ENHANCING BIOPROCESS DEVELOPMENT THROUGH AUTOMATED SPENT MEDIA AND TITER ANALYSIS BY LC-MS WITH DATA CONNECTIVITY VIA STATISTICAL SOFTWARE

CONNECTING SCIENCE
To Solve Problems That Matter

Waters™

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INTRODUCTION

Automation in bioproduction sample preparation and LC-MS analysis is crucial for improving efficiency and accuracy. It helps teams access critical data faster, reduce errors, minimize variability, and lessen the need for constant monitoring. Integrating these workflows with data analytics software enhances insights for better decision-making in bioreactor studies and campaigns.

This work describes integrating sample preparation of clarified bioreactor samples with automated sample preparation with direct integration and execution of LC or LC-MS based assays using a compact LC-MS system. The system consists of an Andrew+™ Pipetting Robot, the Andrew+ Benchhub, the Andrew Plate Sealer+ and a BioAccord™ LC-MS System equipped with the Automation Portal (figure 1).

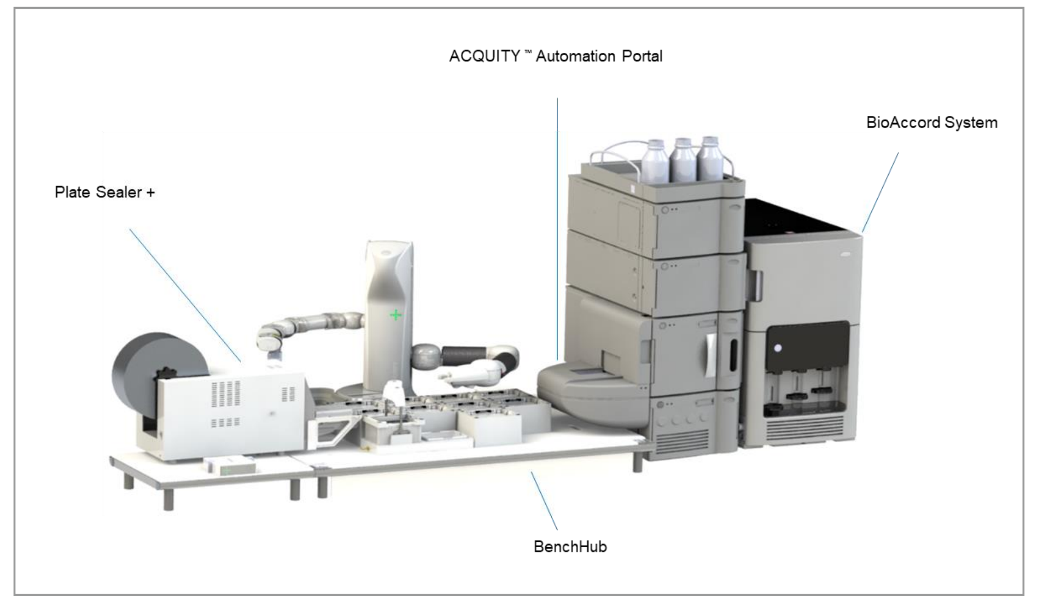
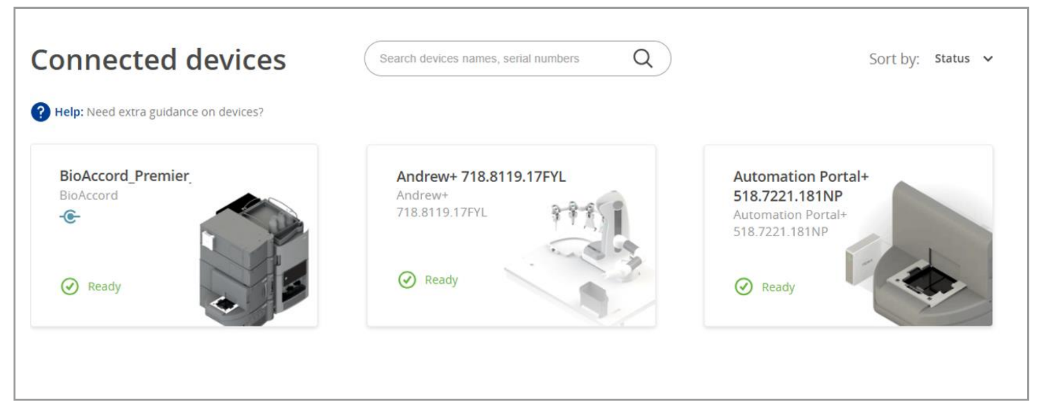


