REAL-TIME MONITORING OF IN-PROCESS SAMPLES BY UPLC

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INTRODUCTION

Process Analytical Technology (PAT) is a critical component of the overall manufacturing process to ensure final product quality and improve process yields. PAT involves taking measurements throughout the production process to verify the quality of in-process batches and understand the critical steps of the process. Many different sensor technologies are employed throughout the manufacturing process to measure the attributes of the in-process batches. Typically process steps such as reaction monitoring and product purification are assessed by spectroscopic sensors which include near-infrared spectroscopy (NIR) or Raman spectroscopy. These techniques have the ability to provide real-time information about the processes but lack the ability to effectively resolve and quantify multiple components in the sample. Therefore, performance of these sensors needs to be benchmarked against a reference standard, which in most instances is high performance liquid chromatography (HPLC) because it is a more selective and sensitive technique with a broader linear dynamic range and the ability to quantify multiple components within complex samples. HPLC is the most widely used technique in pharmaceutical QC laboratories, however the long run times and complex system operation have prevented it from being routinely used for at-line or on-line analysis. With the introduction of UltraPerformance LC® (UPLC®), it is now possible to achieve near real-time LC analysis for in-process samples. Integrated hardware and software offer a simple design which requires little to no user input. The PATROL™ UPLC Process Analyzer brings the reference standard methodology to the manufacturing floor, eliminating the need to calibrate spectroscopic sensors or send suspect samples to the off-line QC laboratory. This paper will discuss implementing UPLC for the on-line reaction monitoring and the monitoring of the effluent from a process purification column.

TECHNOLOGY

UltraPerformance LC (UPLC) is based upon the use of sub-2 µm particles and the technology to take advantage of the benefits of these particles. Since its introduction, many users have transferred their HPLC QC methods with great success, realizing tremendous improvements in throughput and resolution. The PATROL UPLC Process Analyzer brings these significant improvements in LC to the manufacturing floor in a manner which allows LC to be used as a real-time sensor. The PATROL UPLC Process Analyzer is a holistically designed system which integrates UPLC technology, control software, and a rugged engineered sample management module for managing the samples and work flow of the in-process environment. The system components along with all the solvents, waste, and standards are contained within an enclosure which is compatible with all levels of the manufacturing environment. The system is designed to be compatible with both on-line and at-line analysis. The On-Line Manager (OLM) can be interfaced with process streams or reactors to provide real-time analysis and quantification without the need for user intervention. Data can be sent to distributed control systems (DCS) or LIMS for completely automated monitoring. The At-Line Manager (ALM) provides for a walk-up interface with barcode scanning capabilities to eliminate the need for information input from the technician.

Figure 1— PATROL UPLC Process Analyzer

ON-LINE MONITORING OF PROCESS CHROMATOGRAPHY

The purification steps of an in-process API determine the purity and yield of the final product. Spectroscopic techniques are not as selective or sensitive as LC for assessing process column effluent during the purification process. By employing UPLC to monitor the process column effluent, the quality of the final product API can be controlled and optimized with greater confidence. To demonstrate the utility of on-line monitoring with the PATROL UPLC Process Analyzer, a process chromatography effluent was simulated using a quaternary gradient pump.

Experimental

To simulate the process column effluent, a gradient profile was developed to mimic the effluent generated by the purification of and API from 2 impurities. The profile was measured at a UV/Vis detector is displayed in Figure 2. The PATROL UPLC Process Analyzer monitored the simulated process column effluent under the following conditions:

Column:	2.1x50 mm BEH C18 1.7 µm
Eluents:	A—0.1% Formic Acid in Water
	B—0.1% Formic Acid in Acetonitrile
Gradient:	10% to 25% over 1 minute; Curve 4
Flow Rate:	1.0 mL/min
Temperature:	50 °C
Inj. Volume:	5 µL
Detection:	243 nm; 40 Hz; Time Constant 0.025 s
Wash:	70:15:15 Acetonitrile/Water/Isopropanol
Purge:	1 mL (4x volume of transfer line)
Run Time:	1 minute
Cycle Time:	2 minutes 40 seconds

