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응용 자료

LC-MS Analytics in Bioprocessing: Automation-Driven Analysis of Product Quality Attributes and Nutrient Monitoring

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Abstract

Integrating and improving automation will enable process groups to obtain earlier access to essential PQA information and information about their process changes through media monitoring. We recently updated the Waters™ Bioprocess Walk-Up Solutions[1] to enable bioprocess teams to gain access to more analytics, faster. Key features and updates of the Bioprocess Walk-Up Solutions with Portal includes:

- Streamlined Sample Handling: Automation accelerates sample preparation, reducing manual effort and potential errors. Samples are rapidly processed and directly submitted for LC or LC-MS analysis.
- Increased flexibility: Integrated platform offers automated sample prep, data acquisition, and reporting. Recent hardware enhancements include direct LC and LC-MS injection capabilities, plate sealing automation, and the addition of Protein A and filtration automated protocols.
- Updated Software: waters_connect™ informatics solution now analyzes and reports percentages for low and high molecular weight species (LMWS/HMWS/aggregation) and includes several other updates for bioprocessing customers.

- Improving analytics earlier in the process ensures safety, efficacy, and consistency of therapeutic production by identifying the best cell lines and conditions, enabling faster time to results, and time to market.

Benefits

- High throughput: Automated sample preparation using the Andrew+™ Pipetting Robot with direct injection to the BioAccord™ LC-MS system orchestrated by OneLab™ software
- Consistent Results: Enabling Protein A, clarification and dilution capabilities, automated plate sealing and immediate loading of plates into cooled sample manager reduce errors, variability and time spent on running analytics
- Connected Reporting: Reporting of glycoforms, LMWS, HMWS or other summary calculations have been enhanced and includes support for automated reporting to Ambr® bioreactor systems[2]

Introduction

Automation plays a pivotal role in enhancing the efficiency and accuracy of LC-MS analysis for bioprocess Product Quality Attributes (PQAs). In biopharmaceutical manufacturing, the measurement of PQAs are critical, as they ensure the safety, efficacy, and consistency of therapeutic products. The integration of automation into LC-MS workflows allows access to critical PQA information earlier in the process. Preparing samples for various LC and LC-MS based analysis is time consuming, requires training and often rate limiting for adoption. Adding automation to rapidly process samples and submit directly for LC or LC-MS analysis with minimal human intervention, reduces the potential for errors and variability in generating data.

This application note describes the enhancements and overall capabilities introduced to the Bioprocess Walk-Up Solutions since the previous system update.[1] This application note will include an overview of:

- Enhanced Hardware: Plate sealing; Direct insertion of prepped samples/plates to the LC or LC-MS system: Ability to perform extractions/filtrations.
- Improved Protocols: Protein A (ProA) cleanup using Magnet+ module for magnet bead based capture and release; Clarification using Extraction+ vacuum filtration module; Addition of plate sealing to all protocols has been enabled.

- Updated Software for Process teams; Ability to report LMWS, HMWS species for SEC assays; Ability to report acidic/basic species for charge variant LC-MS assays; and method settings to improve automated processing.
- Updated LC-MS Methods: New fast nine minute cell culture media method with comprehensive library updates.
- Data Integration/Visualization Enhancements: Tools for visualization, support for external data packages and control software.

Figure 1. System overview of interface between Andrew+ Pipetting Robot with sealing and filtration capability and BioAccord LC-MS System via the ACQUITY™ Automation Portal plate device. Additional Andrew+ components supported include the Andrew+ BenchHub, Andrew+ Active Rotating System (ARS) and Plate Sealer+.

Collectively, these automation and platform capabilities enable less interaction by the analyst and quicker time to results by automating more of the analytics, interpretation, and reporting at the end of required sample preparation.

Experimental

Detailed experimental details for LC and LC-MS methods can be found in the companion application notes that outline specifics for each PQA or cell culture media metabolite analysis.[3,4,5]

Andrew+ Pipetting Robot System Details

Please refer to system solution documentation and OneLab library protocols for complete/up to date list of dominos and consumables required (OneLab Protocol: Intact and Cell Culture Media Workflows < https://onelab.andrewalliance.com/app/lab/GK6ovDkA/library/intact-mass-and-cell-culture-media-workflows-X5nPLgng)>). The list below contains new items/consumables now supported by Bioprocess Walk-up solutions in this update.