Figure 1: Bioprocess Walk-up Solution integrating Andrew+ Pipetting Robot with the BioAccord LC-MS System

The system is controlled with OneLab™ Software which controls all automation and LC-MS analytical devices from a single easy to use interface. (figure 2).


Connected devices

Search devices names, serial numbers

Sort by: Status

Help: Need extra guidance on devices?

BioAccord_Premier
BioAccord
Ready

Andrew+ 718.8119.17FYL
Andrew+
718.8119.17FYL
Ready

Automation Portal+ 518.7221.181NP
Automation Portal+
518.7221.181NP
Ready

Figure 2: LC. MS, Automation Portal, and Andrew+ Pipetting Robot and associated devices are all controlled via OneLab Device Interface.

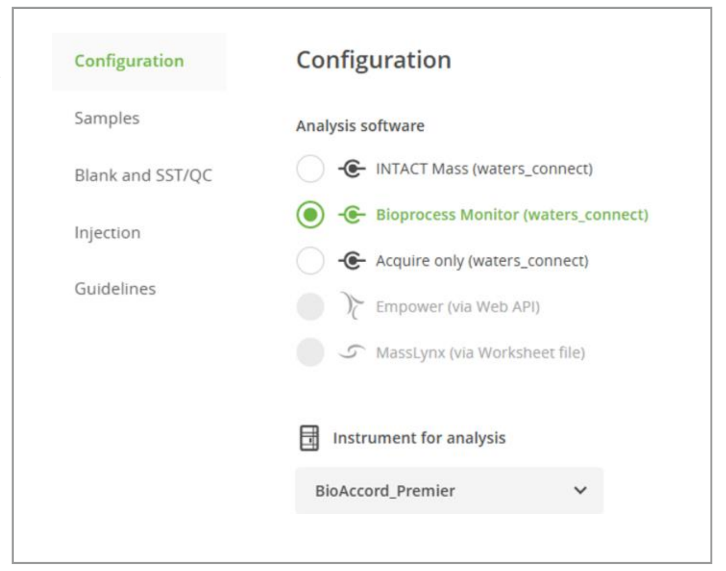
METHODS

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.

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). The OneLab Software was setup to enable direct monitoring of bioreactor aspiration files via an integrated file watcher. Once aspirated files appeared, the OneLab Software notified the user to run the appropriate assay. LC-MS configuration file is shown in figure 3. This enabled the workflow to be directly connected to the appropriate LC-MS system, assay and folders for data results.

Figure 3: LC-MS system and assays are linked and control via the OneLab Software and embedded within the sample prep protocol itself



