The development of a point-of-need miniaturized ESI-MS for upstream bioprocessing applications

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Overview

A point-of-need miniaturized ESI-MS MiD® ProteinID, in conjunction with Proof-of-Concept (PoC) workflows, have been developed to analyze cell culture samples obtained from a bioreactor.

Introduction

New regulatory demands for analytics during the manufacture of biologic drugs can be satisfied by Mass Spectrometry, which can provide context-rich and real-time information at the point-of-need. A deployable MS that samples the media from a bioreactor in real-time can provide important process monitoring, and critical quality attributes of the product. This information can then be used to maximize biologic yields by optimizing the cell media, cell feeding, and harvesting strategies of the target biologic product. In addition, MS point-of-need analysis can provide timely safety assurance as any harmful product post-translation modifications, and dangerous host cell proteins can be controlled and minimized upstream. We describe the development of a miniaturized MS to meet these complex needs.

Methods

Four shake flask cultures of anti-Her2 IgG1 expressing CHO cells were cultivated for 14 days at the CPI under favorable and unfavorable conditions.

- Flask 1 & 2: Unfavorable conditions of excess glucose with wide limits and high percentage of added feed.
- Flask 3 & 4: Favorable conditions of tight glucose control and low percentage of added feed.

Figure 1: Microsaic MiD® ProteinID mass spectrometer based on miniaturized chip-based technology with an adjustable mass range between 50 and 3200 m/z the monitoring of small molecules in cell culture media and intact IgG is possible with this mass spectrometer. The MS features a miniaturized quadrupole mass filter and microflow electrospray source. The resulting small footprint (55 x 35 x 25 cm), low maintenance and ease of use allows it to be easily used at-line and at the point-of-need.

Samples from the cell cultures were first analyzed by a Cedex HT Bioanalyzer for metabolites (lactate, ammonia glutamate, glutamine, glucose and lactate dehydrogenase). Retains of 0.5 ml were taken on days 0, 3, 5, 7, 9, 11, 13 and 14. Following freezing at -80 °C these retains were stored for further analysis using the MiD® ProteinID and PoC workflows.

To analyze the IgG product and cell media two PoC workflows were tested with the ProteinID MS system:

1. IgG Product Analysis

Direct injection of retains onto a Protein A column. Bind and elution undertaken with volatile buffers to allow direct observation of non-binding components and intact IgG eluting from the column in both the bind and elution phases.

2. Cell Media Analysis

Retains mixed with highly organic buffer and clarified to remove precipitates. Separation of this mixture using hydrophilic interaction chromatography (HILIC) allowed direct observation of cell media components. In addition, a PoC quantitation method was assessed that allowed the measurement of metabolite concentrations without the use of expensive isotopically labelled standards.

Results

1. IgG analysis

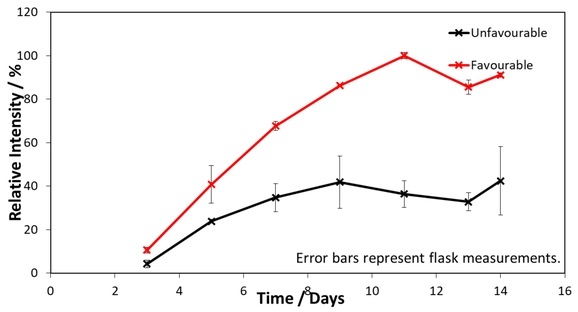

Figure 2: Inferred titers by monitoring the area of selected peaks associated with the IgG. Average IgG mass under unfavourable conditions was measured to be 147.87 kDa and 147.82 kDa under the favourable conditions. This difference in masses was found to be significant at the 1% level.

