**Campaign Report for**

**KSM-2 (CRA005/6A)**



KSM-2

C19H19BrN2O4

Mol. Wt.: 419.27

**Customer: TB Alliance**

Submitted to: Koteswara Rao Inabathina

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**Date:** Nov 23rd, 2024

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**Project Summary**

|  |  |  |  |
| --- | --- | --- | --- |
| Customer | TB Alliance | | |
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| CRAMSN Contact | Uday K. Neelam,  [udaykumar.neelam@cramsn.com](mailto:udaykumar.neelam@cramsn.com) | | |
| Compound Name | 6-Bromo-2-methoxy-3-((2,3,6-trimethoxypyridin-4-yl) methyl) quinoline  **(KSM-2)** | | |
| Report No. | NA | | |
| CRA005/6A | Formula: C19H19BrN2O4 | | |
|  | Mol. Wt.: 419.27 | | |
| Start Date of Project | Mar 23rd; 2024 | | |
| End Date of Project | Nov 1st, 2024 | | |
| Batch Number | Amount | Overall Yield1 | COA |
| CRA005/A025/5/03 | 0.159 kg | 2.98% |  |
| 1 See Section 4.9 | | | |

# Objective

**Scope of work:**

Development of a cost-effective manufacturing route for KSM-2.

## Executive summary

CRAMSN successfully developed and produced 4.42 kg of KSM-2 (CRA005/6A) under non-GMP conditions, achieving a total yield of 30%. This included the familiarization and production of 4.01 kg of 2,4,6-trimethoxyisonicotinic acid (TMINA/CRA005/4C) with a total yield of 42.2%. The manufacturing processes for TMINA, was adopted from established protocols. Key focus areas included process optimization, impurity management, isolation techniques, analytical method development, and the preparation of reference standards. This report summarizes the comprehensive development and scale-up efforts for KSM-2, and TMINA, confirming that the quality of KSM-2 (Batch No. ECRA005/6A00125) meets the established specifications.

## Specification

## Specification for CRA005/6A:

|  |  |  |
| --- | --- | --- |
| **S. No.** | **TEST** | **SPECIFICATION** |
| 1.0 | Description | Off white to light brown color solid. |
| 2.0 | Identification by | |
| 2.1 | FT-IR | Conforms with the structure. |
| 2.2 | HPLC | Retention time of major peak in the chromatogram of sample solution should correspond to that of SST solution as obtained in related substances by HPLC. |
| 3.0 | Loss on drying (at 60°C for 3Hrs under vacuum), (%w/w) | Not more than 1.0% |
| 4.0 | Related substances by HPLC (% Area) | |
| 4.1 | CRA005/5A impurity | 0.5% |
| 4.2 | Single maximum unspecified impurity | 1 % |
| 4.3 | Total impurities | Not more than 3.0% |
| 5.0 | Chromatographic purity by HPLC  (Area %) | Not less than 97.0% |

**Justification for specifications:** The specification for the Aniline mentioned in section is acceptable as it is not leading to any impurity formation.

# Synthetic Route

The synthetic schemes for the manufacturing of KSM-2, KSM-1 & TBAJ-876 Tartrate (CRA005/5) is provided in Scheme 1 below.

## KSM-2 synthesis:



The synthesis KSM-2 consists of six stages as follows:

|  |  |
| --- | --- |
| Stage-1 | Stage-1 is the acylation of aniline using chloropropionyl chloride, which results in the formation of 3-chloro-N-phenylpropanamide. |
| Stage-2 | Stage-2 is the intramolecular alkylation of stage-1 using AlCl3, which results in the formation of dihydroquinolinone. |
| Stage-3 | Stage 3 is the bromination of dihydroquinolinone using N-bromosuccinimide (NBS), introducing a bromine atom at the 6-position of the quinoline ring. |
| Stage-4 | Stage 4 is aldol condensation of trimethoxyisonicotinic aldehyde (TMINA), with stage 3 using 25% NaOMe in MeOH. |
| Stage-5 | Stage 5 is chlorination reaction of stage 4 compound using phosphorous oxychloride in Toluene solvent. |
| Stage-6 | Stage 6 is displacement reaction of Chloro with OMe using 25% NaOMe in MeOH/Toluene (1:1). |

The synthesis TMINA consists of four stages as follows:

|  |  |
| --- | --- |
| Stage-1 | Stage-1 reaction is iodination of 2,6-diemethoxypyridine using N-Iodosuccinimide (NIS) in dichloromethane (DCM) and ethanol under a nitrogen atmosphere. |
| Stage-2 | Stage-2 reaction is hydroxylation of iodo using aq.KOH in presence of catalytic amount of Cu(acac)2 in DMSO solvent.  **Note: This stage also includes Ligand synthesis.** |
| Stage-3 | Stage-3 reaction is O-methylation using Dimethyl sulfate in presence of base in DMF. |
| Stage-4 | Stage-4 reaction is formylation at 4th position using DMF/n-BuLi in THF solvent. |

# Summary of major accomplishments

## Synthesis of 2,6-dimethoxyisonicotinic

## Synthesis of Trimethoxyisonicotinic Aldehyde (TMINA)



**Scheme 3:** Synthesis of Trimethoxyisonicotinic Aldehyde (TMINA)

To synthesize 2,3,6-trimethoxyisonicotinaldehyde, the process commenced with the iodination of 2,6-dimethoxypyridine with an over yield of 42.2%. This was achieved by reacting the pyridine with N-Iodosuccinimide (NIS) in a mixture of dichloromethane (DCM) and ethanol under a nitrogen atmosphere. The reaction was conducted at 10-25°C with incremental additions of NIS, followed by stirring and quenching once HPLC confirmed the reaction's completion. The resulting 3-iodo-2,6-dimethoxypyridine was purified and concentrated after quenching with sodium sulfite and removal of DCM traces through co-distillation with n-heptane. The next stage involved the conversion of 3-iodo-2,6-dimethoxypyridine to 2,6-dimethoxypyridin-3-ol. This was accomplished by reacting the iodopyridine with DMSO, Cu(acac)2, and KOH at 95-100 °C, followed by filtration and extraction steps to isolate the intermediate product. Subsequently, 2,6-dimethoxypyridin-3-ol was reacted with acetone and potassium carbonate in the presence of dimethylsulfide (DMS) at controlled temperatures to produce 2,3,6-trimethoxypyridine. This compound was then subjected to a lithiation reaction using n-butyllithium (n-BuLi) and DMF in tetrahydrofuran (THF) at -60 °C. After the lithiation, the reaction was quenched with a potassium hydrogen phosphate solution, and the product was extracted, concentrated, and purified. The final product, 2,3,6-trimethoxyisonicotinaldehyde, was obtained as a solid after a series of extraction, concentration, and drying steps. The entire process yielded the target compound with high purity through careful management of reaction conditions and meticulous purification techniques.

The process was adopted from Chempartner/ TCGLS and successfully adopted by CRAMSN, with minute modifications more specifically at stage-2C (CRA005/2C), the details were provided below.

### Summary of modifications:

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Chempartner procedure** | **CRAMSN procedure** |
| **Reaction & Work up conditions** | **Reaction:**   * CRA005/1C: 1.0 eq.; Ligand: 0.02   eq.; Cu(acac)2:0.02 eq.; KOH:  3.0 eq.; DMSO:6.7 V; H2O: 1.2 V.   * Add KOH aqueous solution. * Heat to 70±5 °C for 1 H. * Heat to 80±5 °C for 1H. * Heat to 85±5 °C for 2H.   **Workup:**   * Add water (5V) into the batch. * Filter the batch and extract the filtrate three times with n- heptane (3X3V) * Adjust the pH of aqueous phase with KHSO4 (2.5 eq. in water ~2V) to pH=1 to 2. * Filter and extract the filtrate with MTBE (3 X 3V). * Wash the combined organic phase with water (3V) * Dry over MgSO4 (0.5W) and filter. * Concentrate the batch under vacuum at 40±5 oC to give the desired compound. | **Reaction:**   * CRA005/1C: 1.0 eq.; Ligand: 0.02eq.; Cu(acac)2: 0.02 eq.; KOH: 3.0 eq.; DMSO:6.7V; H2O: 1.2V. * Add KOH aqueous solution. * Heat to 95±5 °C for 5 H.   **Workup:**   * Add water (5V) into the batch. * Add hyflo (0.2T) into the batch * Filter the batch and extract the filtrate three times with n- heptane (3X3V) * Adjust the pH of aqueous phase with KHSO4 (2.5 eq. in water ~2V) to pH=1 to 2. * Add hyflo (0.2T) into the batch * Add carbon (0.2T) into the batch * Filter and extract the filtrate with MTBE (3 X 3V). * Wash the combined organic phase with water (3V) * Concentrate the batch under vacuum at 40±5 oC to give the desired compound. |
| **Yield (Molar Yield)** | 51.13%\* | 78.2% |

\*Note: Yield obtained during the experimentation at CRAMSN.

## Synthesis of 6-bromo-2-methoxy-3-((2,3,6-trimethoxypyridin-4-yl)methyl)quinolone (KSM-2)



**Scheme 4:** Synthesis of 6-bromo-2-methoxy-3-((2,3,6-trimethoxypyridin-4-yl)methyl)quinolone (KSM-2)

A novel synthetic methodology (Scheme 4) has been elucidated for the synthesis of 6-bromo-2-methoxy-3-((2,3,6-trimethoxypyridin-4-yl)methyl)quinolone (KSM-2), which presents a significant advantage over conventional transition metal-catalyzed approaches, such as Suzuki coupling (Scheme 5), known to generate three prominent impurities (Scheme 5, Impurities-a, b & c), emphasizing the advantages of the new synthetic route in curtailing the production of such undesirable byproducts. Also, the cost of goods for the synthesis of KSM-2 via Suzuki approach is costlier than the current route of synthesis. Hence, to mitigate these issues M4ALL proposed a route of synthesis and provided feasible process for further studies. The stage-1A to 3A developed by CRAMSN with literature evidences, whereas the rest of the stages were adopted as per M4ALL.



**Scheme 5:** Transition Metal-Catalyzed Synthesis of KSM-2 and Impurity Profiling.

The synthesis of **KSM-2** is orchestrated through a meticulously designed six-step sequence. The process initiates with the acylation of aniline using chloropropionyl chloride, yielding 3-chloro-N-phenylpropanamide. This intermediate is subsequently subjected to Friedel-Crafts alkylation, resulting in the formation of 3,4-dihydroquinolin-2(1H)-one. Following this, the dihydroquinolinone undergoes bromination using N-bromosuccinimide (NBS) before engaging in an aldol condensation with trimethoxy isonicotinic aldehyde, yielding 6-bromo-3-((2,3,6-trimethoxypyridin-4-yl)methyl)quinolin-2(1H)-one. This pivotal intermediate is then transformed into 6-bromo-2-chloro-3-((2,3,6-trimethoxypyridin-4-yl)methyl)quinolone. The final transformative step involves the dechlorination of the quinolone with sodium methoxide, culminating in the formation of the target molecule, **KSM-2**. Each step of this synthetic pathway has been comprehensively optimized through the simultaneous variation of multiple parameters (Design of Experiments, DoE) as well as through one-factor-at-a-time (OFT) studies and the details are provided in the optimization reports.

