

Nota applicativa

Simplifying Bioreactor In-Process Monitoring with Waters Bioprocess Walk-Up Solutions

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

A walk-up solution that will enable bioprocess engineers to collect attributes easily and rapidly for process related monitoring and optimization is described. The Waters™ bioprocess walk-up solutions afford simple and single point access for automated sample preparation and data acquisition. It manages sample information coming from bioreactors, creates a LC-MS sample run list, executes automated sample preparation and, initiates analysis and intact protein data processing. Automated sample preparation and data acquisition for both intact protein and cell culture media is achieved back-to-back on a single system, using a predefined workflow, to maximize ease of use and minimize human intervention.

Benefits

- Walk-up system for single access to conduct automated sample preparation and LC-MS data acquisition
 - Ease-of-use in obtaining intact protein and cell culture media sample data using well-established methodologies
 - Automated and user-friendly sample preparation and LC-MS platform to obtain high quality data in process
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and product related monitoring including intact protein analysis, cell culture media analysis and many other product analytics when desired

Introduction

Cell culture or microbial based inoculation for protein production is a time-consuming process, typically lasting about two weeks. It is increasingly desirable to routinely monitor critical process and product attributes such as changes in nutrient profiles and high level glycoform information for the drug substance. Monitoring feed and metabolite components will aid in designing or optimizing feed strategies for nutrient replenishment; detect and quantify the formation of toxic metabolites; and can be used to elucidate what reactor conditions are conducive to optimal cell growth and bioproduction. Higher monitoring frequency of the protein formation in the process enables determination of most favorable cell culture duration; yield optimization; and most importantly ensure that the drug substance quality is within the specification criteria. Moving these assays and technologies in the hands of bioprocessing groups facilitates faster access to more in-depth information for the process engineers. However, to enable non-MS experts to perform routine applications of this technology, we need to ensure the systems are easy to use and quickly provide informative results and reporting outputs. In previous Waters application notes, we have published automated sample preparation and LC-MS analytical methods for both protein and culture media analysis.¹ In this technology brief, the bioprocess walk-up solutions are described as a step forward towards automated LC-MS analyses in bioprocess laboratories. A single user interface, based on OneLab™ software, provides a seamless connection between sample input, automated sample preparation and LC-MS data acquisition and analysis (Figure 1). In addition, users of Sartorius Ambr® 15 and 250 High Throughput bioreactors can utilize the data interface to automatically upload all sample information and return their results back to the Ambr software for further evaluation. The interface, combined with predeveloped analytical workflows for critical product and process quality attributes, are designed to allow bioprocess engineers to perform assays generating high quality data with minimal training or knowledge in LC and MS technologies.

