

Real-TIME LC Monitoring of Process Streams for Automating the Development of Flow Reactions

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APPLICATION BENEFITS

The PATROL UPLC® Process Analysis System aids in the development of continuous flow reactions. The system provides automated, online LC monitoring of sample stream, in a flexible deployment format, for the quantification of product yield or purity. Kinetics can be determined for all reaction components, including low-level impurities and/or intermediates as reaction conditions are optimized, resulting in reduced commercialization investment.

WATERS SOLUTIONS

Real-TIME LC™

UPLC®

PATROL UPLC Process Analysis System

Empower® 2, Empower 3 Software

ACQUITY UPLC® Column Chemistries

Connections INSIGHT®

KEY WORDS

Online, atline, flow reaction,
flow chemistry, scale-up

INTRODUCTION

In an effort to optimize chemical synthesis processes, manufacturers are deploying new technologies such as continuous flow chemistry systems. The benefits of continuous flow reactions significantly surpass batch reactions and include improved product throughput and yield, easier scalability, better temperature control, and a safer manufacturing process. Determining the optimal conditions of a flow reaction is crucial to ensure not only the highest yield of the compound of interest, but also to minimize the formation of secondary products that may necessitate isolation or purification steps on the final product. As part of flow reaction development, the final product reaction kinetics are characterized with respect to temperature, pressure, coil residence time, and reactant stoichiometry. However, this information is typically obtained post-reaction by liquid chromatography (LC) analysis, making the development process non-continuous and often time-consuming. Offline analysis can compromise the results, as the aliquots may continue to react or may be altered because of exposure to air. To improve development timelines and quantitative reliability, it is advantageous to deploy an online monitoring technique. Spectroscopic or UV methods have been used to acquire quantitative information about the main product. However, these methods yield little quantitative information about low-level impurities or intermediates, and the kinetics of formation. By employing a quantitative separation technique such as online LC, information can be revealed about the final product and low-level impurities while, simultaneously, automating the flow reaction optimization. Additionally, greater understanding of a reaction improves the scale-up process to manufacturing. This ensures maximum yield and purity.

The benefits of UltraPerformance LC® (UPLC) are well suited to monitoring the optimization of flow reactions. This chromatographic technique provides better resolution, sensitivity, and throughput. As the diameter of the particles in a chromatographic column decreases, the efficiency achieved for an analytical separation increases. Additionally, with the smaller particle size, the optimized linear velocity for that separation increases. Therefore, the chromatographic separation can run much faster without compromising resolution, while providing the inject-to-inject cycle times necessary for the technique to be used for Real-TIME LC analysis. This allows the kinetics in the flow reaction to be characterized quickly and accurately, yielding a robust design space for the final reaction conditions.

To demonstrate the power of UPLC as an online sensor, the optimization of a continuous flow reaction is described. Deploying UPLC as a sensor for reaction optimization first requires the automated development of an analytical method for monitoring the reaction species. This is accomplished by collecting the effluent from a plug flow reaction and analyzing it using atline analysis. Once the UPLC method conditions are established, the UPLC system is directly configured to the flow reactor for online sampling. The reaction conditions are varied to quantify the impact of each of the variables on the final concentrations of the final product and any possible process impurities. This data is then used to determine the optimal flow reaction conditions. The same analytical UPLC method can be used to monitor the scale-up of the flow reaction from benchtop scale to pilot scale, and finally to manufacturing scale.

The PATROL UPLC Process Analysis System, with automated sample dilution, is designed for online and/or atline UPLC analysis during process method development. All methods are directly transferable to the manufacturing floor.

EXPERIMENTAL

The reaction used to demonstrate automated flow reaction development was the addition of morpholine to 3,4-difluoronitrobenzene under elevated temperature in dimethylformamide (DMF), as seen in Figure 1. The flow reaction was run on the Uniqsis FlowSyn System, and the effluent was sampled and monitored using the PATROL UPLC Process Analysis System. For analytical method development, the FlowSyn was configured for plug flow analysis by injecting the reactants onto the reaction coil followed by collecting the fractions on the optional fraction collector as shown in Figure 2. The samples were then analyzed atline to develop the UPLC method for quantification of the starting material, final product, and potential impurities. Once the analytical method was optimized, the PATROL UPLC Pilot Process Analysis System was directly configured to the outlet of the FlowSyn System. Aliquots were automatically sampled and injected onto the UPLC system in an online mode as seen in Figure 3.