Key Consumables	Part Number
Magne® protein A beads:	G8781 (Promega)
Clear heat seal film:	4ti-0540 (Azenta)
AcroPrep cell clarification and sterile filtration Plate, 24 well, 7mL:	97026 (Cytiva, Pall Life Sciences)
twin.tec PCR Plate 96, skirted, 200 µL/well:	951020443 (Eppendorf)

LC-MS System Details

LC details:	Acquity Premier BSM with Automation Portal
MS details:	BioAccord LC-MS System

Data Management

Chromatography software:	Waters_connect version 3.3

MS software applications:	Intact mass 1.8, UNIFI™ 1.9.4
Informatics:	OneLab version 1.20.3

Results and Discussion

Protein A Protocol and Analysis

The solution is capable of processing and running either raw harvested samples (with clarification/dilution) and/or Protein A purified (using magnetic bead-based cleanup) followed by injection of the sample on the BioAccord for LC-MS analysis. Both modes of operation offer the user different and useful information. Raw sample analysis has been previously described (ref) and enables quick, easy glycoform profiling and light chain estimation with minimal sample handing/processing. Adding ProA purification to extract the Fc based therapeutics enables the best profiling accuracy, best used for when titer measurement at lower levels (< 1g/L) is desired and/or when it is a prerequisite for additional assays (for example SEC, IEX, subunit, peptide mapping).

In figure 2, we show a typical day 10 result compared after running the dilution protocol or running the Protein A protocol. Figure 2 a shows raw harvested sample analysis following clarification and a 1/20 dilution step and Figure 2 b) shows the sample sample after protein A purification. Both are analyzed using the same Intact Mass LC-MS acquisition method. Depending on the project's needs, quick IgG glycoprofiling, light chain assessment and/or Protein A purified data is available and can be reported.

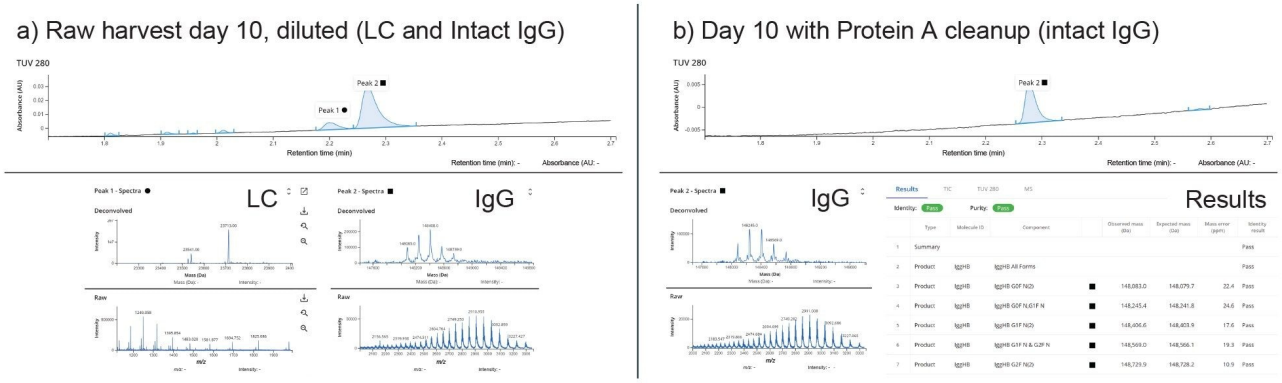

Figure 2. Left panel (a): Day 10 harvested sample, UV chromatogram with spectra for light chain and main IgG peak. Right panel (b): Day 10 protein A purified sample, showing IgG.

Clarification Protocol

Removing cell debris quickly and effectively is important both for preserving integrity of the therapeutic by limiting degradation, and to clarify samples prior to some analytic methods such as LC-MS. Filtration protocols using the two stage 24 well AcroPrep™, (Cytiva) plates were tested. Samples were filtered using the Andrew Extraction+ module, representative profile is shown in figure 3 and can be adjusted for more/less sheer-sensitive cell types. Samples were compared by measuring cell counts prior and after various filtration (and centrifugation) approaches, summarized in table 1. For centrifuged conditions, samples were centrifuged at 800 g for 5 minutes and filtered (1.25 µm). Cell counts were measured using a Vi-CELL counter (Beckman Coulter). Best conditions were found by also prediluting harvested samples using 1:1 PBS buffer.*