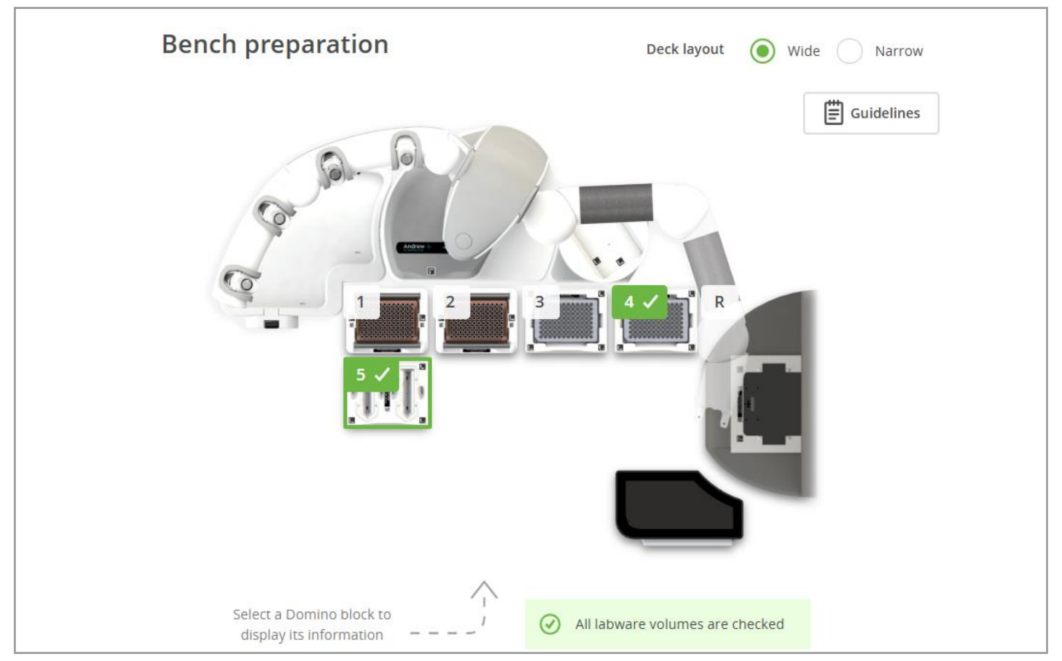
Configuration

Analysis software

○ INTACT Mass (waters_connect)
● Bioprocess Monitor (waters_connect)
○ Acquire only (waters_connect)
○ Empower (via Web API)
○ MassLynx (via Worksheet file)

Instrument for analysis

BioAccord_Premier


Bench preparation

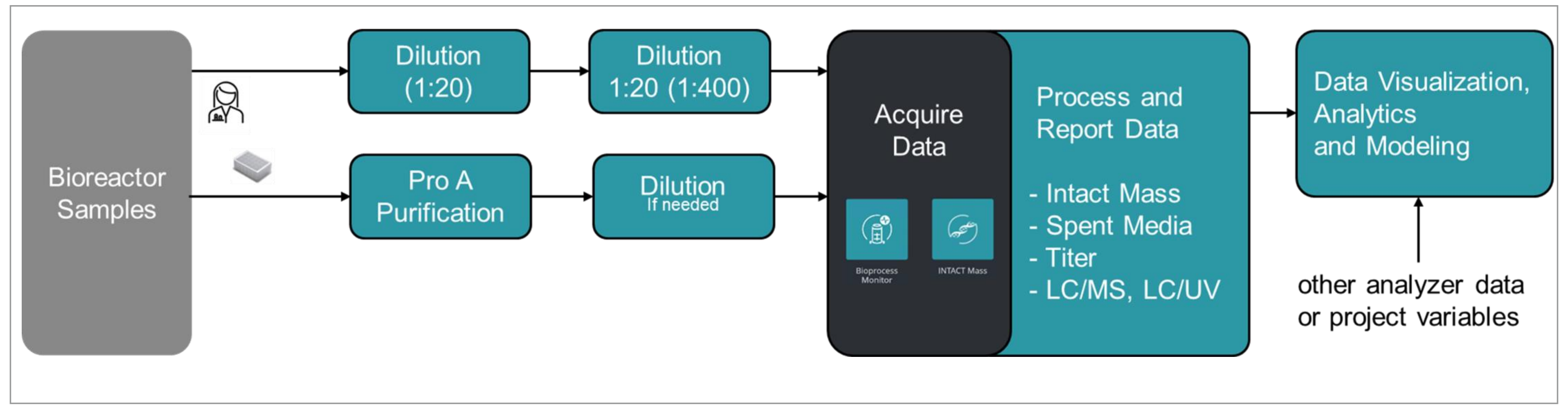
Deck layout ● Wide ○ Narrow

✓ All labware volumes are checked

Figure 4: Device layout and bench preparation checks for assay 1 and assay 2.

