TECHNOLOGY TRANSFER REPORT

The Medicines for All Initiative
Continuous Synthesis of Nevirapine and Reaction Monitoring with Online PAT

NEVIRAPINE

11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one

November 30, 2015
Executive Summary

An efficient continuous synthesis has been developed for nevirapine drug substance to convert starting materials into product in high yield (87% overall). The process employs inexpensive raw materials in flow to produce an active pharmaceutical ingredient (API) that meets or exceeds all USP specifications. This process utilizes two flow reactors; a Synthetron a spinning disk reactor and a Vapourtec E-Series flow reactor.

This process was initially developed for batch operations as previously reported, has also been implemented in a flow chemistry platform by the Medicines for All team at Virginia Commonwealth University. In addition, inline Process Analytical Technology (PAT) has been implemented by MarqMetrix Inc. (Seattle, WA), which will become the basis for a fully automated system. The ultimate goal of PAT is to establish the ability to distinguish reaction pathways, as well as to assess conversion, side reactions, control and confirm real-time quality assurance for pharmaceutical processes. The results presented herein demonstrate the proof of concept for interfacing continuous API production with online process monitoring to yield a fully integrated manufacturing unit.

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INTRODUCTION:

Nevirapine (Figure 1) is currently a major component of frontline treatment in HIV combination drug therapy. Furthermore, it is one of the most effective treatment options in parasit HIV applications. Though its prominence as a frontline therapy may be supplemented by new drug alternatives, the World Health Organization forecasts that over 600 metric tons per year will be required to treat existing patients for the foreseeable future.

Recently, the Medicines For All (MFA) team developed new low cost batch process to produce nevirapine from two highly substituted pyridine precursors: methyl 2-cyclopropylamino nicotinate (MeCAM) and 2-chloro-3-cyano-4-picoline (CAPIC). The two pyridine starting materials are converted into nevirapine by two discrete chemical reactions that are carried out under strongly basic conditions in high yield. By employing a common solvent system, these reactions have been consolidated into a single process step, which significantly reduces the number of manufacturing unit operations and lowers operating costs.

Herein we report a continuous synthesis of nevirapine using the same precursors MeCAN and CAPIC, which were used for the batch process. In the continuous process, 2-(Cyclopropylamino)-nicotinamido-3’-amino-2’-chloro-4’-methylpyridine (CYCLOR) is produced using a spinning disc reactor in combination with a continuous stirred tank reactor (CSTR). The CYCLOR solution produced in flow is then passed through a packed column of NaH at an elevated temperature, which results in ring closure to produce crude nevirapine. This crude nevirapine employed the same final purification to produce final API which meets all United States Pharmacopeia (USP) specification.

The overall yield for the new process is 87% and the total raw material cost for the new process is estimated to be $58.4/kg based on Chemical Marketing Reporter/ISIS pricing information for commodity based raw materials as well as from individual suppliers. This new streamlined process represents a substantial improvement over the existing five step process that has an overall yield of 59%. Furthermore, the nevirapine API produced from this new process contains no detectable new impurities while the impurity profile and chromatographic purity meets or exceeds USP specifications.

The continuous nevirapine process consists of the following steps:
Step. 1: Rapid synthesis of CAPIC-Na in flow from CAPIC and NaH
Step. 2: Conversion to CYCLOR via a CSTR by addition of CAPIC-Na directly onto MeCAN
Step. 3: Ring closure of CYCLOR to form nevirapine crude in flow through NaH column
Prior Art

Nevirapine has been made commercially by at least two alternative reaction pathways. The first generation method that was used for the nevirapine launch in 1996 (US 5620974) employed 2-chloro-nicotinic acid (2-CAN) and 2-chloro-3-amino-4-picoline (CAPIC) as a starting materials and is provided in Scheme 1.

Scheme 1: First Generation Nevirapine Process

A second generation method was developed that uses 2-cyclopropylamino-nicotinic acid as an alternative starting material (US 6680383) and is provided in Scheme 2. The innovator drug company, Boehringer Ingelheim Pharmaceuticals currently practices the second generation process at a cost in excess of $800/kg. However, the original process is currently being used in other countries to supply the majority of the current nevirapine market. For these suppliers, the selling price ranges from $225/kg to less than $140/kg.
Medicines for All Batch Process

The new nevirapine process entails two reaction steps that can be carried out in a single unit operation (Scheme 3). This alternative approach requires the use of methyl 2-cyclopropylaminononicotinate (Me-CAN) and 2-chloro-3-amino-4-picoline (CAPIC) as starting materials in a single solvent system with a common strong base, sodium hydride. A final purification step is used that is identical to the ones practiced in the current commercial processes in order to ensure that we are able to reproduce all of the solid state properties of the API including crystal morphology, particle size distribution, and bioavailability.

