

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 ($\leq 0.10\%$).

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.

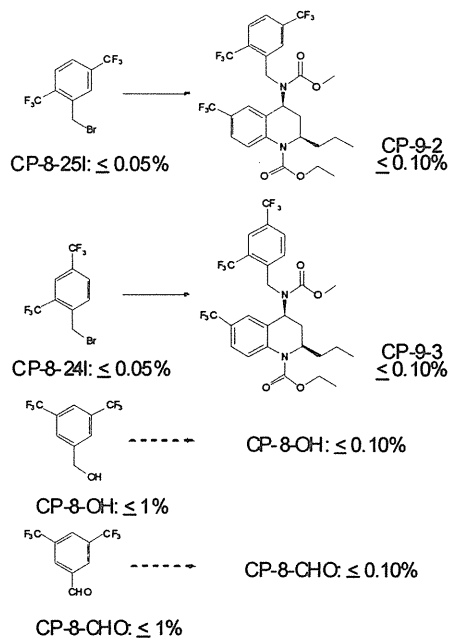
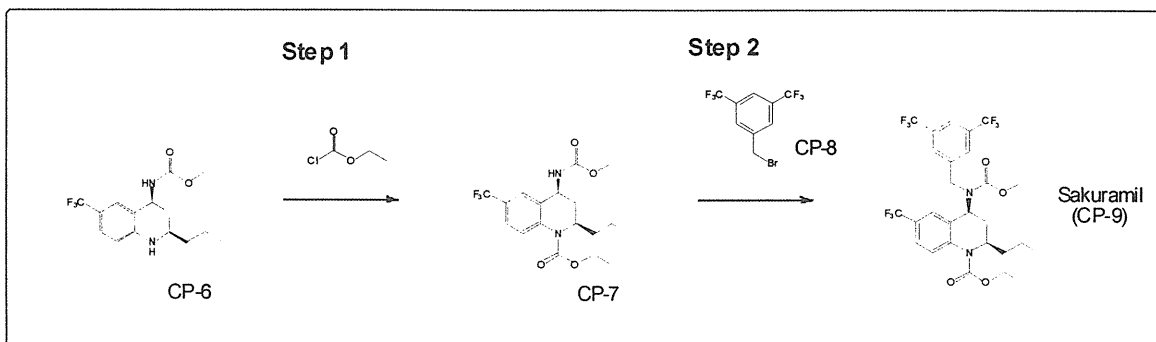


Figure 2.3.S.2.6-8 Fate and Purge of Related Substances in CP-8 in the Manufacturing Process of Sakuramil

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 $\leq 0.05\%$ 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:

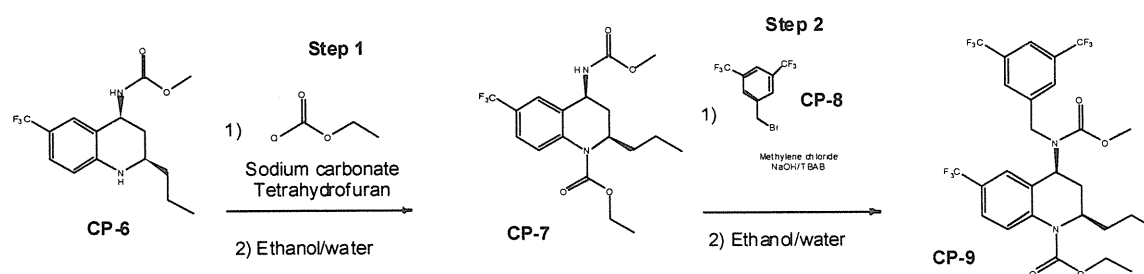
CP-8-OH and CP-8-CHO are not important material attributes. Both of these impurities do not react in the final step and have significant purge factors in the final isolation of drug substance. Fate and purge has demonstrated that 3% is easily purged to $\leq 0.1\%$ unspecified level in drug substance.

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 Substance



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 characteristics 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.

