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.

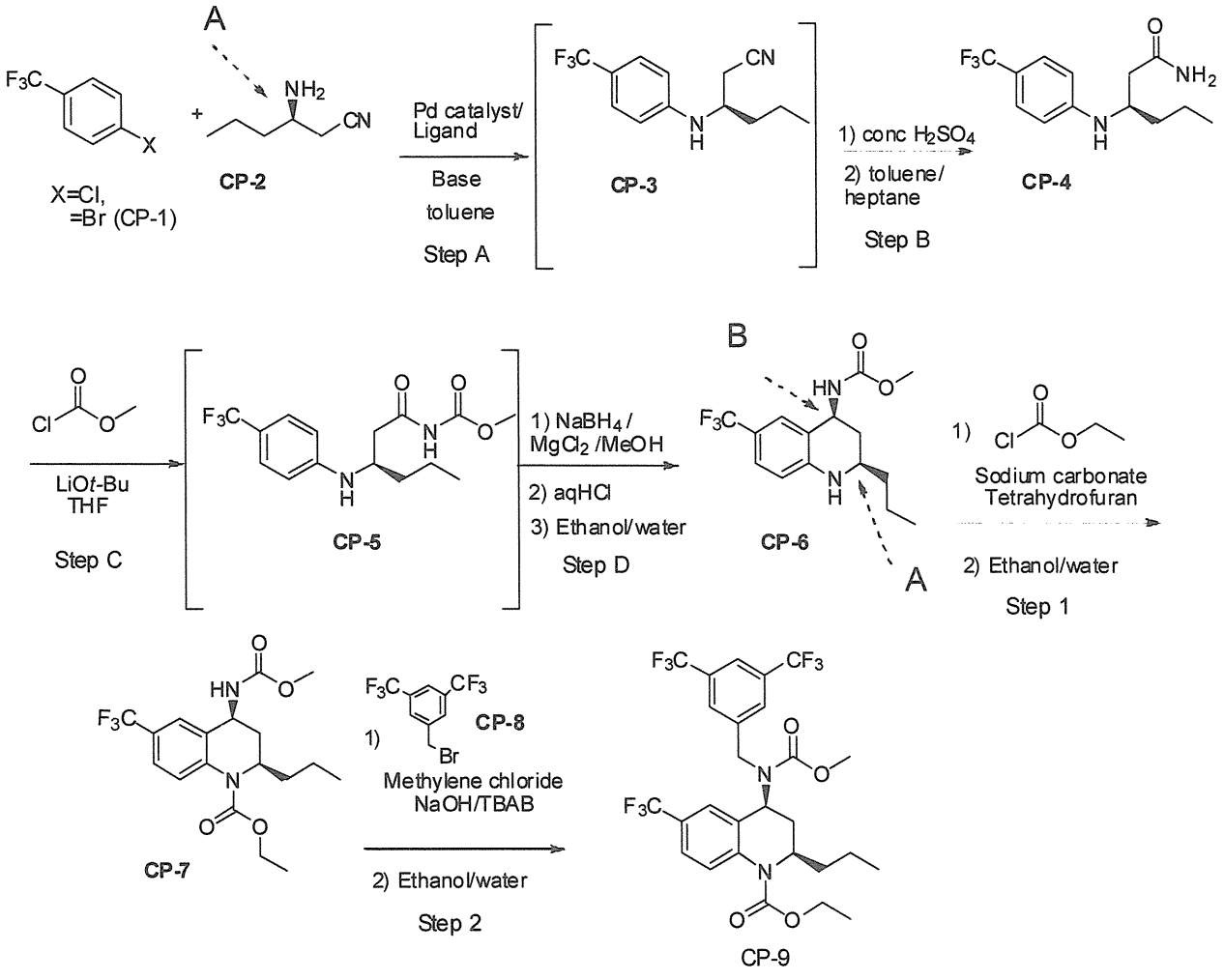


Figure 2.3.S.2.6-1 Sakuramil Manufacturing Scheme

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.