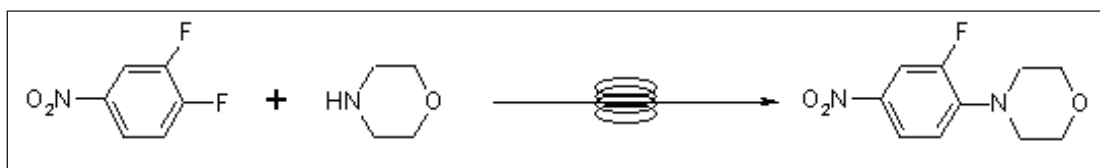


Figure 1. The addition of morpholine to 3,4-difluoronitrobenzene.

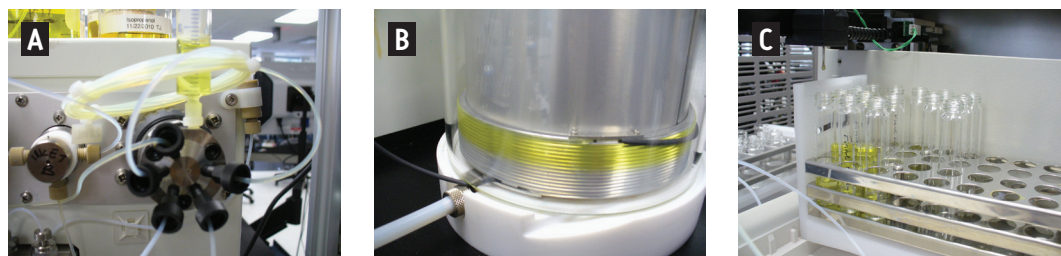


Figure 2. To develop the analytical method, the Uniqsis FlowSyn System was configured for plug flow analysis. The reactants were injected with the sample coil (A) onto the reaction coil (B). The effluent was collected directly into sample vials in the fraction collector (C). The fractions were then used as atline samples in the PATROL UPLC Process Analysis System to develop the analytical method for online monitoring.

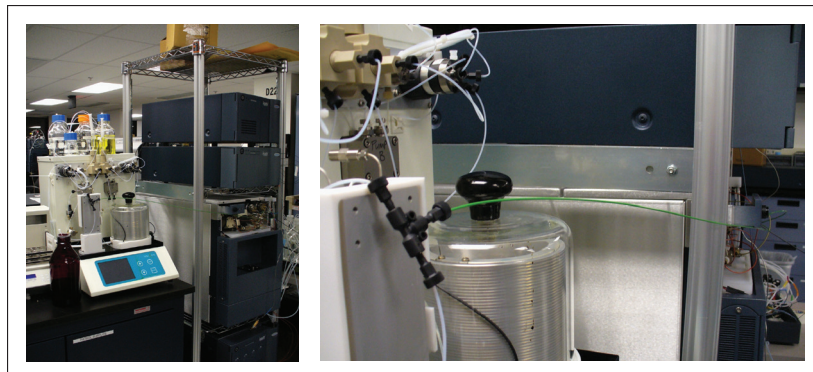


Figure 3. For flow reaction optimization, the PATROL UPLC Process Analysis System was configured with the Uniqsis FlowSyn System for automated online analysis of the flow reactor effluent.

METHOD CONDITIONS

Chromatographic conditions for analytical method development

System:	PATROL UPLC Process Analysis System
Data:	Empower 2 Software
Columns:	ACQUITY UPLC BEH C ₁₈ 2.1 mm x 50 mm, 1.7 µm ACQUITY UPLC BEH Phenyl 2.1 mm x 50 mm, 1.7 µm ACQUITY UPLC HSS T3 2.1 mm x 50 mm, 1.8 µm ACQUITY UPLC HSS C ₁₈ 2.1 mm x 50 mm, 1.8 µm
Sample:	Reaction aliquots in DMF
Injection vol.:	2 µL
Temp.:	40 °C
Flow rate:	600 µL/min
Eluent A1:	10 mM ammonium formate pH 3
Eluent A2:	10 mM ammonium bicarbonate pH 10
Eluent B1:	Acetonitrile
Eluent B2:	Methanol
Gradient:	5% to 95% B over 2 min
Detection:	245 nm, 20 Hz normal
Sample flush:	30 s
Dilution:	100 x
Needlewash:	5 s of 60:20:20 ACN/DMF/water

