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응용 자료

Tanya Tollifson

Waters Corporation

Abstract

The PATROL UPLC Process Analysis System brings reference-standard methodology to process development and is directly scalable through commercial operations, eliminating the need to calibrate spectroscopic sensors or to send suspect samples to an off-line QC laboratory. This application note discusses the use of UPLC for monitoring effluent from a process purification column.

Benefits

The PATROL UPLC Process Analysis System, with automated online and atline analyses, aids in manufacturing throughput and yield by providing real-time information regarding the process effluent. The creation of multi-point calibration curves from a single standard vial allows for real-time decision making on effluent fraction collection based on quantitative data of target product as well as process impurities.

Introduction

For pharmaceutical and biopharmaceutical companies, among other industries, Process Analytical Technology (PAT) is a critical component of the overall manufacturing process, relied upon to provide richer process understanding and consistent product quality at maximum yields with minimal waste.

PAT involves taking timely measurements throughout the production process to verify the quality of in-process batches and to understand performance in each of the critical steps of that process. Many different sensor

technologies are employed throughout the manufacturing process to measure the attributes of the in-process batches. Deployment of appropriate sensors to monitor identified critical quality attributes (CQAs) can aid in maintaining process control and functioning well within the established design space of the operation.

Typically, process steps such as 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 purification process but lack the ability to effectively resolve and quantify multiple components in the effluent stream.

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 has the ability to quantify multiple components within complex samples.

HPLC is the most widely-used technique in pharmaceutical QC laboratories. However, its long run times and complex system operation have prevented it from being routinely used for atline or online analysis.

With the introduction of Waters UltraPerformance LC (UPLC) technology, it is now possible to achieve near real-time chromatographic analysis for in-process samples. UPLC is delivered in a system that includes integrated hardware and software, offering a simple design that requires little to no user input.

The PATROL UPLC Process Analysis System brings reference-standard methodology to process development and is directly scalable through commercial operations, eliminating the need to calibrate spectroscopic sensors or to send suspect samples to an off-line QC laboratory. This application note discusses the use of UPLC for monitoring effluent from a process purification column.

Experimental

The PATROL UPLC Process Analysis System monitored the simulated process column effluent under the following conditions:

Column:	Waters ACQUITY UPLC BEH 2.1 mm x 50 mm, C_{18}, 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
Temp.:	50 °C
Inj. Volume:	5 µL
Detection:	243 nm; 40 Hz; Time Constant 0.025 sec
Wash:	70:15:15 Acetonitrile/ water/isopropanol
Purge:	1 mL (4x volume of transfer line)
Run time:	1 min
Cycle time:	2 min, 40 sec

Results and Discussion

Methods

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 online monitoring with the PATROL UPLC Process Analysis System, a process chromatography effluent was simulated using a quaternary gradient pump.

To simulate the process column effluent, a gradient profile was developed to mimic the effluent generated by the purification of an API from two impurities. The profile measured with a UV/Vis detector is shown in Figure 1. The separation profile has one impurity that is well resolved from the API and a second impurity that is not completely resolved from the API.