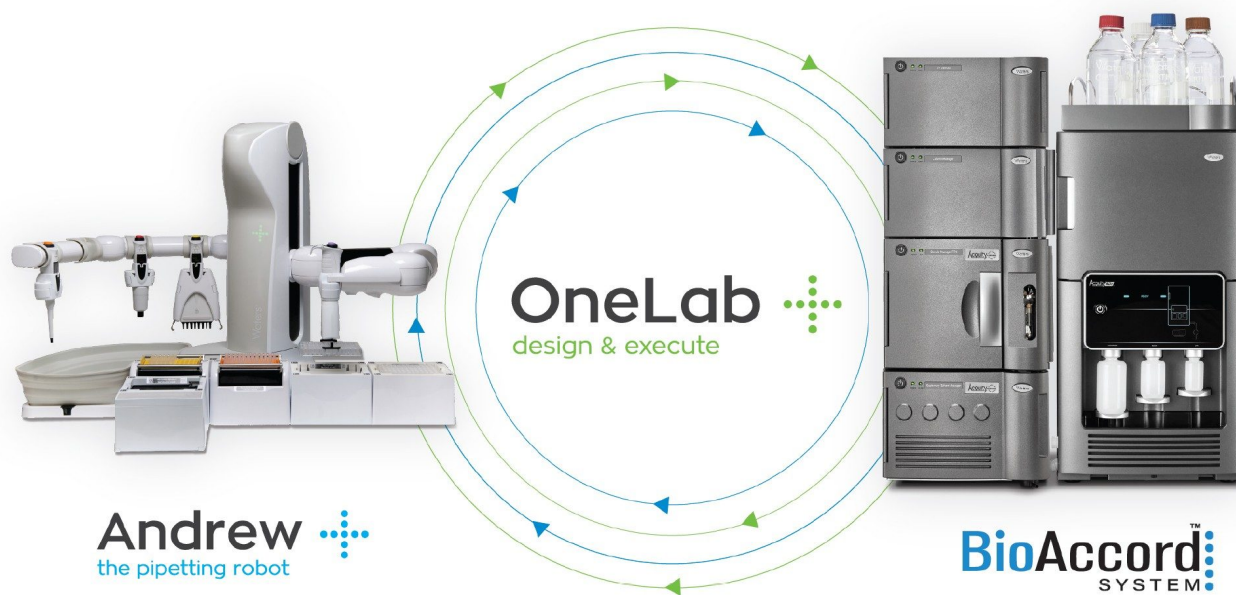


Figure 1. The Waters bioprocess walk-up solutions provide a single access OneLab software platform for automated sample preparation and LC-MS data acquisition and reporting.

Results and Discussion

I. A General Description of Waters Bioprocess Walk-Up Solutions

An integrated walk-up system intended for bioprocess engineers to automatically carry out sample preparation and LC-MS data acquisition has been developed. The Waters bioprocess walk-up solutions are based on OneLab software that enable bioprocess engineers to initiate automated sample preparation using the Andrew+™ Pipetting Robot and subsequent LC-MS data acquisition and processing. This walk-up solution is designed to ease the burden of routine process monitoring that is typically carried out by core analytical labs and allow bioprocess engineers, who are not experienced LC-MS users, to automatically retrieve this data at point of need in their lab. A top-level flow chart of the solution is shown in Figure 2. Specifically, the OneLab LC-MS interface will perform the following:

1. Select test protocol, e.g., intact mass and/or cell culture media analysis. The protocol is a collection of

methods used for sample preparation using Andrew+ Pipetting Robot and LC-MS analysis/reporting using the BioAccord™

2. Enter sample information from the bioreactors. For users running Ambr 15 or 250 HT systems, a OneLab file watcher will automatically import sample information files
3. Enter analysis name and associated information, by clicking “continue”, it will initiate sample preparation using the Andrew+ Pipetting Robot (Figure 2C)
4. When the sample preparation is completed, it instructs the user to place the sample in the BioAccord LC-MS System. Upon clicking “continue”, it will automatically start BioAccord data acquisition (Figure 2D)
5. Access from OneLab for users to activate viewing of intact mass results and the status of their cell culture media data acquisition

In the background, the OneLab interface interacts with waters_connect™ software for BioAccord instrument control and data acquisition. The current OneLab interface will perform intact mass and culture media analyses back-to-back in a single run through column switching using a two-column compartment column manager. A description of Andrew+ sample preparation and LC-MS method using the BioAccord LC-MS System have been described in a separate application note.¹ A summary list of consumables needed for sample preparation is shown in the Appendix.