The overall yield for the new process is 87% with an average yield per step of 93%, which represents a major improvement over the current commercial processes. By using a common solvent for both reaction steps, we were able to able to avoid costly solvent exchanges that add significant process complexity requiring waste treatment, remediation, or recycling. Approximately 80% of the diglyme solvent is recovered and recycled as part of the process. The nevirapine API produced from this process was tested for chromatographic purity as well as impurity profile. There were no measurable new impurities by LC/MS and the API meets or exceeds all US Pharmacopeia standards.
Scheme 3: Medicines for All Nevirapine Batch Process

Overall Yield 87%

Medicine for All Flow Chemistry to produce nevirapine continuously:

While the one-pot batch synthesis of nevirapine API developed previously is highly effective, there are specific operational advantages for the development of a continuous flow synthesis of the API. Continuous chemical processes are inherently more reproducible (4 to 5 sigma) compared with batch processing (2 sigma) leading to fewer failed batches and reworking of off spec product. An alternative continuous process for the preparation of nevirapine from CAPIC and Me-CAN has been developed and is provided in Scheme 2 with an overall yield of 87%.

In the initial reaction sequence of the continuous nevirapine process, the sodium salt of CAPIC is reacted with Me-CAN to produce CYCLOR in flow. The CYCLOR solution is then passed through NaH packed column which is heated to 165°C using a Vapourtec E-Series flow reactor. The ring closure reaction is virtually instantaneous and the conversion to the crude nevirapine is almost quantitative. The nevirapine solution in diglyme is worked up similar to batch process to isolate the crude nevirapine. The quality of crude nevirapine is comparable to the MFA batch process. The crude nevirapine is then converted to pure nevirapine using same procedure as described in batch process. The purity and quality of pure nevirapine which is produced with flow chemistry is equivalent to batch process.
Scheme 4: Medicines for All Nevirapine Continuous Process

Procedure for preparation of nevirapine in flow

Table 1 Reagent

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
<th>Mol. Wt. / density</th>
<th>mmol</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPIC</td>
<td>14.25 g</td>
<td>142.59</td>
<td>100</td>
<td>3 M</td>
</tr>
<tr>
<td>NaH (lot 1)</td>
<td>6.6 g</td>
<td>23.99 / 60% suspension</td>
<td>165</td>
<td>2 M</td>
</tr>
<tr>
<td>MeCAN</td>
<td>19.2 g</td>
<td>192.21</td>
<td>100</td>
<td>neat</td>
</tr>
<tr>
<td>NaH (Lot 2)</td>
<td>10 g</td>
<td>23.99 / 60% suspension</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Glass beads</td>
<td>35 g</td>
<td></td>
<td></td>
<td>n/a</td>
</tr>
<tr>
<td>Diglyme</td>
<td>21 ml for CAPIC</td>
<td>76 ml for NaH(1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EXPERIMENTAL PROCEDURE:

Procedure for preparation of NaH Column

1. To a dry Omnifit column (15 mm x 150 mm), the lower plunger was attached and threaded.
2. Coarse filter material (Kimwipe or glass wool) was applied as a thin layer to the bottom of the column.
3. NaH (Lot 2) and glass beads (Sigma Aldrich, 425-600 µm) were added as alternating, thin bands (500 mg of NaH : 1571 mg glass beads per band) in the column such that a glass bead layer formed the top and bottom layer of the column.
4. Coarse filter material was applied to the top layer.
5. The column was fitted into the column heater (Soham Scientific) and the top plunger was attached and threaded.
6. The column and column heater unit was attached to the Vapourtec E-Series.
7. The column was flushed with anhydrous diglyme via the Vapourtec system.

**Procedure for preparation of Nevirapine in flow**

1. The reactors were assembled according to Scheme 1 and two reagent stream stocks were prepared: one, 3 M solution of CAPIC in anhydrous diglyme and one, 2 M slurry of NaH in anhydrous diglyme.
2. CAPIC and NaH were pumped via an alternating syringe pump (Kloehn) into a Kinetchem Synthetron spinning disk reactor, heated to 95 °C, at 2.02 mL min⁻¹ and 5.00 mL min⁻¹, respectively.
3. The CAPIC-Na stream was diverted to waste until CAPIC-Na was produced at a steady state.
4. The CAPIC-Na stream was dropped to MeCAN (1.0 eq, neat), stirring in a nitrogen purged 500 mL round bottom flask.
5. The reaction mixture was stirred at 65 °C for 2 hours until complete formation of CYCLOR. (80-85% CYCLOR was observed by high performance liquid chromatography (HPLC) (conversion to 98% by ¹H NMR).
6. While the CYCLOR reaction mixture stirred, the NaH column was heated to a temperature of 165 °C.
7. The CYCLOR stock solution was pumped using the E-Series Vapourtec system into the NaH column top-down at a flow rate of 1 mL min⁻¹ and the nevirapine crude product stream until a steady-state conversion (by HPLC or ~96% by ¹H NMR,) was observed and collected in a flask.