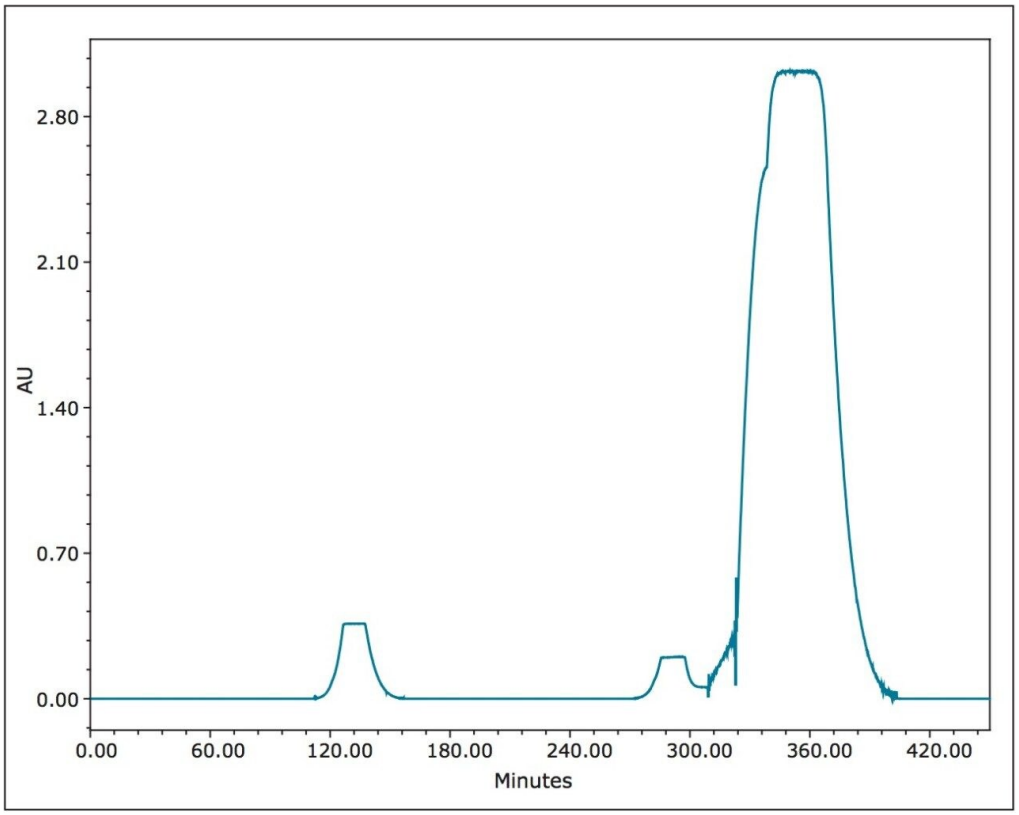

Figure 1. UV/Vis trace of simulated process column effluent (243 nm).

UPLC Technology

UPLC is based upon the use of sub 2-µm column particles and system technology that takes advantage of the benefits of these particles. Since its introduction, many users have transferred their HPLC QC methods to UPLC with great success, realizing tremendous improvements in both throughput, sensitivity, and resolution.

The PATROL UPLC Process Analysis System brings these significant improvements to the manufacturing floor in a manner that allows LC to be used as a real-time sensor. It is a holistically-designed system that integrates UPLC technology, control software, and a ruggedly-engineered sample management module for managing the samples and workflow in an in-process environment. The system's components, along with all solvents, waste, and standards, are contained within an enclosed case that is compatible with all requirements of a manufacturing environment.

The system is designed to be compatible with both online (direct automatic sampling from a process) and atline (manual sample drawn from a process) analysis. The ACQUITY UPLC Process Sample Manager (PSM) 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. For atline applications the system features a walk-up interface with barcode scanning capabilities that eliminates the need for information input from the technician. It also provides sample chain of custody with 21 CFR Part 11 compliant-ready Empower Software.

Application

A system suitability standard (API) was injected from a vial prior to the beginning of the process column simulation. The repeatability of 10 replicate injections (Table 1) was well within the method requirements. A tee was placed in the effluent line to draw sample through a transfer line to the injection valve of the ACQUITY UPLC PSM.

	Retention time %RSD	Peak area %RSD	Peak height %RSD
API (phenacetin)	0.08%	0.06%	0.14%

Table 1. Repeatability of 10 replicate injections.

The PATROL UPLC Process Analysis System was able to monitor the effluent off the simulated process column and successfully quantify all three peaks to generate the same profile as was observed by the UV/Vis detector (Figure 2).