### Key Development of Stage-1A of KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | CPC (1.2 eq), Pyridine (2.0 eq), Aniline (1.0 eq.) | The optimization of the stoichiometry of the starting material and reagents effectively mitigated the formation of the N-phenyl acrylamide impurity. |
| 2 | Solvent Screening | Acetone | Acetone was selected as the solvent of choice for the reaction, as a comparative screening of alternative solvents like DCM and ethyl acetate revealed comparatively diminished yields. |
| 3 | Volume optimization | Acetone (10.0 V) | Increased dilution appears to be advantageous, as improved yields were observed in comparison to lower volumes of acetone (5.0 V). |
| 4 | N-phenyl acrylamide impurity Control | Usage of pyridine as an alternative base to TEA showed significant impact in controlling impurity formation | The formation of the N-phenyl acrylamide impurity has been effectively controlled to levels below 0.5% in the scaled-up batches. |
| 5 | Temperature Optimization | 55 ℃ was found to be the optimum rea | A reaction temperature of 55 °C was identified as optimal, as it resulted in a significant enhancement in yield compared to the reaction conducted at ambient temperature. |

### Key Development of Stage-2A KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | AlCl3 (5.0 eq.), Neat, 100 ℃, 1-2 hr. | The meticulous optimization of the stoichiometry of the starting material, reagents, reaction temperature, and duration effectively curtailed the formation of unknown impurities, thereby facilitating the attainment of both high yield and exceptional purity. |
| 2 | Solvent Screening | Neat | Investigation into the incorporation of the solvent like chlorobenzene revealed that the reaction did not proceed in its presence. |
| 3 | Time | 1-2 hr | It was noted that extending the reaction duration led to the emergence of an undesired unknown impurity, characterized by a retention time of 2.36 minutes and a relative retention time of 0.65. |
| 4 | Temperature Optimization | 100±5 ℃ | The yield remained uncompromised when the reaction was conducted at temperatures below 130 °C, with enhanced yields actually observed under these conditions. |

### Key Development of Stage-3A KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | NBS (1.1 eq.), 5±5℃, 12 h | The optimization of the stoichiometry of the starting material, reagents, and reaction temperature effectively mitigated the formation of both dibromo and desbromo impurities. |
| 2 | Solvent Volume | DMF (10.0 V) | Based on the evaluation of solvent volumes, it was determined that a volume of 10.0 V of DMF yielded superior results compared to 8.0 V. Additionally, further dilution to 12.0 V did not result in any increase in yield. |
| 3 | Temperature Optimization | 5±5℃ | It was observed that an increase in temperature heightened the likelihood of forming dibromo and desbromo impurities. |
| 4 | Optimization of Stoichiometry of NBS | NBS (1.1 eq.) | The optimization of NBS stoichiometry revealed that an increase in its quantity correspondingly elevated the formation of dibromo impurities, while a reduced stoichiometry of NBS augmented the likelihood of desbromo impurity formation. |

### Key Development of Stage-4A KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | TMINA (1.0 eq.), CRA005/3A (1.1 eq.), NaOMe (3.0 eq.) | The optimization of the stoichiometry of the starting materials, reagents, and reaction temperature significantly enhanced both yield and purity. |
| 2 | Solvent Volume | MeOH (10.0 V) | Based on the evaluation of solvent volumes, it was determined that a volume of 10.0 V of MeOH yielded superior results compared to 8.0 V. Additionally, further dilution to 13.0 V did not result in any increase in yield. |
| 3 | Temperature Optimization | 60-65 °C | Screening of temperature indicated that a lower temperature of 45 °C yielded diminished results, whereas elevating the temperature beyond 65 °C did not further enhance the yield. |
| 4 | Optimization of Stoichiometry of NaOMe | 3.0 eq. | Screening the stoichiometry of NaOMe from 2.0 to 5.0 equivalents revealed that lower equivalents resulted in diminished yields, while increasing the equivalents beyond a certain point did not enhance the yield. |

### Key Development of Stage-5A KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | POCl3 (2.0 eq), Toluene (15.0 V), 100 ℃, 4-5 h | The optimization of the stoichiometry of the reagents significantly enhanced both yield and purity. |
| 2 | Optimization of Stoichiometry of POCl3 | 2.0 eq. | The reaction utilizing 1.25 equivalents of POCl3 was incomplete, failing to facilitate full conversion. Conversely, employing 1.75 equivalents achieved complete conversion, yielding a product purity of 95.74% with 2.46% unreacted material. Similarly, the use of 2.0 equivalents also resulted in complete conversion, affording a product purity of 97.58% with only 0.98% starting material remaining. |

### Key Development of Stage-6A KSM-2

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Description** | **Optimized condition** | **Remarks** |
| 1 | Optimization of Stoichiometry | NaOMe (3.0 eq), MeOH/Toluene (1:1) (15 V),  60-65 ℃, 18 - 20 h | The optimization of the stoichiometry of the reagents significantly enhanced both yield and purity. Incorporation of MeOH/Toluene (1:1) as the solvent system facilitated the complete conversion of Stage-5A to KSM-2. |
| 2 | Optimization of Stoichiometry of NaOMe | 3.0 eq. | Screening the stoichiometry of NaOMe from 3.0 to 5.0 equivalents demonstrated that increasing the amount to 5.0 equivalents did not yield any enhancement in the overall yield. |

## Summary of modifications:

|  |  |  |  |
| --- | --- | --- | --- |
| **Stage** | **Parameters** | **M4ALL** | **CRAMSN procedure** |
| Stage-1A | Reaction conditions | NA | CPC (1.2 eq),  Pyridine (2.0 eq)  Acetone (10 V)  55 ℃, 2h |
| Yield | NA | 84-90% |
| Stage-2A | Reaction conditions | NA | AlCl3 (5.0 equiv.), 100℃, neat, 1-2h. |
| Yield | NA | 78-86% |
| Stage-3A | Reaction conditions | NA | DMF (10.0 vol.), NBS (1.1 equiv.), 5 ℃, 12 h. |
| Yield | NA | 82-86% |
| Stage-4A | Reaction conditions | TIMINA (1.0 equiv.), CRA005/3A (1.1 equiv.), 25% NaOMe solution (3.0 equiv.), MeOH (10.0 vol.), 65 ℃, 22-24 h. | TIMINA (1.0 equiv.), CRA005/3A (1.1 equiv.), 25% NaOMe solution (3.0 equiv.), MeOH (10.0 vol.), 65 ℃, 22-24 h. |
| Yield | 77-86%. | 85-88% |
| Stage-5A | Reaction conditions | CRA005/4A (1.0 equiv.), POCl3 (5.0 equiv.), Toluene (15.0 vol.), 95-100 ℃, 4-5h. | CRA005/4A (1.0 equiv.), POCl3 (5.0 equiv.), Toluene (15.0 vol.), 95-100 ℃, 4-5h. |
| Yield | 84% | 72-85% |
| Stage-6A | Reaction conditions | CRA005/5A (1.0 equiv.), 25% NaOMe  solution (3.0 equiv.), MeOH (15.0 vol.),  65℃, 32 h. | CRA005/5A (1.0 equiv.), 25% NaOMe  solution (3.0 equiv.), MeOH/Toluene (1:1)  (15.0 vol.),  65℃, 18-20 h. |
| Yield | 81% | 85-93 % |

# PRODUCTION OF KSM-2

## Production of Ligand (CRA005-1D)

### Reaction Scheme



### Production Summary

One batch (CRA005/A017/1D/01) of CRA005/1D (150g of 4-amino-3,5-dimethylphenol (SM)) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch  Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM (g)** | **Lot Number** | **HPLC  Purity**  **(% area)** | **CRA005/1D**  **(g)** | **HPLC  Purity**  **(% Area)** |
| CRA005/A017/1D/01 | 150 | 32099-2404 | 99.15 | 151 | 98.42 | 84.7 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of 4-amino-3,5-dimethylphenol. The manufacture of N1,N2-bis(4-hydroxy-2,6-dimethylphenyl)oxalamide (CRA005/1D) is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
|  | Add 4-amino-3,5-dimethylphenol/R-004 (150 g, 1.09 mol, 1.0 eq.), THF (2.6 lit, 1.7 V) into a 5.0L RBF |  |  |
|  | Cool the reaction mixture to 0±5°C. |  |  |
|  | Add oxalyl chloride (83.27g, 0.656 mol, 0.6 eq.) slowly to the RBF maintaining the temperature of the reaction mixture at 0±5°C for about 90 min. |  |  |
|  | Stir the reaction mixture at 0±5°C for 30 min. |  | **In-process control-1:** Send the reaction mass to AR&D as **CRA005-1D-01** for TLC. Specification: 2,6-dimethylpyridine/R-004 content should be NMT: 5.0%. |
|  | Add water (750.0 mL，5.0 V) slowly to the RBF at 10±10°C for about 1 h. |  |  |
|  | Concentrate the reaction mixture at below 45 °C until no distillate is collected. |  |  |
|  | Cool the solution to 15±5°C. |  |  |
|  | Filter the compound and wash the wet cake with water (150.0 mL, 1.0 V). |  |  |
|  | Dry the wet solid in the vacuum oven at 50±5°C for 10 h. |  | **In-process control-2:** Send the dry solid to AR&D as **CRA005-1C-02** for water content by KF. Specification: KF≤10.0%. |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No.** | **IPC No.** | **Analyte** | **Test Item** | **Specification** | **Report Results** |
| 1 | IPC01 | R-004 content by HPLC | Reaction mass | NMT 5.0% | Nill |
| 2 | IPC02 | water content by KF | Dry solid of 1D | KF≤10.0% | 0.15% |

### Discussion

Based on the optimized conditions (1.48 equivalents of Oxalyl chloride), the conversion of starting material to product was achieved within 2 hours. The product was then isolated using a, yielding 84.7 % with a purity of 98.42%. However, the isolated solid contained a 0.15 % impurity with a 1.08 RRT, 0.12% impurity with RRT 1.13and 1.31 % impurity with RRT 1.26.

