COMBINING INTACT GLYCOPROFILING AND CELL CULTURE MEDIA ANALYTICS FOR IN-PROCESS MONITORING OF AN AUTOMATED HIGH-THROUGHPUT MULTI-PARALLEL BIOREACTOR SYSTEM

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INTRODUCTION

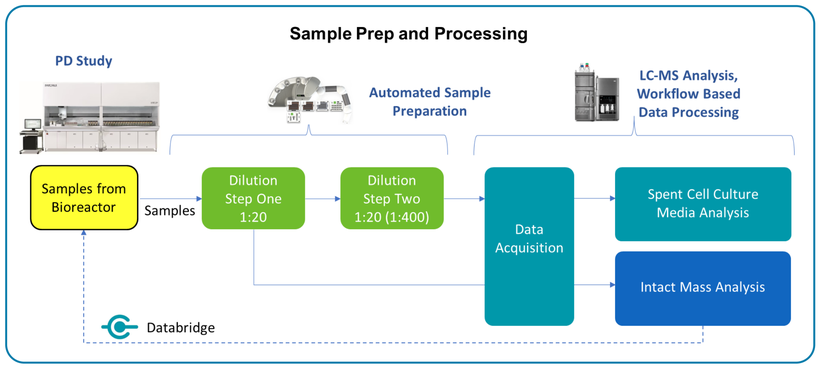
Rapid collection of process parameters and critical product attributes is of key interest in bioprocessing optimizations. LC-MS based analytics has been shown to provide measurement of critical quality parameters and flux of metabolites (consumption / turnover of feed components, as well as production of metabolic by-products) during bioprocessing.

Bringing LC-MS analytics directly into upstream batch monitoring provides insight to where processes could be improved or better controlled, rather than waiting to perform LC-MS assays as part of release assays or determination of quality post campaign.

Here we demonstrate integration of intact glycoform profiling and cell culture media analysis for LC-TofMS based screening of in-process samples from a high throughput multi-bioreactor system.

METHODS

Five bioreactors were sampled in a CHO-cell based mAb process optimization experiment on the Ambr® 250 High-Throughput 12 Station Bioreactor System (Sartorius Stedim, USA). Samples were collected at days 4, 6, 8, 10 and 12 and were immediately centrifuged and filtered using a 0.2 µm syringe filter. The spent media were then diluted at 1:20 (V/V) for direct intact mAb analysis and 1:400 (v/v) for spent media analysis using an Andrew+™ pipetting robot (Waters Corporation, USA). Samples were also further processed for subunit analysis (poster ThP624). LC-MS analysis was performed on the BioAccord™ LC-MS System (Waters Corporation, USA). Data acquisition and processing were performed using waters_connect™ Informatics System (Waters Corporation, USA). The same mobile phases were used for both intact and media nutrient analysis: Mobile phase A was 0.1%FA; Mobile phase B was 90%ACN/ 10%IPA/ 0.1%FA. Additional data acquisition and processing parameters were published previously.[1,2] Glycoprofiling data was transferred back to Ambr® Control Software using the Databridge interface.



RESULTS: INTACT PROTEIN ANAYSIS

Direct intact protein analysis was carried out using 1:20 (V/V) diluted spent media solution without affinity based sample preparation. The mass ranged used for the acquisition was 500-7000 m/z. The total ion chromatogram in Figure 1 showed major compounds present in the spent media including light chain, intact mAb, and a late eluting broad matrix peak. Extracted ion chromatogram of the intact mAb mass region (Figure 1, insert) showed excellent separation and peak shape. Trend plot of % glycosylation provided direct comparison of protein modifications as a function of bioreactors and over time (Figure 2). The % glycosylation data obtained from LC-MS analysis was readily transferred to Ambr software through Databridge in waters_connect for direct data viewing by process engineer, which enabled key LC-MS outcomes to be viewed directly by the engineer along side other routine bioprocess analytics (Figure 3).