2. Cell Media Analysis

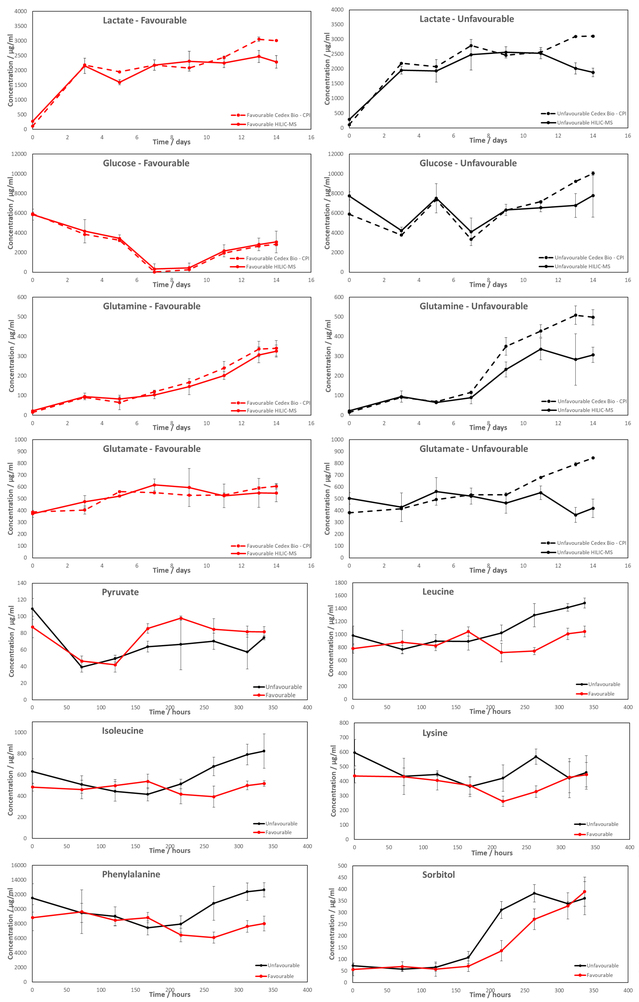

Figure 3: Results from the MiD® ProteinID MS comparing quantified lactate, glucose, glutamine, and glutamate variation in retain samples over time compared to the Cedex HT Bioanalyzer. Pyruvate, leucine, isoleucine, lysine, phenylalanine and sorbitol also measured using the MS. Error bars represent 95%. CI.

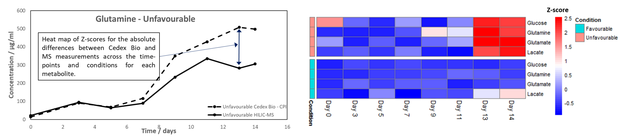

Figure 4: Good correlation between Cedex HT Bioanalyzer and MS measurements was observed. However, deviation between the Cedex HT Bioanalyzer and MS measurements was observed after ~11 Days for the unfavourable samples and not the favourable samples. This discrepancy in behaviour between favourable and unfavourable conditions can clearly be seen with a heat map of z-scores for the difference between Cedex HT Bioanalyzer and MS measurements. The cause of this effect is unknown but may be linked to an increase in by-products from cell death in the unfavourable samples, these by-products leading to interference in the photometric assays.

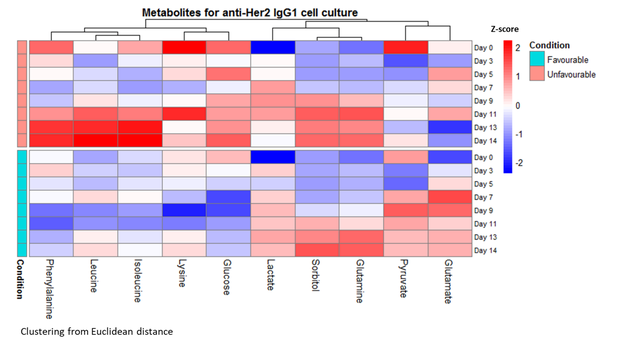

Figure 5: Distinctive metabolite profiles from each condition can be seen. Clear differences in temporal profiles between the conditions can be seen for many metabolites. Clear behaviour for isoleucine, leucine and phenylalanine across the duration of the culture, resulted in the clustering of these species. Heat map and clustering suggests opposing behaviour of glutamate and pyruvate to isoleucine, leucine and phenylalanine.

Conclusions

1. The Microsaic MiD® ProteinID coupled to an automated protein A workflow made IgG measurements from cell media much simpler and strong IgG signals were obtained at titers above 200 µg/ml.
2. HILIC-MS cell media measurements correlate well with conventional measurements. Distinctive behavior of metabolites between the favorable and unfavorable conditions observed.
3. Improvements in measurement speed, precision and accuracy would be expected with automation of sample preparation and bespoke protein A and HILIC configurations using smaller sample volumes and flows.

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