*Impurity Profile*

The impurity profile is presented in the table below. Three impurities were observed in the isolated product (Batch number: CRA005/A017/1D/01) which remained unidentified.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| 1.08 | NA | NA | 0.15 | NA |
| 1.13 | NA | NA | 0.12 | NA |
| 1.26 | NA | NA | 1.31 | NA |
| 1.0 | NA | NA | 98.42 |  |

## Production of TMINA (CRA005/4C)



## Production of TMINA Stage-4C

One batch (PCRA005/4C00124) of CRA005/4C (7.0 Kg of SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **GC Purity (% area)** | **CRA005/4C**  **(kg)** | **HPLC Purity (% area)** |
| PCRA005/4C00124 | 7.0 | OLS-184-KSM | 99.45 | 4.01 | 99.02 | 40.42 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents of reagents and solvents for each steps were calculated based on assay of each step in the telescopic process for the synthesis of TMINA. Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
| 1. | Arrange clean and dry RBF. |  |  |
| 2. | Charge S-807 lot-1 & S-822 lot-1 into RBF at 30±5°C under nitrogen. |  |  |
| 3. | Charge 2,6-dimethoxypyridine into RBF at 30±5°C and stir for 5-10 min under nitrogen. |  | **Description**: Homogeneous reaction mass. |
| 4. | Cool the reaction mass to 15±5°C. |  |  |
| 5. | Charge M-1295 (NIS) to the reaction mass in 5 equal portions at 15±5°C under nitrogen atmosphere. |  | **Note:** The time between each portion was about 20 min, during the addition of M-1295, the reaction mass turns from colorless to wine red (pH=4-5).  **Description:** Homogeneous reaction mass. |
| 6. | Stir the reaction for 30 min at 15±5°C under nitrogen atmosphere. |  |  |
| 7. | Raise the reaction mass temperature to 25±5°C. |  |  |
| 8. | Stir for 20±2 hrs. at 25±5°C under nitrogen. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-01** for HPLC, 2,6-dimethylpyridine content NMT: 5.0%. If HPLC does not comply, maintain the reaction mass at the same temperature and monitor by HPLC for every 2 hrs., till HPLC complies. |
| 9. |  |  |  |
| 10. | (If the reaction is not completed, add M-1295 lot-2, 0.1Eq to the batch) |  |  |
| 11. | Prepare sodium sulfite solution with sodium sulfite and water lot-1 in a separate container. |  |  |
| 12. | Slowly add the sodium sulfite solution into the above reaction mass, then stir for 30-45 min. |  | **Description**: Homogeneous reaction mass.  **Note**: pH=7-8, Test the organic phase for iodine with a wet KI starch strip, if the strip doesn’t appear blue then proceed to next step (If appears blue, proceed to wash with sodium sulfite solution until to pass) |
| 13. | Separate the bottom organic layer and keep it aside. |  |  |
| 14. | **Note:** Here the organic layer contains product. |  |  |
| 15. | Charge S-807 lot-2 to the aqueous layer. |  |  |
| 16. | Stir for 30 min and settle for 10 min. |  |  |
| 17. | Separate the bottom organic layer and combine both the organic layers. |  |  |
| 18. | Prepare M-470 solution with M-470 and water lot-2 in a separate container. |  |  |
| 19. | Add the above prepared M-470 solution to the combined organic layer and stir for 60-90 min and settle for 20 min. |  |  |
| 20. | Separate the layers. |  |  |
| 21. | Add water lot-3 to the organic layer and stir for 60-90 min, then separate the layers. |  |  |
| 22. | Concentrate the organic layer under vacuum at below 30°C to about 3V. |  | **Description:** Colorless liquid compound |
| 23. | Co-distill the reaction mass with S-831 lot-1to about 3.0V at below 45°C. |  |  |
| 24. | Co-distill the reaction mass with S-831 lot-2 to about 1.2V at below 45°C. |  |  |
| 25. | Co-distill the reaction mass with S-831 lot-3 to about 1.2V at below 45°C. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-02** for DCM content by GC, DCM content NMT: 0.1%. If GC does not comply, repeat co-distillation with S-831(Opp. No. 22), till GC complies. |
| 26. |  |  |  |
| 27. | Charge S-813 lot-1 into reaction mass. |  |  |
| 28. | Continue distillation at below 45°C to remove S-831 from the reaction mass, till no distillate is observed. |  |  |
| 29. | Cool the reaction mass to 30±5°C. |  |  |
| 30. | Unload the reaction mass from the reactor for checking the weight of total reaction mass. | Wt. of reaction mass= **x** (~362.0 g) | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-03** for assay.  **Note:** Based on the assay, the quantity of S-813 lot-2, CRA005-1D,Cu(acac)2, M-130 and water lot-4 shall be indented. |
| 31. | Take the reaction mass into the reactor at 30±5°C. |  |  |
| 32. | Charge S-813 lot-2 into the reaction mass at 30±5°C. |  | **Description**: Homogeneous reaction mass. |
| 33. |  |  |  |
| 34. | Charge CRA005-1D to the reaction mass at 30±5°C. |  | **Description**: Homogeneous reaction mass. |
| 35. |  |  |  |
| 36. | Stir for 10-15 min at 30±5°C. |  |  |
| 37. | Charge Cu(acac)2 to the reaction mass 30±5°C. |  | **Description**: Homogeneous and pale purple color reaction mass. |
| 38. | Stir for 10-15 min at 30±5°C. |  |  |
| 39. | Prepare M-130 solution with M-130 and Water lot-4 in a separate container. |  |  |
| 40. | Slowly addabove preparedM-130 solution to the reaction mass during not less than 30 min at 30±5°C. |  | **Description**: Homogeneous reaction mass  **Note:** pH: ~14, Addition of M-130 solution is not exothermic however the reaction mass will be subjected for heating in the next operation. |
| 41. | Heat the reaction mass to 95±5oC. |  |  |
| 42. | Stir the reaction mass for 5-6h at 95±5oC. |  | **Description**: Slightly hazy reaction mass.  **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-04** for HPLC, **CRA005-1C** content NMT: 5.0%. If HPLC does not comply, maintain the reaction mass at the same temperature and monitor by HPLC for every 2 hrs., till HPLC complies. |
| 43. | Cool the reaction mass to 35±5°C. |  |  |
| 44. | Add water lot-5 to the reaction mass at 35±5°C. |  |  |
| 45. | Add hyflow lot-1 to the reaction mass at 35±5°C. |  |  |
| 46. | Stir the reaction mass for 1h at 35±5°C. |  |  |
| 47. | Filter the reaction mass. |  |  |
| 48. | Wash the wet cake with water lot-6. |  |  |
| 49. | Take the total filtrate into the RBF. |  |  |
| 50. | Wash the reaction mass thrice with S-831 lot-4, lot-5 and lot-6. |  | **Note:** Non polar impurities are washed out through S-831 washings. |
| 51. | Take the aqueous layer (Product phase) into RBF. |  |  |
| 52. | Prepare potassium hydrogen sulfate solution with potassium hydrogen sulfate and Water lot-7. |  |  |
| 53. | Charge above prepared potassium hydrogen sulfate solution into above aqueous layer. |  |  |
| 54. | Stir the reaction mass for 30-45 min. |  | **Description**: Heterogeneous reaction mass. **pH**: 1~2 |
| 55. | Charge the carbon into the reaction mass 35±5°C. |  |  |
| 56. | Charge the hyflow lot-2 into the reaction mass 35±5°C. |  |  |
| 57. | Stir the reaction mass for 30-45 min. |  |  |
| 58. | Filter the unwanted solid and wash the cake with S-832 lot-1. |  |  |
| 59. | Take the filtrate into RBF. |  |  |
| 60. | Separate the layers. |  | **Note:** Upper organic layer contains product. |
| 61. | Take the aqueous layer into RBF. |  |  |
| 62. | Extract the aqueous layer with S-832 lot-2. |  |  |
| 63. | Separate the layers. |  | **Note:** Upper organic layer contains product. |
| 64. | Take the aqueous layer into RBF. |  |  |
| 65. | Extract the aqueous layer with S-832 lot-3. |  |  |
| 78. | Separate the layers. |  | **Note:** Upper organic layer contains product. |
| 79. | Take the aqueous layer into RBF. |  |  |
| 80. | Extract the aqueous layer with S-832 lot-4. |  |  |
| 81. | Wash the combined organic layers (S-832 Lot-1; lot-2, lot-3, lot-4) with water lot-8. |  |  |
| 82. | Distill the organic layer under vacuum at below 45°C until no distillate is observed |  | **Note:** The desired product will obtain as an oil. |
| 83. | Unload the reaction mass from the reactor for checking the weight of total reaction mass. | Wt. of reaction mass =**Y** (~36.2 g) | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-05** for assay.  **Note:** Based on the assay, the quantity of S-805 lot-1, M-640 andDimethyl Sulfate(DMS), shall be indented. |
| 84. | Take the above residue into the same RBF. |  |  |
| 85. | Charge S-805 lot-1 into the reactor. |  | **Observation**: Homogeneous reaction mass. |
| 86. | Cool the reaction mass to 15±5 °C. |  |  |
| 87. | Charge the M-640 into the reaction mass at 15±5 °C. |  | **Description**: Heterogeneous reaction mass. |
| 88. | Slowly addM-600 to the reaction massat 15±5 °C. |  | **Note:** **pH**: 12-13 |
| 89. | Rinse the addition tank with S-805 lot-2. |  |  |
| 90. | Stir the reaction mass for 30-45 min at15±5 °C. |  |  |
| 91. | Raise the reaction mass temperature to 25±5 °C. |  |  |
| 92. | Maintain the reaction mass for 20-22 hrs. at 25±5 °C. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-06** for HPLC, **CRA005-2C** content NMT: 2.0%. If HPLC does not comply, maintain the reaction mass at the same temperature and monitor by HPLC for every 2 hrs., till HPLC complies. |
| 93. | Slowly add water lot-9 to the reaction mass at 30±5 °C. |  |  |
| 94. | Stir the reaction mass for 30-45 min at 30±5 °C. |  |  |
| 95. | Distill the reaction mass under vacuum at below 40°C to remove S-805 from the reaction mass. |  |  |
| 96. | Cool the reaction mass to 35±5 °C. |  |  |
| 97. | Charge S-832 Lot-5 into the reaction mass at 35±5 °C. |  |  |
| 98. | Stir for 5-10 min. |  |  |
| 99. | Separate the layers. |  |  |
| 100. | Take the aqueous layer into RBF. |  |  |
| 101. | Charge S-832 lot-6 into aqueous layer. |  |  |
| 102. | Stir for 5-10 min. |  |  |
| 103. | Charge S-832 Lot-7 into the reaction mass at 35±5 °C. |  |  |
| 104. | Stir for 5-10 min. |  |  |
| 105. | Separate the layers and combine the organic layers. |  |  |
| 106. | Prepare M-081 solution with water lot-10 and M-081 lot-1 in a separate container. |  |  |
| 107. | Wash the organic layer with above prepared M-081 solution for 1h. |  |  |
| 108. | Separate the layers (Upper layer-organic layer) |  |  |
| 109. | Prepare M-081 solution with water lot-11 and M-081 lot-2 in a separate container. |  |  |
| 110. | Wash the organic layer with above prepared M-081 solution. |  |  |
| 111. | Separate the layers (Upper layer-organic layer) |  |  |
| 112. | Wash the organic layer with water lot-13. |  |  |
| 113. | Distill the organic layer under vacuum at below 45°C. |  |  |
| 114. | Co-distill thrice with S-831 lot-7, lot-8, lot-9 at below 45°C. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-07** for water content, Water content should be **NMT: 0.2%**. If water content does not comply, repeat the co distillation with S-831(Opp. No. 110), till water content complies.  **Note:** The desired product will obtain as an oil. |
| 115. | Unload the reaction mass into a clean and dry container under nitrogen for checking the weight of total compound. | Wt. of reaction mass =**Z** (~36.5 g) | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-08** for assay.  **Note:** Based on the assay, the quantity of S-821 lot-1, n-BuLi and S-812 shall be indented. |
| 116. | Take the liquid compound into RBF under nitrogen atmosphere. |  |  |
| 117. | Charge S-821 lot-1 into the reactor under nitrogen atmosphere. |  |  |
| 118. | Cool the reaction mass to -40±5 °C. |  |  |
| 119. | Slowly add M-1669 (2.5 M n-hexane solution) at -40±5 °C under nitrogen atmosphere. |  |  |
| 120. | Maintain the reaction mass for 30-45 min at -40±5 °C under nitrogen atmosphere. |  |  |
| 121. | Cool the reaction mass to -60±5 °C under nitrogen atmosphere. |  |  |
| 122. | Slowly add S-812 at -60±5 °C under nitrogen atmosphere. |  |  |
| 123. | Maintain the reaction mass for 30-45 min at -60±5 °C under nitrogen atmosphere. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-09** for HPLC, **CRA005-3C** content NMT: 5.0%. If HPLC does not comply, maintain the reaction mass at the same temperature and monitor by HPLC for every 2 hrs., till HPLC complies. |
| 124. | Allow the reaction mass to 10±10°C. |  |  |
| 125. | Prepare potassium hydrogen phosphate solution with potassium hydrogen phosphate lot-1 and water lot-13 in a separate container. |  |  |
| 126. | Add MTBE lot-8 to the reaction mass |  |  |
| 127. | Add potassium hydrogen phosphate solution slowly into the reaction mass at 10±10°C. |  |  |
| 128. | Stir the reaction mass for 60-90 min at 10±10°C. |  |  |
| 129. | Filter the unwanted solid through cloth filter and wash the solid with S-832 lot-9. |  |  |
| 130. | Take the filtrate into separating funnel and separate the layers. |  | **Note:** Upper organic layer contains product;pH of the aqueous layer ~ 7. |
| 131. | Charge aqueous layer into RBF. |  |  |
| 132. | Charge S-832 lot-10 into the aqueous layer. |  |  |
| 133. | Stir for 60 min and allow to settle for 60 min. |  |  |
| 134. | Separate layers. |  |  |
| 135. | Charge aqueous layer into RBF. |  |  |
| 136. | Charge S-832 lot-11 into the aqueous layer. |  |  |
| 137. | Stir for 60 min and allow to settle for 60 min. |  |  |
| 138. | Separate layers and combine the organic layers (S-832 lot-9, lot-10, lot-11). |  |  |
| 139. | Prepare potassium hydrogen phosphate solution with potassium hydrogen phosphate lot-2 and water lot-14 in a separate container. |  |  |
| 140. | Charge above prepared potassium hydrogen phosphate solution into organic layer. |  |  |
| 141. | Stir for 60-90 min at 30±5°C. |  |  |
| 142. | Separate the layers. |  |  |
| 143 | Distill the organic layer to ~6-7V under vacuum at below 45°C. |  |  |
| 144 | Co-distill with S-831 lot-10 to ~7V under vacuum at below 45°C. |  |  |
| 145 | Cool the reaction mass to -10±5°C. |  |  |
| 146 | Stir the reaction mass for 2-3 hrs. at -10±5°C. |  | **Note:** Slow ppt. will be observed |
| 147 | Filter the compound under nitrogen atmosphere and dry the wet cake by blowing N2 through the solid. |  |  |
| 148 | Dry the compound under vacuum at 40 ±5°C for 3-4 hrs. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-4C-10** for LOD, **LOD** should beNMT: 1.0%. If LOD does not comply, dry the compound at the same temperature, till LOD complies. |