Final chromatographic method

System:	PATROL UPLC Process Analysis System
Data:	Empower 2 Software
Column:	ACQUITY UPLC HSS T3 2.1 mm x 50 mm, 1.8 µm
Sample:	Reaction aliquots in DMF
Injection vol.:	2 µL
Temp.:	60 °C
Flow rate:	1 mL/min
Eluent A:	10 mM ammonium formate pH 3
Eluent B:	Methanol
Gradient:	35% to 75% B over 30 s; Hold for 15 s
Detection:	245 nm, 40 Hz Normal
Sample flush:	30 s
Dilution:	100 x
Needlewash:	5 s of 60:20:20 ACN/DMF/water

RESULTS AND DISCUSSION

The first step in the automated development of the flow reaction was to develop the UPLC method to be used for monitoring the flow reaction stream. The flow reactor was plumbed for a plug flow experiment in which both of the reactants were injected onto the reaction coil and the effluent was collected directly into sample vials. The reaction conditions do not need to be ideal for this experiment, but must contain unreacted starting material, final product, and any reaction side-products (typically these conditions can be estimated from the batch reaction conditions). The sample vials containing the effluent were barcoded and placed into the PATROL UPLC Process Analysis System. With the optional column manager, the system is capable of selecting between four different column chemistries. ACQUITY UPLC Binary Solvent Manager (BSM) has four different solvents available for method development (two aqueous and two organic eluents). The use of available ACQUITY UPLC Quaternary Solvent Manager (QSM) configurations enables further depth of method development separation condition exploration, with the quaternary solvent combinations and additional six solvent select valved into the fourth line. When the atline sample is introduced into the system, the barcode on the sample vial is read and the system enables a previously designed analytical screening protocol within the Empower Software that examines the impact of method pH, organic modifier, and column chemistry on the final separation of the reaction

components. The level of automated dilution for the sample is also indicated by the barcode to ensure the concentrations of the injected sample components are within the linear range of the PATROL UPLC Process Analysis System. The resulting chromatograms from the screening protocol can be processed to determine the conditions that yield the best resolution of the reaction components, as seen in Figure 4. Once the column and the eluents have been selected, a secondary screening protocol is run, which optimizes the separation temperature, gradient conditions, and flow rate. From these two screening protocols, a fast UPLC method that yields quantification of all the reaction components (including high concentration final products and low-level impurities) is developed. For this assay, morpholine does not have a chromophore and, therefore, cannot be analyzed by UV detection. However, because it is added in excess, it is less critical to monitor it in this flow reaction. It could be analyzed by adding a complementary detection technique such as evaporative light scattering (ELS) detection or mass spectrometry. For this reaction, the initial screening results indicated that the best separation was achieved on an HSS T3 chemistry with the low pH buffer and methanol as the organic eluent. Further optimization with the secondary screening protocol yielded a method with a run time of 45 seconds as shown in Figure 5.

