5 Strategy for Potential CQA of Sakuramil Drug Substance

The design space and control strategy for the commercial manufacture of Sakuramil drug substance has been established relative to its critical quality attributes of the drug substance. Understanding and control of impurities as they progress through the Sakuramil process will lead to control of the impurity critical quality attributes of the drug substance.

The following strategic CQA strategies have been considered in the initial risk assessment as potential sources of impurities in the Sakuramil synthesis that may result in critical quality attributes of the drug substance.

- Control of related substances
- Chirality- control of stereoisomers
- Known genotoxic intermediates
- Metal residues
- Residual solvents

The overall synthesis of Sakuramil drug substance (pre and post starting material) is provided to assist with the justification.

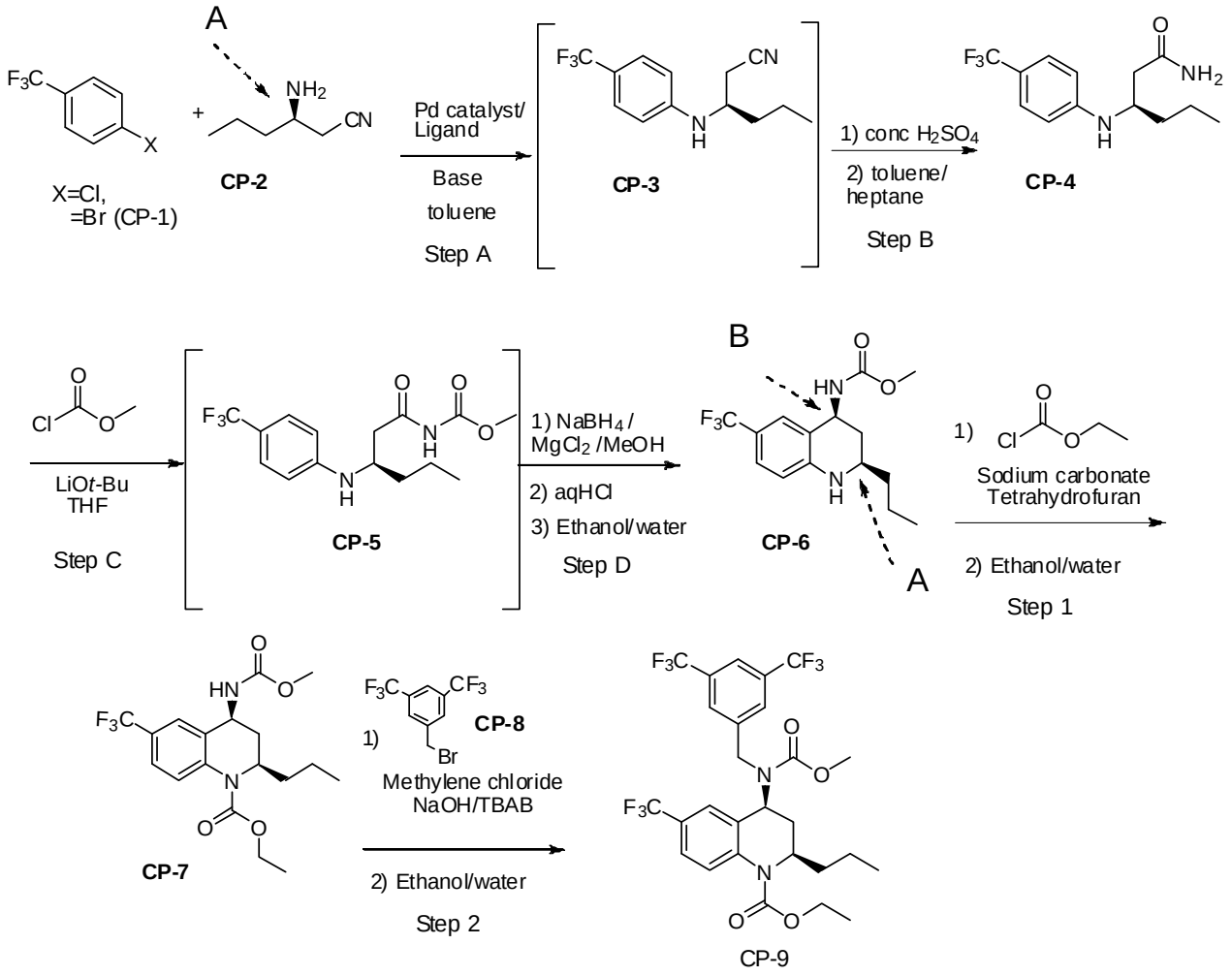


Figure 2.3.S.2.6-1 Sakuramil Manufacturing Scheme

1)-6 Chiral control strategy for Sakuramil

Stereoisomers of CP-6, and thus Sakuramil, are controlled at levels that assure the production of drug substance with high chiral purity. The first chiral center is purchased from the "chiral pool" of chemical commodities as CP-2 (depicted as "**A**"), the second chiral center (depicted as "**B**") is generated in a well precedented, highly stereoselective cyclization reaction to produce CP-6.

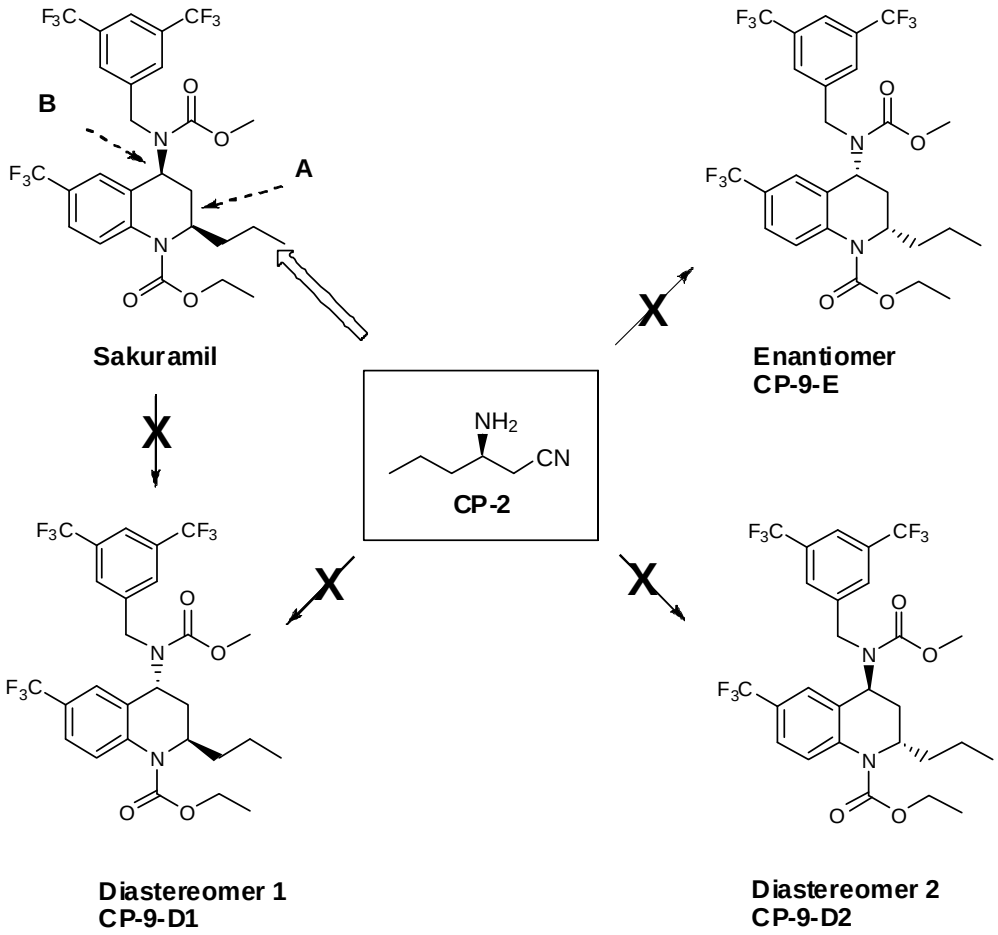


Figure 2.3.S.2.6-2 Chiral Control: Possible Enantiomers and Diastereomers

Enantiomer discussion: The stereoisomer quality of CP-6, and thus Sakuramil, is thus controlled in accordance with the vendor specifications of CP-2 (≤1.5 % of enantiomer). Any levels of the enantiomer of CP-2 introduced into Step 1 of the process would result in enantiomer CP-9-E as an impurity. CP-9-E has been demonstrated to purge to less than 0.10% in the downstream crystallizations of CP-6, CP-7, and/or Sakuramil. To further demonstrate this control, 5% of the off enantiomer of CP-2 was introduced into Step A of the synthesis during a development campaign and was carried through the 6 step process to produce Sakuramil with ≤0.1% of CP-9-E.

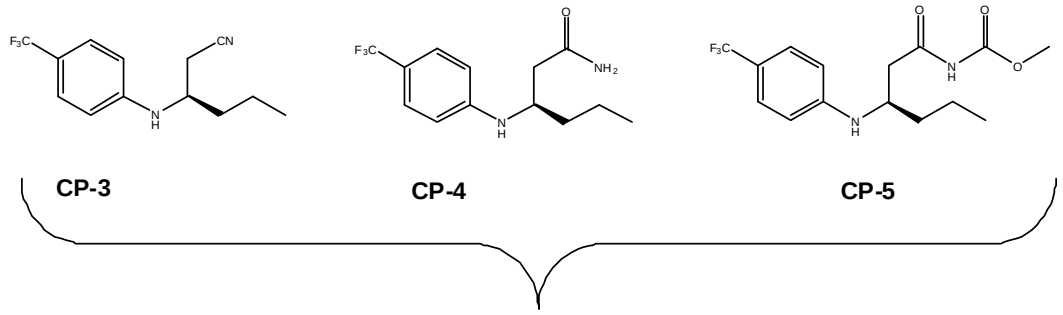
Diastereomers discussion: Trans isomer CP-9-D1, in theory, could come from two possibilities. The first would come from the "disallowed" cyclization reaction to give the trans configuration of the two chiral centers (studies and literature demonstrated this is not possible), and second, from racemization of center "**B**" of CP-6, CP-7, and/or Sakuramil; all of which have been studied throughout the course of development and not observed. The second possible trans isomer CP-9-D2 could not be present since it would result from the wrong enantiomer of CP-2 and the disallowed cyclization selectivity and/or racemization, both of which have been demonstrated not to be possible.

Analytical proof of chiral control strategy: Nevertheless, to confirm the chiral control theory described above, during the course of development all three stereoisomers of Sakuramil, and selected intermediates, have been manufactured with the appropriate analytical methods developed specific for all their detection at various intermediates and in Sakuramil drug substance. All the batches of Sakuramil manufactured have provided Sakuramil with ≤0.1% of each stereoisomer. Changes, such as racemization, in stereochemistry during the synthesis development of Sakuramil have not been observed. This is consistent with the chemical knowledge and literature support that these two centers are not prone to racemization, and they are stable.

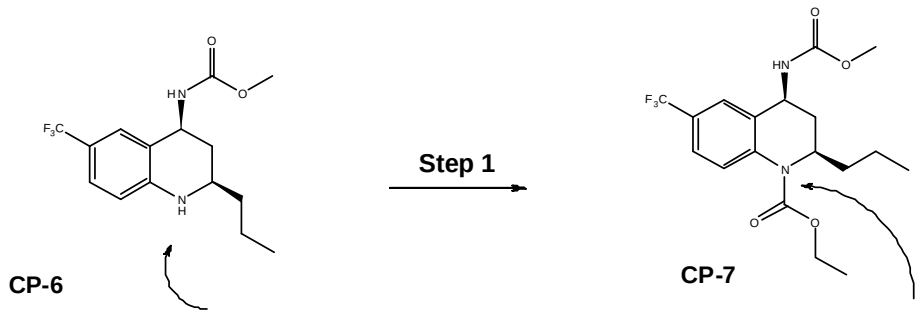
A fate and purge study demonstrated that 1% of the enantiomer (CP-6-E) and the diastereomer (CP-6-D) in CP-6 resulted in <0.1% (less than LOQ of 0.05%) in drug substance. In addition, there is no erosion of the chirality in Steps 1 and 2 even when applying stressing conditions.

1)-7 Control strategy for genotoxic impurities

The synthesis of Sakuramil has been assessed to identify potential genotoxic impurities. CP-6 and CP-4 have been tested positive in the Ames Assay. The intermediate precursors CP-5 and CP-3 (non isolated intermediates) exhibited positive structural alerts in a structure activity relationship database with the same aniline functionality. However, the commercial manufacturing synthesis was designed such that these three intermediates and CP-6 are reacted in Step 1 to eliminate the aniline functionality responsible for the genotoxic characteristics. The reactivity of these genotoxic impurities and intermediates together with the hydrophobicity of formed CP-7 and CP-9 (Sakuramil), in addition to, the Steps 1 and 2 crystallizations having excellent purge of the resulting unreacted anilines and byproducts, have consistently enabled control of these genotoxic impurities in Sakuramil drug substance to a total for the four (CP-6, CP-5, CP-4, CP-3) of ≤25 pm (25 ppm is the TTC for Sakuramil based on the highest dose. Data will be provided and justified in the multivariate development data in this section.)



Genotoxic-precursor intermediates and potential impurities controlled in CP-6



Aniline functionality responsible for genotoxic mecahnism

Aniline functionality reacted in step 1 to form carbamate functionality that is non-genotoxic

Figure 2.3.S.2.6-3 Genotoxic Intermediates and Reactivity

Interpretation:

For genotoxic impurities, European Medicines Agency (EMA) finalized a guideline[1] in 2006 and started the operation of it in 2007. In addition, FDA also released a draft guidance in 2008, and currently it is discussed as an ICH multidisciplinary guideline "ICH M7".

In the EMA guideline, it is determined first whether an impurity that shows genotoxicity has a "genotoxic mechanism related to the threshold" or not. If there is evidence of such mechanism, permitted daily exposure (PDE) is obtained from no observed effect level (NOEL) and uncertainty factor (UF) to evaluate safety. If there is no evidence of such mechanism, it is evaluated first, based on pharmaceutical investigation, whether the impurity can be removed or not. If the impurity level can be no more lowered, it is evaluated whether the level exceeds 1.5 μg/day (threshold of toxicological concern, TTC) or not. If the level does not exceed 1.5 μg/day, the impurity is regarded as negligible risk. If the level exceeds 1.5 μg/day, it is determined whether the level is acceptable or not based on dosage and dose regimen and characteristics of the relevant drug.

TTC represents the upper limit of permissible daily intake which does not give risk to human health. Normally, it is the value estimating "virtually safe dose" for carcinogenic risk not exceeding one millionth throughout the human lifetime. TTC was developed for evaluation of food contamination chemicals or food additives, and is utilized by FDA and Joint FAO/WHO Expert Committee on

Food Additives. Its concept is to establish the threshold of human exposure with no hazard risk for the relevant chemical material based on existing toxicity data and chemical structure even when no toxicity studies have been performed. The TTC value for each person is defined as 1.5 μg/day for non-carcinogens and non-genotoxic carcinogens and 0.15 μg/day for genotoxic carcinogens based on data of hundreds of carcinogens and non-carcinogens.

The TTC value of 1.5 μg/day for genotoxic impurities for medical products has been established 10 times looser than that for genotoxic carcinogens taking into consideration the medical benefit.

The concentration limit of Sakuramil is calculated as below.

Maximal daily dose of Sakuramil: 60 mg (= 0.06 g)
TTC value: 1.5 μg/day
Concentration limit of genotoxic impurities of Sakuramil = TTC / maximal daily dose
= 1.5 μg/day / 0.06 g/day
= 25 ppm

When more than one genotoxic impurity is present in the drug substance, the TTC Value of 1.5 μg/day can be applied to each individual impurity only if the impurities are structurally unrelated[2]. However, if the genotoxicity-related structure is similar, like aniline functionality through CP-3 to CP-6 in the case of Sakuramil, it can be assumed that the impurities act by the same genotoxic mode of action and have the same molecular target, and thus might exert effects in an additive manner. Therefore, it is designed that the total of CP-3 to CP-6 becomes not more than the TTC value (1.5 μg/day; 25 ppm in Sakuramil drug substance).

Upon establishing of ICH M7, the provisions of the guideline should be followed.

[1] Guideline on the Limits of Genotoxic Impurities, EMEA/CHMP/QWP/251344/2006
[2] Questions and answers on the "Guideline on the limits of genotoxic impurities', 23 September 2010, EMA/CHMP/SWP/431994/2007 Rev. 3

2) Development History

2)-1 Route A: First Generation Synthesis

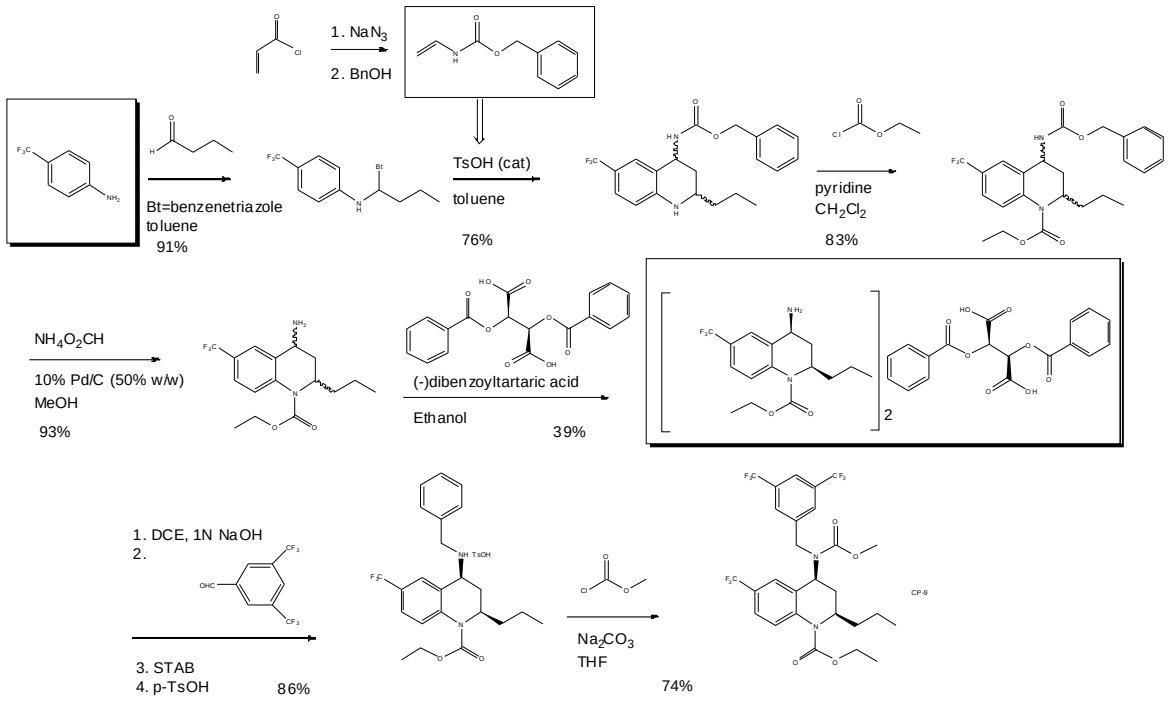


Figure 2.3.S.2.6-4 Route A: First Generation Synthesis

- Disadvantages
    - Resolution discards 50% of the wrong enantiomer: not reasonable for high volume products
- Safety : Use of hazardous reagents/chemistry
    - Trifluromethylbromoaniline (potential HF generation)
        - N-vinylcarbamate (polymerizes and generates impurity)
    - Azide chemistry (explosive)
- Suppliers for this route are limited due to the hazards associated to this chemistry
    - Long term supplies questionable.