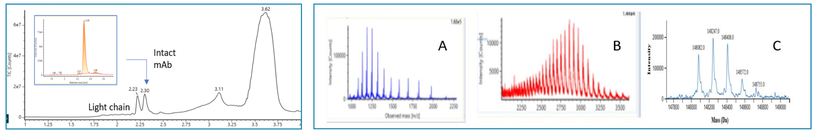


Figure 1. Observed TIC chromatogram and MS spectrum of intact mass analysis of spent media sample. (A) observed MS spectrum of light chain, (b) observed MS spectrum of intact mAb, and (c) deconvoluted spectrum.

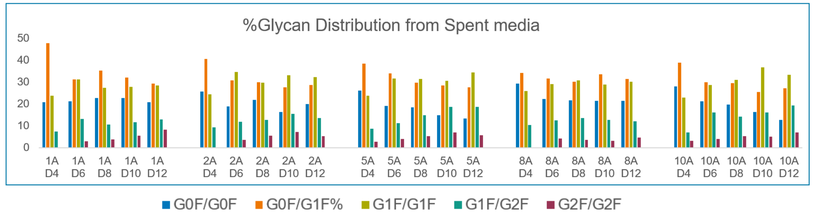


Figure 2. Bar plot of % glycan modifications across bioreactors and over time.

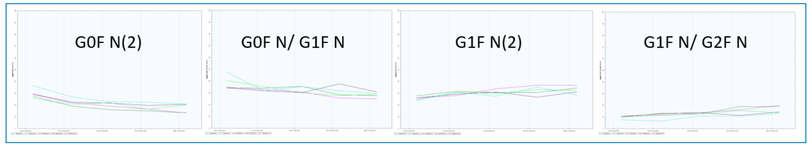


Figure 3. Overlaid plots of major % glycan modifications as a function of bioreactor and over time transferred using Databridge connectivity. Glycan profile data can be easily transferred to Ambr® Software for process control or monitoring.

RESULTS: CELL CULTURE MEDIA ANALYSIS

Spent media nutrient and metabolite analysis were carried out direct using 1:400 (V/V) diluted spent media solution without further derivatization. Based on 200+ compound library provided, ~100 nutrients and metabolites are detected. Compound distribution as defined by compound class in the supplied library is shown in Figure 4. Quantitative and relative media changes plotted by bioreactor and over time are shown in Figure 5. The data was readily exported for further analysis or statistical MVA processing.

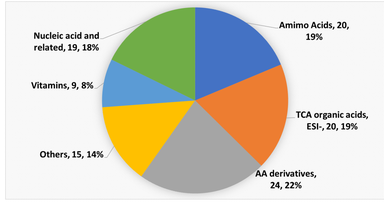


Figure 4. Compound class distribution for the detected compound in the spent media solution.

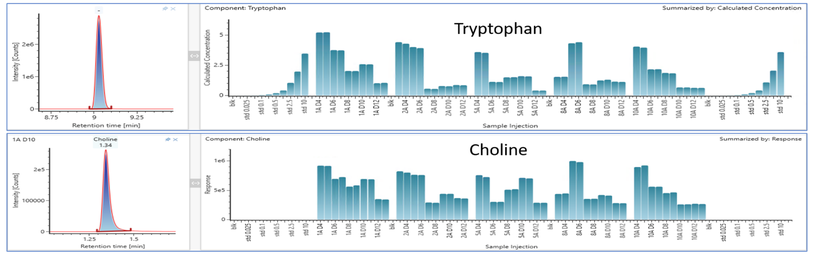


Figure 5. Representative trend plots of metabolites displaying bioreactor sampling over time. For all metabolites included in the calibration standards, concentration (mM) is reported and Tryptophan is shown as a representative sample. For all other detected components, observed response is plotted (Choline is shown).

CONCLUSIONS

- **Automated sample preparation for both intact mAb and media components analysis streamlined sample handling**
- **Direct intact mAb analysis for modifications from sampled bioreactor**
- **Direct media nutrient and comprehensive metabolites analysis in cell culture media sampled from bioreactor**
- **Compact HRMS platform produced excellent data quality and throughput**

References

(1) INTACT Mass™ - a Versatile waters_connect™ Application for Rapid Mass Confirmation and Purity Assessment of Biotherapeutics, 720007547

(2) Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System with ACQUITY Premier, 720007359

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