End-to-End Continuous Manufacturing of Pharmaceuticals: Integrated Synthesis, Purification, and Final Dosage Formation**

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**Process description and experimental**

Unless otherwise noted, all chemicals are obtained from Sigma Aldrich and are used without further purification. DI water (Milli-Q, Millipore) is used. A detailed process flow chart is given in Figure S1. Photographs of the entire process are shown in Figure S2.

**Figure S1.** Process flow diagram with control loops. P – pump; M – mixer; R – reactor; TC – temperature controller; PC – pressure controller; CT – concentration transmitter; FT – flow transmitter; RC – ratio controller; S – separation; Cr – crystallization vessel; LC – level controller; PT – pressure transmitter; W – filter/wash; D – dilution tank; CC – concentration controller; FC – flow controller; E – extruder; MD – mold; sp – control set point.
Figure S2. Photographs of the a) front half of the process and b) back half of the process.

1 and 2 (provided by Novartis) are manually fed into two separate melting tanks (1 L jacketed vessels) as a dry white powder. The tanks are held at 130 °C, mixed with an overhead stirrer, and material is pumped out of the tanks with two heated pumps (Quizix C-5000-10K, Vindum Engineering) located in a 130 °C oven. The two streams are then pumped into a separate reactor oven shown in Figure S3 (Friction-Aire, Blue M) at 100 °C where they are initially mixed in a cross fitting with a stream of 3 and then mixed with an inline, helical static mixer, M1, (3.3 mm ID tube with 27 elements). The reagents are added in a molar ratio of 1:10:1 of 1:2:3. The mixed stream then enters an 11.7 mm ID stainless steel tube, R1, with
a residence time of 4 h at the nominal process throughput. This residence time was selected to be long enough that dispersion did not affect the performance of the equilibrium reaction. A detailed analysis of the reactor performance will be presented in a future publication. The reactor outlet is mixed with ethyl acetate and water to achieve 12 wt% of 4 in the organic phase and 20 wt% of 2 in the aqueous phase (based on an expected yield of 90 % and conversion of 95 %). The two-phase product then passes through a backpressure regulator (Swagelok) set to 7 bar and cools to near room temperature before entering the liquid-liquid separation system, S1. The two-phase stream is split into twelve equal branches, each with a membrane separator (Pall Zelfuor 1 μm). The outlets of the membrane separators rejoin and the pressure is controlled (EB1, Equilibar) to ≈ 700 Pa higher pressure in the aqueous stream. The aqueous phase is sent to waste while the organic phase is passed through a flow IR cell (Mettler Toledo ReactIR 15 with DS Micro Flow Cell) to monitor the concentration of 1 and 4.

The organic phase is pumped into Cr1, a 15 L jacketed glass vessel, which is controlled at 5 °C through a chiller. Nucleation and crystal growth occur inside the crystallizer and a slurry is formed. The level is controlled to maintain a nominal 4 h residence time. The slurry is transferred into Cr2 through a peristaltic pump (Masterflex L/S) and the flow rate is controlled by a feedback control loop through the control system. The level is monitored by ultrasonic level sensors (Omega Engineering, Inc.). An anti-solvent stream (heptane) is
introduced to Cr2, a 15 L jacketed glass vessel, and the flow rate of the anti-solvent is set to control the mass ratio of 1:3 of heptane:ethyl acetate. The temperature of Cr2 is set at 5 °C. At the end of the crystallization, the slurry of 4 is pumped into a custom-designed continuous filter (W1) where the crystals of 4 are filtered and washed with ethanol and ethyl acetate, Figure S4. The flow rate of the slurry is controlled by a similar feedback control loop to maintain a constant level corresponding to a nominal residence time of 4 h. The filter works by applying a thin layer of slurry on top of a rotating, porous plate. Wash solvent is dripped on the slurry and pulled through the plate by a vacuum. The washed slurry is then scrapped from the surface and conveyed by an auger into the next vessel.

Figure S4. Photographs of a) continuous filter and b) filtration and wash of a wet cake.