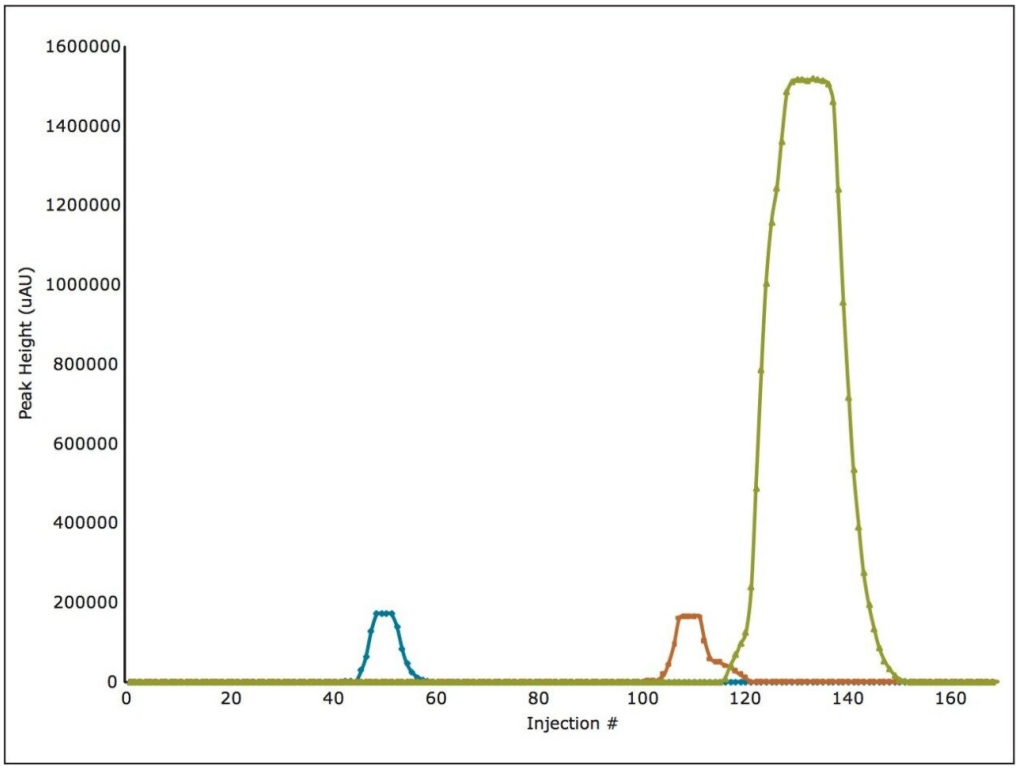

Figure 2. A summary of the peak heights generated by UPLC monitoring models the trace generated by UV/Vis, but also allows for the quantification of individual peaks when there is not baseline resolution.

Three extracted chromatograms from different time points during the simulation (Figure 3) demonstrate the ability to quantify each of the components throughout the progression of the purification process. The most significant benefit of monitoring by UPLC over another spectroscopic method is the ability to determine the optimal time to begin collection of the API.