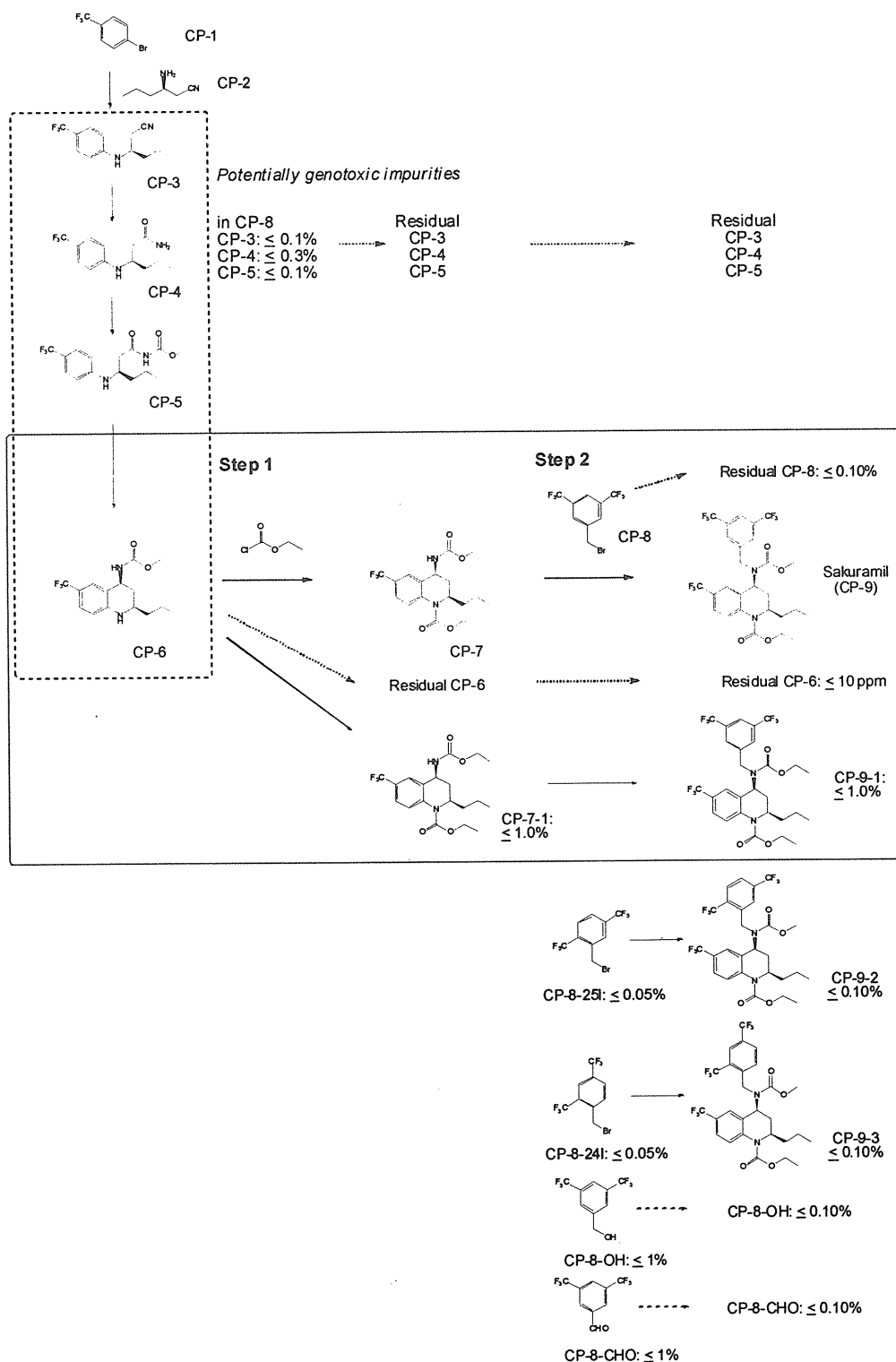
All process related impurities present in CP-6 at levels greater than 0.1% have been identified, and appropriate limits may be established based on demonstrated purge data in subsequent synthetic steps. Individual unspecified impurities are controlled in CP-6 to levels of NMT 0.1%. CP-6 related impurities 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 substance 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.