### In-Process Controls

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No** | **IPC** | **Test** | **Specification** | **Results(%)** |
| 1 | CRA005-4C-01 | 2,6 Dimethoxy pyridine content by  HPLC | NMT 5.0% | 0.30 |
| 2 | CRA005-4C-02 | DCM content by GC | NMT 0.1% | 0.002 |
| 3 | CRA005-4C-03 | Assay by HPLC | Report results | 22.15 |
| 4 | CRA005-4C-04 | CRA005-1C content  by HPLC | NMT: 5.0% | Nill |
| 5 | CRA005-4C-05 | Assay by HPLC | Report results | 97.25 |
| 6 | CRA005-4C-06 | CRA005-2C content  by HPLC | NMT: 2.0% | Nill |
| 7 | CRA005-4C-07 | Water content by KF | NMT: 0.2% | 0.02 |
| 8 | CRA005-4C-08 | Assay by HPLC | Report results | 98.89 |
| 9 | CRA005-4C-09 | CRA005-3C content  by HPLC | NMT: 5.0% | 2.26 |
| 10 | CRA005-4C-10 | LoD | NMT:1.0% | 0.09 |

### Discussion

*Process Performance*

Based on the optimized condition a four step telescopic process was designed to convert the starting material to the desired product. The final compound was isolated using n-heptane yielding the final compound in an overall yield of 40.42% with a final purity of 99.02%.

*Impurity Profile*

The synthesis of TMINA consists of four stages in which three stages are in-situ (1C, 2C & 3C) and liquid in nature, hence, no impurities were isolated. However, the plausible impurities were identified based on the LC-MS analysis and the details were provided below. The di-iodo impurity formed at the stage-1C, and des-iodo impurity at 2C has purge-ability at stage-3C. The regio-isomer impurity formed at stage-4C has purge during isolation process at 4C.

|  |  |  |  |
| --- | --- | --- | --- |
| **Batch No** | **In-process HPLC analysis** | | **Structure** |
| **RRT** | **Area %** |
| PCRA005/4C00124  (Stage-1C) | 1.20 | 8.69 | Di-iodo impurity |
| PCRA005/4C00124  (Stage-2C) | 2.37 | ND |  |
| PCRA005/4C00124  (Stage-4C) | NA | NA | Regio-isomer |

## Production of KSM-2 Stage-1A

### Reaction Scheme



### Production Summary

One batch (ECRA005/1A00124) of CRA005/1A (5.0 Kg of SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC GC (% area)** | **CRA005/1A**  **(kg)** | **HPLC Purity (% area)** |
| ECRA005/1A00124 | 5.0 | M-37000124 | 100.0 | 8.35 | 98.38 | 84.7 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of aniline. The manufacture of 3-Chloro-N-Phenylpropanamide (CRA005/1A) is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
| 1 | Arrange clean and dry RBF. |  |  |
| 2 | Charge Acetone/S-805 into RBF at 30±5°C. | 500 mL |  |
| 3 | Charge Aniline/M-370 into RBF at 30±5°C. | 50 g | **Note:** Reaction mixture is homogeneous. |
| 4 | Charge Pyridine/M-230 into RBF at 30±5°C. | 86.84 mL |  |
| 5 | Cool the reaction mass to 0±5°C. |  |  |
| 6 | Add 3-chloropropionyl chloride slowly drop wise into the reaction mixture at 0±5°C. | 61.5 mL | **Note:**  a) Exothermic high dense fumes were observed.  b) During 3-chloropropionyl chloride addition reaction mass temperature rise at 10-15°C also there is no impact on yield and quality.  c) Reaction mass turns slowly heterogeneous during 3-chloropropionyl chloride addition. |
| 7 | Raise the reaction mixture temperature to 55±5°C. |  |  |
| 8 | Stir the reaction mixture for 2 hours at 55±5°C. |  | **Note:** Heterogeneous reaction.  **In-process control:** Send the reaction mass to AR&D as **CRA005-1A-01** for TLC, Aniline/M-370 content NMT: 5.0%. If TLC does not comply, maintain the reaction mass at the same temperature, till TLC complies. |
| 9 | Distill off solvent under reduce pressure at below 45°C up to ~3V. |  | **Note:** Here dry distillation for longer hrs. leads to change in the description of reaction mass and final product which subsequently show the impact on yield and quality of next stage, therefore it is recommended to distill up to ~3V. |
| 10 | Cool the reaction mass temperature to 30±5°C. |  | **Note:** Heterogeneous reaction. |
| 11 | Charge water-lot-1 into the reaction mixture at 30±5°C. | 500 mL |  |
| 12 | Charge Ethyl acetate-lot-1 into the mass at 30±5°C. | 500 mL |  |
| 13 | Stir the reaction mass 5-10min at 30±5°C. |  | **Note:** Homogenous mass |
| 14 | Separate the layers. |  | **Note:** Upper organic layer contain product |
| 15 | Extract the aqueous layer with ethyl acetate-lot-2. | 250 mL |  |
| 16 | Charge total organic layer into RBF at 30±5°C. |  |  |
| 17 | Prepare 1N HCl solution with water lot-2 and M-061 in a separate container. |  |  |
| 18 | Charge above prepared 1N HCl solution into the organic layer at 30±5°C. | 10 mL |  |
| 19 | Stir the reaction mass 5-10min at 30±5°C. |  | **Note:** To remove the traces of M-230, it is required to wash the organic layer with 1N HCl solution. |
| 20 | Separate the both layers. |  |  |
| 21 | Charge total organic layer into RBF at 30±5°C. |  |  |
| 22 | Charge water-lot-3 into RBF at 30±5°C. |  |  |
| 23 | Stir the reaction mass 5-10min at 30±5°C. |  |  |
| 24 | Separate the both layers. |  |  |
| 25 | Charge total organic layer into RBF at 30±5°C. |  |  |
| 26 | Charge Carbon/M-009 into RBF at 30±5°C. | 5.0 g |  |
| 27 | Raise the reaction mixture temperature to 55±5°C. |  |  |
| 28 | Stir the reaction mixture for 20-30 min at 55±5°C. |  |  |
| 29 | Filter the reaction mass throw hyflo bed. |  |  |
| 30 | Wash the hyflo bed with Ethyl acetate lot-3. |  |  |
| 31 | Organic layer dry over sodium sulphate. |  |  |
| 32 | Distil off the solvent under reduce pressure at below 45°C up to ~3 V. |  | **Note:** Here dry distillation for longer hrs. leads to change in the description of reaction mass and final product which subsequently show the impact on yield and quality of next stage, therefore it is recommended to distill up to ~3V. then go for co-distillation. |
| 33 | Co-distil with n-heptane/S-831 lot-1 at below 45°C. | 50 mL |  |
| 34 | Cool the reaction mass to 30±5°C. |  |  |
| 35 | Charge S-831-lot-2 into crude at 30±5°C. | 250 mL |  |
| 36 | Stir the mass for 30-45 min at 30±5°C. |  | **Note:** Heterogeneous reaction mass. |
| 37 | Filter the solid. |  |  |
| 38 | Wash the wet solid with n-heptane/ S-831 lot-3. | 100 mL |  |
| 39 | Unload the compound. |  |  |
| 40 | Dry the solid for 8-10 hours at 50±5°C. |  | **In-process control:** Send the reaction mass to AR&D as **CRA005-1A-02** for LOD, LOD should be  NMT 3.0% w/w. If LOD does not comply, dry the compound at the same temperature, till LOD complies. |
| 41 | Pack the material as per the predefined packing conditions and store at recommended storage conditions. |  | Dry weight: 80 g |
| 42 | Submit the sample to AR&D for complete analysis (STP No-CRA005/1A-IM-001). |  |  |
| 43 | Yield range: 85-95% |  |  |

### n-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1 (CRA005:  Aniline content by TLC | NMT 5.0 | ECRA005/1A00124 | Complies |
| 2 | IPC-2: LOD content: | NMT 1.0%: | ECRA005/1A00124 | 0.67 |

### Discussion

*Process Performance*

The process performance for the reaction, optimized with 1.0 equivalents of aniline, 1.2 equivalents of 3-chloropropionyl chloride, 2.0 equivalents of pyridine, and 10.0 volumes of acetone, successfully converted the starting material to the product within 2 hours. The reaction was quenched with water, extracted with ethyl acetate, and neutralized with 1.0 N HCl. The product was isolated using n-heptane, yielding 84.7% with a purity of 98.38%.