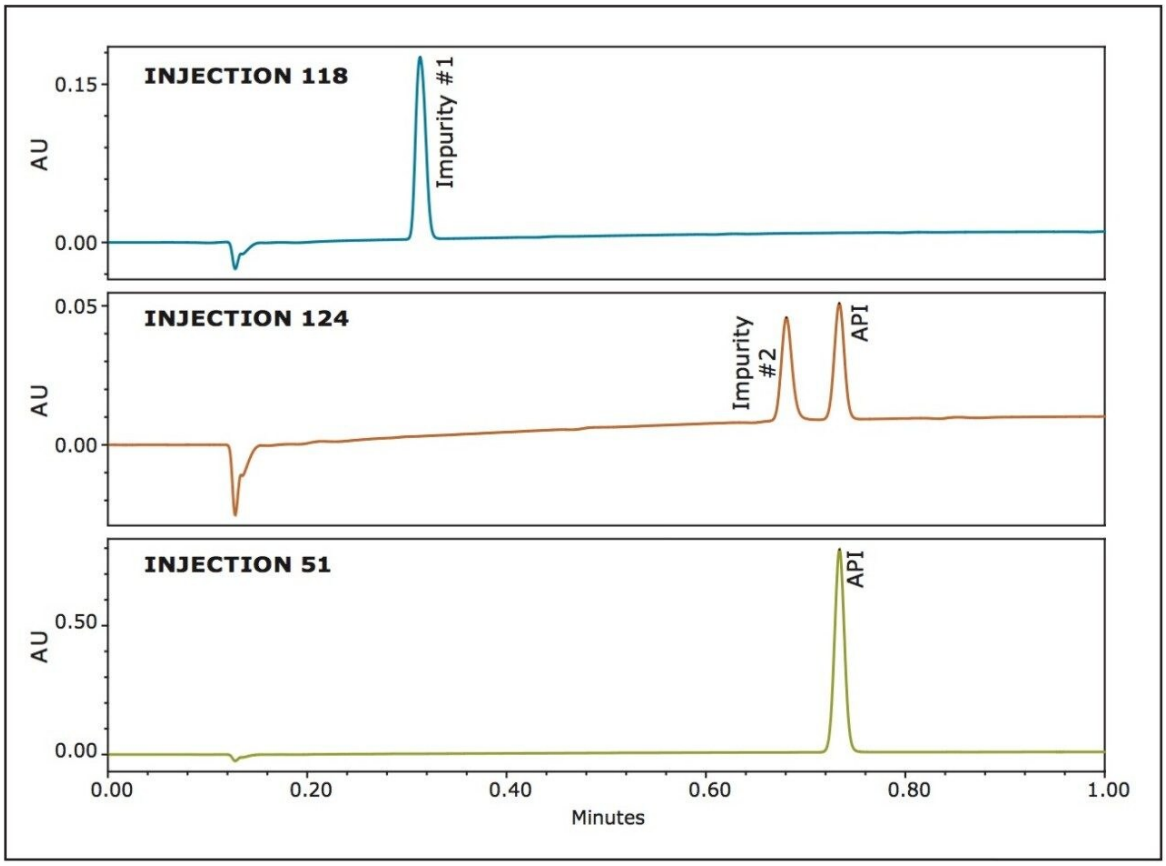

Figure 3. Individual chromatographic traces collected from the simulated process column effluent allow for purity assessment of the API and are used to trigger its collection.

Figure 4 overlays all of the injections from the time point of the apex of the impurity 2 peak on the process column simulation to the time point of the apex of the API. The peaks are well resolved on the UPLC column and can be easily quantified, even at very different levels. Table 2 summarizes the peak areas that were calculated for each of the individual injections on the PATROL UPLC Process Analysis System. The point at which all of the impurity has eluted off the process column can be determined even when it is in very low levels compared to the API (Figure 5).

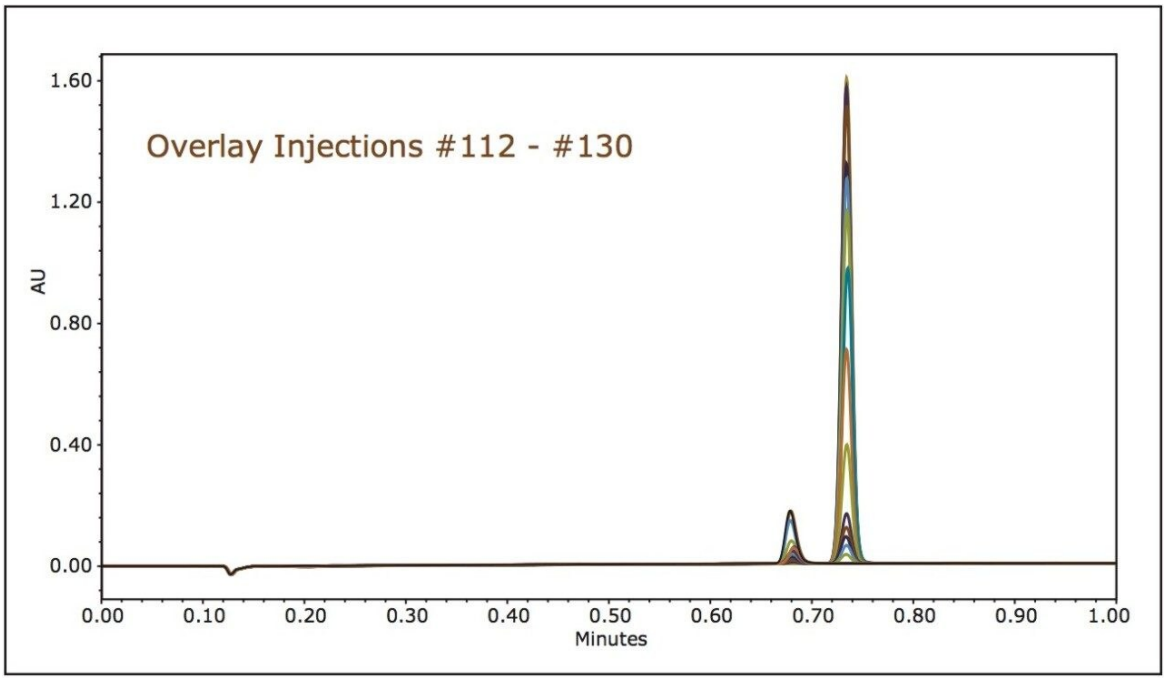

Figure 4. Overlay of all injections from the time point of the apex of the impurity 2 peak on the process column simulation to the time point of the apex of the API.