The wet cake out of the filter is collected in a 5 L vessel, D1. A side stream is pumped out of the tank, through a density flow cell (Anton Paar DPRn 417) and returned to the vessel.
The density reading is used to calculate the solid loading in the vessel and then control addition of ethyl acetate into the vessel to maintain a solid loading of 26.5 wt% of 4. A peristaltic pump (Masterflex L/S) equipped with two heads then pumps the slurry into the second reactor that consists of two parallel reactors run with each pump head. The slurry stream is mixed in a tee with 16 equivalents of 37 wt% hydrochloric acid and reacted in a 4.9 m length of 40 mm ID PFA coiled tubing, R2. At the outlet of the reactor, 25 wt% NaOH is mixed to quench the reaction. The quenched stream passes through a pH flow cell (Hamilton Polilyte Plus) that is used in a feedback control loop to adjust the NaOH flow rate to maintain a pH of 12.

The two phase reaction product is sent into a 2 L settling tank, S3, where the bottom aqueous phase is pumped to waste while the top organic phase with 5 is pumped out into a mixing tee to mix with ethyl acetate controlled by a UV flow cell located downstream. The stream (containing solid NaCl that precipitates after dilution) is then filtered through 14 microfiltration membranes, S4, (0.45 μm Pellicon XL 50, Millipore). After filtration, the stream passes through a UV flow cell (HR2000+, Ocean Optics) that feeds back the concentration of 5 to the ratio control loop involving the dilution pump prior to the microfiltration membranes. The concentration is maintained at 6 wt%. The organic stream then passes through 2 × 35 L packed columns, S5, of 3 Å molecular sieves in series to remove any residual water that is present in the stream. The size of the column is selected such that it will contain all of the water for a given run (up to two weeks of operation). Longer continuous operation could be enabled by switching between smaller columns run in parallel. The stream then exits the column into a break tank where it is metered into the next crystallization process. It passes through another UV flow cell where the concentration of 5 is again measured and fed forward to control the pump delivering fumaric acid into Cr3, a 15 L jacketed glass vessel.

The reagents are fed in a molar ratio of 0.55:1 of fumaric acid:5. The temperature in Cr3 is controlled at 20 °C. The salt formation reaction and the crystallization of the final drug substance occur in Cr3 at the same time. The slurry is transferred into Cr4, another 15 L jacketed glass vessel, with a peristaltic pump (Masterflex L/S) and the flow rate is controlled by a feedback control loop through the control system to maintain a level corresponding to a nominal residence time of 4 h. The temperature of Cr4 is controlled at −10 °C. At the end of the crystallization, the slurry of the final drug substance is pumped into another continuous filter (W2) where the crystals are filtered and washed with ethyl acetate. The flow rate of the
slurry is controlled by the feedback level control loop in Cr4 to maintain a nominal residence time of 4 h.

The wet cake out of the filter is collected in a 5 L vessel, D2. A side stream is pumped out of the tank, through a density flow cell (Anton Paar DPRn 417) and returned to the vessel. The density reading is used to calculate the solid loading in the vessel and then control addition of ethyl acetate into the vessel to maintain a solid loading between 10–15 wt% 6. A peristaltic pump (Masterflex L/S) then pumps the slurry into the continuous dryer. A second pump meters a 2.5 wt% slurry of fumed SiO2 (Sigma-Aldrich #S5505) such that the mixture with the 6 slurry is 2.5 wt% SiO2 on a dry basis.