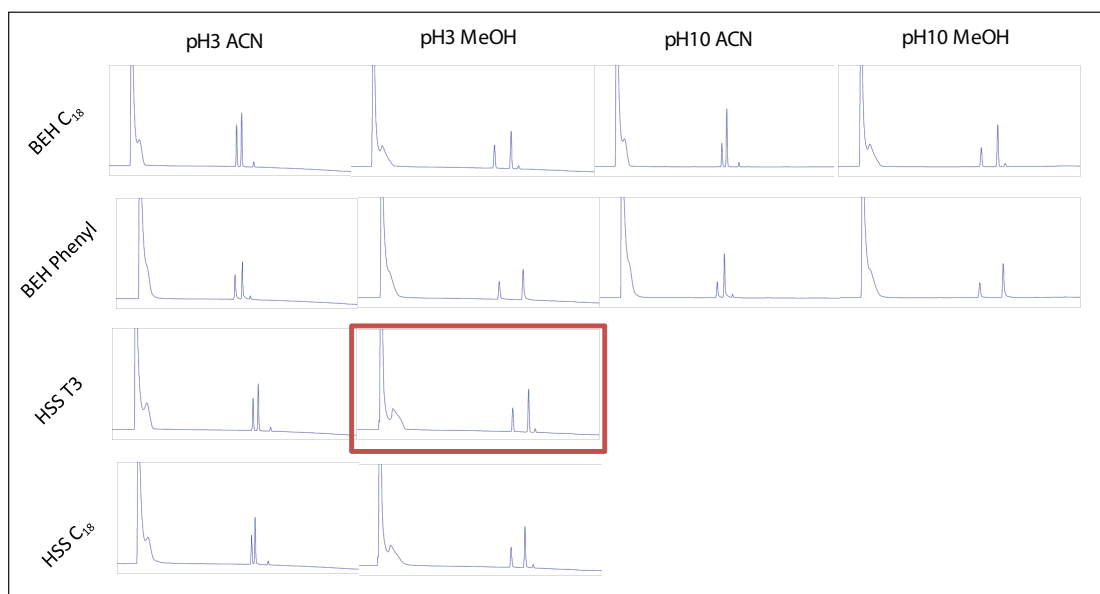


Figure 4. Screening results from the analysis of the plug flow experiment sample. From this data, the separation conditions resulting in the best peak shape and resolution are selected for further optimization.

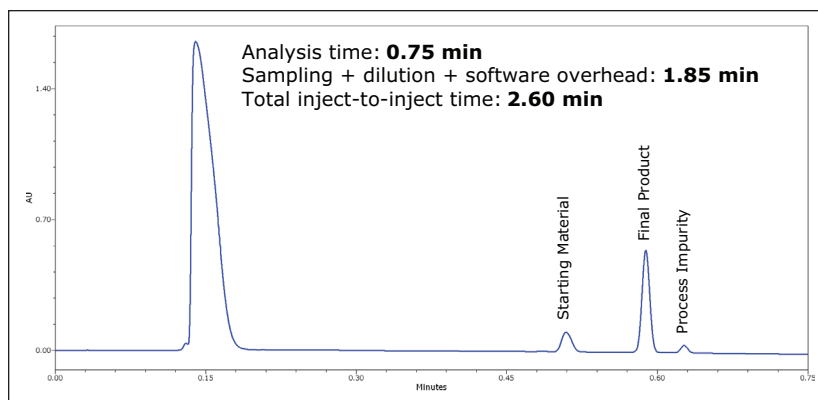


Figure 5. Final gradient separation of the flow reaction media.

The Uniqsis FlowSyn System is designed for automated flow reaction development. A matrix of experiments can be programmed to evaluate the effects of coil temperature, reaction stoichiometry, coil residence time on the yield, and purity of the final product. By interfacing this system with the PATROL UPLC Process Analysis System, examining each of these different reaction conditions can be entirely automated. It is important to collect a sufficient number of samples for each set of conditions at the reaction steady state to ensure that repeatable quantification is achieved as shown in Figure 6. This will yield the best results for chemometric analysis of the data. The time to reach reaction steady state depends on the reaction coil geometry (length and inner diameter) as the laminar flow of the fluid through the open tube disperses the reactants at the start and end of the experiment. Therefore, it is important to understand the amount of time required to achieve steady state conditions. This time will then be added to the time required to achieve sufficient replicate injections at steady state to determine how often reaction conditions can be altered in the automated experiment. The final run time of the UPLC method was 45 seconds, however the additional time required for sampling and dilution increased the inject-to-inject cycle time to 2.6 minutes. In the automated experiment, each set of reaction conditions was held for 25.0 minutes to ensure a sufficient number of injections at steady state.

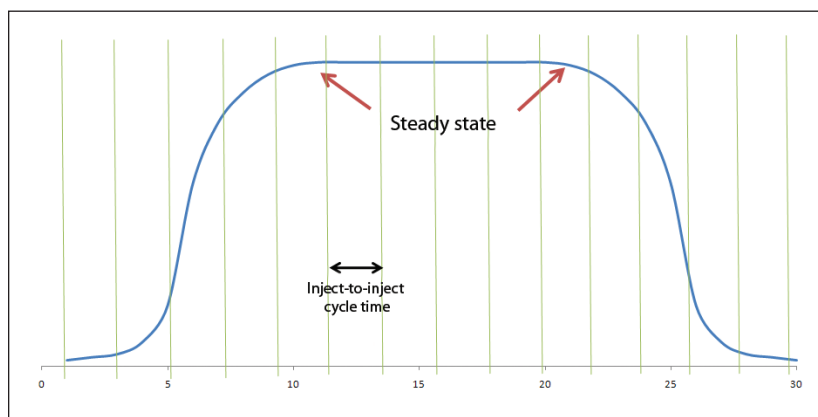


Figure 6. It is important to ensure that a sufficient number of aliquots at each set of reaction conditions were analyzed at steady state. A minimum of three samples at steady state will enable repeatable quantification of each of the reaction components.