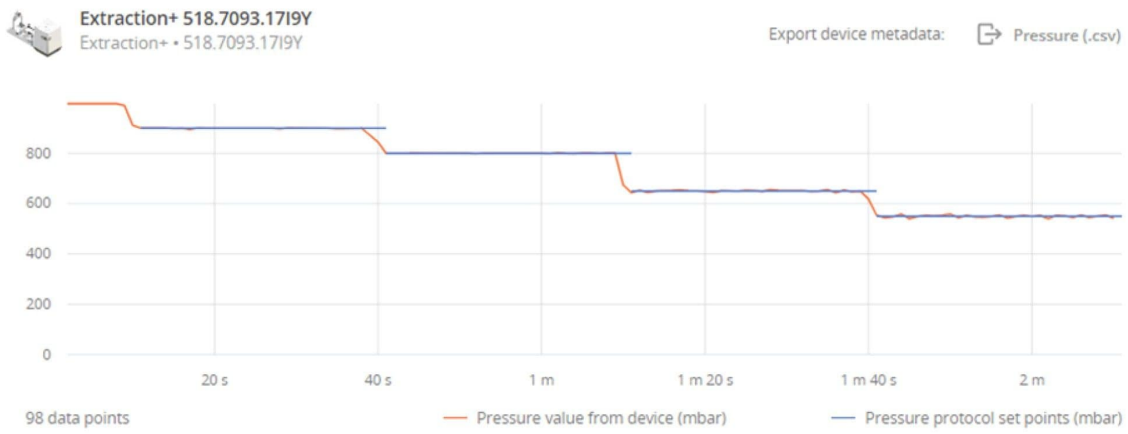

Figure 3. Example vacuum profile showing stepped filtration pressures (expected vs measured during protocol execution) ensuring complete, reproducible, and effective handling of samples has occurred.

Sample	Sample prep	Cell counts	Cell removal
24 million counts	Harvested sample	24.9×10^6	
(batch fed)	Centrifuged and 1.25µ filtered	0.56×10^6	98%
	AcroPrep filtered	0.0047×10^6	>99%
+100 million counts	Harvested sample	~110×10^6	
(perfusion)	AcroPrep filtered	0.0047×10^6	>99%

Table 1. Cell counts reported for batch fed (medium density) and perfusion samples (high density) samples after AcroPrep filtration with Extraction+ on the Andrew+ Pipetting Robot.

**Note: For best and most consistent analytical results, removal of cell debris and media matrix should be performed as soon as possible. If samples cannot be run immediately, rapid centrifugation to quickly remove cell debris post-harvest is still desired.*

SEC Analysis and Reporting

Protein A prepared samples may either be run using RP method (above) or run under SEC conditions to further

separate the mAb from low molecular and high molecular weight species to assess aggregation and fragmentation. New enhancements in Intact Mass 1.8 also enables automatic reporting of a single LMWS and HMWS percentage for simple reporting. These values are also compatible with Sartorius Ambr® Data Interface, previously described. Figure 4 shows an example of SEC analysis reported in the Intact Mass application.

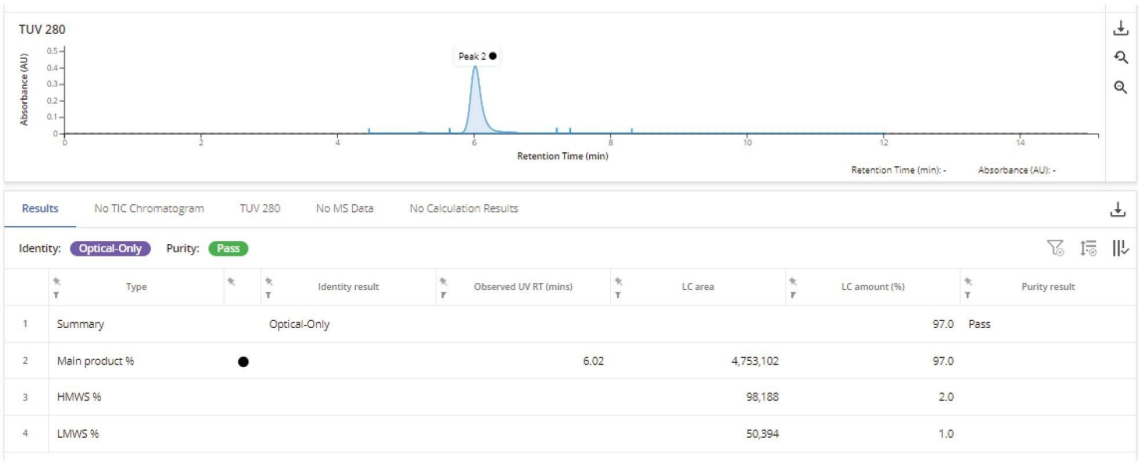

Figure 4. Example mAb SEC analysis with HMWS% and LMWS% table calculations performed using Intact Mass Application.