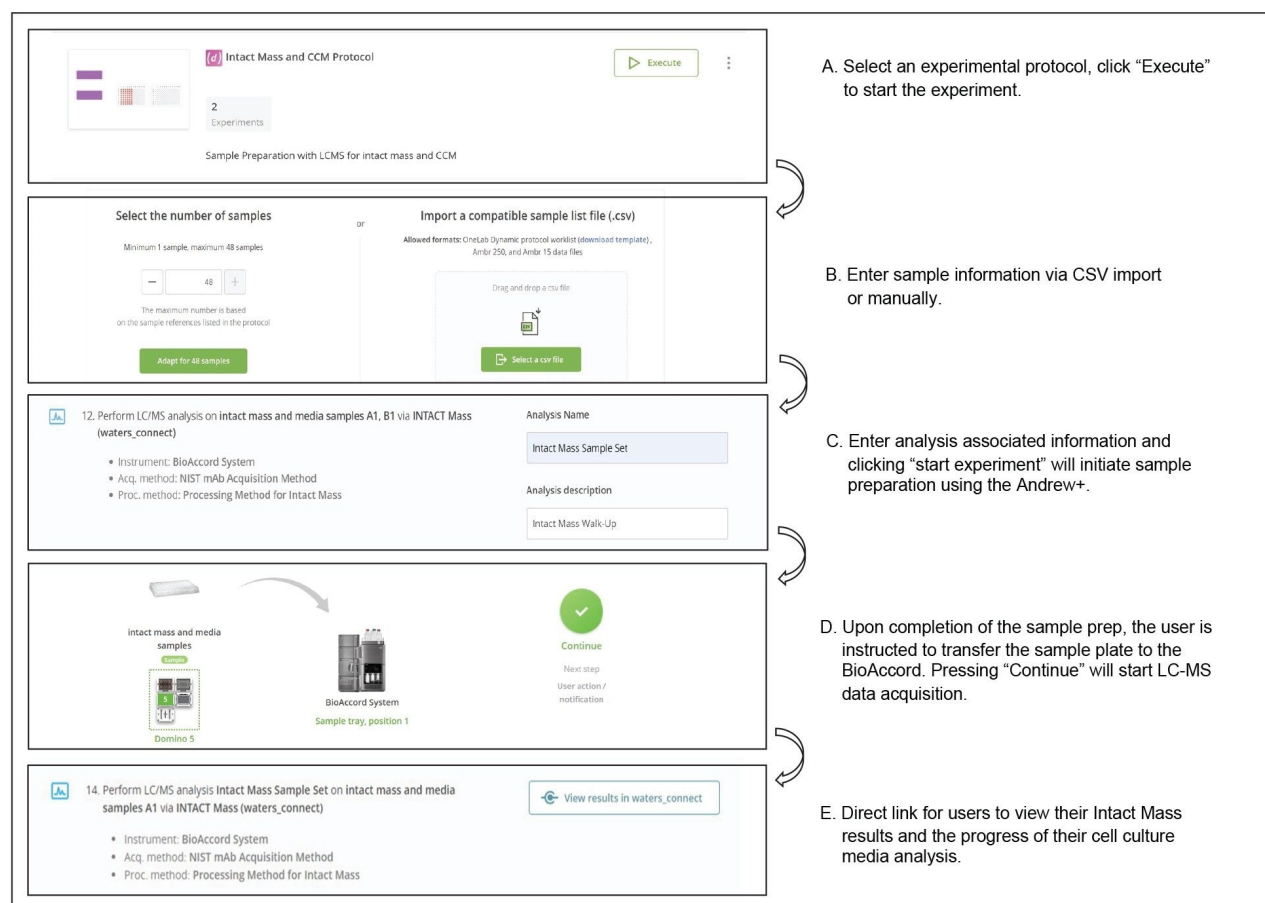


Figure 2. Top level flow chart for the bioprocess walk-up solution using single access in the OneLab software platform.

II. Intact Protein Analysis Results

For intact protein analysis, the acquired data is automatically processed using the Intact Mass™ App. The Intact Mass App is an easy-to-use dedicated data processing application that will automatically process raw spectral data (MS deconvolution) and then perform a determination of protein modifications (major glycoforms) and molecular weight identification (pass/fail) of monoclonal antibody (mAb) biotherapeutics. Detailed description of the Intact Mass App can be found in a previously published Waters application note.² Figure 3 is the resulting display for an analyzed mAb sample. In the top-level dashboard display, all samples are visualized with a color-coded system with green showing passing status which indicates that the sample identification meets criteria set

in the analysis method. By clicking on each sample, sample level information is displayed such as LC-MS chromatogram, protein modification result table, observed mass spectrum, and deconvoluted spectrum (Figure 3 B-D). In data export, the results are exported in a format that can be readily read into Ambr software or other third-party software for further data integration, viewing, and analysis. An example of Ambr data display showing an overlay of %modifications as a function of incubation time and bioreactors can be found in a previously published application note.¹