2)-2 Route B: Second Generation Synthesis

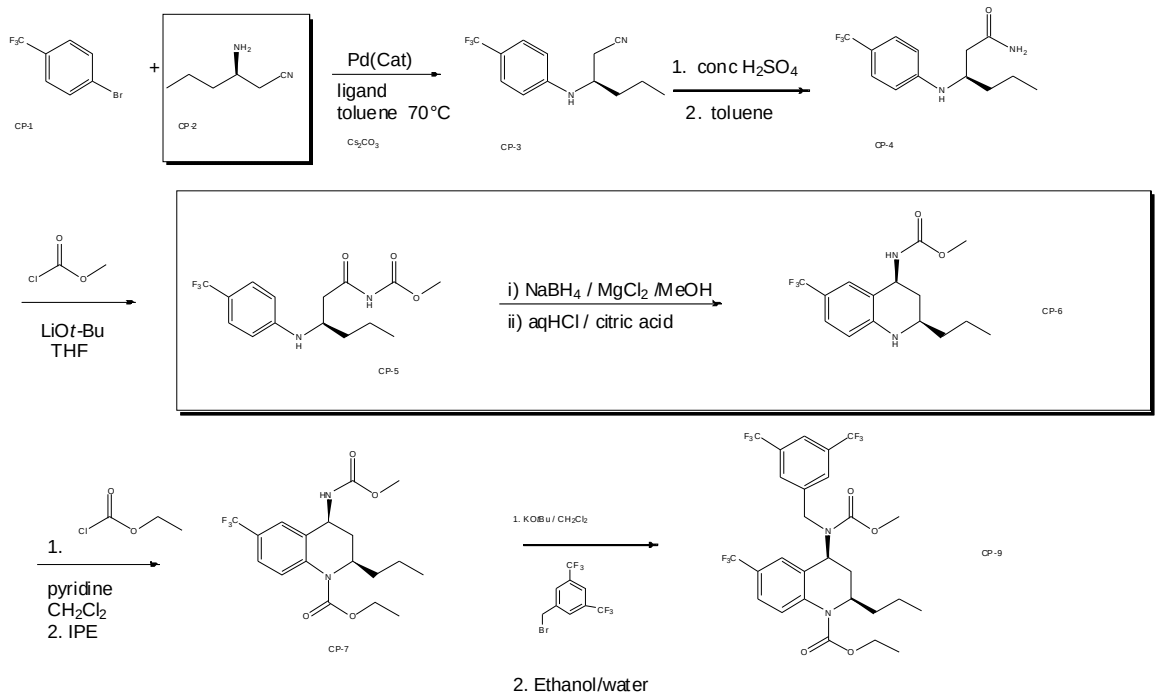


Figure 2.3.S.2.6-5 Route B: Second Generation Synthesis

Improvements from Route A

- Use safer and reliable technology that reduces the use of hazardous reagents
- Elimination of resolution step
- Technology that can be run in a general purpose plants
- More efficient capacity utilization for large volume product
- Multiple vendors and the supplier option

Process highlights that needed to be addressed for commercialization

- **Step CP-6 to CP-7**
    - Reaction done in DCM with IPE as the isolation solvent
    - Pyridine use as base for the reaction with ethylchloroformate
    - Reaction done at a low temperature to reduce decomposition
    - Reaction typically resulted in 2% unreacted starting material

- **CP-7 to Sakuramil**
    - Potassium t-butoxide reacts with methylene chloride when CP-7 is absent, potential safety issue identified.
    - Potassium t-butoxide must be titrated to reaction to avoid formation of impurities difficult to purge
    - Two solid charges / worker exposure potential.
    - Large volumes of DCM also needed
    - The process requires a significant excess of CP-8 (1.4 eq.) an expensive reagent classified as a sensitizer (P-4).
    - The reaction typically leaves 1 to 2.5% of starting material

2)-3 Route C: Third Generation Synthesis

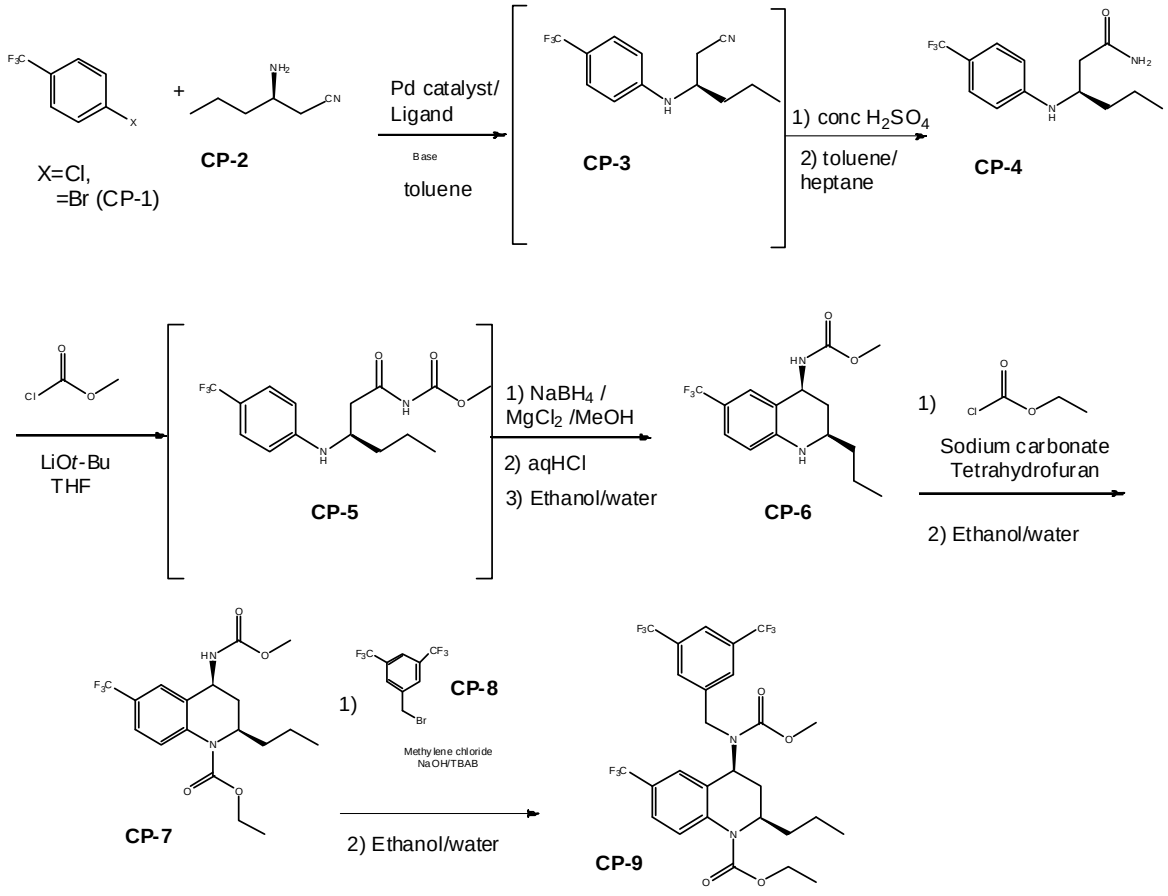


Figure 2.3.S.2.6-6 Route C: Third Generation Synthesis

Key Improvements to Commercial Process

- **Step CP-6 to CP-7**
    - Reaction done in THF with Ethanol/water as the isolation solvent
    - Pyridine was substituted by tribasic sodium phosphate and / or sodium carbonate *
        - No stability issue
        - Reaction typically resulted in ≤0.5% unreacted starting material

- **CP-7 to Sakuramil**
    - Reaction is done using 50% NaOH and a phase transfer catalyst
    - DCM still needed but with significantly volumes reductions
    - No stability or incompatibility issues between reagents/solvents
    - The process requires only 1.03 equiv of CP-8 (vs. 1.4 eq. Old process) an expensive reagent classified as a sensitizer (P-4).
    - The reaction typically leaves ≤0.5% of starting material

** Sodium phosphate and sodium carbonate were both part of the design space development and presented as options in S.2.2.*

3) Starting Material Justification and Commercial Manufacturing Process Selection

IROHA-corp proposes that CP-6 and CP-8 serve as structurally distinct and suitable Starting Materials (SM), or "Regulatory Starting Materials" (RSM). The proposed "Regulatory Synthesis" is convergent, consisting of two synthetic steps, wherein significant molecular fragments are incorporated to create Sakuramil drug substance. The proposed "Regulatory Synthesis" was selected after extensive laboratory and manufacturing experience established a high degree of process understanding. The appropriate specifications for SM have been established to assure the quality of Sakuramil drug substance.

CP-6 that was manufactured by the three development routes, as outlined in that section above, have all produced Sakuramil drug substances that meets the current specifications. In addition, batches of CP-6 have been manufactured by multiple suppliers using second and third generation synthesis. All of these resulted in CP-6 and Sakuramil drug substance with similar impurity profiles and of high quality. There were not any impurities from all of these batches or route modifications that resulted in any impurity above the 0.15% level in Sakuramil drug substance.

Interpretation:

Selection and control of SM are important factors in establishing quality of the drug substance. SM in S.2.2 description of manufacturing process represents the starting point of GMP operation. To assure that the validity of drug substance CQA functionally related to SM material attributes (MA) is justified, maintained and controlled, SM high or moderate risk MA are reflected in specifications (see 2.3.S.2.3). For example, impurities in SM are controlled because they may impact quality of the final drug substance by being included in the manufacturing process of the drug substance.

3)-1 Justification of CP-6

CP-6 is well characterized, physically and chemically stable and is manufactured by IROHA-corp and several qualified external manufacturers. The stability is suitable for shipment and management.

CP-6 have been manufactured by IROHA-corp, NIHO-corp – Italy, HETO-cop – Germany and CHIRINU-corp – France. CP-6 manufactured at commercial scale has been converted to Sakuramil drug substance that has been used in Phase 3 clinical trials and ICH drug substance stability studies. The quality of CP-6 is tightly controlled in accordance with the commercial specification delineated in Table 2.3.S.2.6-2.

The desired impurity profile of CP-6 is achieved through the use of a reproducible process and a robust crystallization using a combination of ethanol and water as the final solvent mixture.

All process related impurities present in the SM, CP-6, at levels greater than 0.1% have been identified and appropriate limits and controls have been established based on demonstrated purge data, design space knowledge, and scale and equipment considerations in the subsequent synthetic

steps. Individual unspecified impurities are controlled in lots of CP-6 to levels of NMT 0.1% (NMT=Not More Than). CP-6 is not a source of impurities (≥ 0.15%) in the drug substance.

Stability evaluation of CP-6 has demonstrated that CP-6 is stable with no observations of significant degradation. An informal study of the stability of CP-6 under conditions of 30°C/65% RH through 18 months showed no degradation product increase or impurity change greater than 0.1%.

Table 2.3.S.2.6-2 Specifications for CP-6

Tests	**A**	**Control Value**[1]	**Actual Value**	**Comments: Importance Designation**
Description	No	White to pale yellow Crystals or crystalline powder	B	Low risk
Identity – IR	No	Consistent with standard	Meets Test	Moderate risk
Related Substances	Yes			
CP-4		≤ 0.3%[2]	≤ 0.01-0.04%	High risk 3000 ppm (0.3%) resulted in < 10 ppm in drug substance
CP-6-E (Enantiomer)		≤ 1.0%	≤ 0.01%	Low risk[4] Controlled by vendors specifications
CP-6-D1 (Diastereomer)		≤ 1.0%	≤ 0.1%	Low risk[4] Controlled by vendors specifications
Other (Individual)[3]		≤ 0.1%	≤ 0.05-0.2%	Moderate risk
Other Impurities Total		≤ 0.5%	0.1-0.2%	Moderate risk
Assay	No	98-102%	97.0%-103%	Moderate risk
Residual Solvents	No	●●	▲▲	
Pd	No	≤ 10 ppm	≤ 1 ppm	Moderate risk

A: Potential for variables to impact quality
B: White to pale yellow crystal or crystalline powder
[1] These acceptance criteria have changed with increasing processing experience and understanding. While some of these lots do not meet current acceptance criteria, all lots have been successfully processed forward to produce drug substance of acceptable quality.
[2] Fate and purge study supports up to 1%.
Fate and purge study: Elevated levels of this impurity is introduced to laboratory batches and then evaluated in the process to identify the fate of the impurity, and the purge factor of the impurity or reacted impurity.
[3] CP-6 regio isomers, CP-3 and CP-5 are monitored and controlled as unspecified "other" impurities.
[4] Include no-risk.

3)-1-1 Importance assessment for CP-6 material attributes

Fate and purge of related substances in CP-6 in the manufacturing process of Sakuramil drug substance is shown in the figure below.

CP-6 and CP-4 have tested positive in the Ames Assay. The intermediate precursors CP-5 and CP-3 exhibited positive structural alerts in a structure activity relationship database, therefore will be controlled as potential genotoxic impurities (CP-6 & CP-4) as well.

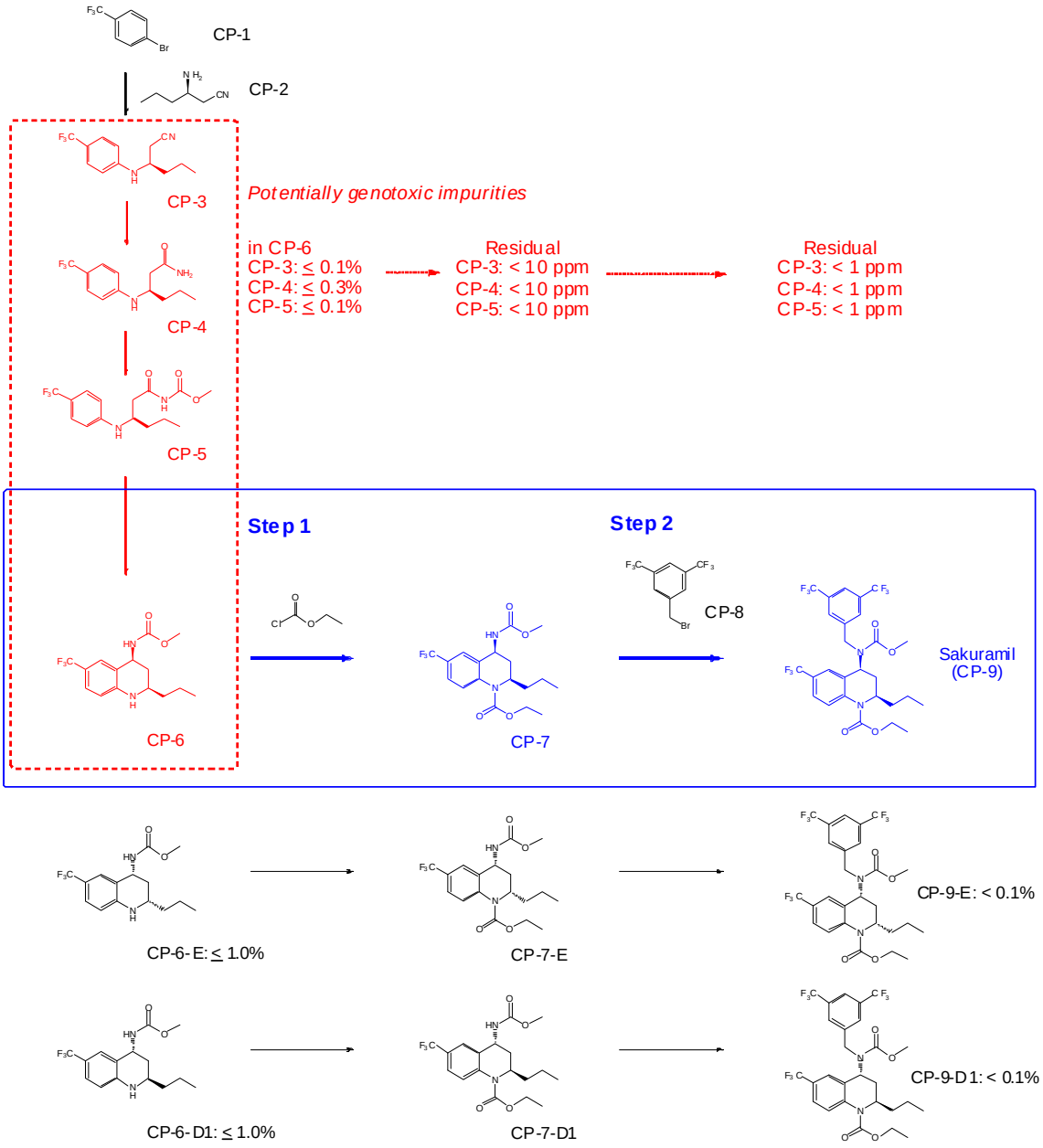


Figure 2.3.S.2.6-7 Fate and Purge of Related Substances inCP-6 in the Manufacturing Process of Sakuramil

3)-1-1-1 CP-6 important material attribute:

CP-4 is designated a high risk material attribute and the specification is established at 0.3% in CP-6. CP-4 is one of the four potentially genotoxic impurities in the process to manufacture Sakuramil drug substance; and all four of these impurities will be controlled as a total in the drug substance to ≤ 25 ppm (which is the established TTC). It should be noted that CP-5 and CP-3 (two other potentially genotoxic impurities) are controlled under the unspecified impurity specification as NMT 0.1% in CP-6 as shown in the table above. However, even at this level of 0.1% for each of these two impurities, the level in Sakuramil drug substance for CP-5 and CP-3 is typically seen well below 1 ppm combined. The reactivity of the functional center responsible for the genotoxic activity (aniline) combined with the purge factors for these two impurities is 100 times greater that in CP-4. Therefore, control at CP-4 is an excellent and appropriate indicating test to assure that all three of these impurities total to not more than 10 ppm when processed through to Sakuramil. In addition, 10 ppm was chosen as the target for the total of the three known impurities in Sakuramil drug substance based on the additive effect and combined control strategy for the last potentially genotoxic impurity; CP-6. This upstream critical control point for CP-4 is part of the combined control strategy for all potentially genotoxic impurities as summarized below (More on the control of CP-6 will be summarized in following sections on design space and control strategy summary).

Summary of Genotoxic Control Strategy

Control Strategy for CP-4, CP-5, CP-3:
In CP-6: Important MA CP-4 (≤ 0.3%) + CP-5 and CP-3 (≤ 0.1% each) = ≤ 10 ppm total for these three in Sakuramil drug substance

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 Sakuramil drug substance: ≤ 10 ppm)

Therefore: Overall Genotoxic Control strategy = the total of these two control points ensure that CP-5, CP-3, CP4, and CP-6 to be < 25 ppm (25 ppm is the concentration limit based on TTC and the daily dose of Sakuramil drug substance).

3)-1-1-2 Control items for CP-6 moderate risk material attributes:

Identity, assay, unspecified impurities, and total impurities have not been identified as important material attributes.