**Step 2: Crude Nevirapine to Pure Nevirapine**

![Nevirapine Crude](image1)

\[ \text{HN} - \text{O} \]

\[ \text{HN} - \text{O} \]

\[ \text{NaOH, pH \sim 5-7} \]

\[ \text{HCl/H}_2\text{O/Carbon} \]

![Nevirapine Pure](image2)

\[ \text{HN} - \text{O} \]

\[ \text{HN} - \text{O} \]
### Materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>Amount</th>
<th>Mwt./Density</th>
<th>Mmole</th>
<th>Equiv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevirapine Crude</td>
<td>10g</td>
<td>266.3</td>
<td>375.5</td>
<td>1</td>
</tr>
<tr>
<td>H₂O</td>
<td>43 ml</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCl (37%)</td>
<td>11.6 ml</td>
<td>36.5</td>
<td>117.5</td>
<td>0.313</td>
</tr>
<tr>
<td>Activated Carbon (Norit)</td>
<td>0.3g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH (50%)</td>
<td>4.64 ml</td>
<td>40/1.515g/ml</td>
<td>176.2</td>
<td>0.469</td>
</tr>
<tr>
<td>Celite</td>
<td>1gm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Procedure:

1. To a 250 ml 3-neck round-bottom flask fitted with magnetic stirrer, thermocouple and addition funnel charge nevirapine crude (10g, 375.5 mmole) and purified water 43 ml.
2. Stir at room temperature and then lower the content temperature to 0-5°C using ice/water bath.
3. Charge HCl (11.6 ml, 117.5 mmole) drop wise to the above slurry while keeping temperature below 5°C. *(Note 1)*
4. After HCl charge is completed, stir the reaction mixture for around 30 minutes at 0-5°C to make sure all nevirapine dissolved. *(Note 2)*
5. After nevirapine is in solution, charge activated carbon (0.3g) and stir the solution for at least another 30 minutes while keeping temperature between 0-5°C.
6. Filter the solution using celite pad to capture all the carbon.
7. Rinse the celite pad with 2x5ml water.
8. Filter clear filtrate using 4 µ filter to remove any insoluble material/fibers etc. before moving to next step to precipitate the final product. *(Note 3)*
9. Take the clear filtrate into 250 ml 3-neck flask fitted with magnetic stirrer, thermocouple and addition funnel and keep the solution between 0-5°C.
10. Charge NaOH (50% solution) drop wise to pH 4-7, while keeping temperature below 5°C. A white precipitate will appear when desired pH is reached. Stir for about 30 minutes. *(Note 4)*
11. Filter the product under vacuo using filter paper. Wash the solid with water 3x10ml. *(Note 5)*
12. Cut the cake and dry the wet cake between 90 -110°C under vacuo to a constant weight.
13. Isolated yield 9.6g; 96%, Purity 100A% by HPLC. See attached chromatogram.

### Process Notes:

1. *We have seen some impurity if temperature is kept above 10C for considerable time.*
2. *The solution may not be completely clear. It may be hazy due to residual oil.*
3. *Since this is final API, filtering with 4 µ filter is needed to capture fibers, etc.*
4. Since this is final API, use of filtered NaOH is recommended. Less than 50% NaOH solution can be used as suitable for filtration.
5. Optimum wash needs to be optimized during scale-up based on residual salt content of final product and specification. Nevirapine is not soluble in water therefore, more washes has no impact on the yield.

OVERVIEW OF PAT

Process Analytical Technology (PAT) is a term encompassing any technologies, measurement schemes, and analytical tools applied to a process for the purpose of real-time or near real-time monitoring, qualification, quantification, or control of a process.

MarqMetrix Inc. specializes in the development of tools for PAT, effective and representative sampling methods, and intelligent application of PAT for process monitoring and improvement. In addition to univariate measurements such as temperature, pressure, flow rate, refractive index, etc., MarqMetrix has a suite of multivariate measurement tools including Near Infrared Spectroscopy (NIRS), Raman Spectroscopy, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), and Nuclear Magnetic Resonance Spectroscopy (NMR). All or a subset of these tools may be used in conjunction to effectively characterize a process in real or near-real time. The different sensitivity and selectivity of these measurements means that nearly all analyses and quantities of interest may be interrogated by concurrent, representative sampling in both batch and flow processes.

In this body of work, Raman Spectroscopy was applied to all reaction steps with excitation in the near infrared region at 998nm to mitigate severe issues with fluorescence. This technique was chosen due its sensitivity and complementry selectivity to infrared spectroscopy. NMR Spectroscopy was not used due to concerns with clogging of the NMR flow probe by NaH slurries. ATR-FTIR was used for initial experiments, but the light source failed midway through experimentation, precluding its full incorporation into the assessment of PAT. However, initial results using the ATR-FTIR before issues with the light source arose were highly promising, especially in conjunction with Raman Spectroscopy. Finally, NIRS was investigated in offline measurements as the immersion probe interface arrived after flow reactions were completed.