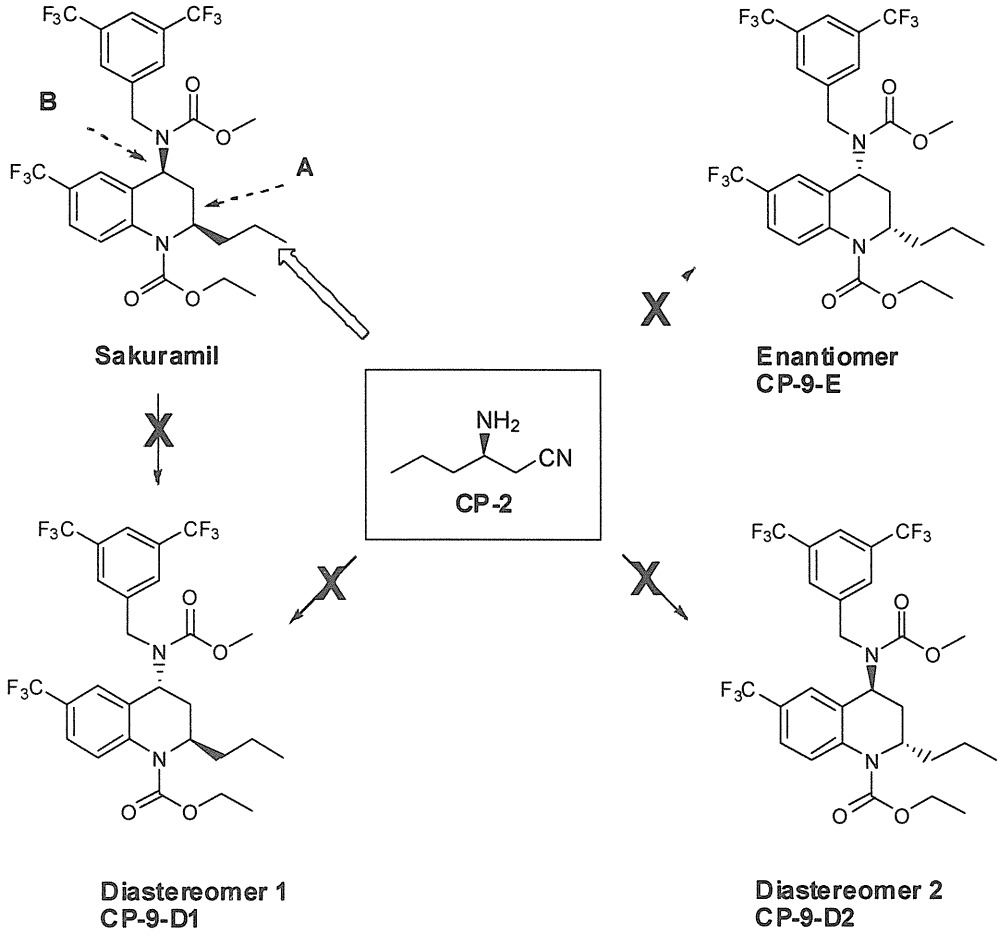


Figure 2.3.S.2.6-2 Chiral Control: Possible Enantiomers and Diastereomers

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.