However, they are important when managing current suppliers or evaluating additional supplier of CP-6. These tests serve as an opportunity to identify any potential new sources of impurities that were not possible to evaluate during the course of development. In addition, the downstream methods for CP-7 and for Sakuramil drug substance are excellent additional orthogonal methods used as well to manage and mitigate risk of unknown impurities' introduced from CP-6.

3)-1-1-3 Control items for CP-6 low risk material attributes:

The enantiomer (CP-6-E) and diastereomer (CP-6-D1) are low risk material attributes. As discussed above, chirality is controlled in the CP-2 and the process and intermediates have no impact on the chiral control. In addition, the process has significant purge of all isomers as well as methods that are specific for, and confirmed, the correct enantiomer of Sakuramil (specifications for intermediate and the drug substance are specific for all chiral isomers). Manufacturing of the enantiomer would therefore not go undetected early in the process. In addition, the drug substance methods are specific for the diastereomers and would be classified as unspecified (≤ 0.10%).

Control of the enantiomer and diastereomer will be maintained within the purchase specification for CP-6 and the manufacturing sites quality systems.

3)-2 Control of CP-8

While the starting material CP-8 exhibited positive structural alerts in a structure activity relationship database, it was determined to be negative in the Ames assay. Therefore, CP-8 is controlled in Sakuramil drug substance as an unspecified impurity with a limit of NMT 0.10%. (NMT= not more than)

CP-8 is a commercial commodity prepared by several suppliers, using their proprietary manufacturing processes. CP-8 is purchased from a number of suppliers in accordance with appropriate specification criteria as provided in Table 2.3.S.2.6-3.

Table 2.3.S.2.6-3 Specifications for CP-8

Tests	A	Control Value	Actual Value	Comments: Importance Designation
Description	No	White to pale yellow Crystals or crystalline powder	B	Low risk
Identity – IR	No	Consistent with standard	Meets Test	Moderate risk
CP-8-OH	No	≤ 1%[1]	≤ 0.2%	Low risk No reaction
CP-8-CHO	No	≤ 1%[1]	≤ 0.2%	Low risk No reaction
CP-8-25I	Yes	≤ 0.05%[2]	≤ 0.02%	High risk React like SM, little purge in drug substance
CP-8-24I	Yes	≤ 0.05%[2]	≤ 0.02%	High risk React like SM, little purge in drug substance
Other (Individual)	No	≤ 0.1%	≤ 0.1%	Moderate risk
Other Impurities Total	No	1.0%	≤ 0.1-0.3%	Moderate risk
Assay	No	≥ 97%	99.7%-100%	Moderate risk

A: Potential for variables to impact quality
B: White to pale yellow crystal or crystalline powder
[1] Fate and purge study supports up to 3%.
[2] 0.1% supported by fate and purge study → not more than 0.1% in drug substance

3)-2-1 Importance assessment for CP-8 material attributes

Fate and purge of related substances in CP-8 in the manufacturing process of Sakuramil is shown in the figure below.

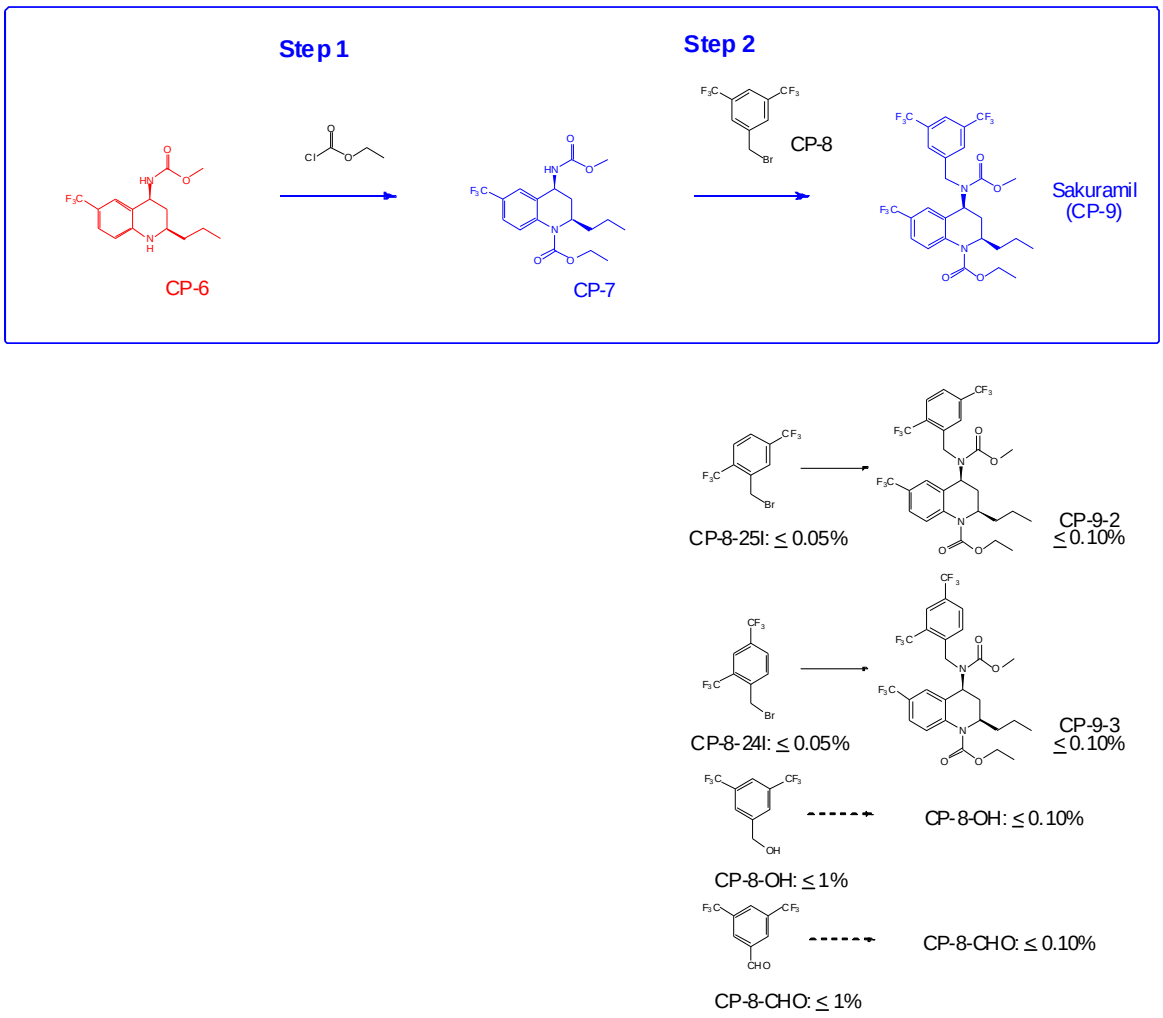


Figure 2.3.S.2.6-8 Fate and Purge of Related Substances in CP-8 in the Manufacturing Process of Sakuramil

3)-2-1-1 CP-8 important material attribute:

The two regio-isomeric benzylbromides are high risk material attributes as designated in the table above. The high risk material attribute justification is warranted due to the fact that they produce to isomers of the drug substance. These two isomers react mechanistically the same as the SM and have very little purge in the final crystallization to isolate Sakuramil drug substance (see fate and purge data in a footnote to the table).

Therefore, the specification (and thus the control strategy) is established as ≤ 0.05% for each to ensure the quality of Sakuramil drug substance.

3)-2-1-2 Control items for CP-8 moderate risk material attributes:

Identity, assay, unspecified impurities, and total impurities have not been identified as important material attributes.

However, they are important when managing current suppliers or evaluating additional supplier of CP-8. This test serves as an opportunity to identify any potential new sources of impurities that are not possible to evaluate during the course of development. In addition, the methods for Sakuramil drug substance are excellent additional orthogonal methods used as well to manage and mitigate risk of unknown impurities from CP-8.

3)-2-1-3 Control items for CP-8 low risk material attributes:

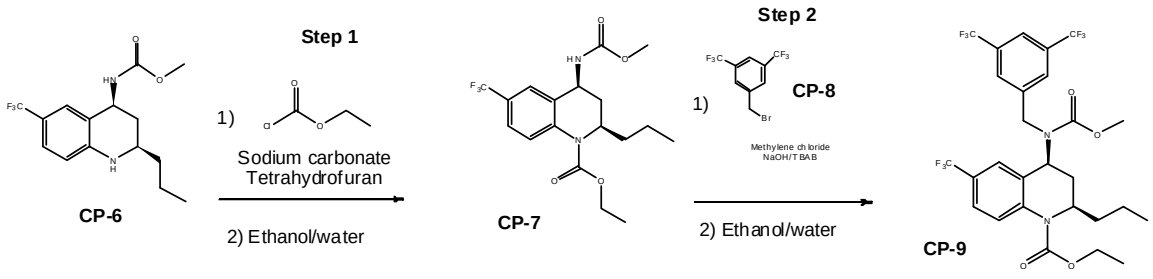
CP-8-OH and CP-8-CHO are not important material attributes. Both of these impurities do not react in the final step and have significant purge factors in the final isolation of drug substance. Fate and purge has demonstrated that 3% is easily purged to ≤ 0.1% unspecified level in drug substance.

The non important tests will be maintained within the purchase specification for CP-8 and the manufacturing sites quality systems.

3)-3 Summary of the commercial manufacturing process selection

CP-6 and CP-8 have been selected as SM. The process steps starting from CP-6 to Sakuramil drug substance will be validated for commercial manufacture.

Commercial Process to Manufacture Sakuramil Drug Substnace



4) Risk Assessment for Knowledge Space and Control Strategy Development

Once the commercial manufacturing process for Sakuramil drug substance was established, increased understanding of the functional relationships between manufacturing process inputs (raw materials, SM, intermediates, etc.) and operating parameters (and their respective levels of criticality) and critical quality attributes of the drug substance was developed. This ultimately led to defining a design space and a control strategy for the manufacturing process.

Initially, a risk assessment process was undertaken to identify potentially critical process parameters and potential scale and equipment dependencies and assess their probable impact on drug substance quality (resulting in an understanding of their criticality and definition of inputs and operating parameters as high risk (critical), moderate risk (key) or low risk (non-critical)). In this risk assessment process, each step of the manufacturing process was considered individually. Firstly, the material attributes of the product for each step were considered with respect to their potential to impact a critical quality attribute of the drug substance. For drug substance processes, this is strictly an impurity exercise until the isolation of the drug substance, where physical characteristic are included. Next, process inputs and process operating parameters for each step were assessed with respect to their potential to impact the important impurity attributes for that step in the process.

This initial structured risk assessment utilized the knowledge that had been gained through development and scale up of the manufacturing process, and a mechanistic and kinetic understanding of the chemistry and the manufacturing process. After identifying the process inputs (raw materials, SM, intermediates, etc.) and process operating parameters and their potential links to drug substance critical quality attributes, an experimental plan was developed, prioritized and executed in order to (a) establish if the identified parameter does impact the quality attributes, and (b) to determine the extent of this impact and identify the design space / proven acceptable ranges (PAR) within which the process can be operated to produce drug substance that meets its specification.

4)-1 Commercial manufacturing process related impurities (including intermediates and diastereomers)

All impurities that may be included in the manufacturing process of Sakuramil drug substance are shown in the following figure of Impurity Cascade for Sakuramil.

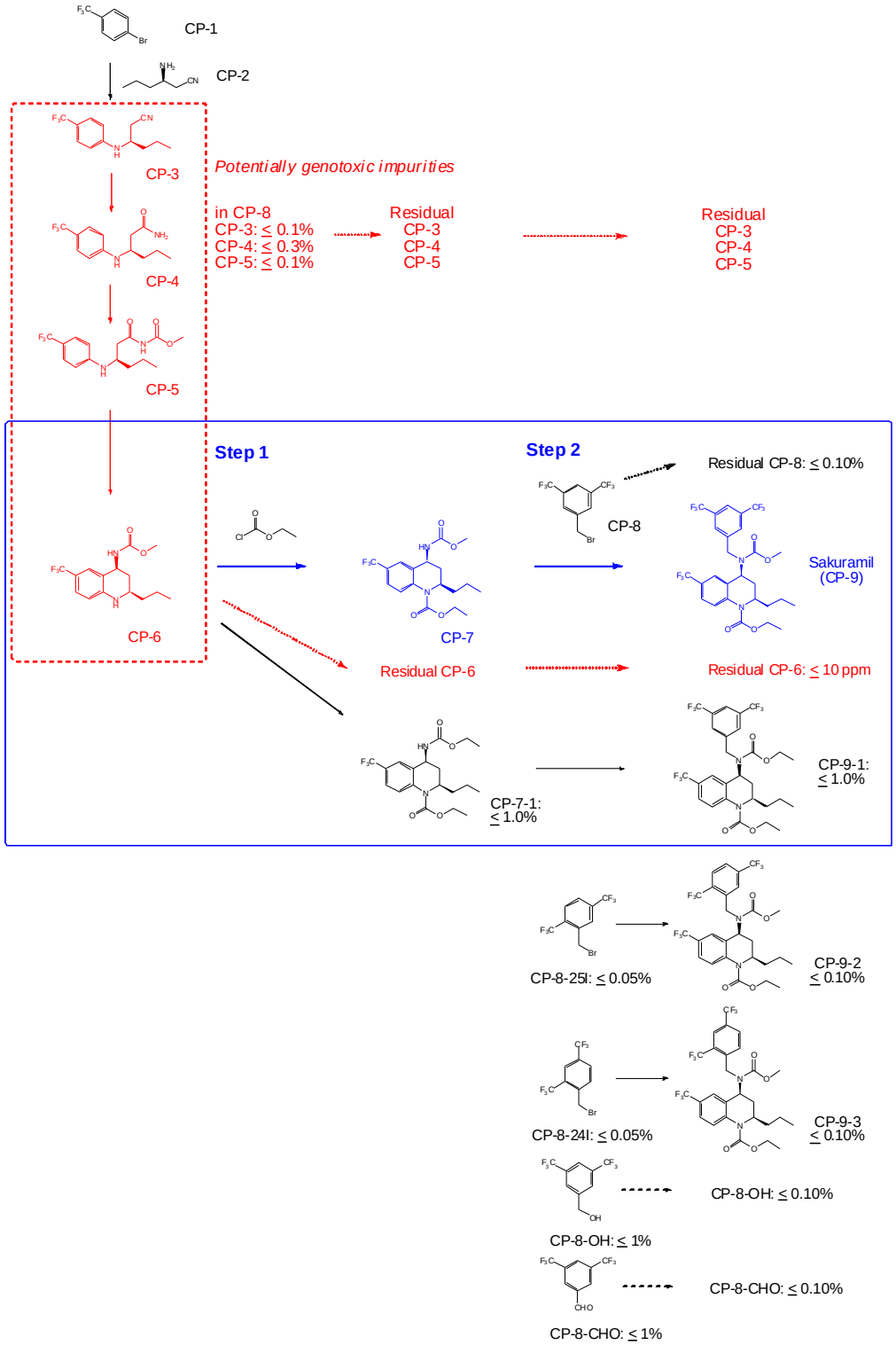
All process related impurities present in CP-6 at levels greater than 0.1% have been identified, and appropriate limits may be established based on demonstrated purge data in subsequent synthetic steps. Individual unspecified impurities are controlled in CP-6 to levels of NMT 0.1%. CP-6 related impurities are not a source of significant levels of impurities in the drug substance. The desired impurity profile of CP-6 and Sakuramil are consistently achieved through a reproducible process and robust crystallizations. Because of the lipophilic nature of all the intermediates and Sakuramil (as a result of the trifluoromethyl functional groups) the crystallizations have historically demonstrated an unprecedented, highly efficient purge of previous intermediates and impurities such that the quality of CP-6 and Sakuramil are historically independent of major and minor changes. This purge stands true for all process related impurities, diastereomers, genotoxic intermediates, and starting material related impurities.

Interpretation:

To assist with the design space development and control strategy options, a holistic impurity grid was mapped (and maintained during development) for the entire process (pre and post commercial manufacturing process) to ensure the QA of the SM and intermediates are well understood. This impurity grid is the foundation for risk assessment and the multivariate experimental phase to establish design space boundaries and control strategy options.

It is summarized in a pictorial summary of each of the impurity fate and purge (with supporting data). Criticality of each process related impurity cannot be established until the functional relationship between each impurity and the CQA of the drug substance are mapped and understood. Fate and purge also play an important role in the final criticality assessment and control strategy for this exercise. For drug substance processes, impurity tracking and knowledge are the primary focus of all experimental designs until the final step that produces drug substance (this is the only place physical characteristics become part of the experimental design space). This grid serves as the QA that are chosen to evaluate during any multivariate design for the Sakuramil process.

Impurities generated in the proposed commercial manufacturing process and their fate were the primary focus of the work conducted to understand the manufacturing process of and determine the design space and control strategy for Sakuramil drug substance.



Note: Dashed arrow - Without structure change. Solid arrow - Reaction with structure change.

Figure 2.3.S.2.6-9 Impurity Cascade for Sakuramil

4)-2 Manufacturing process impact on CQA of Sakuramil Drug Substance

Impact of SM and manufacturing steps on the CQA of Sakuramil drug substnace are shown in Table 2.3.S.2.6-4.

Table 2.3.S.2.6-4 Impact of SM and Manufacturing Steps on Drug Substance CQA

CQA	Tests	CP-6 (SM)	CP-8 (SM)	Step 1	Step 2
ID	IR, chiral HPLC	No	No	No	Yes
Potency	Assay	No	No	No	Yes
Purity	Related Substances	No	Yes	Yes	Yes
	Genotoxic Impurities	Yes	No	Yes	Yes
	Residual Solvents	Yes	No	Yes	Yes
	Metal Impurities	Yes	No	No	No
Optical Activity	Stereoisomers	Yes	No	No	No

4)-2-1 Material Attribute (MA) to be evaluated: Related Substances

The following figure shows the impurities deriving from the manufacturing steps which could highly potentially impact CQA of Sakuramil. In Step 1, ethyl homolog CP-7-1, the single impurity in the entire manufacturing process, is formed. CP-7-1 reacts and is converted to CP-9-1 in Step 2 and ultimately remains in the drug substance. CP-9-1 has very little purge in the crystallization in Step 2. Therefore, it is required to identify the process parameters which impact the formation and purge of CP-7-1 in Step 1.

In Step 2, unreacted SM CP-8 remains in the drug substance. Therefore, it is required to identify the process parameters which impact the formation and purge of CP-8 in Step 2.

SM CP-6, a genotoxic impurity used in Step 1, is discussed in the section for genotoxic impurities.

4)-2-2 Material Attribute (MA) to be evaluated: Genotoxic Impurities

SM CP-6, and CP-3, CP-4 and CP-5, potential impurities in SM, are genotoxic impurities. It is required to investigate the process parameters which impact the purge of these impurities.