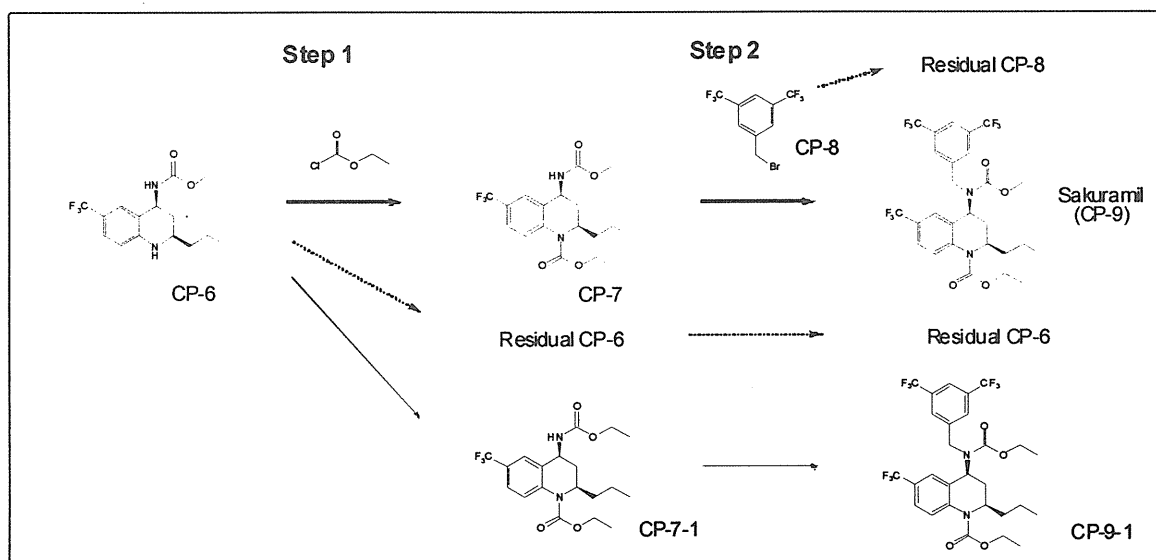
4)-2-4 Material Attribute (MA) to be evaluated: Residual Solvents

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	Filtration	Quench, Phase sep.	Crystallization	Filtration	Drying	Reaction	Quench, Phase sep.	Distillation	Filtration	Crystallization	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.

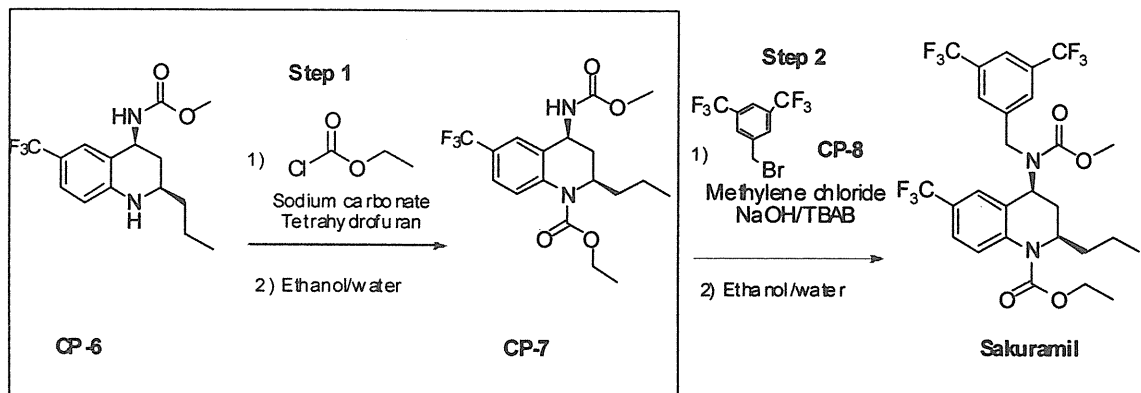


Figure 2.3.S.2.6-11 Step 1 Process

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.

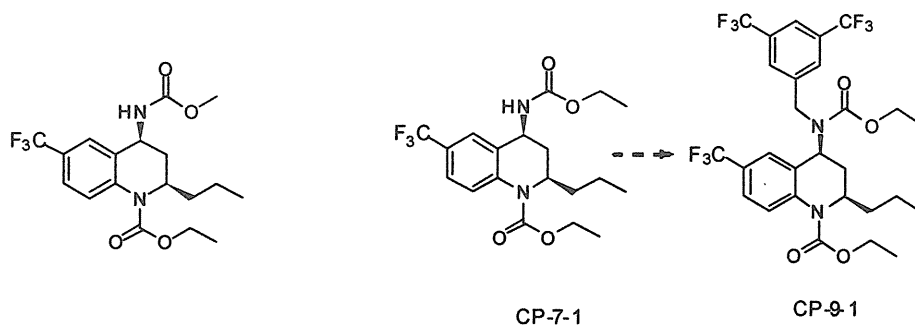


Figure 2.3.S.2.6-12 CP-7-1, Ethyl Homolog

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.

