AUTOMATING SAMPLE PREP AND DEMYSTIFYING LC-MS ASSAYS FOR FASTER DATA ANALYTICS IN BIOPRODUCTION

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INTRODUCTION

Automation is highly desired for the preparation of analytical samples to measure product quality attributes (PQAs) and process monitoring variables in bioproduction campaigns. Process development teams aim to access critical data earlier, reduce errors, minimize data variability, and lessen the burden on teams typically needing to monitor bioreactors 24/7 to ensure quality during long studies and production campaigns. LC-MS systems provide high-quality answers for many desired process quality attributes (PQAs) and process parameter monitoring assays. Herein, we discuss our latest efforts to integrate automated sample preparation with automated LC-MS analysis to support daily monitoring of attributes and nutrient profiles. Integrating workflows with data analytics software enables insights critical for feedback loops and decision outcomes for bioreactor studies and campaigns.

METHODS

Daily harvested samples were prepared using the Andrew+™ Pipetting Robot (Waters Corporation) to provide critical sample prep capabilities for samples harvested during typical batch production campaigns. These prepared samples were delivered directly to the BioAccord™ system, an LC-MS analyzer and several assays were run automatically (figure 1).

Bioreactor samples were prepared using various protocols required for typical LC-MS preparation, including clarification, dilution, and affinity cleanup, followed by automated plate sealing and introduction of the sample to the LC-MS system.

These protocols were developed, tested, and made available for download on the Onelab™ protocol library (Waters Corporation). Software enhancements for the laboratory control software enabled direct monitoring of bioreactor aspiration files to detect new samples and run the appropriate assay (Ambr® 15 and 250 HT system, Sartorius Stedim Biotech, NY, USA) automatically.

Figure 1: Bioprocess Walk-up Solution integrating Andrew+™ Pipetting Robot with the BioAccord™ LC-MS System

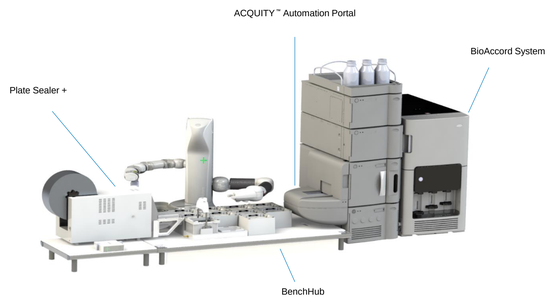
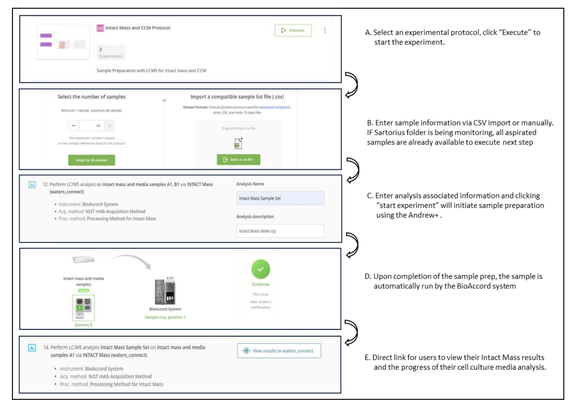
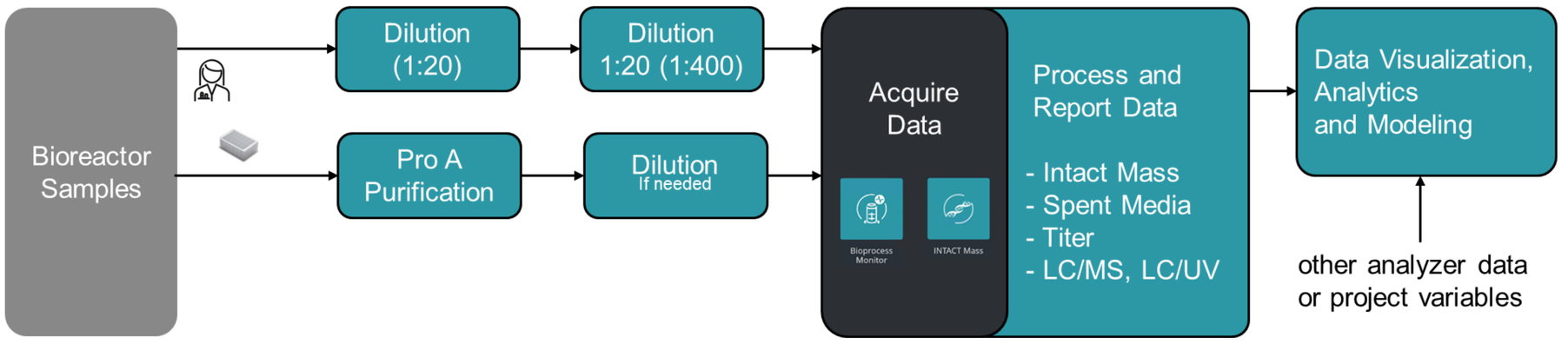