**Note: SEC-MS compatible methods are preferred to ensure non-volatile buffers do not enter the mass spectrometer. If LC only based SEC is desired (ie phosphate-based buffers), user must divert the MS flow to waste.*

Data visualization

When running multiple bioreactors with multiple variables (clones, conditions), generating multiple PQA datasets and reviewing data holistically across many bioreactors, conditions and/or timepoints is a challenge for users. Waters supports several data export strategies for data acquired by the BioAccord to fit various customer needs and scenarios. Waters has also recently generated a simple MS excel based macro tool that automatically displays overlay plots for metabolites and/or glycoforms, making it possible to generate quick overly plots of metabolites to compare bioreactor conditions and time courses.

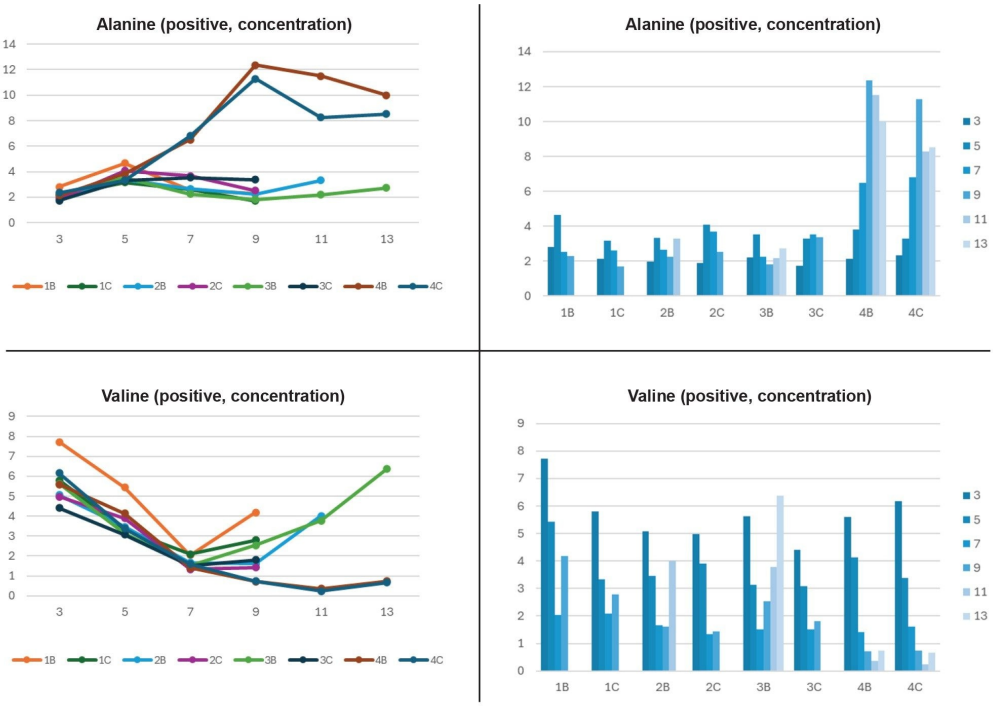

Figure 5. Visualizing differences in bioreactor conditions using excel response analysis tool. Reported concentrations (mM) shown for amino acids, alanine and branched chain amino acid, valine. Left panel (line plots) show overlay of eight bioreactor conditions, right panel (bar plot) shows time course separated by time.

Simple .CSV reports are also available for all workflows, which can be imported in various software packages such as JMP, SIMCA, Spotfire, EZinfo, etc.

Conclusion

Waters solutions are designed to simplify, automate, and accelerate bioprocessing teams' access to high quality LC and LC-MS data for any step that benefits from faster LC and LC-MS derived PQAs using low sample volumes. This enables teams to generate information more quickly to inform cell line selection and cell line development triage steps and process development optimization steps.

References

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2. Alelyunas YA, Prochaska C, Kukla K, Dunning C, Hanna C, Wrona MD. Monitoring Intact Glycoprofiles and Spent Media Metabolites in Samples from Sartorius Ambr 250 High Throughput Bioreactor System to Support Upstream Process Development. Waters Application Note 720008042, 2023.
3. Alelyunas YA, Gray J, Wrona MD, Boyce P. Introducing a Rapid Throughput LC-MS Method for Cell Culture Media Nutrient and Metabolite Analysis Supporting Upstream Bioprocessing. Waters Application Note, 720008170, 2024.
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5. Koza SM, Jiang AHW, Yu YQ. Rapid SEC-UV Analysis of Monoclonal Antibodies Using Ammonium Acetate Mobile Phases. Waters Application Note, 720007852, 2022.

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waters_connect Software Solutions <https://www.waters.com/nextgen/global/products/informatics-and-software/waters_connect.html>

720008487, September 2024