A matrix of reaction variables was created to examine the impact of coil residence time, reaction temperature, and reaction stoichiometry. The method development matrix is listed in Table 1. These variables were programmed into the Uniqsis FlowSyn System and the sequence was initiated. The automated acquisition of the online samples by the PATROL UPLC Process Analysis System was also started and allowed to run unattended.

When the sequence was completed, both systems were programmed to automatically flush and shut down. The resulting chromatographic data was analyzed to determine the impact of each reaction variable on the yield of both the final product and the process impurity. The resulting relative amounts of each of the final product and process impurity are listed in Table 1. The trends for each of these variables are displayed in Figure 7. This data can then be used for chemometric analysis to determine the optimal reaction conditions. The final reaction conditions were determined to be a 1.5x excess of morpholine, a coil residence time of 11 minutes, and a coil temperature of 100 °C. This resulted in the greatest yield of final product with a minimized formation of the process impurity.

	Residence time (min)	Coil temp. (°C)	Reactant ratio	Ratio of final product	Ratio of process impurity
Trial #1	10	100	1:1	52.13	6.25
Trial #2	10	100	2:1	64.71	11.99
Trial #3	10	100	1.5:1	60.71	9.20
Trial #4	8	100	1:1	47.82	6.04
Trial #5	12	100	1:1	56.11	6.41
Trial #6	12	80	1.5:1	53.86	8.51
Trial #7	12	100	1.5:1	64.80	9.47
Trial #8	12	120	1.5:1	69.16	13.46

Table 1. Flow reaction optimization variables and the impact they had on the relative amount of final product and the process impurity.

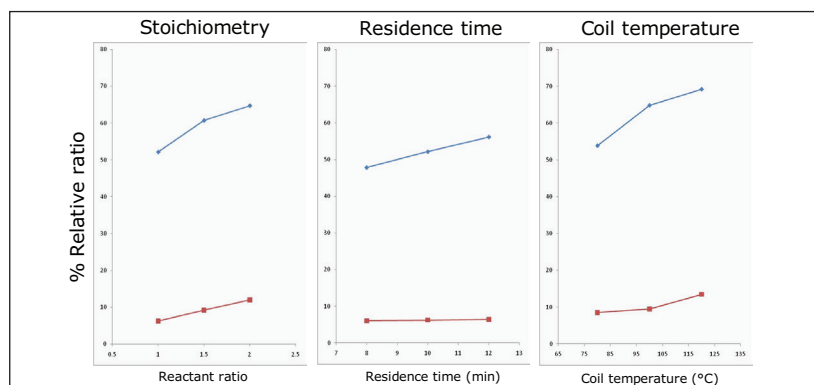


Figure 7. Trend plots for each of the flow reaction variables and their impact on the formation of the final product (blue) and the process impurity (red).

The final flow reaction conditions can then be easily scaled-up to pilot, plant, or manufacturing scale, and the PATROL UPLC Process Analysis System can be used for monitoring on the manufacturing floor. By monitoring the scale-up process using UPLC and the final process on the production floor, the information gathered in the flow reaction development is directly transferrable to understanding process deviations during the manufacturing process.

CONCLUSIONS

The PATROL UPLC Process Analysis System is ideally suited for monitoring the development and optimization of flow reactions by analyzing aliquots of the reaction effluent. The results are used to map the kinetics of all species in the reaction including low-level process impurities. The system is easily interfaced with the Uniqsis FlowSyn System, an automated benchtop flow reactor. The methods developed to monitor the flow reaction can then be transferred to the PATROL UPLC Process Analysis System for monitoring the final flow reaction, with simplified user interface for easy deployment on the production floor.

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