Figure 2: Workflow in Onelab software showing major workflow steps to import and execute aspirated samples and send to the BioAccord™ system for automatic injection acquisition and processing



RESULTS

Figure 3: High level schematic of integrated sample preparation through processing and formatting of the data for easy entry to common data visualization packages



Bioreactor aspirated sample lists were monitored and automatically displayed in Onelab software, where information was transcribed to generate an LC-MS sequence list, ready for execution. Basic information about the sample analysis names entered by the user, followed by execution of the protocol to both prepare samples for the appropriate assay and execute the LC-MS analysis and processing as part of a single unified protocol using Onelab software workflow (figure 2).

Figure 3 shows the overall workflows run on the platform. Data obtained from analytical workflow were further funneled into additional software packages for visualization and made available for additional analytics and modeling. All data from assays 1-3 were analyzed using Waters_connect™ software, Intact Mass and Bioprocess Monitor applications.

Several protocol/assay combinations were implemented and tested and two are described here.

Assay 1 – Intact Glycoform monitoring

Clarified samples were run using simple dilute and shoot method (figure 4a). For this experiment, an aliquot was also purified using a protein A preparative protocol on the Andrew+™ pipetting robot for comparison (figure 4b).

Assay 2 – Aggregation monitoring

Protein A purified samples were also run using an SEC analysis (UV only) for aggregation (figure 5). Intact Mass software was used to integrated and automatically calculate % high molecular and low molecular weight species (HMWS/LMWS).[1]

Assay 3 – Spent Media analysis

Using a second dilution step (also automated), and additional sample was prepared at 1/400 dilution and run using the BioAccord system. Data were processed using the Bioprocess Monitor software application (figures 6 and 7).[2]

Figure 4: Left panel (a): Day 10 harvested sample, UV chromatogram with spectra for light chain and main IgG peak. Right panel (b): Day 10 protein A purified sample, showing IgG.

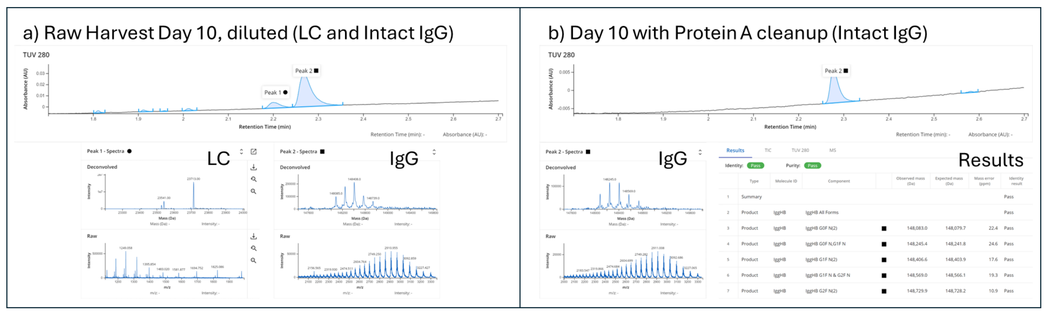


Figure 5: mAb SEC analysis with HMWS % and LMWS % table calculations performed using Intact Mass application