RESULTS

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. Key system components and system layout for Intact and Spent Media assays shown in figure 4, during the bench preparation check run prior to any analysis.


Bioreactor Samples

Dilution (1:20)

Dilution 1:20 (1:400)

Pro A Purification

Dilution If needed

Acquire Data

Process and Report Data
- Intact Mass
- Spent Media
- Titer
- LC/MS, LC/UV

Data Visualization, Analytics and Modeling

other analyzer data or project variables

Figure 5: High level schematic of integrated sample preparation through processing and formatting of the data for easy entry to common data visualization packages

Figure 5 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, results from each assay are show in figures 6 through 8

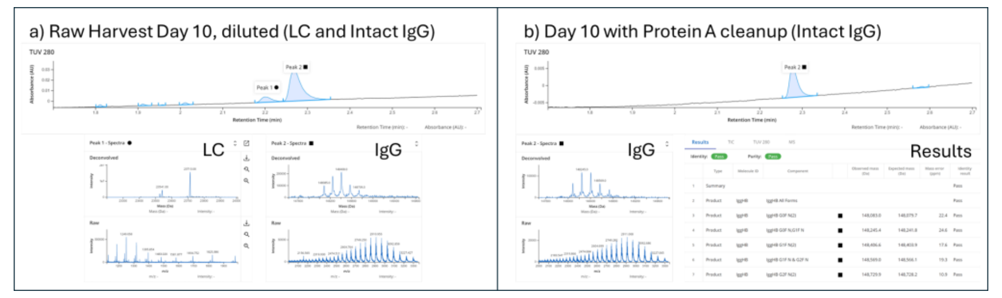


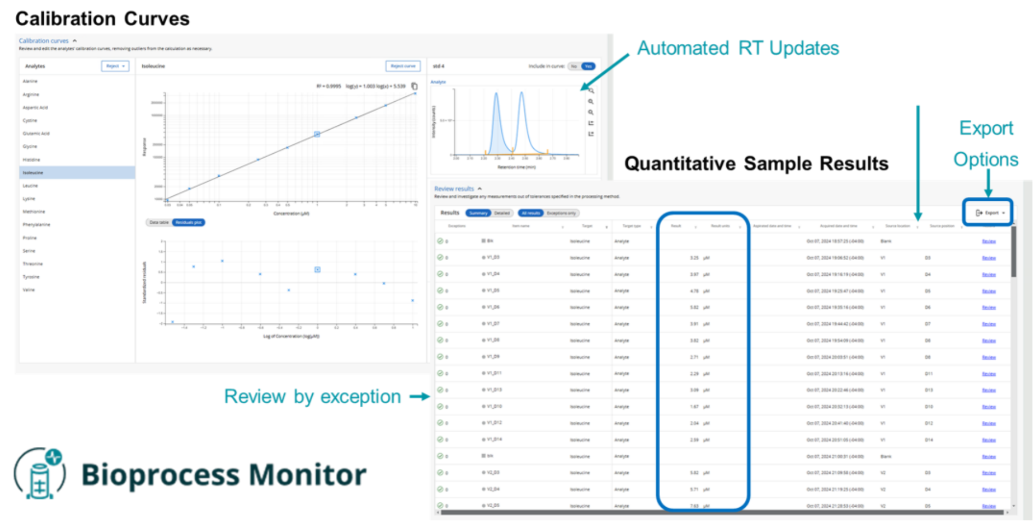
Figure 6. Assay 1 – Intact Glycoform monitoring

