Online UPLC Method for the Support of Cleaning of Validation

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INTRODUCTION

During the manufacturing of active pharmaceutical ingredients (APIs), the formulation of drug substances, and therapeutic fill and finish, the removal of drug residues from the equipment is usually performed by a series of cleaning procedures. It is imperative that the production equipment used in this process be properly cleaned in order to avoid cross-contamination of drug products.[1-3] The safety acceptance criteria for API residues vary with drug substance. More potent compounds will require a lower acceptance limit. In general, most processes aim to have a lower safety limit in the 10 ppb – 1 ppm range (10 ng/mL – 1 µg/mL). In order to achieve these limits, sensitive analytical techniques are required.[4] Typically these samples (either swabs or wash solvents) are taken to an off-line QC laboratory for analysis. The time it takes to receive results from the off-line laboratory can range from hours to days. During this time, the production equipment must sit idle. If laboratory results are positive for API residues, the cleaning process and subsequent off-line QC testing must be repeated, increasing the amount of time the manufacturing equipment sits idle. This poster describes a fast, online, Ultra Performance Liquid Chromatography® (UPLC®) method which monitors wash solvents directly from a sampling point on the manufacturing equipment. By monitoring wash solvents online, the point at which the API has been removed from the production equipment can be determined, thus reducing the volume of wash solvent required and substantially reducing the time that the equipment must be taken off-line before a new batch can be initiated. The results from the online method are compared to those obtained by testing swabs and wash solvents at an off-line UPLC system. The PATROL™ UPLC System (Figure 1) was designed to be utilized in a manufacturing environment and provides near real-time analysis of inprocess samples, both online and atline.

Figure 1. PATROL UPLC System

EXPERIMENTAL

Reaction Conditions

Cleaning was performed on reaction vessels used for the conversion of acetylsalicylic acid (ASA) to salicylic acid.[5] A solution of 0.3 g/L ASA in water was prepared in a 1L reaction vessel. Nitric acid (10mL) was added to the reactor which was placed in a heated bath @ 75°C. After 2 hours the temperature was reduced to 7°C and after 2 additional hours the reactor was removed from the bath. The reactor was then emptied in preparation of cleaning.

Cleaning Procedure

The final cleaning procedure included 3 wash steps using 100mL of 50/50 Water/Methanol to clean the inside of the reactor and 2 wash steps to clean the exit port of the reactor using 200mL of the same solvent. Wash solvents after each step were sampled and analyzed to monitor the cleaning progress. Swabs were used to assess the reactor cleanliness throughout the procedure and also after the final cleaning step to ensure levels were below acceptable limits.

Quantitative Methodology

Calibration curves for the starting material and final product were based upon 4 standards at levels ranging from 10ng/mL to 50µg/mL, depending on which step in the cleaning process was being assessed. The determination of limit of detection (LOD) @ s/n=3, limit of quantification (LOQ) @ s/n=10, and linear range was determined by analyzing 12 standards across the entire concentration range.

Chromatographic Conditions

System:	PATROL™ UPLC® Process Analyzer Online System
	ACQUITY UPLC® System (off-line analysis)
Column:	2.1 x 50mm ACQUITY UPLC® HSS T3 1.8µm
Column Temp:	50°C
Flow Rate:	1.0 mL/min
Mobile Phase:	75/25 Water/Acetonitrile + 0.1% Formic Acid
Inj Volume:	1µL
Weak Wash:	95/5 Water/Acetonitrile (if necessary)
Strong Wash:	70/15/15 Acetonitrile/Isopropanol/Water
Wavelength:	230 nm
Data Rate:	10 Hz
Time Constant:	0.2s (Normal)
Run Time:	1 minute

RESULTS AND DISCUSSION

Chromatographic Method

A fast isocratic method was developed for online monitoring of the wash solvents. The final method had a 60 second run time with an inject-to-inject cycle time of 160 seconds resulting in near real-time analysis. It provided excellent resolution of the starting material, final product, and the two critical process impurities. An example of the chromatography for a standard and a wash step are shown in Figure 2.

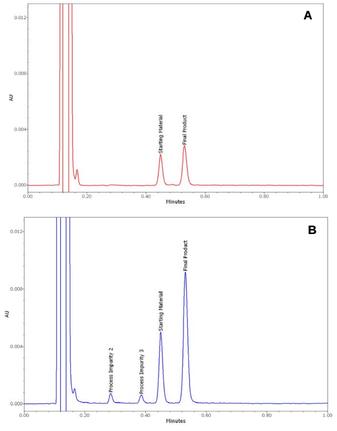

Figure 2. Example chromatograms for a standard (A); and the first wash step (B) containing starting material, final product, and 2 process impurities.

Limits of Detection / Quantification and Linear Range

To ensure the method met sensitivity requirements and that the linear range was sufficient to quantify across the required range, a calibration curve was generated from 10 ng/mL to 50 µg/mL. The calibration curve used a 1/x weighting to ensure good quantification at low levels. Exceptional linearity was observed with R^2 values in excess of 0.999 for the curve which extended across more than 3 orders of magnitude (Figure 3). The final method had excellent limits of detection, as low as 24 ng/mL (See Table 1). LOD and LOQ were determined by plotting the amount versus s/n for the low level standards.

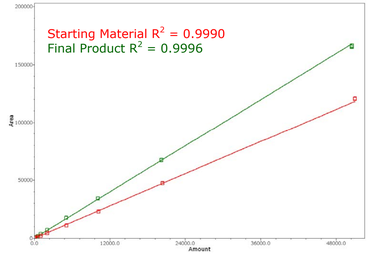

Figure 3. Calibration curves for the starting material and final product (10ng/mL - 50µg/mL).