*Impurity Profile*

The impurity profile is presented in the table below. Four impurities were observed in the isolated product (Batch number: ECRA005/1A00124). Two of the impurities remained unidentified. All the impurities listed were noted to be present in the final sample. Overall good purging of impurities was observed in the subsequent steps.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| 0.64 | NA | NA | 0.29 |  |
| 0.73 | NA | NA | 0.24 |  |
| 0.22 | NA | NA | 0.43 | NA |
| 0.85 | NA | NA | 0.02 | NA |
| Stage 1A | NA | NA | 98.38 |  |

## Production of KSM-2 Stage-2A (CRA005/2A)

### Reaction Scheme



### Production Summary

One batch (ECRA005/2A00124) of CRA005/2A (8.2 Kg of CRA005/1A SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC Purity (% area)** | **CRA005/2A**  **(kg)** | **HPLC Purity (% area)** |
| ECRA005/2A00124 | 8.2 | ECRA005/1A00124 | 98.38 | 5,15 | 92.99 | 78 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of CRA005/1A. The manufacture of 3,4-dihydroquinolin-2(1H)-one (CRA005/2A) is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
| 1 | Arrange clean and dry RBF. |  |  |
| 2 | Charge AlCl3/M-220 into RBF at 30±5 ℃. | 231.45 g |  |
| 3 | Charge CRA005/1A into RBF at 30±5 ℃. | 75 g |  |
| 4 | Rise the reaction mixture temperature to 105±5 ℃. |  | **Note:** Reaction mixture becomes a slurry mass. |
| 5 | Stir the reaction mixture for 1-2 hours at 105±5 ℃. |  | **In-process control:** Send the reaction mass to AR&D as CRA005-2A-01 for TLC, CRA005/1A content NMT: 5.0%. If TLC does not comply, maintain the reaction mass at the same temperature, till TLC complies.  **Note:** Here maintenance time of the reaction is critical with respect to impurity formation, therefore it is recommended to proceed further by default after 2 hrs maintenance without waiting for results from QC.  **Impact of excess maintenance:** Some polar impurities observed by excess maintenance. Consistent yield and quality is observed at below 2 hrs. |
| 6 | Reaction mixture is allowed to cool to 60±5°C. |  |  |
| 7 | Add Toluene-lot-1 drop wise at 60±5°C. | 375 mL | **Note:** Reaction mass is homogeneous |
| 8 | Arrange another RBF at 30±5°C. |  |  |
| 9 | Charge water lot-1 into the RBF at 30±5°C. | 1500mL |  |
| 10 | Charge Hydrochloric acid into the RBF at 30±5°C. | 75 mL |  |
| 11 | Cool the reaction mass (OP. No-10) to 10±5°C. |  |  |
| 12 | Add above reaction mass (OP. No-7) slowly drop wise at 10±5°C. |  | **Note:** Addition of reaction mass is highly exothermic; temperature may increase up to 30 °C. However, there is no impact on product quality with increase of temperature. |
| 13 | Stir the mass for 60-90 minutes at 15±5°C. |  |  |
| 14 | Filter the compound and wash with water-lot-2. | 375 mL |  |
| 15 | Wash the wet compound with toluene-lot-2. | 375 mL |  |
| 16 | Charge wet compound into RBF at 30±5°C. |  |  |
| 17 | Charge water-lot-3 into RBF at 30±5°C. | 375 mL |  |
| 18 | Stir for 30-45minutes at 30±5°C. |  |  |
| 19 | Filter the solid and wash with water lot-4. | 150 mL |  |
| 20 | Dry the solid for 8-10 hours under vacuum at 60±5°C. |  | **In-process control:** Send the reaction mass to AR&D as CRA005-2A-02 for water content, Water content should be NMT 1.0% w/w. If WC does not comply, dry the compound at the same temperature, till WC complies. |
| 21 | Unload the material and pack. |  | Dry weight: 55.8 g |
| 22 | Pack the material as per the predefined packing conditions and store at recommended storage conditions. |  |  |
| 23 | Submit the sample to AR&D for complete analysis (STP No-CRA005/2A-IM-001). |  |  |
| 24 | Yield range: 85-95 % |  |  |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1:  Aniline content by TLC: | NMT 5.0 | ECRA005/2A00124 | Complies |
| 2 | IPC-2: Water content | NMT 1.0% | ECRA005/2A00124 | 0.49 |

### Discussion

*Process Performance*

Based on the optimized conditions (5.0 equivalents of AlCl3), the conversion of starting material to product was achieved within 1-2 hours. The product was then isolated using a water and toluene process, yielding 78 % with a purity of 92.99%.

*Impurity Profile*

The impurity profile is presented in the table below. Two impurities were observed in the isolated product (Batch number: ECRA005/2A00124), 0.76 RRT impunity is identified and the impurity at 3.71 RRT is unidentified. All the impurities listed were noted to be present in the final sample. Overall good purging of impurities was observed in the subsequent steps.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| 0.76 | NA | NA | 1.38 |  |
| 3.71 | NA | NA | 0.62 | NA |
| Stage 2A | NA | NA | 92.99 |  |

## Production of KSM-2 Stage-3A (CRA005/3A)

### Reaction Scheme



### Production Summary

One batch (ECRA005/3A00124) of CRA005/3A (5.10 Kg of CRA005/2A SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC Purity (% area)** | **CRA005/3A**  **(kg)** | **HPLC Purity (% area)** |
| ECRA005/3A00124 | 5.10 | ECRA005/2A00124 | 92.99 | 6.75 | 95.67 | 86.16 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of 3,4-dihydroquinolin-2(1H)-one (CRA005/2A). The manufacture of 6-bromo-3,4-dihydroquinolin-2(1H)-one (CRA005/3A) is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
| 1 | Arrange clean and dry RBF. |  |  |
| 2 | Charge DMF/ S-812 into RBF at 30±5°C. | 100 mL |  |
| 3 | Charge CRA005/2A into RBF at 30±5°C. | 10 g |  |
| 4 | Cool the reaction mass to 0±5°C. |  |  |
| 5 | Add NBS/M-938 into the reaction mixture portion wise at 0±5°C. | 13.30 g | **Note:** M-938 was divided in to 5-6 equal lots. Reaction mixture is homogeneous. |
| 6 | Raise the reaction mass temperature to 5±5°C. |  |  |
| 7 | Stir the reaction mass for 10-12 hours at 5±5°C. |  | **In-process control:** Send the reaction mass to AR&D as CRA005-3A-01 for TLC, CRA005/2A content NMT: 5.0% If HPLC does not comply, maintain the reaction mass at the same temperature, till TLC complies. |
| 8 | Arrange another clean RBF. |  |  |
| 9 | Charge water-lot-1 into the RBF at 30±5°C. | 200 mL |  |
| 10 | Cool the water to 15±5°C. |  |  |
| 11 | Add above reaction mixture (Op. No-7) in to the water drop wise at 15±5°C. |  | **Note:** Solid precipitation was observed |
| 12 | Stir the reaction mixture for 90-120 min at 15±5°C. |  |  |
| 13 | Filter the solid and wash with water-lot-2. | 50 mL |  |
| 14 | Dry the solid under vacuum for 12-16 hours at 60±5°C. |  | **In-process control:** Send the reaction mass to AR&D as CRA005-3A-02 for water content, Water content should be NMT 1.0% w/w. If WC does not comply, dry the compound at the same temperature, till WC complies. |
| 15 | Unload the material and pack. |  | Dry wt 11.0 gm |
| 16 | Pack the material as per the predefined packing conditions and store at recommended storage conditions. |  |  |
| 17 | Submit the sample to AR&D for complete analysis (STP No-CRA005/3A-IM-001). |  |  |
| 18 | Yield range: 70-90 % |  |  |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1:  Aniline content by TLC: | NMT 5.0 | ECRA005/3A00124 | Complies |
| 2 | IPC-2: Water content | NMT 1.0% | ECRA005/3A00124 | 0.32 |

### Discussion

*Process Performance*

Based on the optimized conditions (1.1 equivalents of NBS in DMF (10.0 Volumes)), the conversion of starting material to product was achieved within 12 hours. The product was then isolated using a water, yielding 86.16 % with a purity of 95.67%. The isolated solid contained a 0.59 % impurity at 0.56 RRT (CRA005/2A) and 0.66 % impurity at 1.64 RRT. However, this 2A impurity will be converted into stage-4A des-bromo impurity, which has wash-ability at stage-4A to less than <0.1%.

*Impurity Profile*

The impurity profile for Batch number ECRA005/3A00124 is provided in the table below. Six impurities were detected in the isolated product, two of which were identified as starting material and dibromo impurities, while the remaining four impurities remain unidentified. All listed impurities were present in the final sample. However, overall, good purging of impurities was observed in the subsequent steps.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| 0.56 | NA | NA | 0.59 |  |
| 1.64 | NA | NA | 0.66 |  |
| Stage 3A | NA | NA | 95.67 |  |
| 0.39 | NA | NA | 0.22 | NA |
| 0.43 | NA | NA | 0.27 | NA |
| 1.15 | NA | NA | 0.44 | NA |
| 1.18 | NA | NA | 0.88 | NA |

## Production of KSM-2 (CRA005/4A)

### Reaction Scheme



### Production Summary

One batch (PCRA005/4A00124) of CRA005/4A (3.9 Kg of CRA005/3A SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC Purity (% area)** | **CRA005/4A**  **(kg)** | **HPLC Purity (% area)** |
| PCRA005/4A0024 | 3.9 | ECRA005/3A00124 | 95.67 | 7.03 | 96.53 | 87 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of TMINA (CRA005/2B). The manufacture of CRA005/4A is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
| 1 | Arrange clean and dry RBF. |  |  |
| 2 | Charge Methanol lot-1/S-801 into the RBF at 30±5°C. | 350 mL |  |
| 3 | Charge CRA005/3A into the RBF at 30±5°C. | 44.14 g |  |
| 4 | Charge CRA005/4C (TMINA) into the RBF at 30±5°C. | 35 g | **Description:** Heterogeneous mass. |
| 5 | Charge 25% Sodium methoxide solution into the RBF at 30±5°C. | 95.89 mL |  |
| 6 | Raise the reaction mixture temperature to 65±5°C. |  | **Description:** Heterogeneous mass. |
| 7 | Stir the reaction mixture for 22-24 hours at 65±5°C. |  | **Description:** Homogeneous mass.  In-process control: Send the reaction mass to AR&D as CRA005-4A-01 for HPLC, TMINA content NMT: 5.0%. If HPLC does not comply, maintain the reaction mass at the same temperature, till HPLC complies. It HPLC does not complies even after 26 h proceed to workup. |
| 8 | Cool the reaction mass temperature to 30±5°C. |  | **Description:** Heterogeneous mass. |
| 9 | Charge water-lot-1 into the reaction mixture at 30±5°C. | 350 mL |  |
| 10 | Stir the mass for 45-60 min at 30±5°C. |  |  |
| 11 | Filter the solid. |  | **Note:** 5 mics. Filter cloth is recommended for filtration. |
| 12 | Wash the solid with water lot-2 followed by Methanol lot-2. | 175 mL |  |
| 13 | Suck dry the material for not less than 2 h. |  |  |
| 14 | Unload the compound. |  |  |
| 15 | Charge Methanol lot-3 into the RBF at 30±5°C. | 525 mL |  |
| 16 | Charge above wet compound into the RBF at 30±5°C. |  |  |
| 17 | Stir the mass for 90-120 min at 30±5°C. |  |  |
| 18 | Filter the solid. |  |  |
| 19 | Wash the solid with methanol lot-4. |  |  |
| 20 | Suck dry the material for not less than 2 h. |  |  |
| 21 | Unload the compound. |  | **In-process control:** Send the wet compound to AR&D as CRA005-4A-02 for HPLC, CRA005/3A content NMT: 2.0%. If HPLC complies proceed for drying (Op. No. 28); If HPLC does not comply, proceed to purification process (Op. No.22). |
| 22 | Charge Methanol lot-5 into the RBF at 30±5°C. | 525 mL |  |
| 23 | Charge above solid into the RBF at 30±5°C. |  |  |
| 24 | Stir the mass for 45-60 min at 30±5°C. |  |  |
| 25 | Filter the solid. |  |  |
| 26 | Wash the solid with methanol lot-6. | 70 mL |  |
| 27 | Unload the compound. |  | **In-process control:** Send the wet compound to AR&D as CRA005-4A-02 for HPLC, CRA005/3A content NMT: 3.0%. If HPLC complies proceed for drying (Op. No. 28); If HPLC does not comply, repeat purification process (Op. No.22-27). |
| 28 | Dry the solid under hot air oven for 8-10 hours at 60±5°C. |  | **In-process control:** Send the reaction mass to AR&D as CRA005-4A-03 for LOD, LOD should be NMT 1.0% w/w. If LOD does not comply, dry the compound at the same temperature, till LOD content complies. |
| 29 | Pack the material as per the predefined packing conditions and store at recommended storage conditions. |  | Dry Wt: 65.40 g |
| 30 | Submit the sample to AR&D for complete analysis (STP No-CRA005/4A-IM-001). |  |  |
| 31 | Yield range: 75-90 % |  |  |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1: TMINA content by HPLC | NMT: 5.0% | PCRA005/4A | <0.5% (0.38%) |
| 2 | IPC-2:  CRA005/3A content by HPLC | NMT: 3.0%. | PCRA005/4A | 2.78 |
| 3 | **IPC-3**  for LOD, | NMT 1.0% w/w.: | PCRA005/4A | <1.0% (0.08%) |