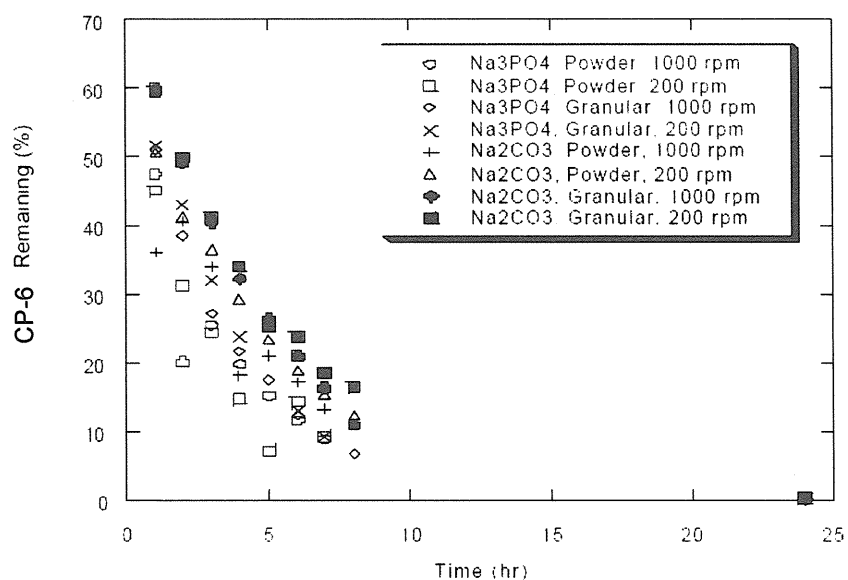


Figure 2.3.S.2.6-13 Impact of Base Particle Size and Agitation Speed on Reaction Rate

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 ₃ PO ₄ or Na ₂ CO 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.

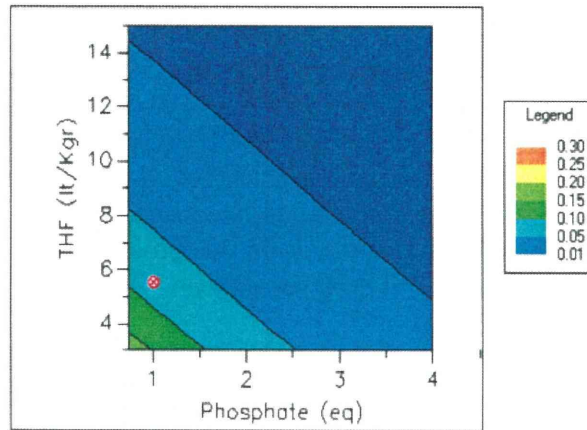
Conclusion for Ethyl Homolog, CP-7-1

- 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°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.