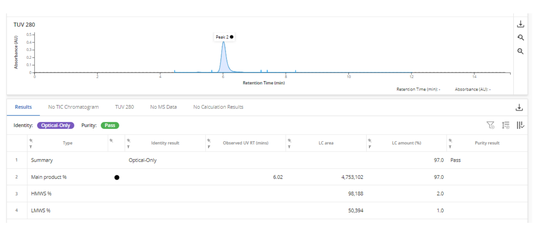


Figure 6 shows results for calibration of method, showing a typical calibration curve in the main calibration set review. 17 amino acids where quantified using the Waters amino acid standard (PN:WAT088122), after which data acquired using the BioAccord system were processed using the Waters Bioprocess Monitor software application. Results from assays 1, 2, and 3 were then exported to csv ready for processing using multivariate data analytics software (MVDS) packages.

Spent media data *.csv was imported to JMP® software (JMP Statistical Discovery LLC, NC, USA), using the Waters Bioprocessing Monitoring – JMP add-in (figure 7) provided on the JMP Marketplace.[3] Results for amino acids and key energy pathways are shown in figure, enabling a quick overview of profile changes and similarities across bioreactors.

Figure 6: Data for spent media were processed using Bioprocess Monitor software. System was calibrated using amino acid standards and Ambr® 250 bioreactor data samples run and results for vessel 1 and 2 shown

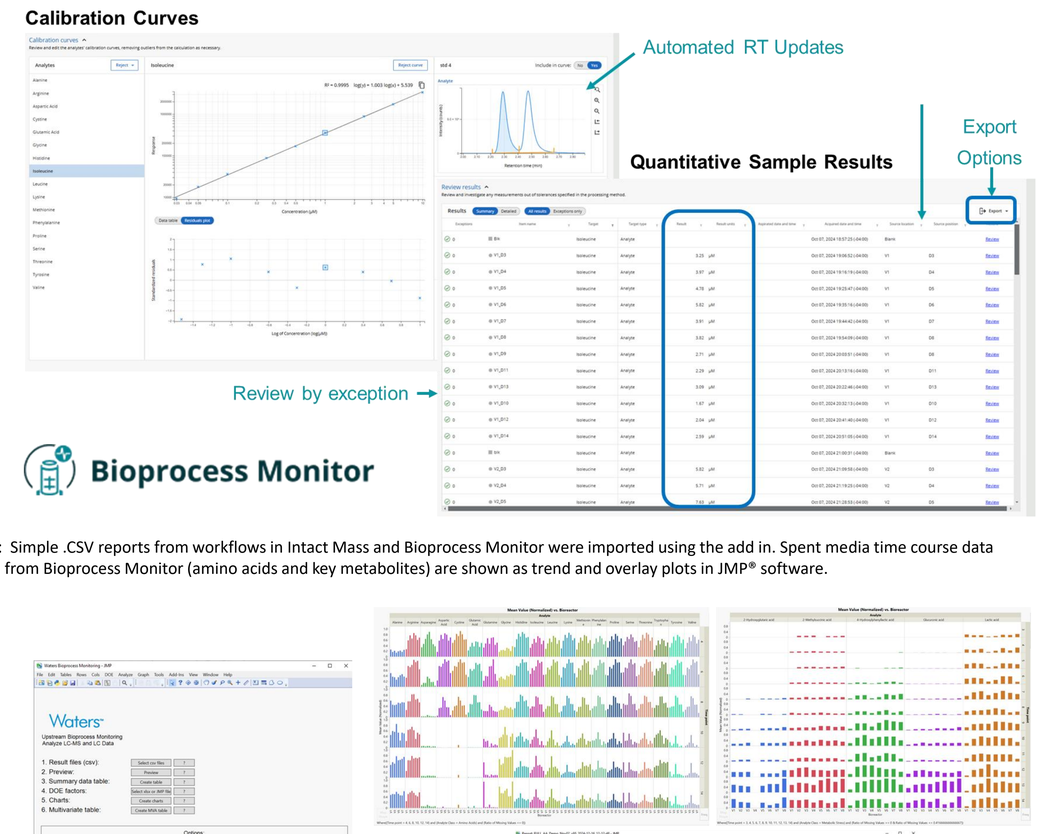
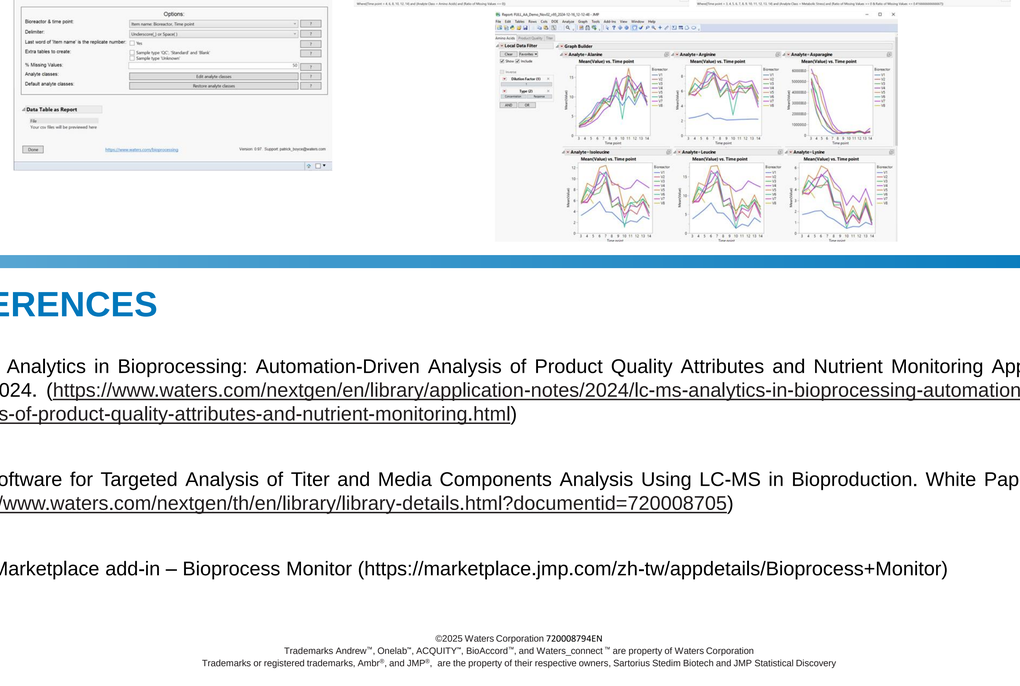