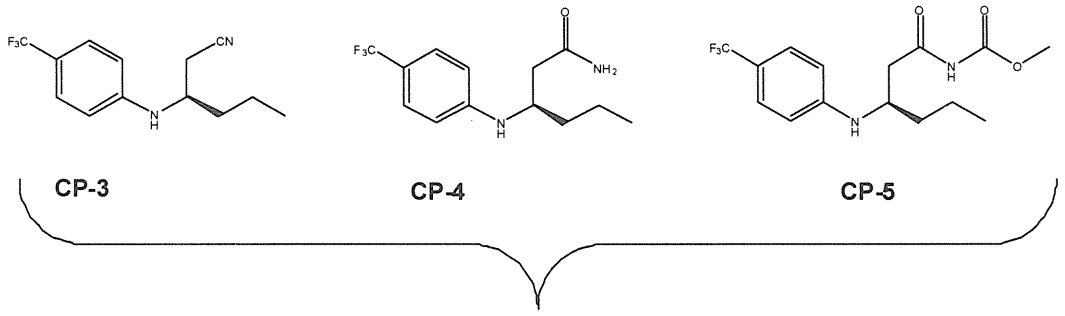
Diastereomers discussion: Trans isomer CP-9-D1, in theory, could come from two possibilities. The first would come from the "disallowed" cyclization reaction to give the trans configuration of the two chiral centers (studies and literature demonstrated this is not possible), and second, from racemization 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.

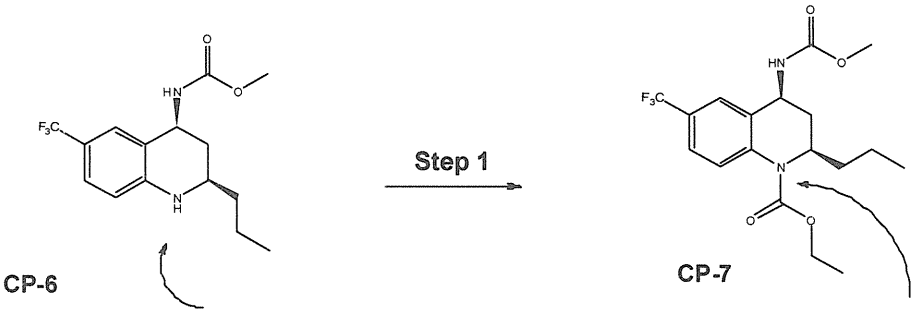
A fate and purge study demonstrated that 1% of the enantiomer (CP-6-E) and the diastereomer (CP-6-D) in CP-6 resulted in <0.1% (less than LOQ of 0.05%) in drug substance. In addition, there is no erosion of the chirality in Steps 1 and 2 even when applying stressing conditions.