**36 hr Ethyl Homolog (%Area CP-7-1)
Contour Plot**



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

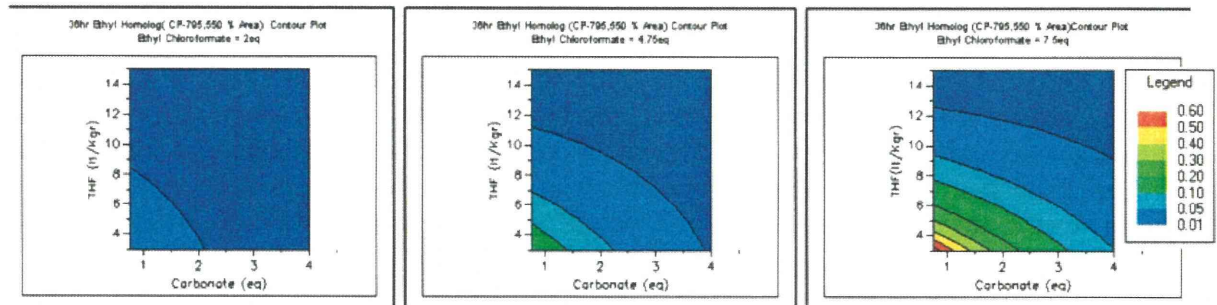


Figure 2.3.S.2.6-15 Carbonate: Contour Plots of Ethyl Homolog at 36 hours

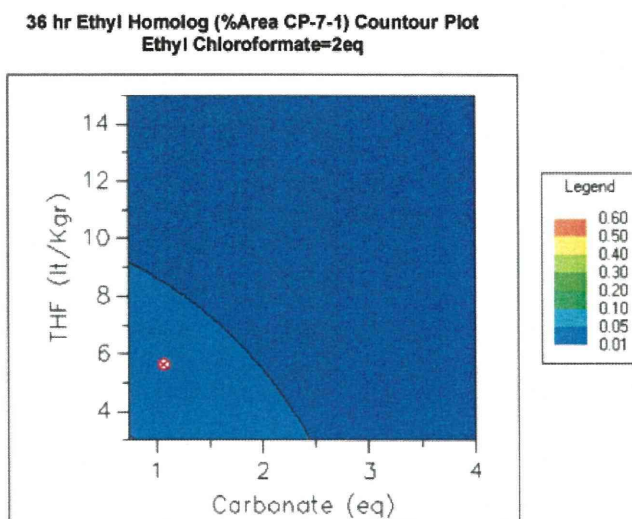


Figure 2.3.S.2.6-16 Carbonate: Contour Plots at 36 hours for the Standard Conditions

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.

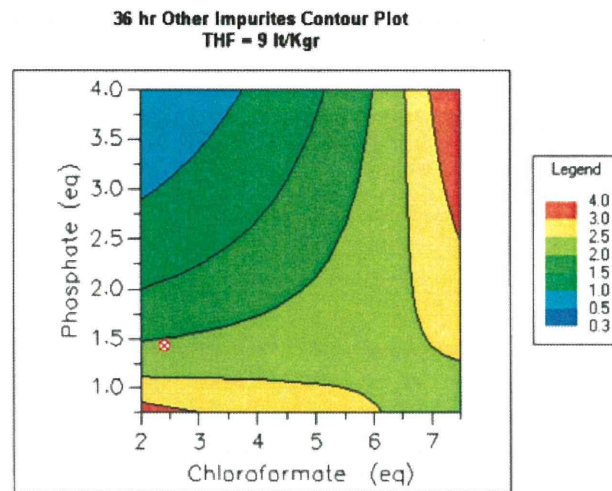


Figure 2.3.S.2.6-17 Phosphate: Contour Plots of “Other” Impurities at 36 hours at 9 Volumes THF

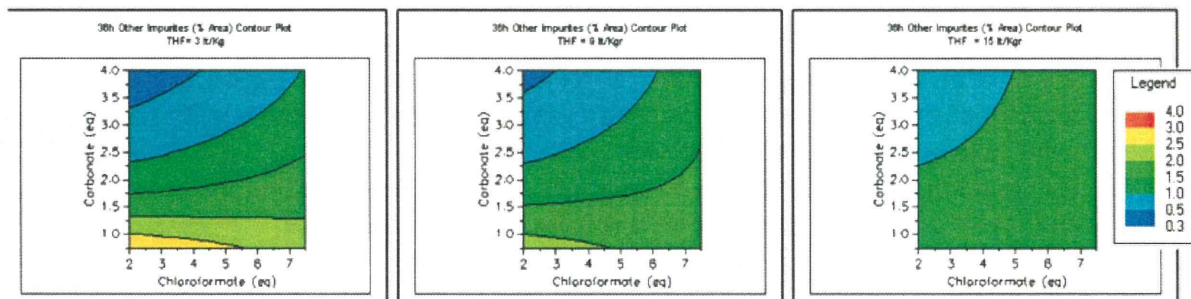


Figure 2.3.S.2.6-18 Carbonate: Contour Plots of “Other” Impurities at 36 hours

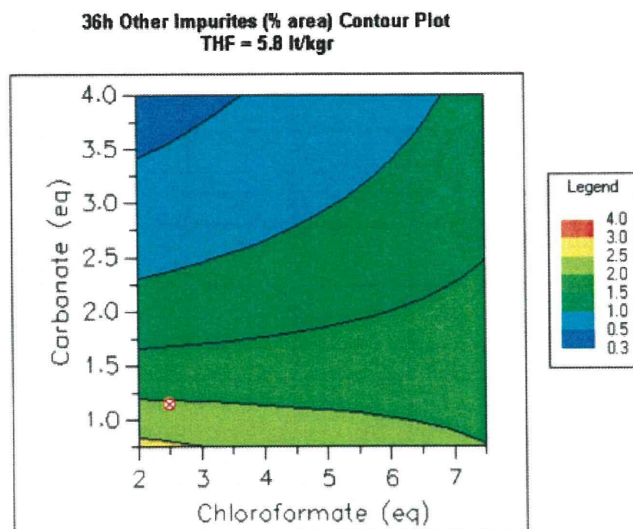


Figure 2.3.S.2.6-19 Carbonate: Contour Plots of “Other” Impurities at 36 hours at Standard THF-Volumes

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.