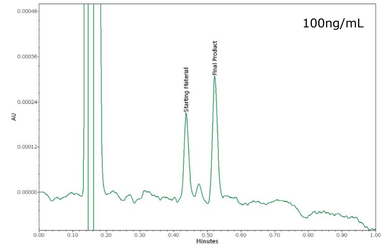

Figure 4. Standard injection near LOQ.

Compound	LOD (s/n = 3)	LOQ (s/n = 10)
Starting Material	31 ng/mL	102 ng/mL
Final Product	24 ng/mL	80 ng/mL

Table 1. LOD and LOQ of the reaction components.

Sample	Wash A1	Wash A2	Wash A3	Wash B1	Wash B2
Online 1	1767	28	—	46	—
Off-Line 1	1416	22	—	47	—
Swab 1	172	—	—	—	—
Online 2	1807	29	—	94	—
Off-Line 2	1443	19	—	83	—
Swab 2	71	—	—	6	—

Table 2. Levels of final product in the wash solvents during the cleaning protocol development. Results from the online method were in agreement with off-line results (both swab and wash solvent). Test performed in duplicate. Levels in ng/mL.

Assessing Online Monitoring by UPLC

To demonstrate the viability of using with the PATROL UPLC Process Analyzer Online System for the support of cleaning validation and the monitoring of wash solvents, equivalency to off-line results must be demonstrated. A protocol was developed to clean the reactor and its efficiency was assessed after each step by both online and off-line analysis. The results in Table 1 demonstrate that if the final product was detected by off-line analysis (wash solvents or swabs) it was also detected by online monitoring. The PATROL UPLC System was an extremely useful tool in developing the cleaning protocol as the level of contamination could quickly and easily be determined at each cleaning step. The final cleaning protocol consisted of 3 wash steps inside the reactor (protocol A) and 2 wash steps at the outlet (protocol B). Once the final cleaning protocol was developed, the repeatability of the PATROL UPLC System to monitor the cleaning process was assessed. The test was performed 4 times and the results of on-line and off-line monitoring were consistent for determining the presence of both the starting material and final product in the reactor (Table 2). The final results indicate that if residue was not detected in the A wash steps, the inside of the reactor was clean, and if residue was not detected in the B wash steps, the outlet of the reactor was clean (as confirmed by swab analysis). Online monitoring of the cleaning of manufacturing equipment is more effective than traditional off-line tests. A reactor can be washed and analyzed until it meets cleaning specifications rather than over washing, wasting solvent and time, or risking the equipment fail repetitive cycles of off-line QC tests and sitting idle while the cleaning process is repeated.

Starting Material in Wash Solvents (ng / mL)

Trial	Sample	Wash A1	Wash A2	Wash A3	Wash B1	Wash B2
Trial #1	Online	1124	48	—	1131	—
Trial #1	Off-Line	1098	56	—	1045	—
Trial #2	Online	2164	24	—	73	—
Trial #2	Off-Line	2023	24	—	67	—
Trial #3	Online	1726	38	—	61	—
Trial #3	Off-Line	1676	45	—	60	—
Trial #4	Online	855	—	—	128	—
Trial #4	Off-Line	816	—	—	118	—

Final Product in Wash Solvents (ng / mL)

Trial	Sample	Wash A1	Wash A2	Wash A3	Wash B1	Wash B2
Trial #1	Online	1580	27	—	60	—
Trial #1	Off-Line	1632	31	—	56	—
Trial #2	Online	1647	19	—	40	—
Trial #2	Off-Line	1647	21	—	40	—
Trial #3	Online	1658	29	—	50	—
Trial #3	Off-Line	1678	32	—	51	—
Trial #4	Online	1619	15	—	127	—
Trial #4	Off-Line	1587	17	—	131	—

Table 3. Levels of starting material and final product (ng/mL) as determined on-line by the PATROL UPLC System and an off-line method. All corresponding swabs after the final wash step were also clear.

Benefits of Online Monitoring by UPLC

Time for Analysis

Online	Near real-time analysis	Typically < 4 minutes
Off-Line	Analysis time includes laboratory activities	2 hours to days

Solvent Consumption

Online	'Clean until Clean'	Wash as long as necessary, no extra solvent consumption
Off-Line	'Clean to Worst Case Scenario'	Consumes excess solvent

Equipment Down Time

Online	'Clean until Clean'	Minimize time to clean equip-ment
Off-Line	'Clean to Worst Case Scenario'	Excess down time; if samples fail QC test, cleaning repeated

CONCLUSION

- The PATROL UPLC Process Analyzer Online System provides a highly effective solution for monitoring wash solvents from the cleaning of manufacturing instrumentation
- The results obtained by the PATROL UPLC System were consistent with those determined by off-line instruments
- The large linear dynamic range of the PATROL UPLC system provides the means to monitor reactions at high concentrations and monitor the low levels required for cleaning protocols on the same instrument
- The PATROL UPLC System was able to monitor the low ng/mL levels required by methods to support cleaning validation
- Monitoring of the cleaning progress by the PATROL UPLC System allows for manufacturing equipment to be continuously washed until cleaning specifications are met, rather than using repetitive cycles of excess wash solvents or risking the idling of the manufacturing process caused by the re-cleaning process if off-line results indicate contamination

References

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