Note: Raman peak assignments are derived either from direct observation of standard spectra and inferences from observing spectral changes, or from the excellent resource, Infrared and Raman Characteristic Group Frequencies: Tables and Charts, by George Socrates (3rd Ed.).
SYNTHESIS OF CAPIC-NA IN BATCH AND FLOW

The synthesis of CAPIC-Na intermediate is the first stage of the nevirapine API synthesis. In batch, this process is time-consuming and troublesome due to the large exotherm generated from the formation of the CAPIC sodamide by the strong base, sodium hydride (NaH). In order to assess the kinetics of this process, and determine spectroscopic regions of interest via PAT, MarqMetrix performed the CAPIC-Na synthesis according to the standard S.O.P. (see Appendix). Subsequently, the standard CAPIC-Na flow synthesis developed by VCU using the Synthetron spinning disk reactor was monitored using PAT.

Equipment and Instrumentation
- Jacketed 3-neck 500 mL flask and inert gas manifold
- Synthetron (Kinetichem) with custom Kloehn syringe pumps
- RXN2 998nm Raman spectrometer (Kaiser Optical Systems Inc.) with MarqMetrix BallProbe (1/4”) sampling interface -or- MarqMetrix Raman Flow Cell sampling interface
- VWR Scientific recirculating heater/chiller
- Data reduction and analysis performed using MatLab (MathWorks) and PLS Toolbox (Eigenvector Research Inc.)

Batch Experimental Procedure

For batch CAPIC-Na synthesis, solid NaH (60% dispersion in mineral oil) and CAPIC were charged into the jacketed flask, followed by anhydrous diglyme. The resulting suspension was stirred and heated incrementally from 30° C to 65° C using a circulating heater.

A thermocouple and ¼” MarqMetrix Raman BallProbe were inserted into a rubber septum and a size 24/40 PTFE fitting with fluoroelastomer gasket respectively, both conferring an airtight seal. Dry N₂ was applied to a third septum via hypodermic needle, and another needle inserted to vent excess N₂ and H₂ evolved from CAPIC-Na formation.

Raman spectra were acquired in 2-minute intervals to track the progress of the reaction. These data are plotted below in figure 2:
Figure 2: (Blue to Red → time=0 to time=final) Evolution of Raman spectra during batch CAPIC-Na synthesis

The evolution of the Raman spectra of CAPIC and NaH in diglyme with time reveals several spectral regions of interest, encapsulating the formation of the CAPIC amide anion and resultant sodium salt, CAPIC-Na. In particular, the region from 600cm⁻¹ to 1100cm⁻¹ provides selective Raman peaks showing a frequency shift in the main 647cm⁻¹ CAPIC peak along with growth of two peaks at 838cm⁻¹ and 1087cm⁻¹, relating to C-NH-Na vibrational modes. This region is plotted below in figure 3, along with an overlay of the Raman spectrum of CAPIC:

Figure 3: (Blue to Red → time=0 to time=final: black – pure CAPIC) Overlay of CAPIC spectrum with Batch CAPIC-Na spectra
As revealed in Figure 3, the shift in the CAPIC band at 647 cm\(^{-1}\) correlates with formation of CAPIC-Na, allowing the peak position to be used to assess reaction completeness. Additionally, the drastic increase in intensity of bands at 838 cm\(^{-1}\) and 1087 cm\(^{-1}\) due to the influence of sodium on the otherwise weak amine modes can be used to follow the reaction progress. These two modes also exhibit notable peak shifts upon formation of CAPIC-Na, further allowing real-time assessment of reaction progress.

It is important to note that the peak position of several of these bands are shifted relative to those of pure, solid CAPIC. Raman Spectroscopy is sensitive to bonds that are polarizable (in contrast to IR spectroscopy, which is sensitive to bond dipole moments), and that the resonant polarizability of a bond is affected, sometimes greatly, by the local chemical environment such as by solvation effects, hydrogen bonding, etc. Unsurprisingly, formation of an ionic analog of the original substrate also yields similar changes in resonant modes.

Kinetic traces of Raman peaks corresponding to newly formed bonds can be derived from these spectroscopic measurements. For example, the aforementioned Raman modes at 651 cm\(^{-1}\), 838 cm\(^{-1}\), and 1087 cm\(^{-1}\) may be plotted as a function of peak intensity vs sample # (time), as shown in figure 4 below:

![CAPIC-Na formation kinetic traces](image)

Figure 4: Kinetics traces of Raman peaks corresponding with formation of CAPIC-Na from CAPIC and NaH in diglyme

From the above plot, it is easy to see that reaction kinetics for this may be easily modeled and obtained. Additionally, this information, as well as general spectroscopic information, may be used to assess whether a continuous process, such as the flow synthesis of CAPIC-Na, has effectively proceeded to completion by the time the product exits the flow reactor.
Flow Experimental Procedure

For synthesis of CAPIC-Na in flow, a solution of 2M NaH in diglyme and 3M CAPIC in diglyme were prepared from anhydrous diglyme. All solutions were kept under an N2 atmosphere, and the NaH solution was stirred such that a uniform, well-suspended slurry was obtained. Both solutions were pumped using a custom Kloehn push-pull syringe pump apparatus made by Kinetichem. In order to obtain a mole ratio of NaH:CAPIC of 1.65:1 equivalents, the NaH solution was delivered at a flow rate of 5mL/min, and the CAPIC at a rate of 2.02mL/min.