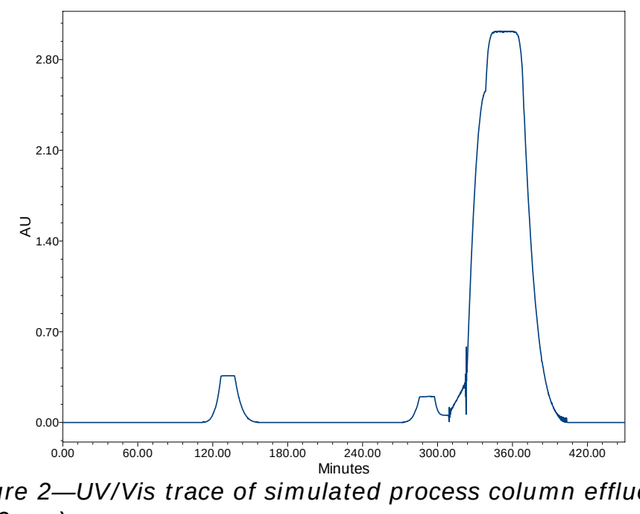

Figure 2—UV/Vis trace of simulated process column effluent (243 nm)

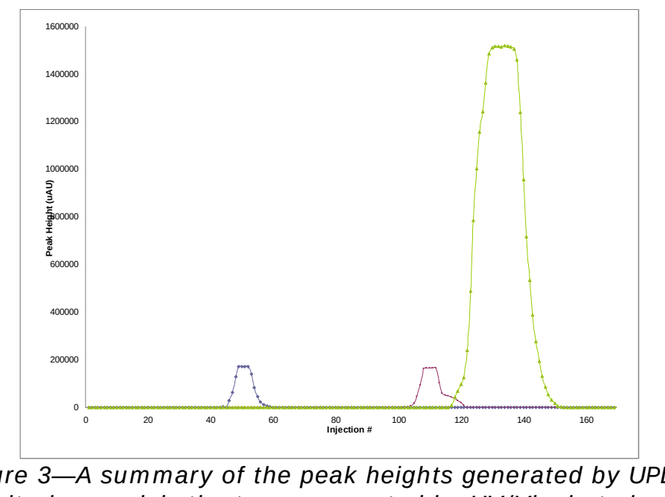

Figure 3—A summary of the peak heights generated by UPLC monitoring models the trace generated by UV/Vis, but also allows for the quantification of individual peaks

Results and Discussion

The API standard was injected from a standard vial prior to the beginning of the process column simulation. The %RSD for 10 replicate injections was 0.06% for peak area and 0.14% for peak height. A tee was placed in the effluent line to draw sample through a transfer line to the injection valve of the OLM. The PATROL UPLC Process Analyzer was able to monitor the effluent off the simulated process column and successfully quantify all 3 peaks to generate the same profile as was observed by the UV/Vis detector (Figure 3). The benefit of monitoring by UPLC over UV/Vis or another spectroscopic method is the ability to determine the time when all of the impurity has eluted off the column before the collection of the API begins (Figure 4). This will result in maximum purity while ensuring highest yield of the final product.

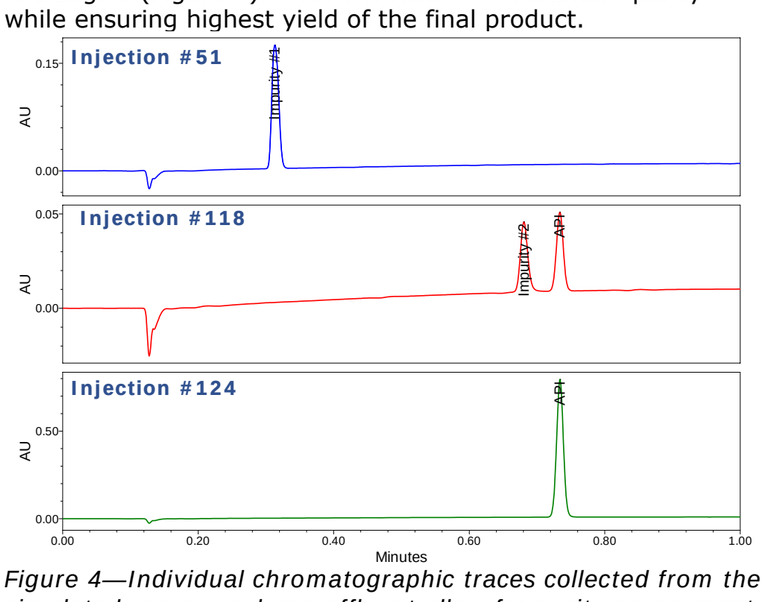

Figure 4—Individual chromatographic traces collected from the simulated process column effluent allow for purity assessment of the API and are used to trigger its collection