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

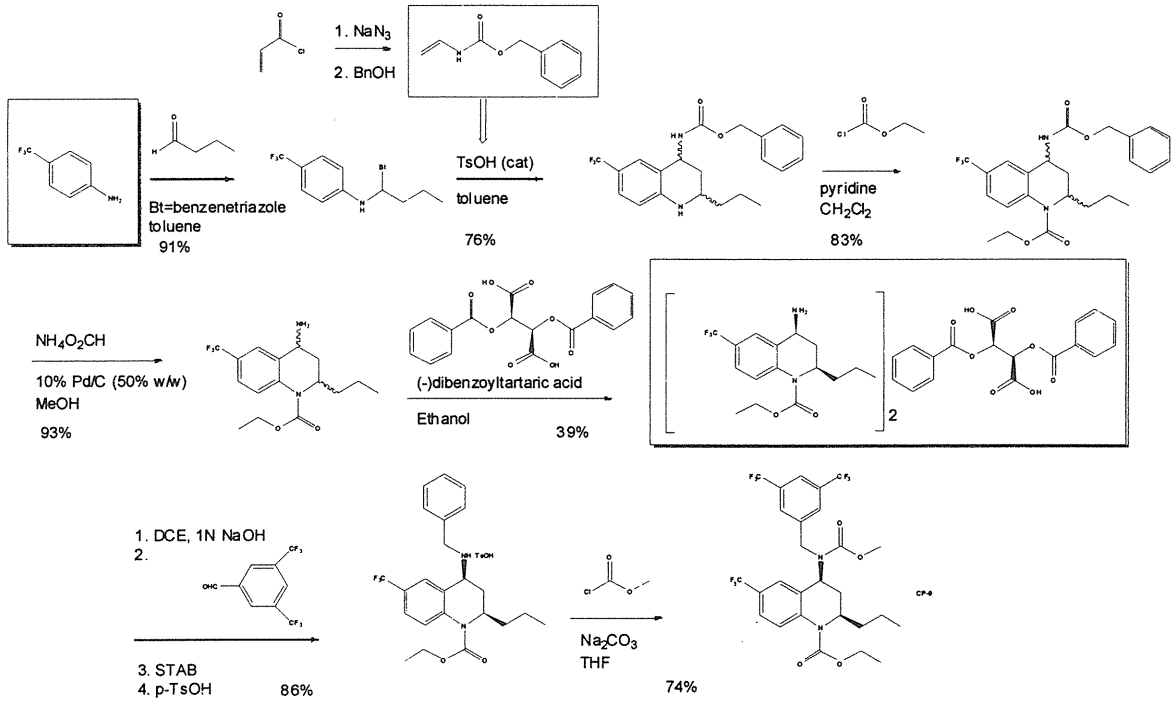


Figure 2.3.S.2.6-4 Route A: First Generation Synthesis

- 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

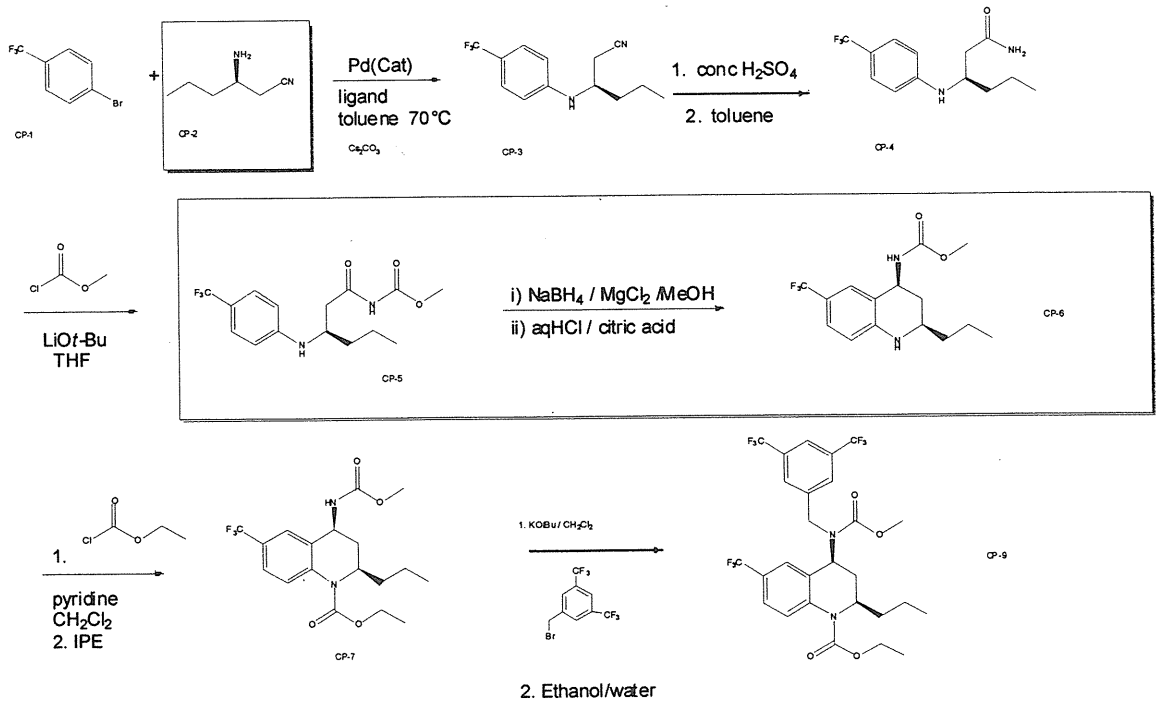