Conversion of CAPIC to CAPIC-Na was performed using a Synthetron spinning disk reactor from Kinetichem. This reactor operates by mixing the two solutions in a heated, jacketed chamber, with a disk rotating at high angular velocity positioned between 100um and 500um above the bottom of the reaction vessel. The total internal volume of the reactor is slightly more than 1mL. The rapid spinning action of the disk results in extremely fast mass and heat transfer, and is able to effectively mix and handle the triphasic reaction (solid NaH, liquid diglyme – CAPIC solution, H2 gas) resultant from the reaction between CAPIC and NaH.

The same Raman instrument used for the batch synthesis of CAPIC-Na was used for monitoring the flow synthesis as well. However, the ¼” BallProbe was replaced by a newly developed MarqMetrix Raman Flow Cell, combining the BallProbe sampling interface with a convenient flow geometry for highly reproducible sampling of fluid flow paths. The circulating heater used for the batch synthesis of CAPIC-Na was also used to maintain temperature of the reaction vessel of the Synthetron at 95°C. Representative spectra from the first flow synthesis of CAPIC-Na are shown in figure 5 below:

![Figure 5: (Blue to Red → time=0 to time=final) Spectra acquired from Raman Flow Cell during flow CAPIC-Na synthesis.](image-url)
Removal of excess H₂ gas from the product stream was attempted via several means, including a membrane separator and an HPLC vacuum degasser, but the most successful removal method consisted of a holding vessel consisting of a stirred 3-neck flask with an inlet from the Synthetron outlet stream, vented through a bubbler, and held under positive pressure with N₂. The outlet of this holding vessel was a section of 1/8” PTFE tubing. A needle valve could be adjusted to change the pressure in the vessel, and match the inlet flow rate to the outlet flow rate based on pressure differential. This arrangement improved the signal level considerably and removed all H₂ gas from the final outlet stream.

SYNTHESIS OF CYCLOR AND MONITORING WITH PAT

CYCLOR is currently synthesized in a CSTR owing to the relatively slow kinetics of formation of CYCLOR from CAPIC-Na and MeCAN, and the need to employ relatively mild conditions to prevent hydrolysis of the methyl ester. Several experiments were performed to synthesize CYCLOR both by adding MeCAN to a suspension of NaH and CAPIC-Na in diglyme, and by adding a slurry of NaH and CAPIC-Na in diglyme to MeCAN oil. For the purposes of PAT assessment, the latter method of adding CAPIC-Na to MeCAN was employed for the sake of monitoring consumption of MeCAN with time, as well as formation of CYCLOR.

Equipment and Instrumentation

- A jacketed 3-neck flask and inert gas manifold
- Synthetron (Kinetichem) with custom Kloehn syringe pumps
- Vapourtec E-Series flow reactor with V3 peristaltic pumps
- RXN2 998nm Raman spectrometer (Kaiser Optical Systems Inc.) with MarqMetrix Raman Flow Cell sampling interface
- VWR Scientific recirculating heater/chiller
- Data reduction and analysis performed using MatLab (MathWorks) and PLS Toolbox (Eigenvector Research Inc.)

CYCLOR Synthesis: Addition of CAPIC-Na to MeCAN

Addition of the coarse CAPIC-Na, NaH slurry was accomplished via cannula transfer from a stirred, heated flask under positive N₂ pressure to the jacketed flask containing MeCAN. All solutions and solvents were held under N₂ prior to reaction and all reactions were performed under a dry N₂ atmosphere. The backpressure of N₂ was adjusted with a needle valve in order to obtain an additional rate of the CAPIC-Na slurry of approximately one drop per second. Slightly more than 1 equivalent of CAPIC-Na relative to MeCAN was added to the flask to ensure consumption of MeCAN.
The solution was sampled using a MarqMetrix Raman Flow Cell with 998nm excitation in a sampling loop configuration, using a Vapourtec V3 pump to pull a sample through the flow loop and subsequently reintroduce the sample into the reaction vessel. Spectra were obtained in 2 minute intervals. The recirculating thermostat was maintained at a temperature of 65°C for the duration of the reaction, and internal temperature of the stirred reactor was not observed to deviate more than 2-3°C of the set temperature.

As in previous plots, temporal spectra plots correlate the transition of blue to red with time. Also to note for analysis is the fact that the CYCLOR produced in this synthesis is a sodium salt, meaning that some peak positions may differ from the standard solid. Spectra obtained over the course of the reaction are plotted in figure 7 below, with standard spectra of pure reagents overlaid for reference:

![](image)

Figure 6: Experimental apparatus for synthesis of CYCLOR and online reaction monitoring with Raman Flow Cell (arrows indicate direction of flow of CAPIC-Na slurry and reaction mixture)

Figure 7: (Blue to Red → time=0 to time=final) Spectra acquired from Raman Flow Cell during addition of CAPIC-Na to MeCAN. Brown curve – MeCAN, black curve – 2-CAN, dark green curve - CYCLOR
In-situ Raman Spectroscopy proved highly effective for monitoring this reaction step, as evidenced by the wealth of information contained in the entirety of the Raman spectra. While there are many regions of interest spectroscopically, several are particularly noteworthy. Specifically, the regions of greatest interest are as follows: 750-1000cm\(^{-1}\), 1000-1100cm\(^{-1}\), 1150-1350cm\(^{-1}\), and finally 1350-1750cm\(^{-1}\). These are investigated in detail in figures 8-11 below:

![Figure 8: (Blue to Red → time=0 to time=final) 750-1000cm\(^{-1}\) region](image)

In the 750-1000cm\(^{-1}\) region, the doublet band present in MeCAN from ~800-840cm\(^{-1}\) (also present in 2-CAN), tentatively assigned as a carboxylic acid mode, is clearly seen to decrease in intensity, with a broad band correlating well with the several CYCLOR peaks at 832cm\(^{-1}\), 860cm\(^{-1}\), and 885cm\(^{-1}\) (depicted in figure 7 above) growing in intensity with time. The band growing at ~900cm\(^{-1}\) is not present in any of the standard spectra in Figure 7, but is tentatively assigned to a red-shifted CYCLOR 917.4cm\(^{-1}\) peak due to the fact that the as-formed CYCLOR is the sodium salt of the amide, CYCLOR-Na.
Figure 9 above depicts the 1000-1100 cm\(^{-1}\) region, showing the redshift of the band assigned to the pyridine ring breathing mode. The small (~5 cm\(^{-1}\)) shift results from the slight change of adjacent bonds relating to the formation of the CYCLOR amide from the MeCAN ester. The position of this peak in MeCAN and CYCLOR is 1062 cm\(^{-1}\) and 1053 cm\(^{-1}\) respectively. The final position of this peak in the reaction spectra is 1057 cm\(^{-1}\), slightly off from that of pure CYCLOR, most likely owing to solvation effects or the influence of the amide anion.

Figure 10 below shows the range from 1150-1350 cm\(^{-1}\). This spectral range is particularly rich with information, showing consumption of MeCAN, formation of CYCLOR, and even formation of 2-CAN. The MeCAN peaks at 1214 cm\(^{-1}\), 1257 cm\(^{-1}\), and 1297 cm\(^{-1}\) are clearly decreasing as MeCAN is consumed, either disappearing entirely from the spectrum, or transitioning into the doublet CYCLOR bands at 1206 cm\(^{-1}\) and 1219 cm\(^{-1}\). The growing peak at 1270 cm\(^{-1}\) can be clearly identified as a characteristic CYCLOR peak, most likely the amide C-N stretching vibration (amide III band), while the growing peak at 1333 cm\(^{-1}\) is most probably assigned as a red-shifted 2-CAN peak of the 2-CAN sodium salt, as carboxylate ion bands often appear in this spectral region.
Finally, Figure 11 above reveals information on the consumption of the methyl ester bands along with formation of the CYCLOR amide, as well as some 2-CAN modes. First, the growth of the peak at 1373 cm\(^{-1}\) most likely correlates with a 2-CAN peak at 1378 cm\(^{-1}\) in the solid state, as it is markedly absent from both the MeCAN and CYCLOR standard spectra. The red shift of this peak may be due to the formation of the sodium salt.
Second, the red shift of the MeCAN 1406cm\(^{-1}\) peak to 1401cm\(^{-1}\) once again correlates with the conversion of MeCAN to CYCLOR. Third, the peak at 1573cm\(^{-1}\), while possibly a blue-shifted 2-CAN peak, is most likely assigned to one of the stronger CYCLOR peaks at 1586cm\(^{-1}\), possibly an amide mode arising from the deprotonation and ionization of the amide hydrogen by NaH.

Finally, the combination peak at \(\sim\)1585-1595cm\(^{-1}\) and the peak at 1696cm\(^{-1}\) both correlate with different modes of the MeCAN methyl ester (C=C=O and C=O), and can be seen decreasing as a function of time.

Importantly, the formation of CYCLOR-Na and not the free amide is confirmed by the marked absence of the 1628cm\(^{-1}\) and 1644cm\(^{-1}\) amide II (N-H) bands from pure CYCLOR. Since the amide is deprotonated, no N-H modes exist due to the lack of hydrogen (CYCLOR has a secondary amide).

Selected kinetic traces of the aforementioned bands revealed in figures 8-11 are shown below in figure 12:

**FLOW SYNTHESIS OF NEVIRAPINE AND MONITORING WITH PAT**

For synthesis of nevirapine in flow, crude CYCLOR-Na was withdrawn directly from the reaction flask and pumped through a column reactor packed with NaH and held at 145°C. The outlet of this packed column was fed through a Raman Flow Cell for sampling the crude product stream, and reaction aliquots were collected for HPLC analysis from the outlet of the flow cell. For the experiments conducted at MarqMetrix, a layered structure consisting of NaH and glass beads to mitigate compaction of the NaH bed and
allow facile flow of the resultant nevirapine sodium salt slurry through the system. This arrangement proved effective at flow rates up to 2mL/min without over pressurization.