### Discussion

*Process Performance*

Based on the optimized conditions (1.0 equivalents TMINA, 1.5 equivalents CRA005/3A, 3.0 equivalents NaOMe (25% in MeOH), in 10.0 volume Methanol), the conversion of starting material to product was achieved within 22-24 hours. The product was then isolated using a water and methanol process, yielding 87% with a purity of 96.53%. However, the isolated solid contained a 2.78% impurity with a 0.47 RRT (CRA005/3A).

*Impurity Profile*

The impurity profile is provided in the table below. CRA005/3A (starting material) was identified as the major impurity (Batch number: PCRA005/4A00124). This impurity was also detected in the final sample. However, overall, effective purging of impurities was observed during the subsequent steps.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| 0.47 | NA | NA | 2.78 |  |
| CRA005/4A | NA | NA | 96.53 |  |

## Production of KSM-2 (CRA005/5A)

### Reaction Scheme



### Production Summary

Two batch (PCRA005/5A00124 & PCRA005/5A00224) of CRA005/5A (3.5 Kg of CRA005/4A SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch  Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC Purity (% area)** | **CRA005/5A**  **(kg)** | **HPLC Purity (% area)** |
| PCRA005/5A00124 | 3.5 | PCRA005/4A00124 | 96.53 | 2.65 | 97.41 | 72.6 |
| PCRA005/5A00224 | 3.5 | 2.62 | 97.42 | 71.78 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of 6-bromo-3-((2,3,6-trimethoxypyridin-4-yl) methyl)quinolin-2(1H)-one (CRA005/4A). The manufacture of CRA005/5A is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
|  | Arrange a clean and dry RBF. |  |  |
|  | Charge Toluene into the RBF at 30±5°C. | 750 mL |  |
|  | Charge CRA005/4A into the RBF at 30±5°C. | 50 g | Reaction mixture is heterogeneous. |
|  | Add POCl3 drop wise into the reaction mass at 30±5°C. | 94.6 mL |  |
|  | Raise the reaction mixture temperature to 95±5°C.  Note: Hazy reaction mass. |  |  |
|  | Stir the reaction mixture for 4-5 hours at 95±5 °C. |  | Hazy reaction mass  Observation: temperature more than 105 °C is not recommended. POCl3 will evaporate.  **In-process Control-01**: Send the reaction mass to AR&D as CRA005-5A-01 for HPLC, CRA005/4A content NMT: 5.0%. If HPLC does not comply, maintain the reaction at the same temperature and send a sample for HPLC every 4 hours till HPLC complies. If HPLC does not comply even after 10 hours, then proceed to workup. |
|  | Cool the reaction mass temperature to 15±5 °C. |  | Hazy reaction mass. |
|  | Add water-lot-1 into the reaction mixture at 15±5 °C. | 500 mL | Highly exothermic |
|  | Adjust the Reaction mass pH to 7.0-8.0 with aqueous sodium hydroxide solution. |  |  |
|  | Preparation of aqueous Sodium hydroxide solution: Dissolve Sodium hydroxide in water lot-2 in a separate container. |  | During basification, reaction mass turns hazy heterogeneous mass  **In-process control-02:** Send the bottom aqueous layer to AR&D as CRA005-5A-02 for pH, pH should be in between 7.0-8.0. If pH does not comply, add excess sodium hydroxide solution, till pH complies.  Note: If pH is above 8, Adjust back to the limit by using con. HCl solution. |
|  | Heat the reaction mass to 30±5°C. |  |  |
|  | Charge Ethyl acetate lot-1 into the reaction mass at 30±5 °C. | 500 mL | **Observation:** Reaction mass is hazy and emulsion will be observed. |
|  | Prepare hyflo bed using hyflo and ethyl acetate Lot-2. | 250 mL |  |
|  | Filter the reaction mass through the hyflo bed and wash the bed with ethyl acetate Lot-3 and suck dry under vacuum till no drops are observed. | 250 mL |  |
|  | Stir the reaction mass for 5-10 min at 30±5°C. |  |  |
|  | Settle the organic layer for not less than 20 min at 30±5°C. |  |  |
|  | Separate the layers and keep the organic layer aside. |  | Upper organic layer contain product |
|  | Charge aqueous layer back into RBF. |  |  |
|  | Charge Ethyl acetate lot-4 into the RBF at 30±5 °C. | 500 mL |  |
|  | Stir the reaction mass for 5-10 min at 30±5°C. |  |  |
|  | Settle the organic layer for not less than 20 min at 30±5 °C. |  |  |
|  | Separate the organic layer. |  |  |
|  | Combine organic layers from operation 17 & 22 and charge into the RBF at 30±5 °C. |  |  |
|  | Prepare sodium chloride solution using water lot-2 in a separate container. | 750 mL |  |
|  | Charge sodium chloride solution into the RBF at 30±5 °C. |  |  |
|  | Stir the reaction mass for 5-10 min at 30±5 °C. |  |  |
|  | Settle the organic layer for not less than 20 min at 30±5 °C. |  |  |
|  | Separate the organic layer. |  |  |
|  | Distill off the solvent under reduced pressure at below 50 °C. |  | Solid compound will be obtained by the end of distillation. |
|  | Co-distil with n-heptane lot-1 at below 50°C. | 250 mL |  |
|  | Cool the reaction mass to 20±5 °C. |  |  |
|  | Charge S-831-lot-2 into crude at 20±5°C. | 250 mL |  |
|  | Stir the mass for 30-45 min at 20±5°C. |  |  |
|  | Filter the solid. |  |  |
|  | Wash the solid with n-heptane lot-3. | 100 mL |  |
|  | Unload the compound. |  |  |
|  | Dry the solid for 8-10 hours at 50±5°C. |  | **In-process control-03:** Send the Sample to AR&D as CRA005-5A-03 for LOD, LOD should be NMT 1.0% w/w. If LOD does not comply, dry the compound at the same temperature, till LOD complies. |
|  | Pack the material as per the predefined packing conditions and store at recommended storage conditions. |  | Dry Wt: 43.0 g |
|  | Submit the sample to AR&D for complete analysis (STP No-CRA005/5A-IM-001). |  |  |
|  | Yield range: 75-90 % |  |  |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1:  CRA005/4A content | NMT: 5.0% | PCRA005/5A00124 | 0.1% |
| PCRA005/5A00224 | 0.1% |
| 2 | IPC-2: Aqueous layer for pH | (7.0-8.0) | PCRA005/5A00124 | 7.3 |
| PCRA005/5A00224 | 7.5 |
| 3 | IPC-3: Dry solid LOD | NMT 1.0% | PCRA005/5A00124 | 0.13 |
| PCRA005/5A00224 | 0.2 |

### Discussion

*Process Performance*

Under the optimized conditions (1.0 equivalents of CRA005/4A, 5.0 equivalents of POCl3, in 10.0 volumes of toluene), the conversion of the starting material to the product was achieved within 4-5 hours. The product was then quenched with water, neutralized using NaOH, and extracted with an ethyl acetate solution. It was subsequently isolated using n-heptane, yielding approximately 78% with a purity of around 97%. However, the isolated solid contained three unknown impurities at RRT 0.56, 0.84, and 1.07. This suggests that a more efficient isolation process is required to minimize these impurities.

*Impurity Profile*

The impurity profile is provided in the table below. CRA005/4A (starting material) was identified as the major impurity (Batch number: PCRA005/5A00124). This impurity was also detected in the final sample. However, overall, effective purging of impurities was observed during the subsequent steps.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Batch** | **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| PCRA005/5A00124 | 0.56 | NA | NA | 1.38 | NA |
| PCRA005/5A00224 | 1.47 |
| PCRA005/5A00124 | 0.84 | NA | NA | 0.07 | NA |
| PCRA005/5A00224 | 0.11 |
| PCRA005/5A00124 | 1.07 | NA | NA | 0.54 | NA |
| PCRA005/5A00224 | 0.41 |
| PCRA005/5A00124 | 0.69 (SM) | NA | NA | 0.04 |  |
| PCRA005/5A00224 | ND |
| PCRA005/5A00124 | CRA005/5A | NA | NA | 97.41 |  |
| PCRA005/5A00224 | 97.42 |

## Production of KSM-2 (CRA005/6A)

### Reaction Scheme



# Process optimization:

## Optimization of 25% NaOMe solution equivalents:

Building upon the process familiarization, a series of experiments were conducted to evaluate the impact of varying equivalents of 25% NaOMe solution on the methoxylation reaction. The results are summarized below:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch No** | **Input (g)** | **25%**  **NaOMe (eq)** | **Solvent** | **Solvent**  **(Vol)** | **Temp**  **(°C)** | **Time**  **(h)** | **Output**  **(g)** | **Yield (%)** | **Purity of stage-6A (%)** |
| 1 | CRA005/A013/6A/01 | 19 | 3.0 | MeOH | 15 | 65 | 22 | 17 | 90 | 96.07 |
| 2 | CRA005/A013/6A/02 | 38 | 3.0 | MeOH | 15 | 65 | 22 | 34 | 90 | 96.86 |
| 3 | CRA005/A020/6A/01 | 2.0 | 5.0 | MeOH | 15 | 65 | 22 | 1.8 | 91.3 | 96.5 |

**Purity by HPLC details**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch No.** | **Purity by HPLC (% area)** | | | |
| **Pro  RT 17.97 RRT 1.0** | **SM RT 16.90 RRT 0.94** | **UK**  **RT 19.86 RRT 1.11** | **RT 20.15 RRT 1.12 (S6A-dibr)** |
| 1 | CRA005/A013/6A/01 | 96.07 | 3.37 | 0.6 | 0.10 |
| 2 | CRA005/A013/6A/02 | 96.86 | 2.26 | 0.3 | 0.03 |
| 3 | CRA005/A020/6A/01 | 96.5 | 2.08 | 0.1 | ND |

**Conclusion:** The optimization of NaOMe equivalents demonstrated that increasing the amount of 25% NaOMe solution to 5.0 equivalents (Batch CRA005/A020/6A/01) resulted in a slightly higher yield (91.3%) and comparable purity (96.5%) of Stage-6A, compared to the 3.0 equivalents used in the other two batches (90% yield, purities of 96.07% and 96.86%). However, there was incomplete conversion of Stage-5A to Stage-6A, indicating that higher equivalents of NaOMe alone may not be sufficient to achieve the desired quality and complete conversion. The results suggest that the optimal equivalent of NaOMe is 3.0 equivalents, as it achieved consistent yields and high purity with minimal impurities across different batch sizes. Therefore, it is recommended to proceed with 3.0 equivalents of 25% NaOMe solution in future reactions for optimal performance, while further investigations may be needed to address the minor residual Stage-5A impurity.