Figure 2.3.S.2.6-5 Route B: Second Generation Synthesis

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

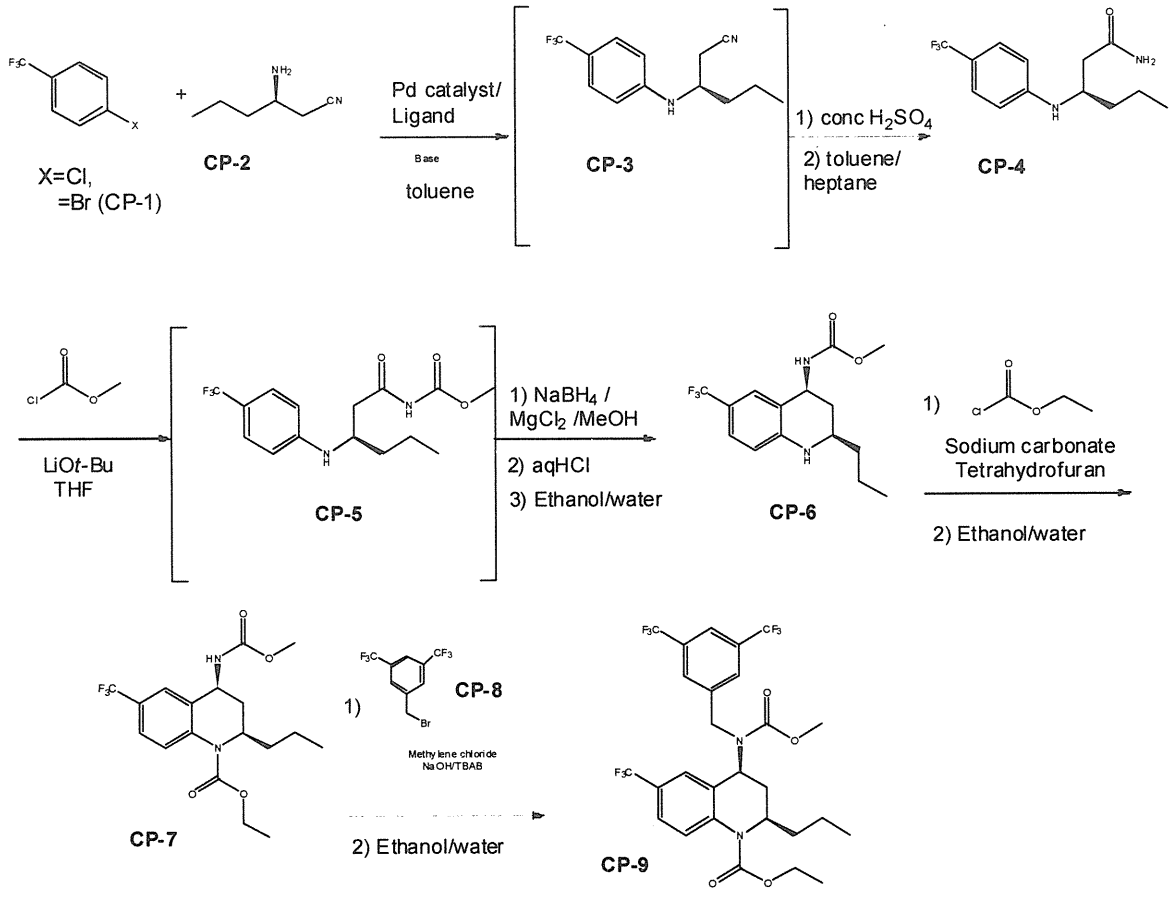


Figure 2.3.S.2.6-6 Route C: Third Generation Synthesis

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.