**Equipment and Instrumentation**

- RXN2 998nm Raman spectrometer (Kaiser Optical Systems Inc.) with MarqMetrix Raman Flow Cell sampling interface
- Vapourtec E-Series equipped with packed NaH column reactor and heating jacket
- Data reduction and analysis performed using MatLab (MathWorks) and PLS Toolbox (Eigenvector Research Inc.)
- Agilent Infinity 1220 HPLC with EMD Millipore Chromolith HiRes C18 column

As with previous experiments, the temporal evolution of the Raman spectra was followed over the course of the flow reaction. In this particular case, as with the synthesis of CAPIC-Na, in situ Raman Spectroscopy is used as a tool to assess the level of conversion of the product output, rather than track reaction kinetics as in the batch synthesis of CYCLOR. The goal with this sensing scheme is to assess whether under particular reaction conditions conversion to the desired product occurs and to what extent conversion has occurred.

Three main spectral regions of interest are shown below in Figures 13 through 15, showing the conversion of CYCLOR to nevirapine as the temperature of the heated NaH column increases to the point at which ring closure occurs. Also note that the final (most red colored) spectra in the figures below contain no nevirapine: this is because of the solvent flush at the end of the experiment.

Figure 13 below reveals a variety of Raman peaks correlating well with those shown in the nevirapine spectrum overlay. Some peaks are unsurprisingly present in the CYCLOR spectrum as well, often with some peak shifts. Of note, two secondary amide bands at 525cm\(^{-1}\) and 620cm\(^{-1}\) are seen relating to N-C=O modes. The two peaks increasing in intensity at 724cm\(^{-1}\) and 870cm\(^{-1}\) are not present in the spectra of pure nevirapine, CYCLOR, MeCAN, or 2-CAN. This is another instance where an ionic versus a free base will display different peaks in the fingerprint region due to the ways that the presence of strong charge separation and much greater mass of the Na ion versus a proton affect the polarizability of various bonds in the vicinity.
In Figure 14, a large number of sharp, well-resolved peaks can be seen correlating well with those present in pure nevirapine, some with slight shifts on the order of 5-10 cm\(^{-1}\). The peak at 995 cm\(^{-1}\) can be assigned as the cyclopropyl ring vibration, and the peak at \(\sim 1046\) cm\(^{-1}\) as a pyridine ring mode. In this region, the amide III (C-N stretch) is present, most likely assigned as the peak at 1204 cm\(^{-1}\) or its shoulder at 1222 cm\(^{-1}\). Additionally, one of the peaks in the 1250-1300 cm\(^{-1}\) range may also be related to the amide.
The final region of interest in the nevirapine flow synthesis Raman spectra is that from 1350cm\(^{-1}\) to 1650cm\(^{-1}\). Several interesting features can be noted. First, the nevirapine peak at 1493cm\(^{-1}\) appears to split into two prominent peaks at 1476cm\(^{-1}\) and 1532cm\(^{-1}\). Once again, these peaks are most likely resultant from the formation of the nevirapine sodium salt and therefore some combination vibrational mode of the amide.

Additionally, the lack of the amide I band (C=O) at ~1650cm\(^{-1}\) is also easily explained by the influence of the sodium cation on the amide nitrogen. Besides these two spectral features, most of the peaks in the spectra from the flow cell correlate very well with pure nevirapine.

HPLC analysis further corroborates the formation of nevirapine shown in the Raman spectra. However, an interesting effect was noted in this process: since the nevirapine sodium salt is insoluble at standard temperature, it was noticed that the tubing on the outlet of the packed NaH column began to take on a deep purple color over time. This correlates with the marked increase in signal intensity from the Raman measurement, most likely due to the precipitation of nevirapine-Na in the tubing and flow cell. A significant amount was still present in the HPLC chromatograms below, but a smaller proportion than residual 2-CAN and CYCLOR. This is easily explained by the “collection” of the majority of product in the fairly long length of tubing needed for the flow cell. Figures 16-18 with HPLC chromatograms below illustrate and confirm these hypotheses:
Figure 16: Chromatogram of pure nevirapine in methanol, RT ~ 3.55 (mins:s)

Figure 17: Chromatogram of pure CYCLOR in methanol, RT ~ 4.30 (mins:s)
Figure 18: Chromatogram of crude output of packed NaH bed from nevirapine synthesis in flow

Vapourtec E-series
Figure 1. CYCLOR reaction mixture

Figure 2. Nevirapine crude product stream

Figure 3. Nevirapine Pure
The UPLC and HPLC methods used to determine chromatographic purity is as follows:

**UPLC** chromatographs were acquired on a Waters Acuity UPLC H-Class system using a Waters BEH C18 column (1.7 µm, 2.1 mm x 50 mm). A gradient of 5% ACN in H₂O to 90% ACN in H₂O was applied from 0.5 min to 9.0 min at a flow rate of 0.6 mL/min, followed by a hold at 90% CAN from 9.0 min to 10.0 min.

**HPLC** chromatographs were acquired HPLC on an Agilent 1260 Infinity system using an Agilent Poroshell 120 EC-C18 column (2.7 µm, 4.6 mm x 50 mm). A gradient of 5% ACN in H₂O to 95% ACN in H₂O was applied from 0.5 min to 6.5 min at a flow rate of 1.5 mL/min.