4)-2-3 Material Attribute (MA) to be evaluated: Chirality (Stereoisomers)

As stated in 1)-6 Chiral control strategy for Sakuramil, no racemization has been observed in the manufacturing process of Sakuramil drug substance. As chirality is controlled in accordance with

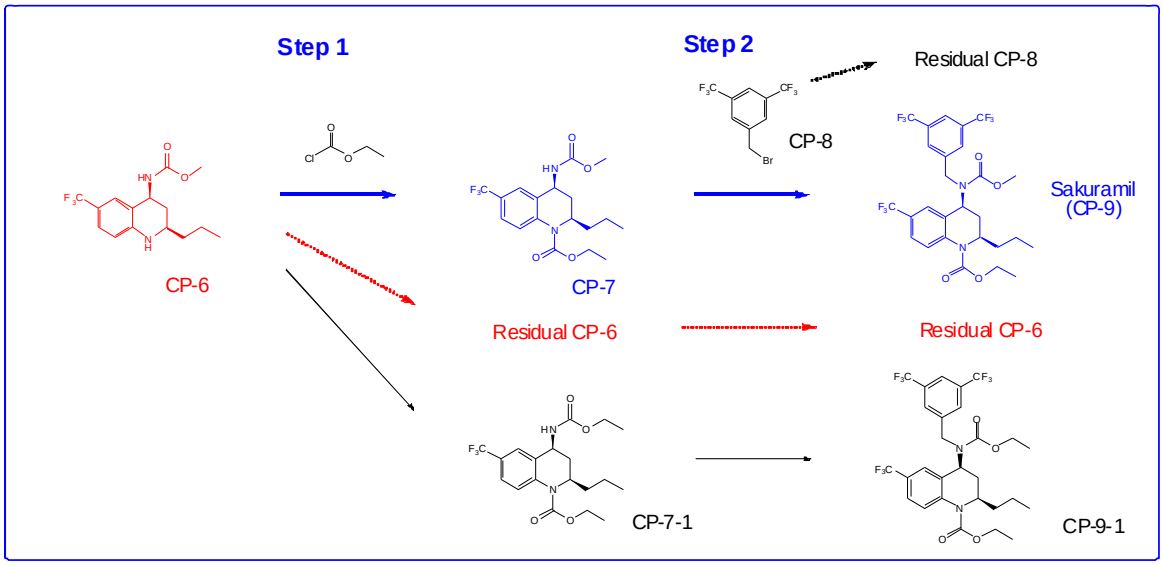
the vendor specifications of CP-2 and the manufacturing process and intermediates do not impact the control of chirality, it is not an important MA.

4)-2-4 Material Attribute (MA) to be evaluated: Residual Solvents

Class 2 solvents THF and *n*-hexane are used in Step 1, but because of two solid-liquid separation operations in the crystallization in Step 1 and Step 2, these solvents have not been detected in the development stage. A Class 2 solvent dichloromethane is used in the reaction step in Step 2, but as it is designed to be submitted for crystallization after dichloromethane is evaporated after the completion of the reaction, dichloromethane also has not been detected in the development stage. Therefore, it is also not an important MA.

4)-2-5 Material Attribute (MA) to be evaluated: Metal Residues

Metal catalysts are not used in the manufacturing process of Sakuramil drug substance. However, Pd catalyst is used in the initial stage of the manufacturing process of SM CP-6. Therefore, Pd has been included in the specifications for SM. As the level of metals does not increase in the manufacturing process of Sakuramil drug substance, Pd is not an important MA.



Note: Dashed arrow - Without structure change. Solid arrow - Reaction with structure change.

Figure 2.3.S.2.6-10 Potential Process-Related Impurities

5) Unit Operation Design Spaces for Each Step of the Drug Substance

For the purposes of defining the design space for the Sakuramil manufacturing process, the impact of the process on the critical quality attributes of Sakuramil was investigated in combination with quality risk management processes and tools. A design space for the commercial manufacture of Sakuramil has been established relative to its critical quality attributes and critical process inputs (material and process parameters).

The following sections (Step 1 to Step 2) provide a summary of the risk based assessment of each step, the experimental work conducted, an assessment of the results and the resultant design space for each step, leading to the overall design space for the manufacturing process.

5)-1 Focus area multivariate protocols, experimental summaries, and conclusions that set up the Design Space for Sakuramil drug substance

Introduction

Once the commercial route to Sakuramil was established as described in the process development history section above, a greater understanding of the functional interrelationships of the manufacturing process parameters on the critical quality attributes of the drug substance was established and this ultimately led to definition of the design space for the manufacturing process.

Initially, a risk assessment process was undertaken to identify all process parameters (including MA and in-process controls) and assess their probable impact on drug substance quality. In order to do this, each step of the manufacturing process was divided into focus areas (FA) and evaluated individually. Step 1 and Step 2 were each divided into FA1 – FA6. Evaluated FA are shown in Table 2.3.S.2.6-5. This initial risk assessment was conducted by means of Cause and Effect (C&E) Matrix. Examples of process parameters assessed are shown in Table 2.3.S.2.6-6.

First, the material attributes of the product for each step were assessed against their potential to impact a critical quality attribute of the drug substance. Next, process parameters for each step were assessed against their potential to impact the important material attributes for that step product. The evaluation points were calculated and risks were classified into three levels (high, medium and low). This initial structured risk assessment utilized the knowledge that had been gained through development and scale up of the manufacturing process, and a mechanistic understanding of the chemistry of reactions and work up procedures.

The quality attributes that gain the highest priority to evaluate were the impurities generated and controlled by the process.

Table 2.3.S.2.6-5 Focus Areas in Risk Assessment for Manufacturing Process of Sakuramil

Focus Area	Step 1	Step 2
FA1	Reaction	Reaction
FA2	Reaction Mixture Filtration	Quench, Phase Separation, Wash
FA3	Quench, Phase Separation	Distillation
FA4	Crystallization	Filtration (remove insoluble material)
FA5	Cristal Filtration	Crystallization
FA6	Drying	Drying

Table 2.3.S.2.6-6 Examples of Process Parameters Assessed at Initial Risk Assessment

1. **Facility construction**
2. **Quality of raw materials**
3. **Input order of raw materials/order of operation**
4. **Duration of material charge/addition speed**
5. **Stirring speed**
6. **Reaction time**
7. **Reaction temperature**
8. **Sampling from reaction solution**
9. **pH of water phase**
10. **Operation of phase separation**
11. **Replacement of solvent**
12. **Concentration at crystallization**
13. **Temperature at crystallization**
14. **Filtration**
15. **Volume of wash solvent**
16. **Drying temperature**
17. **Degree of vacuum**
18. **SOP**
19. **Training for operator/tester**

After the risk assessment was completed for the manufacturing process, the focus areas where impurities were generated and/or controlled were identified as the foundation of the design space requiring more process understanding to assess the impact on the critical quality attributes of Sakuramil drug substance. An experimental plan was developed for each of these focus areas to evaluate process parameters and quality attribute links. They were prioritized and executed in order to (a) examine if the identified parameters do impact the quality attributes, and (b) to determine the extent of this impact and identify the proven acceptable ranges (PAR) within which the process can be operated to produce Sakuramil drug substance that meets the specification.

The focus areas identified for the Sakuramil process are summarized in Table 2.3.S.2.6-7 and Table 2.3.S.2.6-8.

Table 2.3.S.2.6-7 Designation of Possibility Which Affect on Sakuramil CQA through a Risk Assessment

Sakuramil CQA	Step 1						Step 2					
	FA1	FA2	FA3	FA4	FA5	FA6	FA1	FA2	FA3	FA4	FA5	FA6
	Reaction	Filtra-tion	Quench, Phase sep.	Crystal-lization	Filtra-tion	Drying	Reaction	Quench, Phase sep.	Distill-ation	Filtra-tion	Crystal-lization	Drying
Chirality	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Specified Impurities												
CP-6	Low	Low	Low	Low	Low	Low	Medium	Low	Low	Low	Medium	Low
CP-8	N/A	N/A	N/A	N/A	N/A	N/A	High	Low	Low	Low	Medium	Low
CP-3	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
CP4	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
CP-5	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
CP-7-1	High	Low	Low	Medium	Low	Low	Low	Low	Low	Low	Low	Low
Total	High	Low	Low	Medium	Low	Low	High	Low	Low	Low	Medium	Low

High Risk: CQA and parameter impacting on product quality
Medium Risk: CQA and parameter potentially impacting on product quality
Low Risk: CQA and parameter not impacting on product quality

Table 2.3.S.2.6-8 Focus Areas Identified for the Sakuramil Manufacturing Process

1. **S2-Reaction**
2. **S1-Reaction**
3. **S2-Crystallization**
4. **S1-Crystallization**

S=step
Material Attributes were included in the risk assessment (RA) and multivariate designs.

The following sections provide a summary of the risk based assessment of each step of the manufacturing processes; the experimental work designed and conducted, an initial risk assessment of the results based on criticality; which led to the overall design space for the manufacturing process.

5)-1-1 Step 1

5)-1-1-1 Multivariate designs for Step 1 reaction

In Step 1, the aniline of CP-6 is reacted with ethyl chloroformate to give the carbamate CP-7. Following the reaction, the mixture is quenched with a sodium hydroxide solution to consume any excess ethyl chloroformate and hexane is added. The hexane layer is separated, exchanged with ethanol, and CP-7 is crystallized from an ethanol/water mixture.

Two focus areas were studied for the Step 1 process: the reaction and the crystallization.

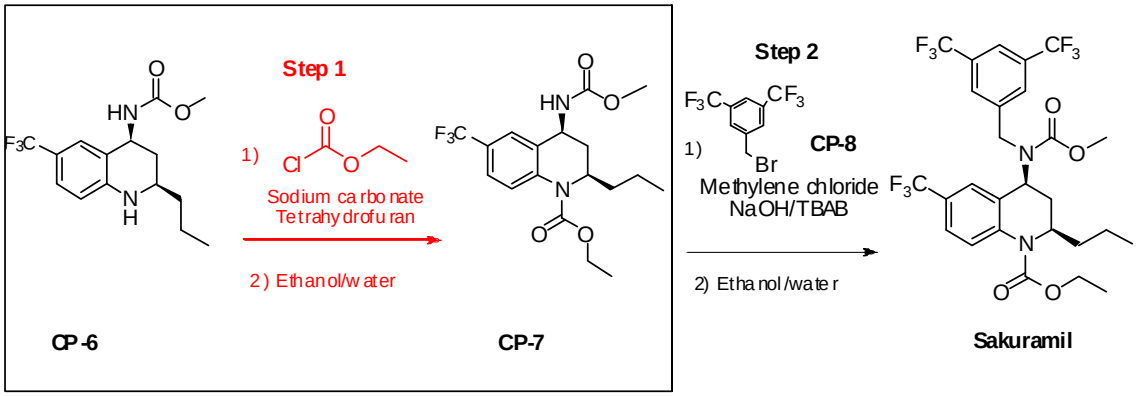


Figure 2.3.S.2.6-11 Step 1 Process

Reaction:

Impurity quality attributes, parameters, and ranges for the Step 1 reaction study:

Only one impurity, ethyl homolog (CP-7-1), has been observed in batches of CP-7 that is formed in the Step 1 process that could potentially impact this critical quality attribute of Sakuramil. This impurity is formed through the reaction of ethyl chloroformate with the nitrogen of the methyl carbamate; and then subsequent loss of the methyl carbamate (or loss of methyl carbamate and the alkylation with ethyl chloroformate). Step 1 is the source of CP-7-1 formation and it is the single impurity in the entire manufacturing process demonstrated to have little purge in the Step 1 and Step 2 crystallizations that give Sakuramil drug substance.

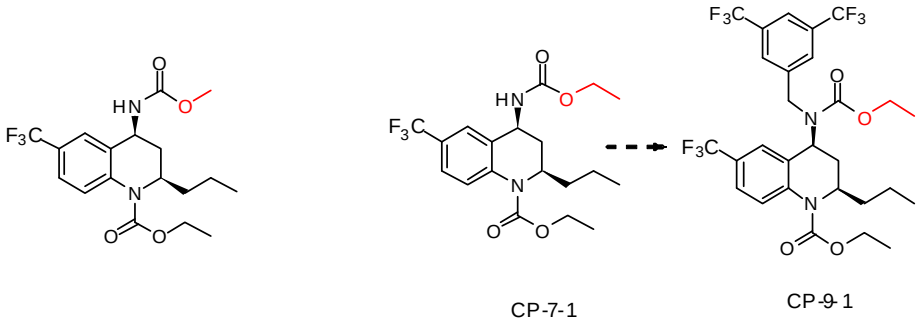


Figure 2.3.S.2.6-12 CP-7-1, Ethyl Homolog

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-7-1 (and total "other" impurities) in CP-7 were identified. This assessment identified that the stoichiometry of ethyl chloroformate and the base(s), and volume of THF were the process parameters that had the highest risk of impacting these quality attributes of CP-7. 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.

In addition, the study included two possible bases for this reaction. Trisodium phosphate and sodium carbonate. The disposal requirements were not fully recognized and both of these bases were considered acceptable for this reaction. They are potential non critical parameters; however, feasibility- cross over study was used to confirm both bases impact.

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

Neither the Trisodium Phosphate or Sodium Carbonate particle size nor the agitation speed had any observable effect upon the reaction rate and product purity profile, indicating that poor mixing upon scale-up should not be a significant concern. Although the rate of the biphasic ethyl chloroformate quench is sensitive to agitation, there were no byproducts or safety concerns to indicate any potential issues upon scale-up related to mixing.

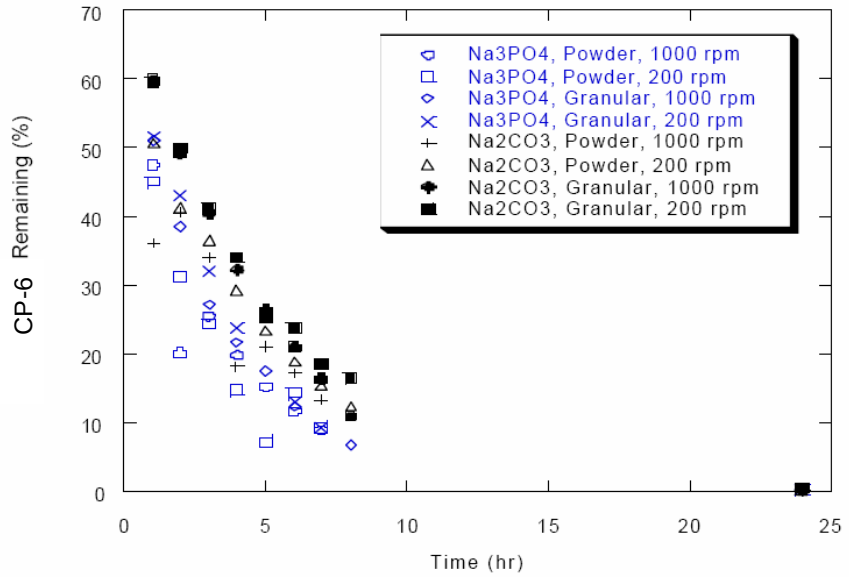


Figure 2.3.S.2.6-13 Impact of Base Particle Size and Agitation Speed on Reaction Rate

Through the course of development there has not been any observed scale and or equipment limitation, which is supported by chemical knowledge. However, additional stressing experiments

are 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 mimic the "worst case" scenario of the proposed commercial equipment and its limitations.

Note: The "worst case" scenario means the laboratory simulation of heating and cooling profiles, etc. of the worst conditions that may occur in the commercial manufacturing facility. For example, whether by-products or degradation products increase or not is confirmed by conducting the reaction at the temperature 10°C higher than the set reaction temperature or for a longer time than the set reaction time, or with reagents in a larger quantity than the set charge-in quantity.

Parameters and ranges explored in the DoE:

A central composite DoE was designed and executed using the following:

Table 2.3.S.2.6-9 Step 1 Reaction DoE

Parameters	Low	Center	High	NOR
Ethyl chloroformate equivalents	2.0	4.75	7.5	2.5
Na_3PO_4 or Na_2CO_3 equivalents	0.75	2.375	4	1.1
Reaction concentration (L/kg, Volumes of THF relative to CP-6	3.0	9.0	15	5.8

** Note: An abnormally high range was chosen for the ethyl chloroformate (ECF) of 7.5 equivalents in an attempt to exaggerate and stress absolute worst case for ethyl homolog formation to achieve better process understanding. This level of ECF is not an option for commercial manufacturing.*

Because the ethyl homolog has very little opportunities for purge in the process, it will be the MA that limits the boundaries of the unit operation design space for Step 1.

The specification of the ethyl homolog derivative in Sakuramil drug substance is 1.0%. The acceptance criteria for the multivariate experiments for the ethyl homolog in the Step 1 reaction and crystallization will be the same as 1.0%; since derivatives from ethyl analogs are only slight purge in Steps 1 & 2 crystallizations of the downstream.

Conclusion for Ethyl Homolog, CP-7-1

- In all experiments using phosphate and carbonate, the level of CP-7-1 was measured to be significantly less than 1.0% (1.0% is the specification for CP-9-1, the ethyl homolog resulting from CP-7-1 in Sakuramil drug substance). In addition the level of CP-7-1 measured in the reaction mixture after 36 hours at the process temperature of 66^{o}C (a worst case scenario) was well below 1.0% as well. Step 2 of the Sakuramil process is not a source of formation of CP-7-1 and the crystallizations of Steps 1 and 2 demonstrate slight purge of this impurity and downstream analog CP-9-1. Therefore, the design space proposed for Step 1 is very much in control of the formation of CP-7-1; well within the critical quality attribute specification limit set for CP-9-1 of 1.0% set in Sakuramil drug substance.

- 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.

Trisodium Phosphate and/or Sodium Carbonate are non critical parameters as shown below.

- For the phosphate series, the most significant factor for the formation of ethyl homolog appears to be the amount of base and concentrations (volumes of THF). Low levels of base and high concentration (low volumes) lead to higher impurity levels. The amount of ethyl chloroformate present in the solution does not statistically affect the level of ethyl homolog.

- For the carbonate series, all three factors are important with the cross interaction term between ethyl chloroform and base to play the biggest role. In general the low amount of carbonate, high concentrations, at high ethyl chloroformate levels lead to increased amount of ethyl homolog. Carbonate appears to generate larger amounts of ethyl homolog under the extreme conditions. However at the standard condition of 2.5 eq ECF, the reaction is extremely clean through out the design space.

- **It should be noted that the highest ethyl homolog values (~0.3%) were obtained after holding at reflux for 36 hours, yet some of these reactions were essentially complete at less than 6 hours.** So scale and equipment time differences are not factors for controlling the generation of the ethyl homolog.