Injection #	Peak area salicylic acid	Peak area phenacetin	Peak area salicylic acid ——————X 100% Peak area p henacetin
112	131278	–	
113	107101	–	
114	57779	–	
115	42602	–	
116	40202	–	
117	33420	420	7957%
118	24705	21240	116%
119	17451	42089	41.5%
120	10129	62435	16.2%
121	3442	83320	4.13%
122	440	114626	0.38%
123	–	273784	
124	–	493515	
125	–	682528	
126	–	818540	
127	–	901826	
128	–	939711	
129	–	1072950	
130	–	1112468	

Table 2. Summary of peak areas that were calculated for each of the individual injections.

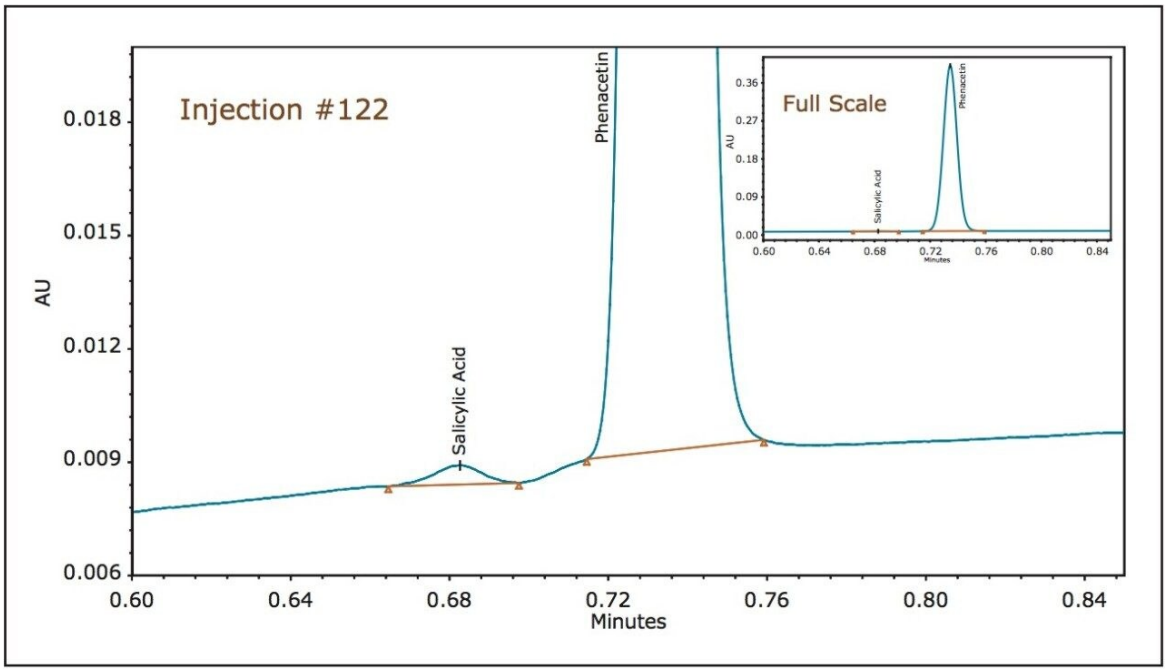

Figure 5. Determining the point at which all of the impurity has eluted off the process column, even when it is in very low levels compared to the API.

Conclusion

- Monitoring of process column effluent by UPLC allows for the simultaneous quantification of APIs and process impurities for maximum product yields and purity.
- Deployment of the PATROL UPLC Process Analysis System on the manufacturing floor promotes process efficiency, while maintaining rich process understanding.
- The PATROL UPLC Process Analysis System was designed with ease-of-use in mind to meet the challenging and diverse applications of in-process samples.
- The fully automated integration with online analysis and walk-up atline analysis reduces risk associated with user handling errors.

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ACQUITY UPLC System <https://www.waters.com/514207>

PATROL UPLC Process Analysis System <https://www.waters.com/10046886>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

Connections INSIGHT <https://www.waters.com/1000345>

NuGenesis SDMS <https://www.waters.com/513068>

ACQUITY UPLC Process Sample Manager <https://www.waters.com/134796101>

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