Figure 7: Simple .CSV reports from workflows in Intact Mass and Bioprocess Monitor were imported using the add in. Spent media time course data exported from Bioprocess Monitor (amino acids and key metabolites) are shown as trend and overlay plots in JMP® software.



CONCLUSIONS

- **Bioprocess Monitor software easily acquires and processes data from titer and spent media metabolite assays**
- **Automation of sample prep using Onelab software and Andrew Pipetting+ Robot provides access to high quality LC and LC-MS data**
- **Protocols automatically read Ambr® 15 and 250HT aspiration files, load protocols and enables user to perform sample prep through analysis quickly**
- **Process optimization and visualization of the data was performed using the JMP ® statistical software packages to gain further insights**

REFERENCES

1. LC-MS Analytics in Bioprocessing: Automation-Driven Analysis of Product Quality Attributes and Nutrient Monitoring Application Note 2024. (https://www.waters.com/nextgen/en/library/application-notes/2024/lc-ms-analytics-in-bioprocessing-automation-driven-analysis-of-product-quality-attributes-and-nutrient-monitoring.html)

2. New Software for Targeted Analysis of Titer and Media Components Analysis Using LC-MS in Bioproduction. White Paper 2025 (https://www.waters.com/nextgen/th/en/library/library-details.html?documentid=720008705)

3. JMP® Marketplace add-in – Bioprocess Monitor (https://marketplace.jmp.com/zh-tw/appdetails/Bioprocess+Monitor)