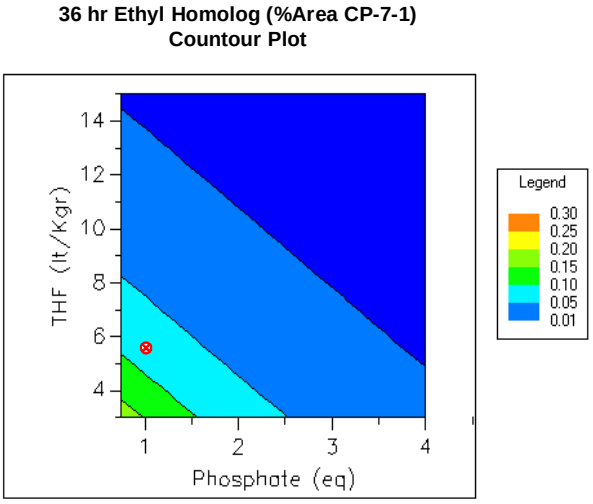


Figure 2.3.S.2.6-14 Phosphate: Contour Plots of Ethyl Homolog at 36 hours (Red Dot Standard Conditions)

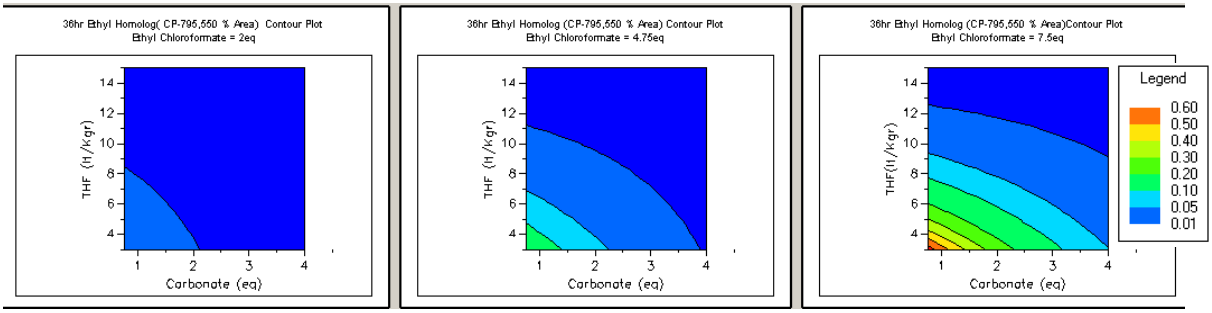


Figure 2.3.S.2.6-15 Carbonate: Contour Plots of Ethyl Homolog at 36 hours

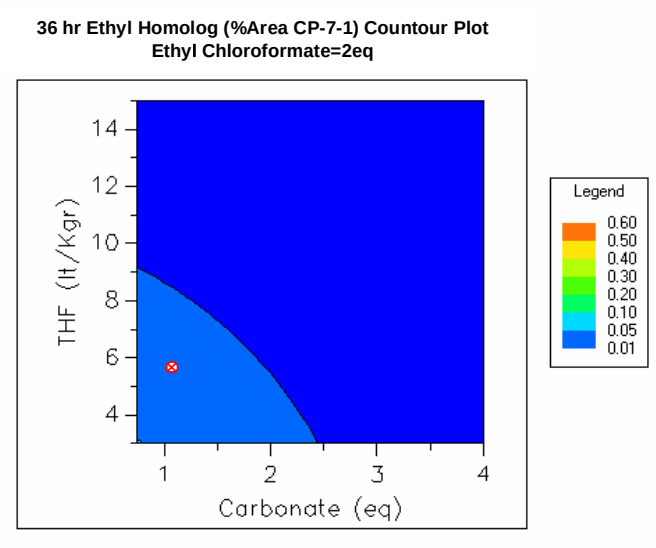


Figure 2.3.S.2.6-16 Carbonate: Contour Plots at 36 hours for the Standard Conditions

Conclusion for "Other" Impurities

- For all experiments the total impurities ranged from 0.3 to 0.4 % taken as area % by HPLC in the reaction mixture after 36 hours. 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.

Additional Observations:

- The standard operating conditions for both bases, in general, generate the highest level of "other" impurities within the design space. This suggests that the standard workup condition should be sufficient purging the other impurities through out the design space.
- Phosphate experiments: Based on the analysis, the level of other impurity does depend strongly on concentration. In general the level of other impurities decreases with a high amount of base and low levels of ethyl chloroformate.
- Carbonate experiments: In general, less concentrated reactions lead to cleaner product.
- Overall the carbonate series appear to be cleaner than phosphate.

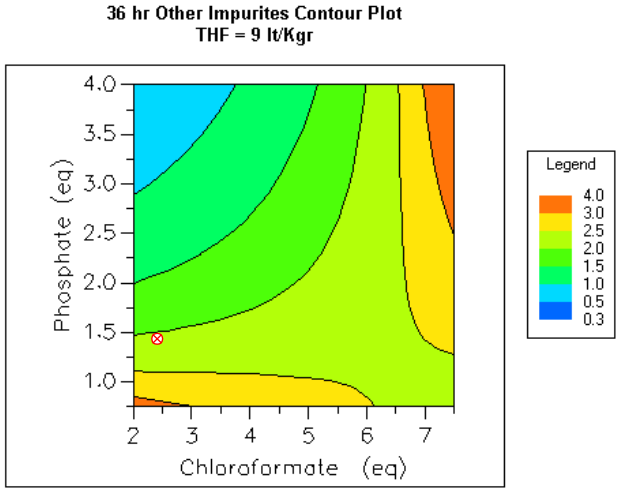


Figure 2.3.S.2.6-17 Phosphate: Contour Plots of "Other" Impurities at 36 hours at 9 Volumes THF

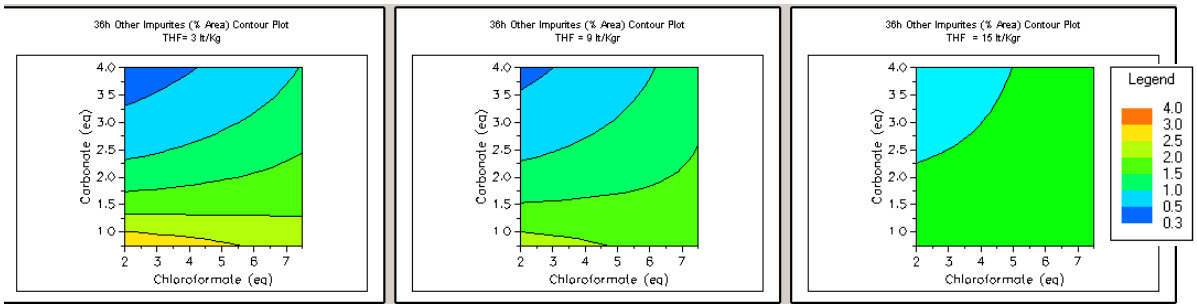


Figure 2.3.S.2.6-18 Carbonate: Contour Plots of "Other" Impurities at 36 hours

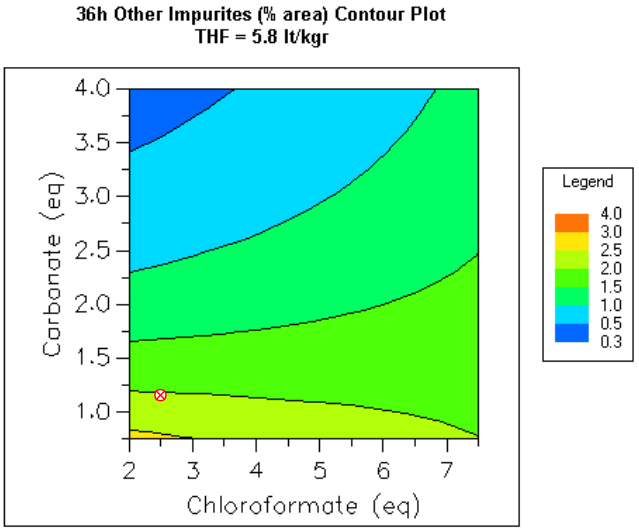


Figure 2.3.S.2.6-19 Carbonate: Contour Plots of "Other" Impurities at 36 hours at Standard THF-Volumes

5)-1-1-2 Multivariate designs for Step 1 crystallization

For the crystallization DoE, the experimental design and analytical strategy required isolation and testing of CP-7. The same measurable responses from the Step 1 reaction of assay and impurity levels were collected and evaluated as well. To eliminate input variability, a crude reaction product that had non typical, higher levels of the ethyl homolog impurity was selected to make it easier to observe and evaluate the impact of the process parameters on the purge for this specific impurity (ethyl homolog CP-7-1). The parameters and ranges for the experimental design are provided in the table below. Ranges were selected based on prior knowledge, realistic manufacturing operability, and desired design space flexibility.

Scale and equipment consideration: 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.

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

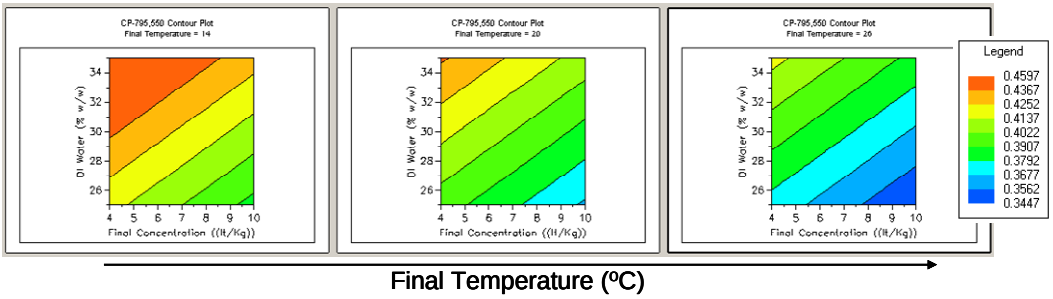


Figure 2.3.S.2.6-20 Ethyl Homolog Levels in Step 1 Crystallization

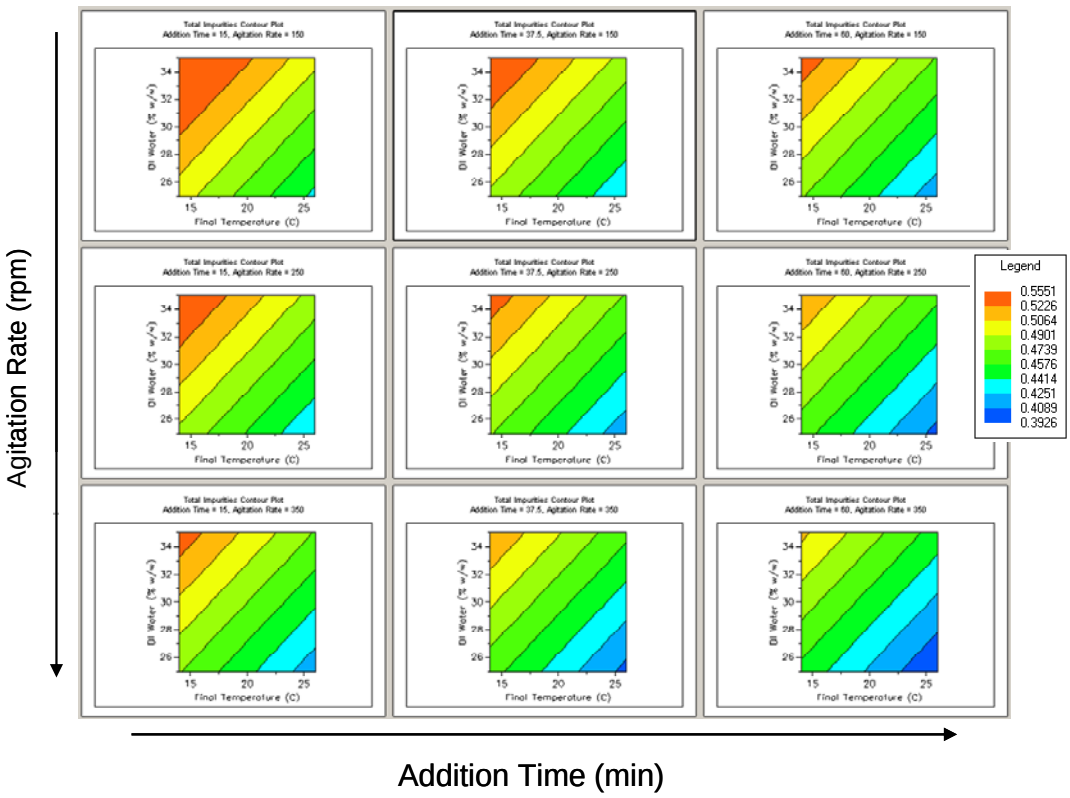


Figure 2.3.S.2.6-21 Total Impurities (%)

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)

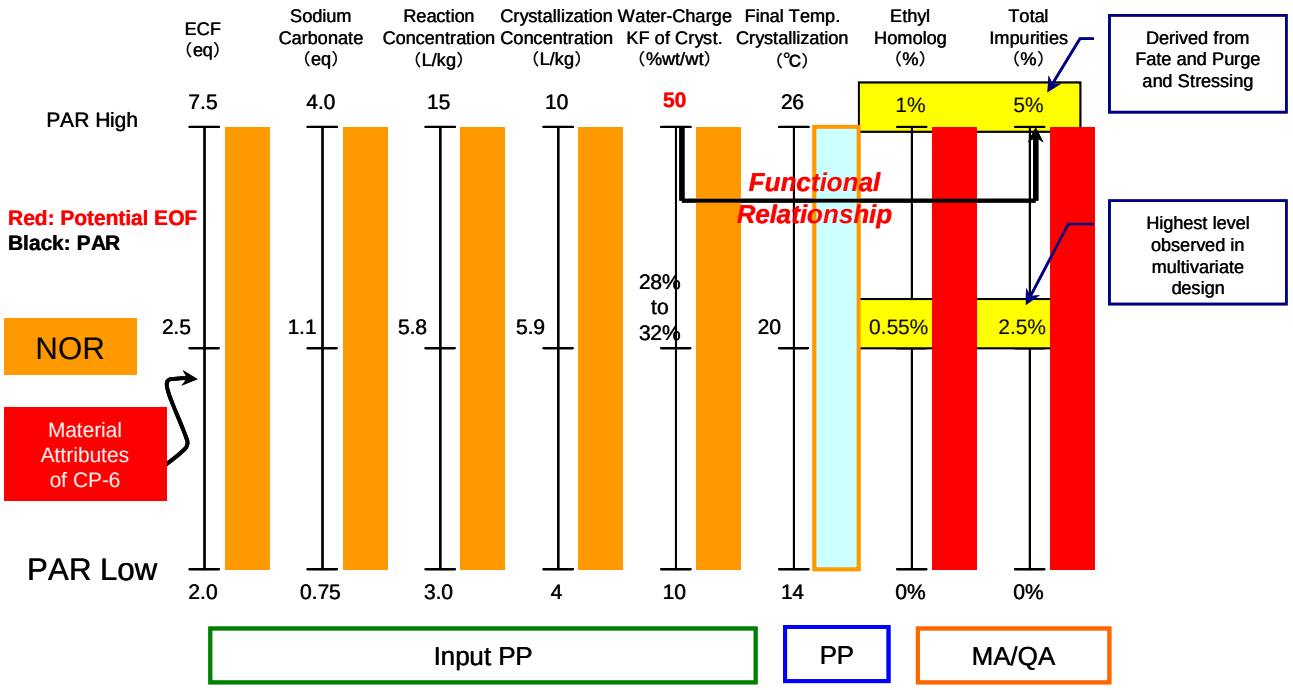
Table 2.3.S.2.6-11 summarizes the output from the multivariate analysis for Step 1 reaction and crystallization.

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 ≥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.



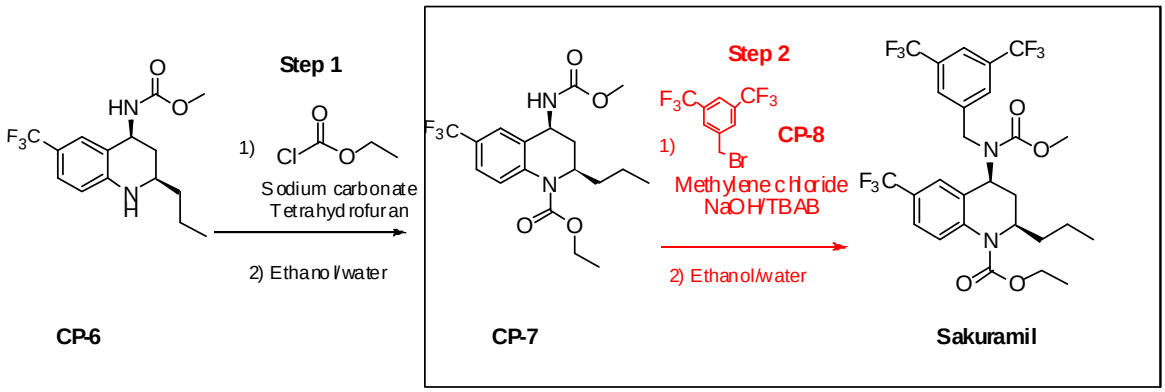
The black arrow shows the functional relationship between QA and PP.

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.

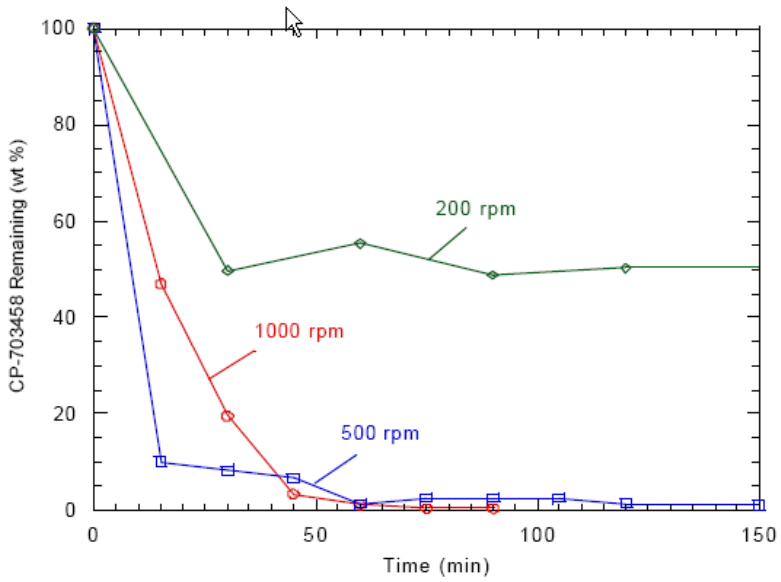
Reaction:

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.

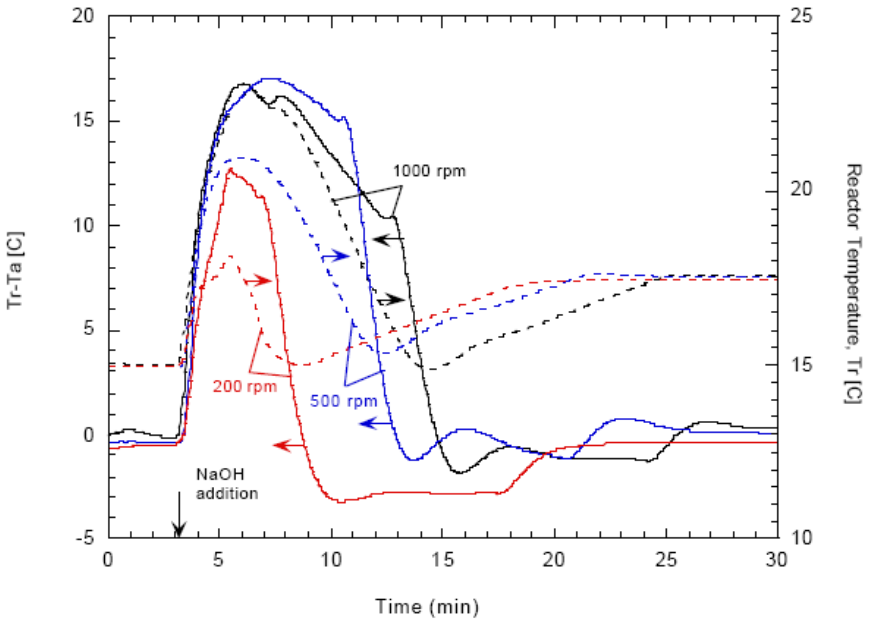
Scale and equipment consideration: (Prior to multivariate design to establish the design space)

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 (≤ 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.