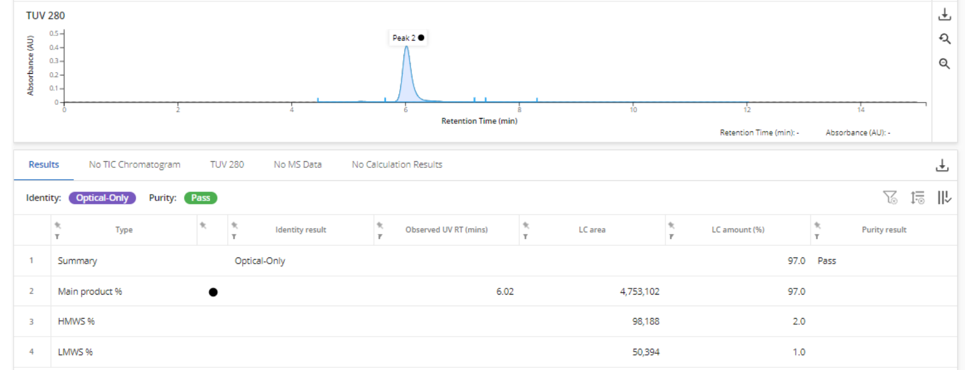
Clarified samples were run using simple dilute and shoot method and compared with an aliquot which was also purified previously using a protein A preparative protocol on the Andrew+ Pipetting Robot for comparison.

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 7 - Assay 2 – Spent Media analysis

Using a second dilution step in the same protocol as for assay 1, and additional series of samples were prepared at 1/400 dilution and run using the BioAccord System using the Bioprocess Monitor Software application.[2] Prior to analysis, LC-MS system was calibrated using amino acid standards (shown). Results for vessel 1 shown on bottom left.





Results | No TIC Chromatogram | TUV 280 | No MS Data | No Calculation Results

Identity: Optical-Only Purity: Pass

	Type		Identity result	Observed UV RT (mins)	LC area	LC amount (%)	Purity result
1	Summary		Optical-Only			97.0	Pass
2	Main product %	●		6.02	4,753,102	97.0	
3	HMWS %				98,188	2.0	
4	LMWS %				50,394	1.0	

Figure 8 Assay 3 – Aggregation monitoring

An additional aliquot of clarified samples were processed using protein A protocol and run using an SEC analysis (UV only) method for aggregation determination. Intact Mass software was used to integrated and automatically calculate % high molecular and low molecular weight species (HMWS/LMWS).2 Figure 5: mAb SEC analysis with HMWS % and LMWS % table calculations performed using Intact Mass 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, Pro A titer, and crude aggregation product quality attribute data was imported to JMP® Software (JMP Statistical Discovery LLC, NC, USA). Waters Bioprocessing Monitoring and INTACT Mass applications along with the JMP Software add-in provided and easy way to review multiple datasets.[3] Results for amino acids and key energy pathways are shown in figure 9, enabling a quick overview of profile changes and similarities across bioreactors.