**RESULTS AND DISCUSSION**

In summary, Process Analytical Technology (PAT) in the form of Raman Spectroscopy has been successfully applied to the continuous synthesis of nevirapine in flow (and batch where applicable). Observation of reaction pathways, kinetics, and other useful information were obtained in real time in all three major reaction steps in the synthesis of nevirapine from methyl 2-cyclopropylamino nicotinate (MeCAN) and 2-chloro-3-cyano-4-picoline (CAPIC).

The ring closure step was highly successful using a packed NaH column and the Vapourtec E-Series with its ability to pump slurries at relatively high pressure (10 bar). Overpressurization issues seen previously were overcome by inclusion of a layered structure to allow easier flow of the solid product synthesized in the final reaction step.

Coupling PAT effectively to this process is also challenging due to the difficulty of handling a slurry and obtaining a reproducible and representative measurement of the resultant multiphasic system. Fortunately, due to the robustness of the BallProbe sampling interface, excellent Raman measurements were obtained, both with an immersion probe and through a flow cell, demonstrating the capability of thoughtfully designed and implemented measurements to add considerable value and understanding to both batch and flow synthetic processes.

**Process Flow Diagram and Mass Balance:**

A process flow diagram of the new nevirapine process in flow is provided in Scheme 4. All processes are in flow except the last step of purification where the product is precipitated and centrifuged.
Scheme 4: Medicines for All Nevirapine Process Flow Diagram
Economic Analysis

The economic analysis of the new nevirapine process would be restricted to raw material cost, since other manufacturing and operating costs would be highly site dependent and less meaningful. A breakdown of raw material costs for the continuous nevirapine process is provided in Table 2, which is similar to batch process.

<table>
<thead>
<tr>
<th>Raw Material (RM)</th>
<th>RM kg / Nev kg</th>
<th>RM $ / kg</th>
<th>RM Cost / kg Nev</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPIC</td>
<td>0.613</td>
<td>19.74</td>
<td>12.099</td>
<td>20.72%</td>
</tr>
<tr>
<td>Sodium Hydride</td>
<td>0.602</td>
<td>7.5</td>
<td>4.513</td>
<td>7.73%</td>
</tr>
<tr>
<td>Me-CAN</td>
<td>0.868</td>
<td>34.70</td>
<td>30.106</td>
<td>51.56%</td>
</tr>
<tr>
<td>Diglyme</td>
<td>0.835</td>
<td>4.80</td>
<td>4.010</td>
<td>6.87%</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>0.798</td>
<td>1.20</td>
<td>0.957</td>
<td>1.64%</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>1.605</td>
<td>2.18</td>
<td>3.500</td>
<td>5.99%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.001</td>
<td>1.09</td>
<td>1.087</td>
<td>1.86%</td>
</tr>
<tr>
<td>Hydrochloric Acid (as 36% aq. Solution)</td>
<td>0.124</td>
<td>0.752</td>
<td>0.093</td>
<td>0.16%</td>
</tr>
<tr>
<td>Activated Carbon</td>
<td>0.031</td>
<td>10.747</td>
<td>0.336</td>
<td>0.58%</td>
</tr>
<tr>
<td>Sodium Hydroxide (as 50% aqueous)</td>
<td>0.147</td>
<td>0.302</td>
<td>0.044</td>
<td>0.08%</td>
</tr>
<tr>
<td>Celite</td>
<td>0.104</td>
<td>15.750</td>
<td>1.641</td>
<td>2.81%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58.385</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Conclusions and Recommendations

Benefits of Continuous Synthesis

- Non isolated intermediate except final product
- Use of PAT to monitor reaction completion
- Reduction in manufacturing cycle time
- Less intermediate testing and release quality control
- Occupy less manufacturing space
- More control of reaction in real time
- Easier regulatory filing and approval
The results presented herein can be summarized as follows:

- Nevirapine was successfully synthesized in flow from CYCLOR passed through a packed NaH column. The process was monitored by Raman Spectroscopy and validated by HPLC.
- CYCLOR is produced in flow from CAPIC-Na and Me-CAN but require a CSTR for manufacturing scale.
- The flow synthesis of CAPIC-Na was demonstrated using a rotating disc reactor.
- PAT was successfully employed in the monitoring and characterization of all reaction steps in the synthesis of nevirapine.
- Both batch reactions and flow reactions can be monitored using PAT.
- Raman Spectroscopy was highly successful for this reaction, and continues to be a powerful tool for monitoring of chemical processes.
- Hydrogen evolution, while difficult for flow measurements, can be mitigated with thoughtful reactor design.
- As expected, the synthesis of CYCLOR from CAPIC-Na and MeCAN could be analyzed in real-time by PAT, showing kinetics and a wealth of spectroscopic information on both product formation as well as side-reactions.
- Finally, we demonstrated synthesis of nevirapine in flow on lab scale which can be scaled up by engineering design to a manufacturing scale.