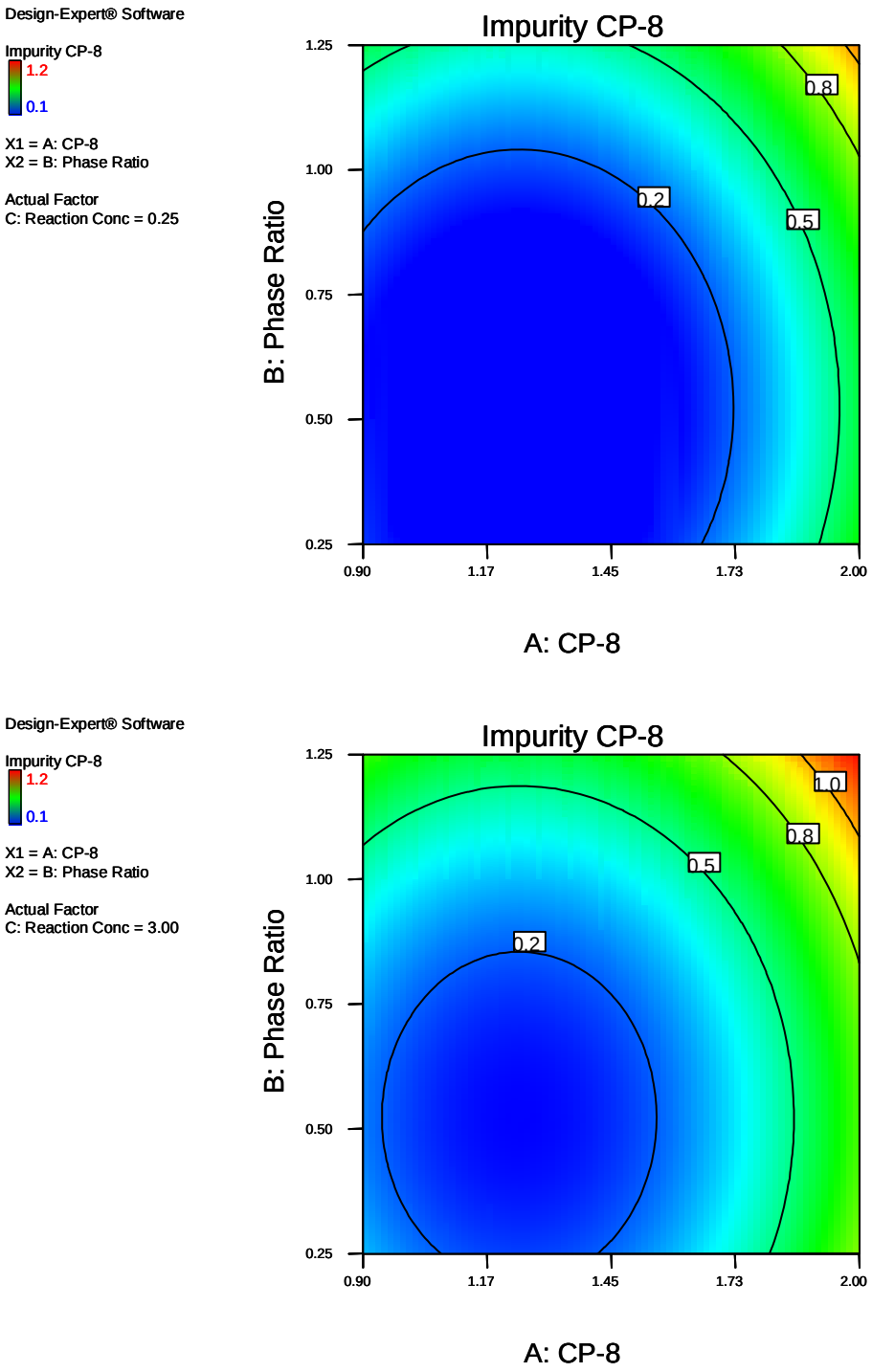


Figure 2.3.S.2.6-25 Relation between Remaining CP-8 and Process Parameters

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 (≤ 0.3%) + CP-5 and CP-3 (≤ 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).

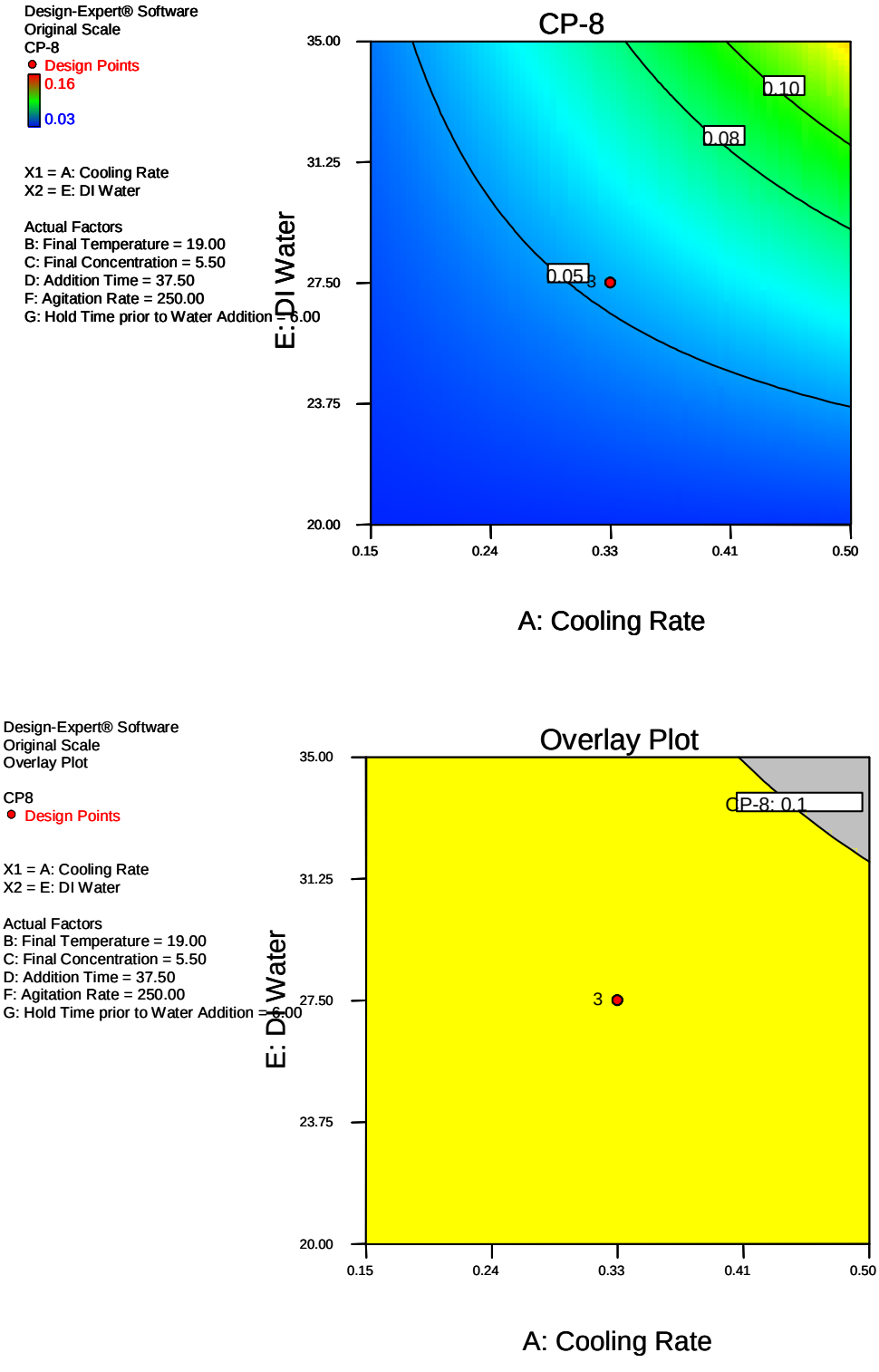


Figure 2.3.S.2.6-26 Relation between Remaining CP-8 and Crystallization Process Parameters

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.

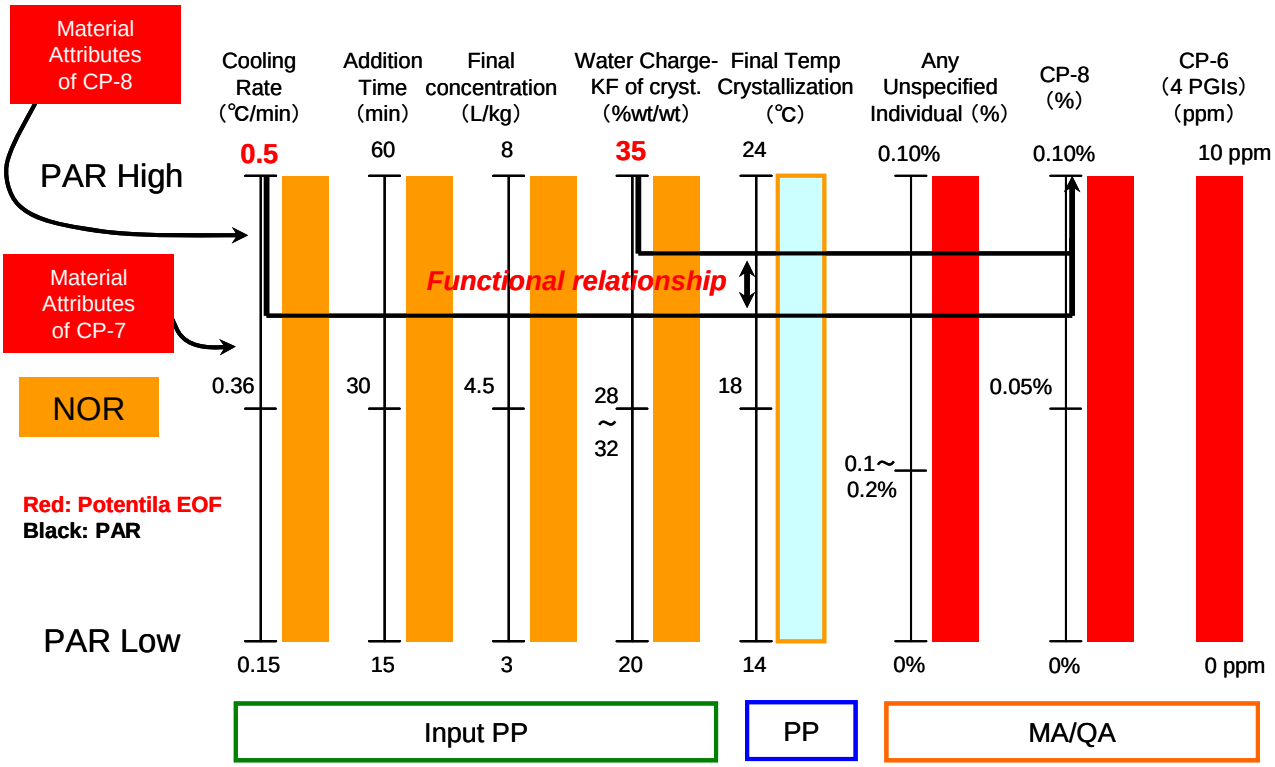
Table 2.3.S.2.6-15 Summary of Multivariate Analysis Results for Step 2

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 iresidual 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 ≤1.0% in drug substance	• Design Space for Step 1 (parametric control) • Specification in CP-7 of ≤ 1% to be used when appropriate. o 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.	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. No edges of failure were identified in the Step 1 design space. This is a very robust process. No CPP's identified for the reaction of Step 1.
Total impurities NMT 5% (Step 1, intermediate MA) And unspecified impurities of ≤0.10% (Step 2, DS CQA) *Intermediate MA & DS CQA's are functionally related*	• Specification for total impurities in Step 1 of ≤ 5% • Specification for unspecified impurities of ≤ 0.10% each in drug substance.	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; • The % water for the crystallizations in Step 1 and Step 2 at the high level are CPP's. o 50% water Step 1 CPP o 35% water Step 2 CPP
CP-8 of ≤0.10% in drug substance	• RTRt: Primary control: PAT: ≤ 1.2% of remaining CP-8 at the reaction completion. o In the event of PAT failure o HPLC can be used as well for determining the level of CP-8 of 1.2% as an in-process analysis o HPLC in drug substance of ≤ 0.10%	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.

	• Crystallization of Step 2 (parametric RTRt): if CP-8 is ≤ 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	• 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): o CP-4 (0.3%) o 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	• Specification in CP-6 o ≤ 1% of enantiomers o ≤ 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:

1. Step 1 is not scale and/or equipment dependent. Changes in scale and equipment will be managed in the quality systems
2. 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.

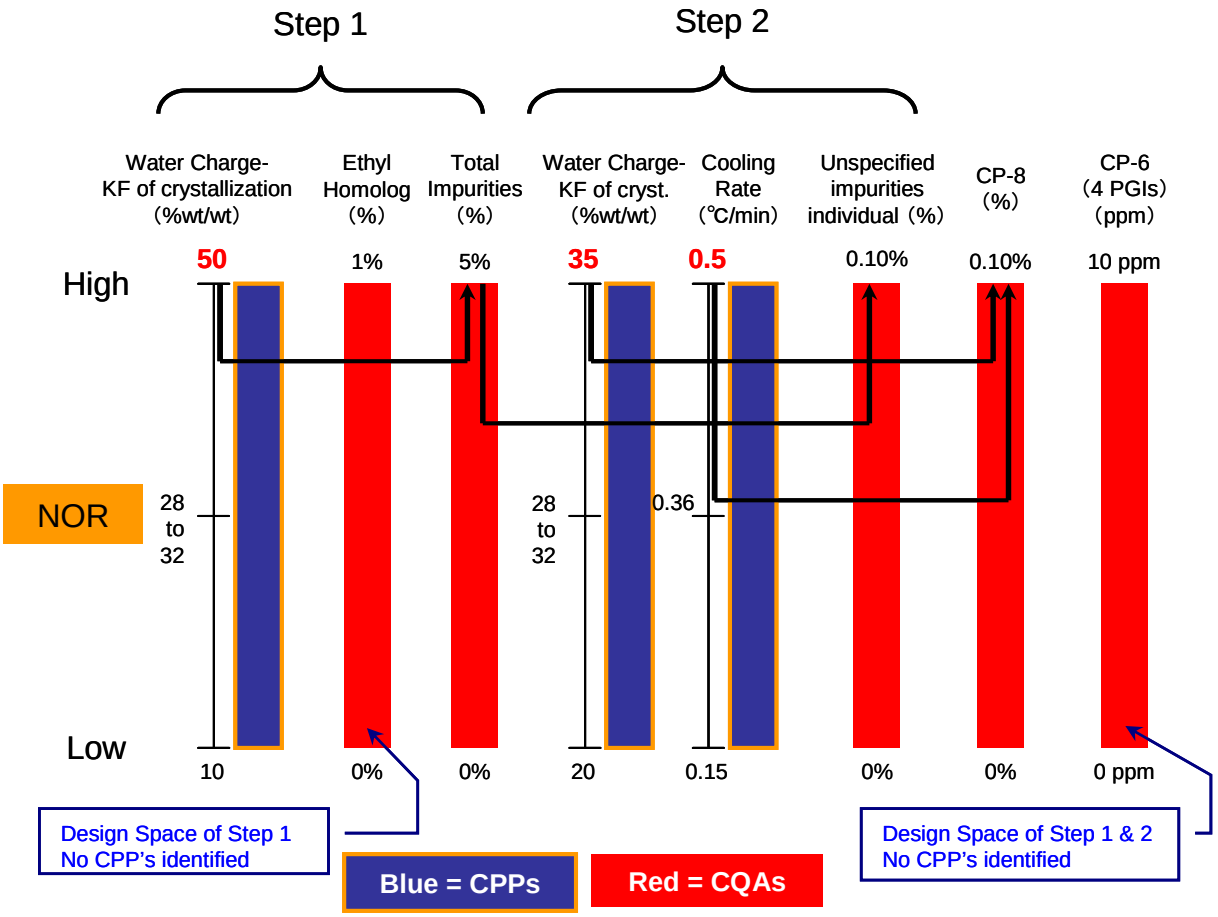
Important Material Attributes: See justification for moderate and low risk matters above in Starting Material Sections.

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

Important MA	Specification	Justification
SM CP-6:		
CP-4	≤ 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	≤ 0.1%	
CP-3	≤ 0.1%	
SM CP-8:		
CP-8-25I	≤ 0.05%	Guarantees ≤ 0.10% in drug substance
CP-8-24I	≤ 0.05%	Guarantees ≤ 0.10% in drug substance

[1] Fate and purge supports up to 1%.

CPP Identified in the Overall Design Space



Black Arrow(→): CPP Functional relationship to CPP's and CQA's

PGIs : Potentially genotoxic impurities

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_2Cl_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.

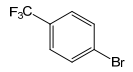
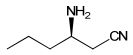
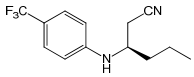
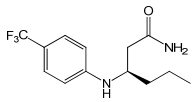
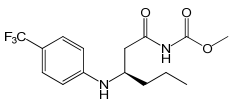
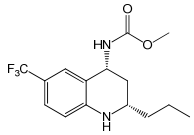
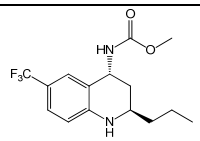
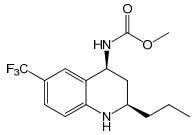
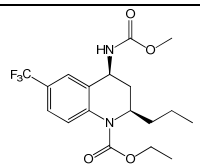
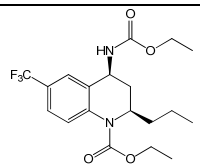
b) Skip testing will be applied.
Test will be conducted every 25th batch or one batch every year, whichever more frequent.