The continuous dryer, Figure S5, combines a rotary drum dryer, S6, and a screw vacuum dryer, S7. The drum dryer includes two horizontal, stainless steel, hollow drums (21.6 cm diameter) rotating at 50 rpm in opposite directions relative to each other. The product is pumped between a 0.1 mm gap between the drums, where it is squeezed while 95 °C air is blown on the product surface on the back side of the drum. The semidried wetcake is broken down into particulates by the action of static stainless steel scraper blades located on the either side of each drum. Particles fall into a hopper that vibrates in order to release the material through a butterfly valve into an airlock before entering a 3-zone vacuum screw dryer. The vacuum screw dryer consists of 3 sections of 6 cm ID × 1.5 m long pipe with acetal screws. The temperature zones in the dryer are controlled by electric heaters at sequentially higher temperatures of 40 °C, 60 °C, and 75 °C. The material is conveyed down the pipes by the auger-type screws. The vacuum level within the screw dryer is < 30 mTorr.
A vacuum conveyor (IEDCO) activates automatically every 2 minutes for 20 seconds to transport the dry API powder into a reservoir (≈ 100 g capacity) located at the top of a gravimetric feeder for the API. Two gravimetric feeders (Schenk AccuRate PureFeed DP-4) supply the API and the excipient material directly into an extruder, E1, at a rate of 45 g h\(^{-1}\) and 85 g h\(^{-1}\), respectively. The excipient is 6,000 Da polyethylene glycol (PEG, Sigma-Aldrich #81260). The co-rotating screws inside the extruder (Leistritz, 16 mm twin screw extruder) rotate at 160 rpm while mixing and pushing material through five temperature-controlled zones. The first acts as the cooling zone to prevent build-up of API or excipient in the extruder hopper and is set at 16 °C, while the remaining four zones are set at 60 °C. The average extruder residence time at steady-state is around 12 minutes.
The extrudate is continuously fed into an integrated hot-runner molding system, MD, (Mold Hot Runner Solutions). The molding unit consists of two heated reservoirs (90 °C), a heated manifold (90 °C), and a heated valve gate region (T = 90 °C). As the material is ejected through the manifold via an injection piston, it travels through the heated valve gates into six cavities that are continuously chilled to approximately 0 °C. The material is cooled for 36 s and the tablets are then ejected via a stripper plate mechanism from the cavities.

The control system is implemented using Siemens PCS7 software. Data from the control system are recorded at a 1 s to 1 min sampling frequency into a central database. All equipment receives set points from the control system and the plant was operated under closed-loop control. Manual interventions are only made in cases where significant deviations would cause hazardous situations or would significantly impair the process performance.

Analytically pure samples of 1, 2, 4, 5, 6, and 7 were provided by Novartis. Identities of compounds are confirmed by matching peaks using validated HPLC methods from Novartis (see HPLC analysis below). Concentrations of reactants, products, side products, and byproducts are monitored by HPLC (Agilent 1100). Residual solvent levels in the dried powders and final tablet are monitored by headspace GC (Agilent 7890A with a G1888 headspace sampler). Differential scanning calorimetry is performed using a TA Instruments DSC Q2000 at 10 °C min⁻¹.

Dissolution testing is carried out using a basket in an Agilent Varian VK 7025 equipped with a Cassini 10-channel fiber optic sampling system (C Technologies Inc.) in combination with an Agilent Cary 50 Bio UV spectrophotometer detection system. Tablets are dissolved in 500 mL of 0.01 M HCl maintained at 37 °C. Each tablet was placed in a rotating basket and submersed in the acid solution. The concentration of API in the vessel was measured using UV detection at 279 nm via a fiber optic probe every one minute for approximately 90 minutes total.

X-ray diffraction (XRD) patterns are recorded with a PANalytical X’Pert PRO Theta/Theta Powder X-ray Diffraction System with a Cu tube and X’Celerator high-speed detector. Tablets are broken up and ground into a powder for measurement. Crystalline 6 is measured by drying a wet cake of 6 produced under process conditions on the bench prior to analyzing.

The value for % content is calculated dividing the amount of a material in a tablet by the declared content in the tablet (112 mg 5). Calibrations for 7 are based on assuming the same absorptivity as 5 to match specifications provided by Novartis.
Startup and operation of the plant

The plant startup was performed sequentially with each unit brought to near steady state prior to starting the next unit. This was done to favor stability and disambiguation of data during startup. Each unit operation was run for at least 1-3 times its residence time before starting the next unit operation. The first set of tablets was made after 200 h corresponding to 4 residence times of the entire process. The extra time was due to restarting some units which were halted during startup (clogs, equipment breakdown, etc.) Figure S6 shows the concentration development in vessels D1 and D2 (key CMAs identified in previous analysis). The front half of the process (through D2) was operated to near steady state within the first 150 h (with two set point changes in the concentration of D2 between 150 and 200 h). The dryer was operated with material from D2 once it was available for periods up to 40 h. The extrusion and molding units were then operated for separate periods of time up to 8 h.
**Figure S6.** Plot of key CMAs a) concentration of 4 in D1 and b) concentration of 6 in D2 throughout the process. Concentration determined by HPLC with \( t = 0 \) set to when feed was started to R1. There were two set point changes between \( t = 150 \) and 200 h in the D2 concentration.