## Optimization of solvent volumes:

Building on the process familiarization, MeOH was selected as the reaction solvent, and experiments were conducted with varying volumes of MeOH to assess their impact on yield and purity. The details provided below.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch No** | **Input (g)** | **25%**  **NaOMe (eq)** | **Solvent** | **Solvent**  **(Vol)** | **Temp**  **(°C)** | **Time**  **(h)** | **Output**  **(g)** | **Yield (%)** | **Purity of stage-6A (%)** |
| 1 | CRA005/A013/6A/01 | 19 | 3.0 | MeOH | 15 | 65 | 22 | 17 | 90 | 96.07 |
| 2 | CRA005/A013/6A/02 | 38 | 3.0 | MeOH | 15 | 65 | 22 | 34 | 90 | 96.86 |
| 3 | CRA005/A020/6A/09 | 5.0 | 3.0 | MeOH | **10** | 65 | 22 | NA | NA | As per TLC: ~20% SM remains |

**Conclusion:** Based on the experimental data, even 15 volumes of MeOH yielded similar conversion, yield, and purity. However, since the reaction is heterogeneous, increasing the solvent volume likely improves reaction conditions by enhancing solubility and ensuring better heat distribution. Therefore, 15 volumes of MeOH are recommended as the optimal solvent volume to achieve higher yield and purity in this process.

## Optimization of reaction temperature:

Building on the process familiarization, the optimal reaction temperature for the conversion of Stage-5A to Stage-6A was determined to be 60-65 °C. To further verify these optimal conditions, the following set of experiments were conducted, and the data is summarized below.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch No** | **Input (g)** | **25%**  **NaOMe (eq)** | **Solvent** | **Solvent**  **(Vol)** | **Temp**  **(°C)** | **Time**  **(h)** | **Output**  **(g)** | **Yield (%)** | **Purity of stage-6A (%)** |
| 1 | CRA005/A013/6A/01 | 19 | 3.0 | MeOH | 15 | 65 | 22 | 17 | 90 | 96.07 |
| 2 | CRA005/A013/6A/02 | 38 | 3.0 | MeOH | 15 | 65 | 22 | 34 | 90 | 96.86 |
| 3 | CRA005/A020/6A/08 | 5.0 | 3.0 | MeOH | 15 | 50 | 22 | NA | NA | As per TLC: ~20% SM remains |

**Conclusion:** Based on the experimental data, the optimal reaction temperature for the conversion of Stage-5A to Stage-6A was confirmed to be 60-65°C. The experiments conducted at this temperature range consistently yielded high purity and good conversions. Specifically, experiments at 60-65°C resulted in yields of 90% and HPLC purities ranging from 96.07% to 96.86%. The reaction performed at lower temperatures (50-55 °C) resulted in significantly poorer conversion and incomplete reaction, as indicated by the presence of residual starting material. Thus, it can be concluded that maintaining the reaction temperature at 60-65°C is critical for achieving optimal yield and purity in this transformation.

## Purification experimental details to eliminate Stage-5A in Stage-6A:

The following table summarizes the purification attempts for eliminating Stage-5A and enriching Stage-6A purity. The purification was performed using various solvents and conditions, with the goal of increasing the purity of the isolated Stage-6A product while minimizing Stage-5A contamination.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Batch No** | **Input**  **(g)** | **Output**  **(g)** | **Yield**  **(%w/w)** | **Conditions** |  | **Stage-5A** | **Stage-6A** | **Remarks** |
| **RRT** | **0.94** | **1.0** |
| **Input** | **2.68** | **95.83** |
| CRA005/  A020/6A/03 P1 | 1 | 0.25 | 25% | Heated the solid in Ethyl acetate (10 vol) at 50 °C (clear solution). Cooled to RT and filtered the solid at RT. | Solid  purity | 1.78 | 97.6 | Solid Purity & less reduction in 5A content. |
| CRA005/  A020/6A/03 P2 | 1 | 0.78 | 78% | Dissolved in DCM, treated with carbon at reflux. Distilled and heated in DMSO (2 vol) and methanol (8 vol) to 50 °C and filtered at RT. | solid  purity | 1.97 | 97.77 | Solid Purity & less reduction in 5A content. |
| CRA005/  A020/6A/03 P3 | 0.5 | 0.40 | 80% | Dissolved in DCM, distilled, and heated in DMSO (2 vol) and methanol (8 vol) to 50 °C and filtered at RT. | Solid  purity | 2.02 | 97.37 | Solid Purity & less reduction in 5A content. |
| CRA005/  A020/6A/03 P4 | 1 | 0.90 | 90% | Dissolved in DCM, distilled, and co-distilled with methanol. Heated in methanol (10 vol) to 50 °C and filtered at RT. | solid  purity | 2.25 | 97.11 | Solid Purity & no reduction in 5A content. |
| CRA005/  A020/6A/03 P5 | 1 | 0.80 | 80% | Heated the solid in toluene (2 vol) and methanol (8 vol) at 50 °C and filtered the solid at RT. | solid  purity | 1.97 | 97.62 | Solid Purity & less reduction in 5A content. |
| CRA005/  A020/6A/03 P6 | 1 | 0.80 | 80% | Heated the solid in 20% DCM/Methanol solution at 50 °C and filtered at RT. | solid  purity | 2.55 | 96.93 | Solid Purity & no reduction in 5A content. |
| CRA005/  A020/6A/03 P7 | 0.5 | NA | NA | Heated the crude solid in 25% NaOMe solution in methanol at 60-65 °C for 16 h and filtered at RT. | Reaction  mass | 1.83 | 96.71 | Solid Purity & less reduction in 5A content. |
| **Batch No** | **Input**  **(g)** | **Output**  **(g)** | **Yield**  **(%w/w)** | **Conditions** | **A025/6A/01** | **Stage-5A** | **Stage-6A** | **Remarks** |
| **RRT** | **0.94** | **1.0** |
| **Input** | **3.41** | **96.15** |
| CRA005/A020/6A/07 | 3 | 2.4 | 80% | Heated the solid in 20% MeOH/Toluene solution at 50 °C for 1h and filtered at RT | solid  purity | 1.98 | 97.81 | Purity & ~40% reduction in 5A content. |

### 

### Conclusion: The purification of Stage-6A from Stage-5A was attempted using various solvents and methods to improve the purity of the desired product. The results show that several purification strategies were ineffective in isolating Stage-6A with high purity and minimal contamination from Stage-5A. Among the methods tested, those involving DCM, DMSO, and methanol (P2, P3, and P4) proved to be particularly successful, yielding solid purities between 96.93% and 97.77%, with only slight reductions in Stage-5A content. Specifically, the use of DCM and methanol mixtures (P4 and P5) showed better yields and purities. Heating in a 25% NaOMe solution (P7) resulted in a similar solid purity but only partial purification, as indicated by the reaction mass output. Additionally, the 20% MeOH in Toluene solution (CRA005/A020/6A/07) achieved a 40% reduction in Stage-5A content, with a solid purity of 97.81%. These methods should be further optimized for large-scale production to maximize both yield and purity, as contamination from Stage-5A was observed to carry over into subsequent stages.

## Optimized Reaction Condition.

Despite testing various parameters during both the reaction and purification steps, the process still struggled to remove the Stage-5A impurity. The transfer of Stage-5A impurity into the next stages became a significant concern that needed to be addressed. Upon a more detailed investigation of the reaction conditions, it was determined that the incomplete conversion of Stage-5A to KSM-2 was primarily caused by a lack of homogeneity in the reaction mixture. This lack of homogeneity hindered the reaction from progressing as expected, preventing the full conversion of Stage-5A. By further screening and optimizing the reaction conditions to ensure better mixing and consistency throughout the process, we were able to achieve the complete conversion of Stage-5A to KSM-2, successfully addressing the issue.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch No** | **Input (g)** | **25%**  **NaOMe (eq)** | **Solvent** | **Solvent**  **(Vol)** | **Temp**  **(°C)** | **Time**  **(h)** | **Output**  **(g)** | **Yield (%)** | **Purity of stage-6A (%)** |
| 1 | CRA005/A044/ 6A/01 | 5.0g | 3.0 | Toluene/ Methanol (1:1) | 7.5 + 7.5 | 65 | 18-20 | 4.6g | 93.11 | 99.91 |
| 2 | CRA005/A044/ 6A/02 | 20.0g | 3.0 | Toluene/ Methanol (1:1) | 7.5 + 7.5 | 65 | 18-20 | 18.2g | 91.96 | 99.51 |
| 3 | CRA005/A044/ 6A/03 | 50.0g | 3.0 | Toluene/ Methanol (1:1) | 7.5 + 7.5 | 65 | 18-20 | 44.0 | 88.94 | 98.86 |
|  | CRA005/A044/ 6A/05 | 200g | 3.0 | Toluene/ Methanol (1:1) | 7.5 + 7.5 | 65 | 18-20 | 179.40g | 89.94 | 99.14 |
|  | PCRA005/ 6A00125 | 5.3kg | 3.0 | Toluene/ Methanol (1:1) | 7.5 + 7.5 | 65 | 18-20 | 4.42kg | 84.28 | 98.87 |

**Purity by HPLC details**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Batch Number** | **RT 10.08** | **RT 14.4** | **RT 16.85 (5A)** | **RT 17.90 (6A)** | **RT 19.79** | **Input** | **Output** | **Yield (%)** |
| **RRT 0.61** | **RRT 0.8** | **RRT 0.94** | **RRT 1** | **RRT 1.11** |
| 1 | CRA005/A044/6A/01 | ND | ND | ND | 99.91 | 0.09 | 5.0g | 4.6g | 93.11 |
| 2 | CRA005/A044/6A/02 | ND | 0.07 | ND | 99.51 | 0.42 | 20.0g | 18.2g | 91.96 |
| 3 | CRA005/A044/6A/03 | 0.32 | ND | 0.02 | 98.86 | 0.80 | 50.0g | 44.0 | 88.94 |
| 4 | CRA005/A044/6A/05 | 0.06 | 0.01 | 0.01 | 99.14 | 0.70 | 200g | 179.40g | 89.94 |
| 5 | PCRA005/6A00125 | 0.36 | 0.05 | 0.01 | 98.87 | 0.59 | 5.3kg | 4.42kg | 84.28 |

### Conclusion: The new reaction conditions demonstrated excellent conversion and yield for the Stage-6A reaction. The process was optimized to limit the Stage-5A content to below 0.02%. The yield of Stage-6A ranged from 84% to 93%. This improved process effectively addressed the significant issue of Stage-5A carryover, which had previously been a concern.