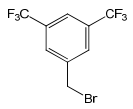
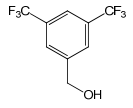
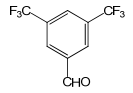
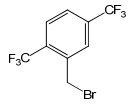
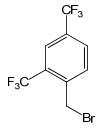
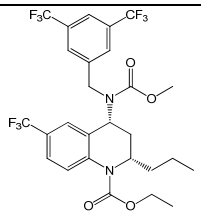
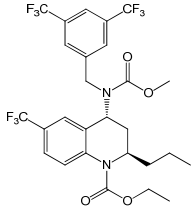
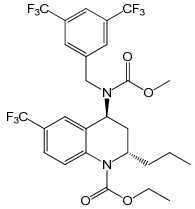
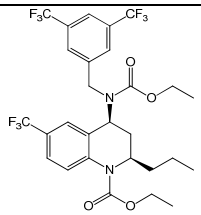
2.3.S.4.5 Justification of Specification

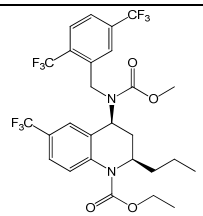
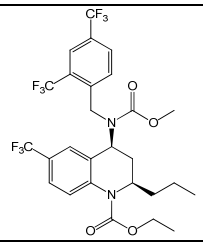
Table 2.3.S.4.5-1 Summary of Control Strategy for Sakuramil (Abstract)

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
- *n*-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		*In situ* 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		*In situ* 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

CP-8		Starting material	Alkyl halide functional group based genotoxicity aerting structure. Negative in the Ames Assay.	Control in accordance with ICH Q3A
CP-8-OH		By-product of CP-8	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-8-CHO		By-product of CP-8	No genotoxicity aerting structure.	Control in accordance with ICH Q3A
CP-8-25I		By-product derived from impurity in raw material of CP-8	Alkyl halide functional group based genotoxicity alerting structure. Common structure with CP-8.	Control in accordance with ICH Q3A
CP-8-24I		By-product derived from impurity in raw material of CP-8	Alkyl halide functional group based genotoxicity alerting structure. Common structure with CP-8.	Control in accordance with ICH Q3A
CP-9-E		Enantiomer of Sakuramil drug substance	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-9-D1		Diastereomer 1 of Sakuramil drug substance	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-9-D2		Diastereomer 2 of Sakuramil drug substance	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-9-1		Ethyl homolog of Sakuramil drug substance	No genotoxicity alerting structure.	Control in accordance with ICH Q3A

CP-9-2		2,5-Regioisomer of trifluoromethyl group	No genotoxicity alerting structure.	Control in accordance with ICH Q3A
CP-9-3		2,4-Regioisomer of trifluoromethyl group	No genotoxicity alerting structure.	Control in accordance with ICH Q3A

Appendix-2 Example of Description of Manufacturing Process in Application Form

Step 1 (Critical Step) (Reaction[1)], Extraction, Purification[2)], Phase Separation, and Drying)

Methyl (2*R*,4*S*)-2-propyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinoline-4-ylcarbamate (CP-6) [1] 『(230 kg)』, tetrahydrofuran 『(1300 L)』, sodium carbonate 『(42.4 kg)』 are combined. Ethyl chloroformate "158~592 kg" is added and the mixture is heated at temperature up to reflux. The mixture is filtered, and the filtrate is quenched with a "50%"[2)] sodium hydroxide solution. To the mixture, *n*-hexane is added and stirred, and the layers are settled and separated. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration 『(1400 L)』). Water ("25 to 35%" weight per weight of ethanol) is added and the mixture is stirred at 『20°C』. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 『42.5°C』 to yield Ethyl (2*R*,4*S*)-2-propyl-4-(methoxycarbonylamino)-6-(trifluoromethyl)-3,4-dihydroxyquinoline-1(2*H*)-Carboxylate (CP-7) [2] (product 253 kg, yield 89%).

1) Ethyl chloroformate quantity, tetrahydrofuran volume and sodium carbonate or trisodium phosphate, dodecahydrate are parameters establishing Design Space which control quantity of CP-7-1.
2) Water quantity relative to ethanol quantity, ethanol volume and crystallization temperature are parameters establishing Design Space which control quantity of total impurities.

Step 2 (Critical Step) (Reaction[3)], Extraction, Purification[4)], Phase Separation, and Drying)

CP-7 [2] 『(250 kg)』 from Step 1 and 3,5-bistrifluoromethylbenzyl bromide (CP-8) 『(215 kg)』 are combined in methylene chloride 『(750 L)』. Tetra-*n*-butylammonium bromide 『(50 kg)』 and "50%"[2)] aqueous sodium hydroxide solution 『(750 L)』 are added and stirred, and then methylene chloride and water are added and stirred. The mixture obtained is settled and the layers are separated. The organic layer is washed with diluted hydrochloric acid. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration 『(1800 L)』). Water (20 to 35% weight per weight of ethanol) is added, and then the mixture is cooled at the rate of 0.15 to 0.5°C per minute, followed by stirring at 『18°C』. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 『42.5°C』 to yield Ethyl (2*R*,4*S*)-4-{[3,5-bis(trifluoromethyl)benzyl](methoxycarbonyl)amino}-2-propyl-6-(trifluoromethoxy)-3,4-dihydroquinoline-1(2*H*)-carboxylate [3] (Sakuramil) (product 360 kg, yield 90%).

3) Quantity of 3,5-bistrifluoromethylbenzyl bromide (CP-8), volume of methylene chloride and volume of aqueous sodium hydroxide solution are parameters establishing Design Space which control quantity of residua l CP-8.
4) Ethanol volume, quantity of water relates to ethanol, cooling rate and cooling temperature are parameters establishing Design Space which control quantity of residual CP-8.

Step 3 (Packaging)

Sakuramil drug substance [3] is packaged in polyethylene bags, closed with a tie-wrap, which is then stored in “fiber drums”.

Alternative manufacturing process

In Step 1, trisodium phosphate, dodecahydrate 『(101.4 kg)』 [1)] can be used instead of sodium carbonate 『(42.4 kg)』 [1)] as alternative base.

Appendix-3 Reference Information: Manufacturing Method in Application Form

Reference only

Step 1 (Critical Step) (Reaction[1)], Extraction, Purification[2)], Phase Separation, and Drying)

Methyl (2*R*,4*S*)-2-propyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinoline-4-ylcarbamate (CP-6) [1] 『(230 kg)』[Note 1)], tetrahydrofuran 『(1300 L)』[Note 1)], sodium carbonate 『(42.4 kg)』[Note 1)] are combined. Ethyl chloroformate "158~592 kg"[Note 2)] is added and the mixture is heated at temperature up to reflux. The mixture is filtered, and the filtrate is quenched with a "50%"[Note 3)] sodium hydroxide solution. To the mixture, *n*-hexane is added and stirred, and the layers are settled and separated. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration 『(1400 L)』[Note 1)]). Water "25 to 35%"[Note 4)] weight per weight of ethanol) is added and the mixture is stirred at 『20°C』[Note 3)]. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 『42.5°C』[Note 3)] to yield Ethyl (2*R*,4*S*)-2-propyl-4-(methoxycarbonylamino)-6-(trifluoromethyl)-3,4-dihydroxyquinoline-1(2*H*)-Carboxylate (CP-7) [2] (product 253 kg, yield 89%).

1) Ethyl chloroformate quantity, tetrahydrofuran volume and sodium carbonate or trisodium phosphate, dodecahydrate are parameters establishing Design Space which control quantity of CP-7-1.
2) Water quantity relative to ethanol quantity, ethanol volume and crystallization temperature are parameters establishing Design Space which control quantity of total impurities.

Step 2 (Critical Step) (Reaction[3)], Extraction, Purification[4)], Phase Separation, and Drying)

CP-7 [2] 『(250 kg)』[Note 1)] from Step 1 and 3,5-bistrifluoromethylbenzyl bromide (CP-8) 『(215 kg)』[Note 1)] are combined in methylene chloride 『(750 L)』[Note 1)]. Tetra-*n*-butylammonium bromide and "50%"[Note 3)] aqueous sodium hydroxide solution 『(750 L)』[Note 1)].are added and stirred, and then methylene chloride and water are added and stirred. The mixture obtained is settled and the layers are separated. The organic layer is washed with diluted hydrochloric acid. The organic layer is concentrated by distillation with ethanol for the solvents exchange (final concentration 『(1800 L)』[Note 1)]). Water (20 to 35% weight per weight of ethanol) is added, and then the mixture is cooled at the rate of 0.15 to 0.5°C per minute, followed by stirring at 『18°C』[Note 5)]. The resulting crystalline precipitates are separated, rinsed with ethanol, and dried at 『42.5°C』[Note 5)] to yield Ethyl (2*R*,4*S*)-4-{[3,5-bis(trifluoromethyl)benzyl](methoxycarbonyl) amino}-2-propyl-6-(trifluoromethoxy)-3,4-dihydroquinoline-1(2*H*)-carboxylate [3] (Sakuramil) (product 360 kg, yield 90%).

3) Quantity of 3,5-bistrifluoromethylbenzyl bromide (CP-8), volume of methylene chloride and volume of aqueous sodium hydroxide solution are parameters establishing Design Space which control quantity of residua l CP-8.
4) Ethanol volume, quantity of water relates to ethanol, cooling rate and cooling temperature are parameters establishing Design Space which control quantity of residual CP-8.

Step 3 (Packaging)

Sakuramil drug substance [3] is packaged in polyethylene bags[Note 6)], closed with a tie-wrap, which is then stored in "fiber drums"[Note 7)].

Alternative manufacturing process

In Step 1, trisodium phosphate, dodecahydrate 『(101.4 kg)』[Note 1)] can be used instead of sodium carbonate 『(42.4 kg)』[Note 1)] as alternative base.

Note 1) Scale dependent values, minor notification matter
Note 2) This quantity is one of parameters establishing Design Space. This parameter is critical, however, risk affecting on DS CQA is low through control strategy established to operate process parameter good enough within the specified range. In consequence, this parameter is defined as medium risk and described as range of notification.
Note 3) Target/Set values (Range is noted and controlled in MBR and SOP)
Note 4) This quantity is one of parameters establishing Design Space. This parameter is critical, however, risk affecting on DS CQA is low through control strategy established to operate process parameter good enough within the specified range. In consequence, this parameter is defined as medium risk and described as range of notification.
Note 5) Temperature is target/set value (Range is described/controlled in MBR/SOP)
Note 6) Material of primary container is described.
Note 7) Secondary container to ensure stability is described

Appendix-4

The flow diagram showing the outline of manufacturing process development indicated in the chapter of the background of manufacturing process development for Sakuramil drug substance (2.3.S.2.6) is provided below. This flow diagram starts from the timepoint of decision of "manufacturing methods for the application," and the following items implemented during the period from early stage development to the decision of manufacturing the manufacturing methods are described as "Prior Knowledge & Experience" : investigation results; risk assessment; change of manufacturing process; selection of the starting materials, etc.

In the development of manufacturing process for Sakuramil drug substance, all elements of so-called QbD approach described as a "more enhanced approach" in ICH Q11 are included.

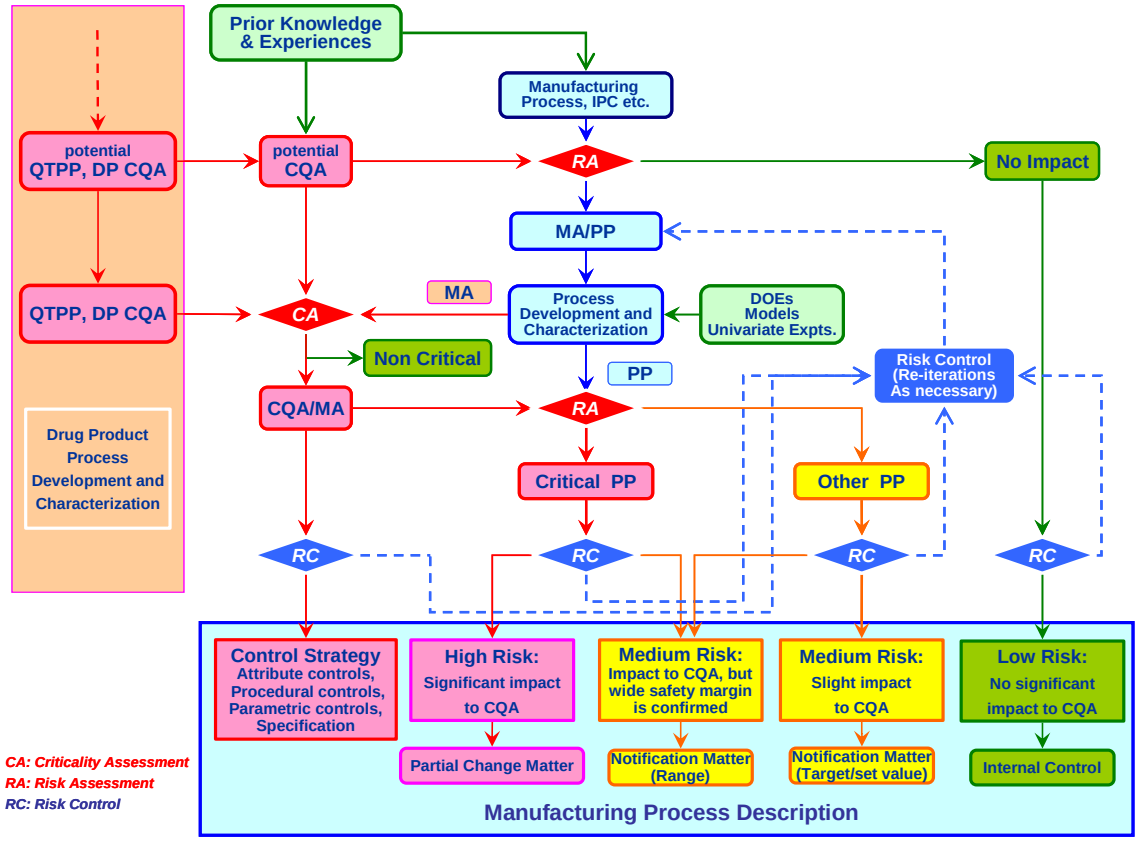


Figure Flow diagram of the outline of manufacturing process development for drug substances

Note: In this flow diagram, we used the terms: Criticality Assessment (CA) for the assessment of Quality Attribute (QA) and Material Attribute (MA). These terms are used for clearly distinguish these from Risk Assessment (RA) based on the concept of POINT TO CONSIDER (*R2*) created by ICH Q-IWG that "Quality Attribute criticality is primarily based upon severity of harm and does not change as a result of risk management."

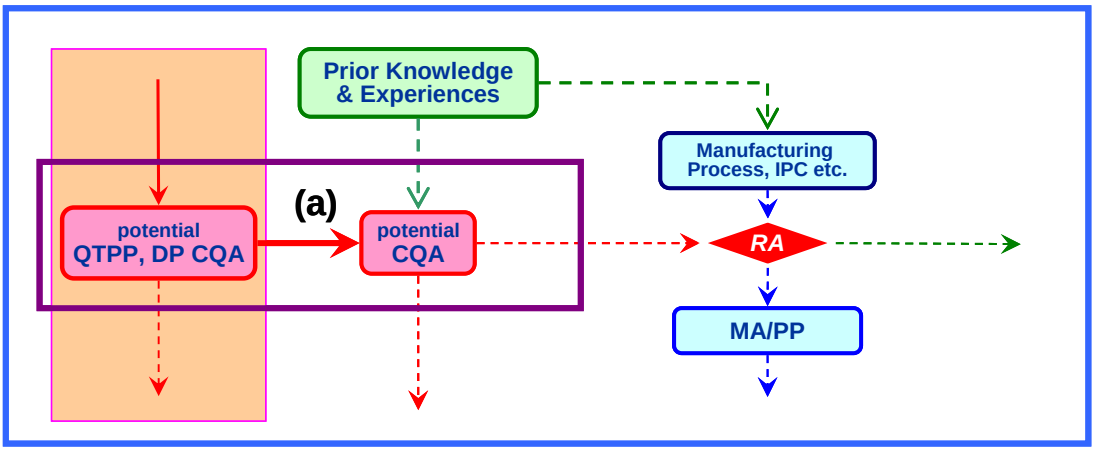
The chapter of the background of manufacturing process development (2.3.S.2.6) in Sakuramil S2 Sample is made up by the following 6 sections, and we indicate the relationship between the content of these sections and the flow diagram of overview in the following.

2.3.S.2.6 Background of manufacturing process development

1) Critical Quality Attribute (CQA) expected for Sakuramil drug substance.

This section corresponds to (a) in the below flow diagram of the outline of manufacturing process development for drug substances.

In this section, the process to specify the expected CQA of drug substance from Quality Target Product Profile (QTPP) of drug product is described. Since Sakuramil drug substance is an insoluble compound, it is formed as tablet after making spray-dry dispersed in-process materials by dissolving the drug substance in manufacturing process. Therefore, physical attributes (crystalline form, particle size distribution) of Sakuramil drug substance have no impact on drug products; and hence the focal point for the development of manufacturing process for Sakuramil drug substance is the control of impurities.

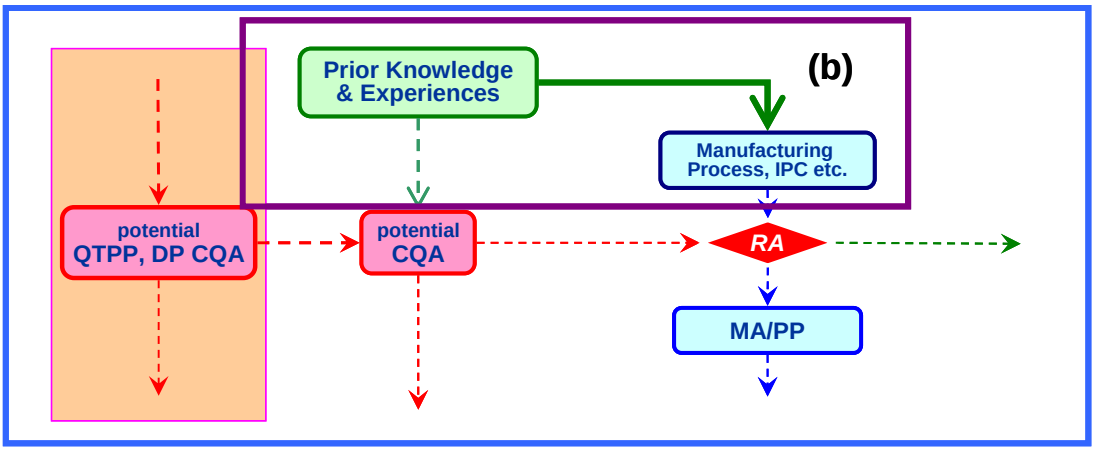


The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

2) Background of development

This section corresponds to (b) (expressed as “Prior Knowledge”) in the below flow diagram of the outline of manufacturing process development for drug substances.

In Sakuramil S2 Sample, we itemize tasks (disadvantages) in each route from manufacturing methods in early development (Route A) through to manufacturing methods for the application (Route C), and indicate how those tasks were improved.

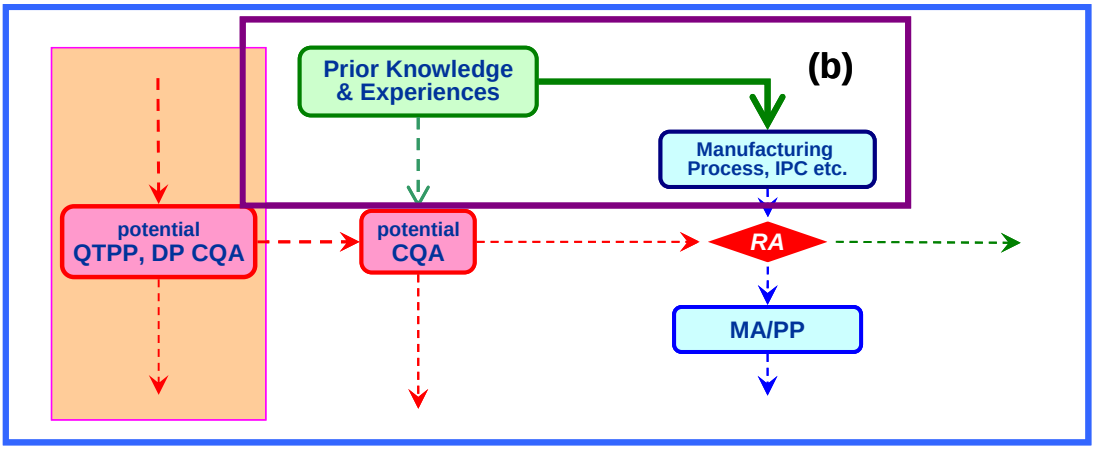


The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

3) Validity of starting materials and selection of manufacturing methods for commercial production

This section corresponds to (b) (expressed as “Prior Knowledge”) in the below flow diagram of the outline of manufacturing process development for drug substances.