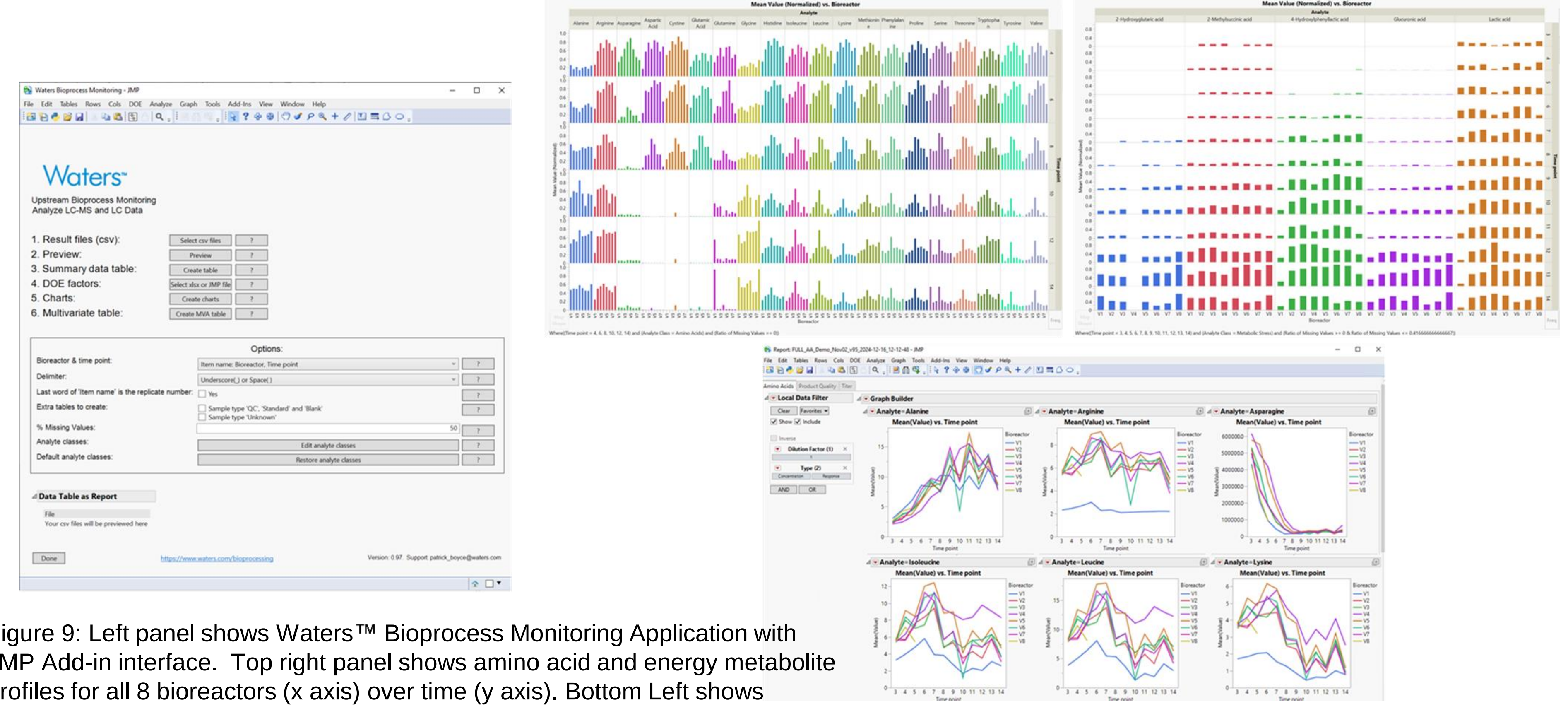


Figure 9: Left panel shows Waters™ Bioprocess Monitoring Application with JMP Add-in interface. Top right panel shows amino acid and energy metabolite profiles for all 8 bioreactors (x axis) over time (y axis). Bottom Left shows overlays for several amino acids. Significant differences for arginine, isoleucine, leucine, and lysine were observed in bioreactor 1.

CONCLUSIONS

Integrating end-to-end analysis and statistical workflows in bioproduction is essential for optimizing processes and gaining deeper insights. In this work we are able to combine the following elements to provide a workflow and results:

- **Automation Tools**: OneLab Software and Andrew Pipetting+ Robot streamline sample preparation, providing high-quality LC and LC-MS data.
- **Protocol Integration:** Automatically reads Ambr® 15 and 250HT aspiration files, loads protocols, and enables quick sample prep through analysis.
- **Waters_Connect and Bioprocess Monitor Software**: Efficiently acquires and processes data from titer and spent media metabolite assays.
- **Statistical Analysis**: JMP® software packages are used for process optimization and data visualization, offering valuable 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)

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