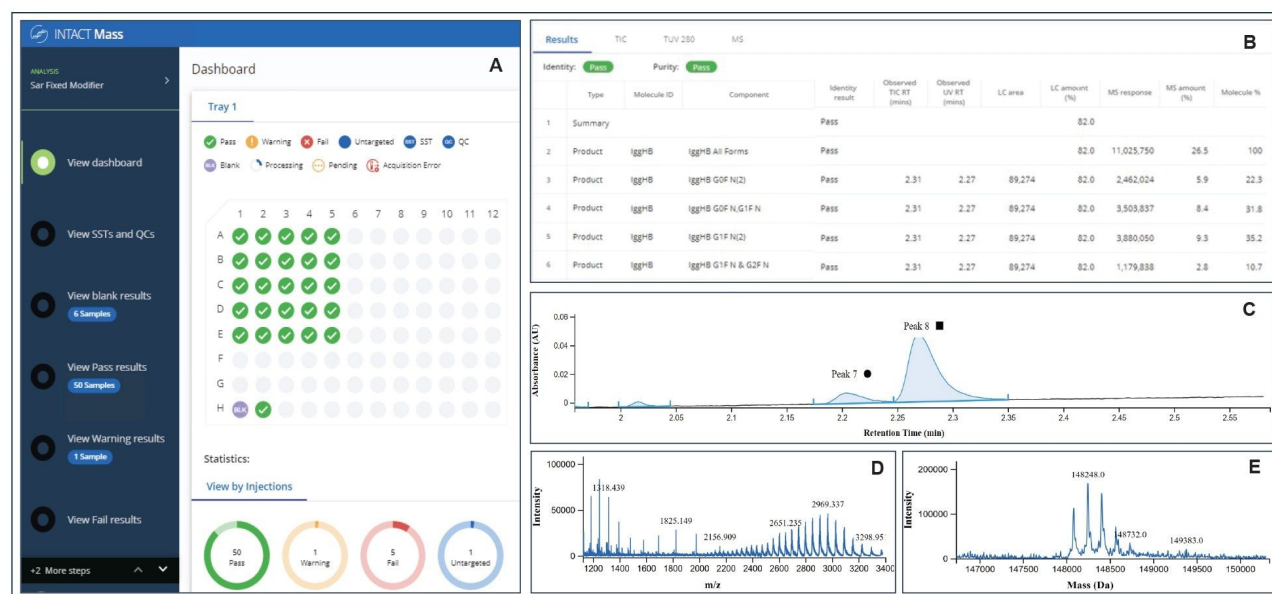


Figure 3. Display of Intact Mass App in the waters_connect™ platform. (A) Dashboard view showing injections and status of mAb detection, green color indicates the sample has passed detection criteria set in the method. (B) Summary table of modifications observed for the mAb produced including r.t., MS response, %modifications and other info. (C) Observed TUV chromatogram, which is used for peak detection. The first peak shown is light chain (LC) of the mAb, second peak is intact mAb. (D) Observed spectrum of the intact mAb peak, showing charge state distribution. (E) Deconvoluted spectrum of intact mAb, exhibiting the expected four major glycan modifications.

III. Cell Culture Media Analysis

For cell culture media nutrient and metabolites analysis, the acquired data is currently processed using the UNIFI™ screening application and workflow in the waters_connect software. The screening workflow performs

small molecule quantitative and qualitative analysis. In this example, for process monitoring, choline and its metabolite, choline phosphate, are monitored for each sampled media solution during the study (Figure 4). A detailed description of cell culture media analysis using UNIFI can be found in a previously published application note.³ The analysis can be easily expanded to additional cell culture components and identified metabolites. Additional tools to mine the data (previously described)³ to enable a deep understanding of compounds present in the media solution and relationships to metabolic and cellular processes through multivariate data analysis (MVDA) approaches.

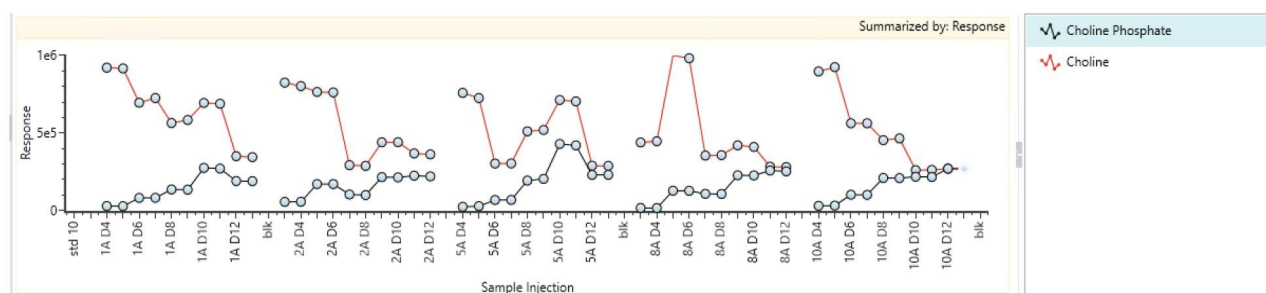


Figure 4. Overlaid trending plots of choline and choline phosphate. X-axis is bioreactor name and sampling date, y-axis is LC-MS response. Red line is choline, black line is choline phosphate.