ON-LINE REACTION MONITORING

The ability to quantitate all of the components in a reactor during a synthesis reaction results in the highest conversion to the target compound with the lowest generation of side products. The real-time monitoring capabilities of the PATROL UPLC Process Analyzer maps out the amounts of all the components in the reactor to determine the optimal time to quench the reaction.

Experimental

The conversion of acetylsalicylic acid (ASA) to salicylic acid was monitored. One liter of ASA was prepared at 0.3 g/L in water. The repeatability of the system was assessed prior the reaction initiation by drawing from the vessel at ambient temperature. The vessel was then placed in a 75°C heated bath and 10mL of nitric acid added. The reaction was sampled and analyzed under the following conditions:

Column:	2.1x50 mm HSS T3 1.8 µm
Eluents:	A—0.1% Formic Acid in Water
	B—0.1% Formic Acid in Acetonitrile
Gradient:	5% - 80% over 2 minutes; Curve 6
Flow Rate:	0.8 mL/min
Temperature:	50°C
Inj. Volume:	1 µL
Detection:	243 nm; 40 Hz; Time Constant 0.025s
Wash:	70/15/15 Acetonitrile/Water/Isopropanol
Purge:	1mL (4x volume of transfer line)
Run Time:	2.5 minutes
Cycle Time:	4 minutes 10 seconds

Figure 5—Chromatograms from the transferred samples allow for quantification of the API and any process impurities.

Results and Discussion

The %RSD for the transfer and analysis of the starting materials prior to reaction initiation was determined to be 0.15% for peak area and 0.19% RSD for peak height. Once the reaction was initiated, the aliquots from the reaction vessel were automatically transferred to the PATROL UPLC Process Analyzer and the amount of each component present was determined. The system was able to separate all the components with good resolution and quantify low level impurities below 0.05% of the API (Figure 5). On-line spectroscopic sensors cannot simultaneously quantify such a complex matrix with such varying concentrations. By plotting the %Area of each of the components from the chromatogram a map of the reaction can be generated (Figure 6). This plot can be used to determine the optimal time to quench the reaction to maximize the yield of the API, maximize the purity of the API or determine the point at which a critical impurity reaches a threshold level.

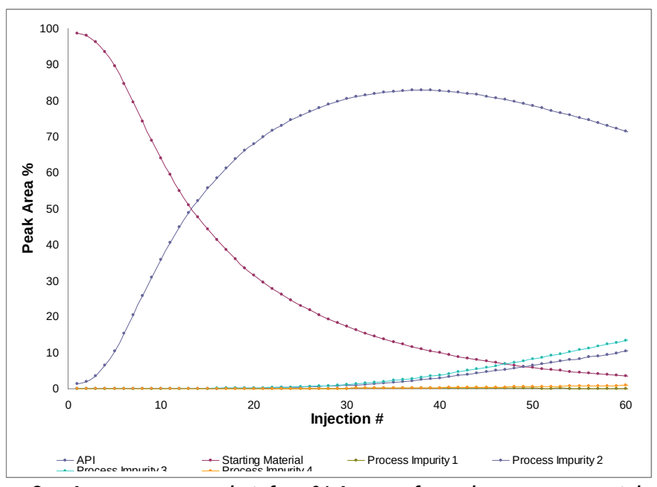

Figure 6—A summary plot for %Area of each component in the reaction vessel for determination of the reaction endpoint.

CONCLUSIONS

- **The PATROL UPLC Process analyzer brings the QC reference standard methodology to the manufacturing floor**
- **The PATROL UPLC Process Analyzer was designed to meet the challenging and diverse applications of in-process samples**
- **The system was designed for fully automated integration with on-line analysis and for walk-up at-line analysis**
- **Monitoring of process column effluent and reaction vessels by UPLC allows for the simultaneous quantification of APIs and process impurities for maximum product yields and purity**

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