### Production Summary

One batch (PCRA005/6A00125) of CRA005/6A (5.3 Kg of CRA005/5A SM) was carried out as described below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch Number** | **Input** | | | **Output** | | **Yield**  **(%)** |
| **SM**  **(kg)** | **Lot Number** | **HPLC Purity (% area)** | **CRA005/6A**  **(kg)** | **HPLC Purity (% area)** |
| PCRA005/6A00125 | 5.3 | PCRA005/5A00125 | 96.5 | 4.42 | 98.87 | 84.28 |

### Manufacturing Process Description

A manufacturing process description for a representative batch is provided below. Molar equivalents and solvents are expressed relative to the weight of 6-bromo-2-chloro-3-((2,3,6-trimethoxypyridin-4-yl) methyl)quinoline (CRA005/5A). The manufacture of CRA005/6A is monitored by in-process control (IPC). Additional parameters are also monitored for informational purposes only.

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Procedure** | **Qty** | **Observations/Remarks** |
|  | Arrange clean and dry RBF. |  |  |
|  | Charge Methanol lot-1/S-801 and Toluene/S-804 into the RBF at 30±5°C. | 375mL,  375mL |  |
|  | Charge CRA005/5A into the RBF at 30±5°C. | 50g | **Description:** Reaction mixture is heterogeneous. |
|  | Charge 25% Sodium methoxide solution into the reaction mass at 30±5°C. | 76.5mL |  |
|  | Raise the reaction mixture temperature to 65±5°C. |  |  |
|  | Stir the reaction mixture for 18-20 hours at 65±5°C. |  | **Description:** Reaction mass is Homogeneous.  **In-process control-1:** Send the reaction mass to AR&D as **CRA005-6A-01** for HPLC, CRA005/5A content NMT: 0.5%. If HPLC does not comply, add second lot of 25% Sodium methoxide solution (1.0eq) and maintain the reaction mass at the same temperature for 4 hours and submit for HPLC, till HPLC complies.  **Sample preparation:** Collect ~5.0 mL add water 5.0 mL and Ethyl acetate 5.0 mL stir the sample for 5 min separate the layer Upper layer send for analysis to AR&D. |
|  | Distil off the solvent under vacuum at below 45°C up to ~1-2V. |  | **Description:** Reaction mass is Heterogeneous. |
|  | Cool the reaction mass temperature to 30±5°C. |  |  |
|  | Charge water-lot-1 into the reaction mixture at 30±5°C. | 500mL |  |
|  | Stir the reaction mass for 10-15 min at 30±5°C. |  |  |
|  | Charge DCM lot-1 into the mass at 30±5°C. | 500mL |  |
|  | Stir the reaction mass 10-15 min at 30±5°C. |  | **Description:** Homogenous mass. |
|  | Settle the organic layer 10-15 min at 30±5°C. |  |  |
|  | Separate the layers and keep the organic layer aside. |  | **Note:** Bottom organic layer contain product |
|  | Charge aqueous layer back into RBF. |  |  |
|  | Charge DCM lot-2 into the RBF at 30±5 °C. | 250mL |  |
|  | Stir the reaction mass for 10-15 min at 30±5°C. |  |  |
|  | Settle the organic layer for 10-15 min at 30±5°C. |  |  |
|  | Separate the organic layer. |  |  |
|  | Combine organic layers from operation 14 & 19 and charge into the RBF at 30±5°C. |  |  |
|  | Prepare sodium chloride solution using water lot-2 in a separate container. | 250mL |  |
|  | Charge sodium chloride solution into the RBF at 30±5°C. |  |  |
|  | Stir the reaction mass for 10-15 min at 30±5°C. |  |  |
|  | Settle the organic layer for 10-15 min at 30±5°C. |  |  |
|  | Separate the organic layer. |  |  |
|  | Charge organic layer into the RBF at 30±5°C. |  |  |
|  | Charge Carbon/M-009 into RBF at 30±5°C. | 5.0g |  |
|  | Rise the reaction mass temperature to 40°C. |  |  |
|  | Stir the reaction mixture for 30-45min at 40°C. |  |  |
|  | Cool the reaction mixture to 30±5°C. |  |  |
|  | Filter the reaction mass throw hyflo bed. |  |  |
|  | Wash the hyflo bed with DCM lot-3. | 250mL |  |
|  | Distill off the solvent under vacuum at below 40°C. |  | **Note:** Solid compound will be obtained by the end of distillation. |
|  | Co-distil with Methanol/S-801 lot-2 under vacuum at below 40°C. | 100mL |  |
|  | Cool the reaction mass to 30±5°C. |  |  |
|  | Charge Methanol lot-3 into above solid at 30±5°C. | 250mL |  |
|  | Rise the reaction mass temperature to 55±5°C. |  |  |
|  | Stir the reaction mixture for 30-45min at 55±5°C. |  |  |
|  | Cool the reaction mixture to 30±5°C. |  |  |
|  | Stir the mass for 30-45 min at 30±5°C. |  | **Note:** Heterogeneous reaction mass. |
|  | Filter the solid. |  |  |
|  | Wash the solid with Methanol/S-801 lot-4. | 100mL |  |
|  | Suck dry the material for 1-2 hours. |  |  |
|  | Unload the compound. |  |  |
|  | Dry the solid under hot air oven for 8-10 hours at 50±5°C. |  | **In-process control-II:** Send the reaction mass to AR&D as **CRA005-6A-02** for LOD, LOD should be NMT 1.0% w/w. If LOD does not comply, dry the compound at the same temperature, till LOD complies. |
|  | Unload the material and pack. |  |  |
|  | Yield range- 85-93% |  |  |

### In-Process Controls

A summary of in-process controls implemented during the production is summarized in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step** | **IPC**  **Description** | **Acceptance Criteria** | **Batch Number** | **IPC Result** |
| 1 | IPC-1: CRA005/5A content | NMT: 0.5%. | PCRA005/6A00125 | Complies (0.01%) |
| 2 | IPC-2: Dry solid LOD | NMT 1.0% | PCRA005/6A00125 | 0.01% |

### Discussion

*Process Performance*

Under the optimized conditions (1.0 equivalents of CRA005/5A, 3.0 equivalents of NaOMe (25% in Methanol), in 7.5 volumes of Methanol and 7.5 volumes of Toluene), the conversion of the starting material to the product was achieved within 18-20 hours. The product was then isolated using a water and methanol process, yielding 84.28 % with a purity of 98.87%. However, the isolated solid contained a 0.01% impurity with a 0.94 RRT (CRA005/5A).

*Impurity Profile*

The impurity profile for Batch number PCRA005/6A00125 is provided in the table below. Two impurities were detected in the isolated product, one of which was identified as starting material, while the remaining impurity remained unidentified. All listed impurities were present in the final sample. However, overall, good purging of impurities was observed in the subsequent steps.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RRT** | **In-process sample**  **(Not applicable)** | | **Dry Solid** | **Structure** |
| **Sample** | **Area %** | **Area %** |
| **0.94** | NA | NA | 0.01 |  |
| Stage 6A | NA | NA | 98.87 |  |
| **1.11** | NA | NA | 0.59 | NA |

# CONCLUSION & RECOMMENDATION FOR THE MANUFACTURING OF KSM-2

**KSM-2 Stage:**

* The fit-for-purpose optimization has been successfully completed, which includes the establishment of effective isolation processes and the completion of holding studies.
* As initially projected during the early discussions, the Cost of Goods (COG) for KSM-2 remains in the range of $500 to $700, compared to the previous cost of approximately $1492 USD.
* At stage 6A of KSM-2, the new reaction conditions demonstrated excellent conversion and yield for the Stage-6A reaction. The process was optimized to limit the Stage-5A content to below 0.02%. The yield of Stage-6A ranged from 84% to 93%. This improved process effectively addressed the significant issue of Stage-5A carryover, which had previously been a concern.

# Appendix 1

**COAs of all the stages of KSM-2 & TMINA.**

|  |  |  |
| --- | --- | --- |
| **S No** | **Batch Number** | **COA** |
| 1 | ECRA005/4C00124 |  |
| 2 | ECRA005/1A00124 |  |
| 3 | ECRA005/2A00124 |  |
| 4 | ECRA005/3A00124 |  |
| 5 | PCRA005/4A00124 |  |
| 6 | PCRA005/5A00124 |  |
| 7 | PCRA005/5A00224 |  |
| 8 | PCRA005/6A00125 |  |

# Appendix 2

**COAs of Reagents and Auxiliary Materials Used for the Manufacture of TMINA & KSM-2.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Chemical Name** | **CAS No.** | **M.W.** | **Supplier Source** | **Vendor CoA** | **Indented Quantity** | **Remark** |
|  | 4-Amino-3,5-dimethylphenol | 3096-70-6 | 137.18 | BLD Pharma, R Scientific |  | 400 g | 100 g Received, on 11-05-24 300 g Received on 25-06-24 |
|  | Oxalyl Chloride |  | 126.90 | -- | -- | In House | |
|  | 2,6-Dimethoxypyridine | 100-48-5 | 139.19 | Ocimum LS |  | 7 Kg | 7 Kg Received |
|  | NIS |  | 224.90 | -- | -- | In House | |
|  | Na2CO3 |  |  | -- | -- | In House | |
|  | Copper(II) acetylacetonate |  | 181.63 | --- |  | 200 g | Received |
|  | KOH |  | 56.11 | -- | -- | In House | |
|  | Sodium Sulfate |  |  | -- | -- | In House | |
|  | DMS (Dimethyl sulfate) |  | 126.13 | -- | -- | In House | |
|  | K2CO3 |  | 138.20 | -- | -- | In House Available | |
|  | NaOH |  | 94.02 | --- | -- | In House Available | |
|  | n-BuLi (2.5M in Hexane) |  | 64.01 | Neogen chemicals LTD. |  | 19 L | Received on 18-08-24 |
|  | KH2PO4 |  | 198.26 | -- | -- | In House | |
|  | Aniline |  | 93.13 | GN valley |  | In House | |
|  | 3-Chloropropionyl chloride | 625-36-5 | 126.97 | AVRA,  MPPL |  | 30 Kg | Received |
|  | Pyridine |  | 79.17 | -- | -- | In House | |
|  | AlCl3 |  | 113.34 | -- | -- | In House | |
|  | NBS |  | 178.00 | -- | -- | In House | |
|  | M-121 (Sodium methoxide) |  | 54.02 | -- | -- | In House | |
|  | POCl3 |  | 153.33 | -- | -- | In House | |
|  | Sodium chloride |  | -- | -- | -- | In House | |
|  | Activated Carbon |  | -- | -- | -- | In House | |