Conclusion

Waters bioprocess walk-up solutions provide a simple and powerful interface for automated sample preparation using the Andrew+ Pipetting Robot and initiating LC-MS data acquisition. The combination of the Andrew+ Pipetting Robot and the BioAccord LC-MS System provides the capability to rapidly process samples from bioreactor systems and easily provide high quality results with minimal user interaction. Highlights and capabilities include:

- A single access and easy to use interface in OneLab, with predeveloped workflows, that will initiate sample preparation and subsequent data acquisition in a walk-up manner
- Automated sample preparation and sample information transfer to maximize productivity and minimize

human error

- Acquisition of both intact mass of protein and cell culture media data back-to-back based on column switching using a two-column compartment column manager
- Seamless collection of protein glycoprofiling and cell culture media composition to aid process monitoring and understanding
- Compact and user friendly BioAccord LC-MS System producing excellent data quality to support the development of more robust processes

In conclusion, Waters bioprocess walk-up solutions will enable process engineers to obtain high quality data easily and routinely on their own to aid in process monitoring and optimization.

References

1. YW Alelyunas, C Prochaska, C Kukla, C Hanna, M Wetterhall, MD Wrona, Monitoring Intact Glycoprofiles and Spent Media Metabolites in Samples From Sartorius Ambr 250 High Throughput Bioreactor System to Support Upstream Process Development, 2023 Waters Appnote.
2. H Shion, P Boyce, SJ Berger, YQ Yu, Intact Mass™ - a Versatile waters_connect™ Application for Rapid Mass Confirmation and Purity Assessment of Biotherapeutics, 2022 Waters Appnote, [720007547](#).
3. YW Alelyunas, MD Wrona, W Chen, Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System with ACQUITY Premier, 2021 Waters Appnote, [720007359](#).

Appendix

Parts description	Part no.	Comments
Dominos and lab ware		
Tip insertion system domino (2)	186009612	Holding 10 µL to 1200 µL optifit non-filtered tips
Microplate domino (2)	186009600	For 350 µL 96 well plate, one each for source and destination plate
8-channel pipette reservoir domino	186009613	Holding two integra 10 mL multichannel reservoir
2 mL HPLC vial rack domino	186010091	Holding 48-well HPLC vial rack, used for standard preparation
Solvent reservoir	Integra Biosciences P/N 4332	10 mL multichannel reagent reservoirs
350 µL 96-well sample collection plate	186002643	Round well, polypropylene, 100/pk
Polypropylene cap mat round well for 96-well plate, 50/pk	186002483	
Polypropylene 12 x 32 mm screw neck vial, 700 µL (no cap)	186005219	
48-well vial rack	700011047	
Pipette and tips		
8-channel Andrew Alliance Pipette, 0.2–10 µL	186009768	
8-channel Andrew Alliance Pipette, 10–300 µL	186009607	
Single-channel Andrew Alliance Pipette, 0.2–10 µL	186009769	
Single-channel Andrew Alliance Pipette, 10–300 µL	186009606	
Pipette adaptor single channel	186009590	
Pipette adaptor multi channel	186009591	
BH Tip 0.1–10 µL, refill (10 x 96) Pk10	700013293	
BH Tip 350 µL, refill (10 x 96) Pk10	700013297	

Summary of consumables used by Andrew Alliance system for intact and media sample and standard preparations.

Featured Products

BioAccord LC-MS System for Biopharmaceuticals <<https://www.waters.com/waters/nav.htm?cid=135005818>>

ACQUITY UPLC Tunable UV Detector <<https://www.waters.com/514228>>

Bioprocess Walk-Up Solutions <<https://www.waters.com/waters/nav.htm?cid=135124488>>

UNIFI Scientific Information System <<https://www.waters.com/134801648>>

waters_connect <<https://www.waters.com/waters/nav.htm?cid=135040165>>

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