**HPLC analysis**

Streams through D1 are analyzed by injecting 3 \( \mu \text{g} \) of 4 (samples are diluted with 1:1 water:acetonitrile) onto an Agilent ZORBAX Eclipse XDB-C18 2.1 mm ID \( \times \) 50 mm, 1.8 \( \mu \text{m} \) particle diameter column maintained at 30 °C. Mobile phase A was 43.8 mM \( \text{H}_3\text{PO}_4 \) in water and mobile phase B was 1:1 \( \text{V} \ \text{V}^{-1} \) acetonitrile:methanol. The mobile phase is changed following the gradient in Table S1 at a constant total flow rate of 0.416 mL min\(^{-1}\). Detection
is performed using UV at 230 nm. Characteristic elution times – blank: 0.35 min; 4: 6.89 min; 1: 8.43 min.

**Table S1**: Mobile phase gradient for detection of 4

| Time / min | % B |
|------------|-----|
| 0          | 45  |
| 5.31       | 70  |
| 6.63       | 80  |
| 7.76       | 80  |
| 7.80       | 45  |
| 10         | 45  |

Streams through S7 are analyzed by injecting 2.26 µg of 5 or 2.5 µg of 6 (samples are diluted with 85:15 V V⁻¹ water:acetonitrile) onto an Agilent Ascentis Express RP-Amide 2.1 mm ID × 50 mm, 2.7 µm particle diameter column maintained at 30 °C. Mobile phase A is 0.1 vol% trifluoroacetic acid in water and mobile phase B is 0.05 vol% trifluoroacetic acid in acetonitrile. The mobile phase is changed following the gradient in Table S2 at a constant flow rate of 0.8 mL min⁻¹. Detection is performed using UV at 230 nm. Characteristic elution times – blank: 0.18 min; 5 and 6: 2.96 min; 7: 3.35 min; 4: 4.10 min.

**Table S2**: Mobile phase gradient for detection of 5 or 6

| Time / min | % B |
|------------|-----|
| 0          | 15  |
| 2.37       | 40  |
| 4.04       | 70  |
| 4.87       | 70  |
| 4.88       | 15  |
| 7.5        | 15  |

The final tablets are analyzed by injecting 2.8 µg of 5 (an entire tablet is diluted with 75:25 V V⁻¹ water:acetonitrile) onto a YmC-Pack ODS-A 4.6 mm ID × 150 mm, 3 µm particle diameter column maintained at 30 °C. An ion pair solution is prepared (30 mM hexanesulfonic acid sodium salt monohydrate 20 mM sodium dihydrogen phosphate monohydrate in water adjusted to a pH value of 2.3 using 85 wt% phosphoric acid) and mixed 80:20 V V⁻¹ ion pair solution:acetonitrile for mobile phase A and 20:80 V V⁻¹ ion pair solution:acetonitrile for mobile phase B. The mobile phase is changed following the gradient in Table S3 at a constant flow rate of 0.8 mL min⁻¹. Detection is performed using UV at 280 nm. Characteristic elution times – blank: 2.1 min; 6: 10.74 min; 7: 13.45 min.
Table S3: Mobile phase gradient for analysis of final tablets

| Time / min | % B |
|------------|-----|
| 0          | 20  |
| 3          | 20  |
| 15         | 70  |
| 30         | 70  |
| 30.1       | 20  |
| 35         | 20  |