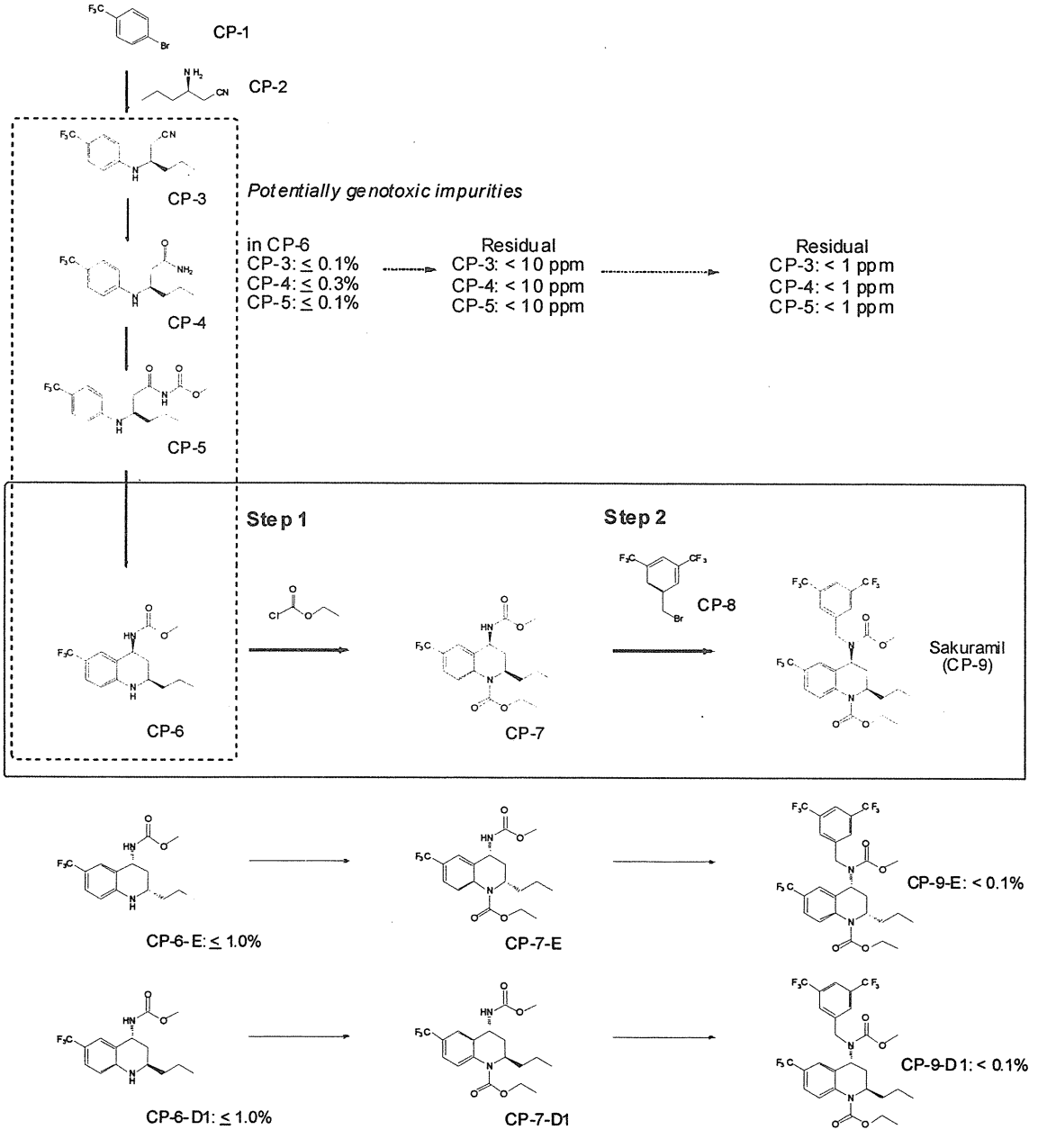


Figure 2.3.S.2.6-7 Fate and Purge of Related Substances inCP-6 in the Manufacturing Process of Sakuramil

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.