Regarding the validity of specifying 2 final reaction processes as the manufacturing methods for commercial production of Sakuramil drug substance, as well as the validity of selecting CP-6 and CP-8 as the starting materials, we discuss the rationale of setting control items/cction limits by reflecting the results of Risk Assessment (RA) for Material Attributes (MA).



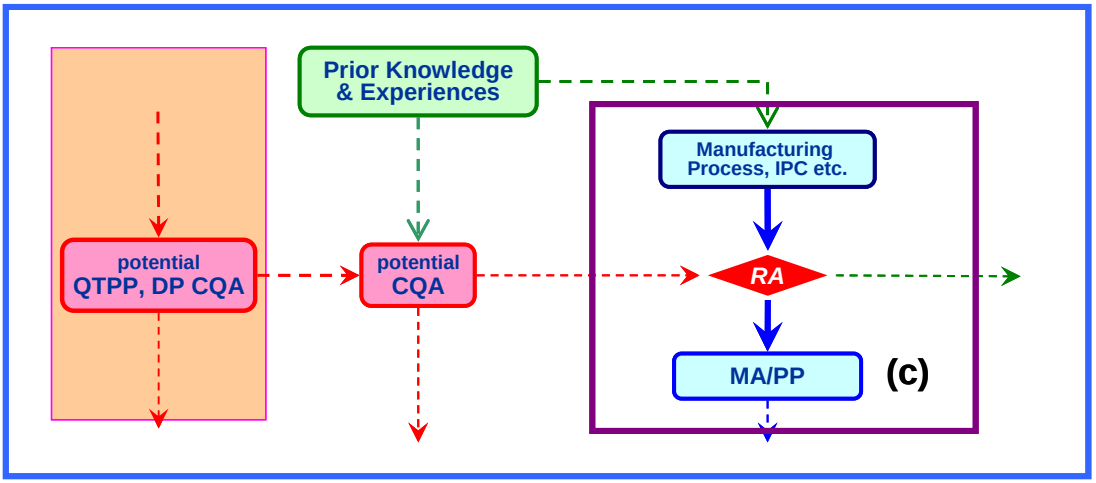
The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

4) Risk assessment for the development of knowledge space and control strategies

This section corresponds to (c) in the below flow diagram of the outline of manufacturing process development for drug substances.

To specify the impact of manufacturing process on Sakuramil drug substance CQA (impurities), we conducted Risk Assessment (RA) for the impact of manufacturing process on drug substance CQA. Further, in order to specify the manufacturing process parameter (PP) which affects drug substance CQA, we divide manufacturing process into unit operations (focus area) and specify the unit operation (focus area) where critical impurities left in the drug substance (drug substance CQA) produce/are introduced/are eliminated by RA.

In addition, there are many cases where RA is implemented repeatedly according to process investigations conducted multiple times, and MA and PP of intermediates are specified by that. In this section, to avoid the scheme to become complicated, we show the one case.



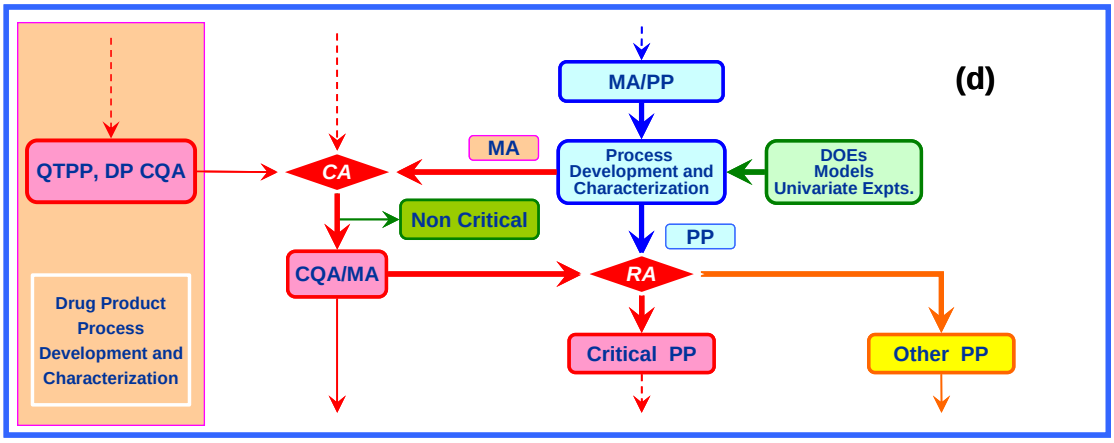
The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

5) Design space of unit operations of each step in drug substance manufacture

This section corresponds to (d) in the below flow diagram of the outline of manufacturing process development for drug substances.

Regarding the focus area (Step 1 and Step 2 of reaction process and crystallized process) specified by risk assessment (RA), we investigate the impact of process parameter (PP) on quality attribute/material attribute by the multivariate design of experiments, and show the investigation result including the establishment of Design space/Knowledge space. Also, we briefly describe the investigation result concerning the experiments assuming the worst case scenario, experiments for addition, scale effect, etc. Further, we conduct RA of criticality for each unit operation (focus area) from the obtained results, and consider Critical Process Parameter (CPP).

We conducted RA for the investigation result on the design of experiments (DOE), and judged PP as CPP in cases where the variation of PP is related to the variation of drug substance CQA with statistical/functional significance, and at the same time, PP has negative impact on drug substance CQA when it is varied within the realistically assumable range. Also, we judged PP as other PP in cases where PP has no negative impact on drug substance CQA unless it is varied with an unrealistic range, as well as in cases where there is no relationship observed between the variation of Pp and drug substance CQA.

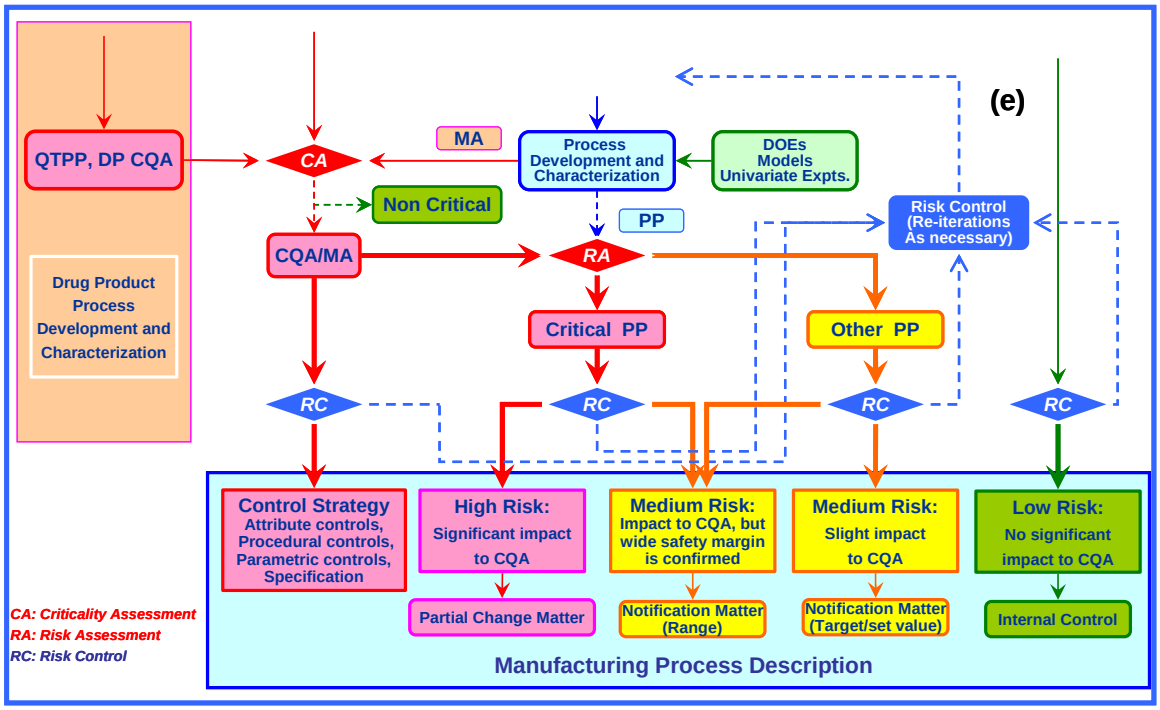


The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

6) Assessment of criticality of manufacturing process

This section corresponds to (e) in the below flow diagram of the outline of manufacturing process development for drug substances.

We considered the final RA results obtained from overall design space and control strategies for the specified CPP and critical quality attributes.



The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

Reference

ICH Q11 DEVELOPMENT AND MANUFACTURE OF DRUG SUBSTANCES

3.1.3 Approaches to Development

An enhanced approach to manufacturing process development would additionally include the following elements:

- Identifying potential CQAs associated with the drug substance so that those characteristics having an impact on product quality can be studied and controlled;
- Defining an appropriate manufacturing process;
- A systematic approach to evaluating, understanding and refining of the manufacturing process, including;
  - Identifying, through e.g., prior knowledge, experimentation and risk assessment, the material attributes (e.g. of raw materials, starting materials, reagemts, solvents, process aids, intermediates) and process parameters that can have an effect on drug substance CQAs;
  - Determining the functional relationships that link material attributes and process parameters to drug substance CQAs;
- Using the enhanced approach in combination with QRM to establish an appropriate control strategy which can, for example, include a proposal for a design space(s) and/or real-time release testing (RTRT).

3.2 Submission of Manufacturing Process Development Information

3.2.1 Overall Process Development Summary

- List of drug substance CQAs;
- Brief description of the stages in the evolution of the manufacturing process and control strategy;
- Brief description of the material attributes and process parameters identified as impacting drug substance CQAs;
- Brief description of the development of any design spaces.

Appendix-5

Regarding regulatory flexibility

In the end of "Introduction" in ICH Q11, regulatory flexibility is described as the following:

> ICH Q11 **DEVELOPMENT AND MANUFACTURE OF DRUG SUBSTANCES** (Excerpt from Introduction)
>
> Introduction
>
> ..... As discussed in ICH Q8 for drug product, a greater understanding of the drug substance and its manufacturing process can create the basis for more flexible regulatory approaches. The degree of regulatory flexibility is generally predicated on the level of relevant scientific knowledge provided in the application for marketing authorisation.

As some proposals expecting regulatory flexibility are also included in Sakuramil S2 Sample, we describe the outline of the proposals in the following. This needs to be understood that these proposals are expecting items discussed by Research on Regulatory Science of Pharmaceuticals and Medical Devices, and that not all applications are approved in the actual application, even though the supporting material for the application is the exactly equivalent to the sample, since the following elements are considered in the actual application: the reliability of methods and tools, the level of facilities conducted R&D, the reliability including the situation of quality risk management and quality system in accordance with ICH Q9 and Q10, etc, implemented by the applicants.

1) Description of manufacturing methods

We indicated the differences in the description of manufacturing methods between the sample and the conventional approach. When using the conventional approach, description of set/target values, etc. for all parameters are required in manufacturing methods of drug substance. We proposed that description of target/set values is not necessary for parameters in cases where the more enhanced approach was used in development such as the sample, since it is clear that these have no impact on quality.

Parameter	Target/Set value	Traditional approach	QbD approach	Justification of QbD
CP-6	230 kg	note	note	Impact on CP-7-1 generation
THF	1300 L	note	note	Impact on CP-7-1 generation
Na_2CO_3	42.4 kg	note	note	Impact on CP-7-1 generation

Ethyl chloroformate	206 kg	note	note	Impact on CP-7-1 generation
Reaction temperature	reflux	note	note	Impact on CP-7-1 generation
50% NaOH aq	1000 L	note	Not noted	No impact on quality
n-Hexane	700 L	note	Not noted	No impact on quality
Concentrated volume	2000 L	note	Not noted	No impact on quality
EtOH volume	1400 L	note	note	Minor impact on impurity residue
Quantity of Water addition	25 - 35%	note	note	Impact on impurity residue **(critical)**
Crystalization temperature	20°C	note	note	Minor impact on impurity residue
Drying Temperature	42.5°C	note	note	Possibility to produce degradation product

QbD approach: more enhanced approach

2) Proposal to specify PP described with range as items that can be changed by simply submitting a minor change notice

We indicate an example where PP described with range are specified as items that can be changed by simply submitting a minor change notice in the description of reference information of manufacturing methods (Attachment-2) in the application form.

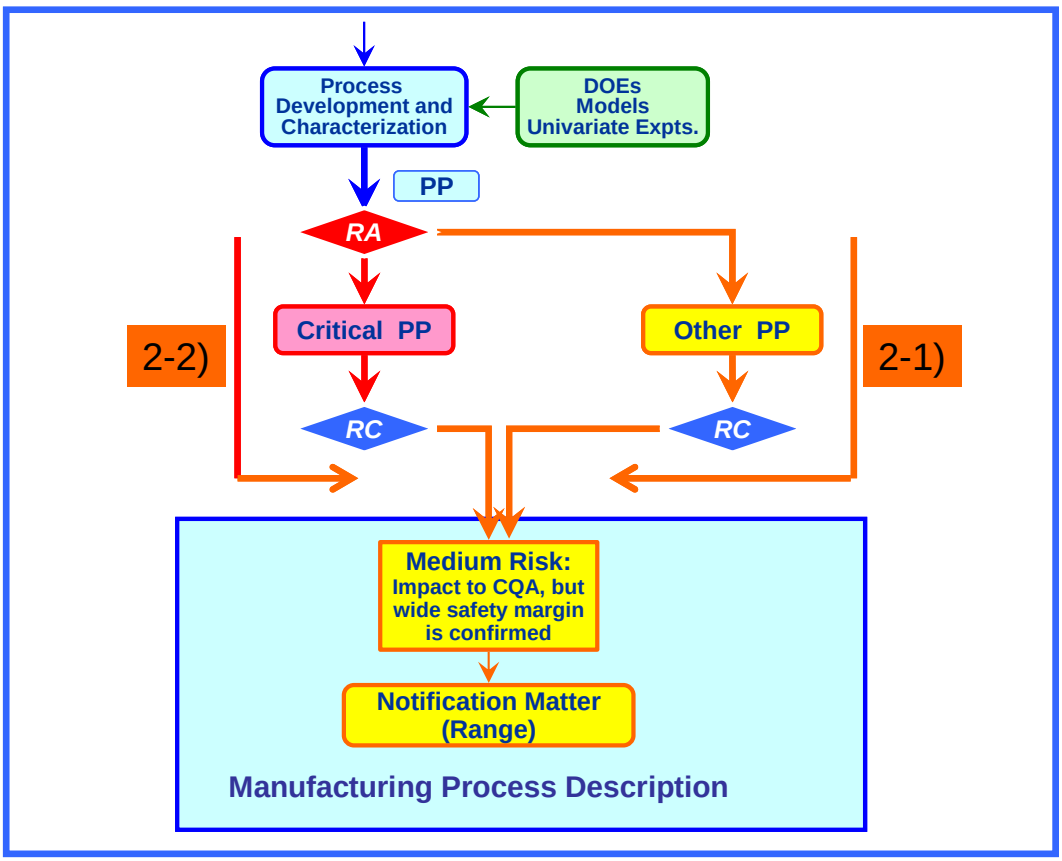
Different from established proven acceptable ranges (PAR) obtained from univariate experimentation, in this case, the impact of PP when it is varied is investigated by the research of DOE. The knowledge concerning the relationship between Edge of Failure (EOF) and PP has been deepened, and this can be considered that risk is sufficiently decreasing. However, as a matter of course, if PP is deviated from pre-determined range, even though deviation is within the range of DS determined by DOE, it is necessary to conduct verification of quality in accord with GMP specifications, and shipment of the products will not be allowed if the deviation is judged inappropriate as a result of verification.

2-1) Range description of other PP which is not specified as CPP

The amount of Ethyl chloroformate in reaction process in Step 1, when it is investigated by the multivariate design of experiments, although it has subtle impact on drug substance CQA, the production of impurities are less than one third of the determined specifications even when using excessive amount which is not used in normal manufacture, and there was no EOF observed in the investigated area. Based on the result, it can be judged that the amount of Ethyl chloroformate is not CPP but other PP ranked as medium risk. Therefore, we proposed the obtained design space as an item that can be changed by simply submitting a minor change notice which can be described with range.

2-2) Range description of CPP which risk is sufficiently decreased

By investigating the amount of water in crystallized process in Step 1 by the multivariate design of experiments, it was specified as CPP because it is statistically/functionally related to drug substance CQA. However, since its risk level is decreased to medium level by a control strategy of setting its area as smaller than the confirmed design space, we proposed it as an item that can be changed by simply submitting a minor change notice.



The corresponding part in the flow diagram of the outline of manufacturing process development for drug substances

3) Specifications of drug substance

We indicated the differences in the setting of specifications of drug substance between the sample and the conventional approach. When using the conventional approach, specified drug substance CQA is set as specifications of drug substance, and tested in drug substance. But when the more enhanced approach was used in development, its relationship with manufacturing PP/material attribute can be clarified. Therefore, we proposed to minimize the specifications set for drug substance by adopting the followings: control using design space, use of in-process control test results, upstream control of material attributes, etc. of starting material and intermediates, etc.

DS CQA	limit	Is CQA tested on drug substance/ included in drug substance specification		QbD Justification
		Traditional approach	QbD approach	
Related Substances (1)				
CP-9-1	≤ 1.0%	Yes/Yes*	No/Yes	DS in Step 1
CP-8	≤ 0.10%	Yes/Yes	No/Yes	DS in Step 2
Related Substances (2)				
Other（each）	≤ 0.10%	Yes/Yes	Yes/Yes	
Total impurities	≤ 0.5%	Yes/Yes	Yes/Yes	
GTIs				
CP-6	≤ 10 ppm	Yes/Yes	Yes/Yes	
Total (CP-3, -4, -5, -6)	≤ 25 ppm	Yes/Yes	No/Yes	DS in Step 2
Residual solvent				
Ethanol	≤ 5000 ppm	Yes/Yes	No/Yes	IPC in Step 2： LOD ≤ 0.40%
Tetrahydrofuran	≤ 720 ppm	Yes/Yes	No/No	After Step 1, this is removed significantly than that of conc limit in Q3C through manufacturing process.
n-Hexane	≤ 290 ppm	Yes/Yes	No/No	After Step 1, this is removed significantly than that of conc limit in Q3C through manufacturing process.
Dichloromethane	≤ 600 ppm	Yes/Yes	Yes/Yes	（Propose skip test）
Assay	98 - 102%	Yes/Yes	Yes/Yes	

QbD methodology：more advanced approach

*CQA is tested in DS or not/CQA is included in DS specification.