Content uniformity

Content uniformity was tested following US Pharmacopeial (USP) (905). Table S1 gives the values of % content of 6 in tablets for 26 tablets assayed during the period shown in Figure 3b. The standard procedure for USP (905) involves testing 10 random tablets from a batch; however, no batches are defined in continuous processes. The data pass the test for most sets of 10 random tablets, but the 5 tablets produced at 200 min have systemically low % content and can cause the test to fail. This is due to all having low tablet mass, nominally 0.400 g, while the composition is correct, nominally 34.1 wt% 6. If these underfilled tablets are removed from the dataset, the test works. If all 26 tablets are subjected to the 30 tablet version of USP (905) with and without 4 additional random tablets selected from within the list (duplicated), then the set also passes. This is not a conservative modification to the test, but the deviation from 30 tablets is small so it is expected that a complete set would pass as well.
Table S4: Tablet % content 6 with tablet mass and wt% 6 for calculation of content uniformity

| $t$ (min) | $C$ (% content) | $m$ (g) | $C$ (wt%) |
|----------|-----------------|--------|-----------|
| 0        | 102.3           | 0.4028 | 34.3      |
| 24       | 97.9            | 0.3925 | 33.7      |
| 24       | 103.6           | 0.4017 | 34.8      |
| 66       | 96.4            | 0.3724 | 34.9      |
| 66       | 91.4            | 0.3539 | 34.8      |
| 66       | 94.3            | 0.3534 | 36.0      |
| 66       | 94.4            | 0.3652 | 34.9      |
| 66       | 89.5            | 0.3486 | 34.7      |
| 66       | 94.4            | 0.3736 | 34.1      |
| 100      | 99.0            | 0.3969 | 33.7      |
| 158      | 97.8            | 0.3949 | 33.4      |
| 200      | 88.2            | 0.3390 | 35.1      |
| 200      | 91.9            | 0.3598 | 34.5      |
| 200      | 89.0            | 0.3468 | 34.6      |
| 200      | 86.3            | 0.3372 | 34.5      |
| 200      | 85.5            | 0.3299 | 35.0      |
| 230      | 98.9            | 0.3998 | 33.4      |
| 277      | 96.5            | 0.3930 | 33.1      |
| 277      | 98.9            | 0.3807 | 35.1      |
| 277      | 97.0            | 0.3740 | 35.0      |
| 277      | 96.7            | 0.3738 | 34.9      |
| 337      | 92.6            | 0.3994 | 31.3      |
| 397      | 96.6            | 0.3939 | 33.1      |

Additional figures

Figure S7. Micrograph of 6 crystals formed in Cr2.
Figure S8. Plot of ethyl acetate content in dried 6 exiting the dryer (S7) with specification limit shown by a dotted line.

Figure S9. Dissolution profile (X, % dissolved) of continuously produced tablet (filled circles) compared with commercial tablet (dashed line with open circles).

Figure S10. XRD patterns of PEG (dotted line), 6 following crystallization, filtration, and washing (dashed line), and formed tablet (solid line).
Table S5: Levels of key impurities throughout process. Values of area% are based on peak area from HPLC. This value is close to the mol% given similar chromophore and high purity of most streams.

| Process position | C / area% product | C / area% impurity |
|------------------|-------------------|-------------------|
| S1 to Cr1[a]     | 89.5              | 4.8               |
| D1 to M3[a]      | 98.7              | 0.18              |
| M4 to S3[b]      | 93.0              | 2.3               |
| D2 to M5[c]      | 99.5              | 0.10              |
| S7 to E1[c]      | 99.7              | 0.11              |
| Final tablet[c]  | 99.7              | 0.13              |

[a] Product is 4 and impurity is 1 which is not observed downstream of R2.
[b] Product is 5 and impurity is 7.
[c] Product is 6 and impurity is 7.

Figure S11. Differential scanning calorimetry of interior of a PEG coated tablet showing PEG melting point at 60 °C and 6 melting point at 117 °C. Arrow shows direction of endothermic heat flow.

References

[1] R. Lakerveld, B. Benyahia, R. D. Braatz, P. I. Barton, AIChE J. 2013, DOI: 10.1002/aic.14107.