Evaluating Traditional Mass Spectrometry Against the New Generation of Emerging Analytical Technology

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Abstract

Analytical instrumentation is essential to evaluate product quality and for the development of process controls in biopharmaceutical drug product manufacturing. Liquid chromatography mass spectrometry (LC-MS) is used in a wide range of various applications across the manufacturing environment from methods related to drug product quality, such as glycans in monoclonal antibodies, and through the process development of cell culture to monitor amino acid and metabolite concentrations in a cell culture feed. While LC-MS has the capacity to address complex questions with flexible application, LC-MS instrumentation requires a user with a high level of technical experience for experimental development and is generally more time-consuming than other analytical methods. Recently, new analytical equipment such as 908devices' REBEL attempts to address the complexity of LC-MS and the growing interest to implement these simplified user-friendly approaches instead of the labor-intensive traditional LC-MS methods. REBEL is an instrument with simple procedures that retains the traditional LC-MS ability to specifically identify and quantitate amino acids and several other important metabolites. REBEL allows for comprehensive analysis and high throughput sample testing with the advantage of minimal method development and sample preparation. Our study aims to compare this new generation of LC-MS analytical platform, 908devices' REBEL, against traditional LC-MS methods and evaluate the quality of data with respect to the trade-offs in operation and resourcefulness between these two analytical instruments.

Introduction

Biopharmaceutical companies have a growing interest to understand media attributes and product quality impacts, but battle against time consuming traditional methods in order to maximize their manufacturing process efficiency. Cell line development and media optimization are a large part of process development because each cell line requires unique and specific supplementation strategies. Traditionally, media nutrient and metabolomic studies are done with liquid chromatography mass spectrometry (LC-MS), which requires a high level of technical expertise and can be time-consuming. However, optimal experiments require in-process analytics to support real-time examination of bioreactor parameters and the outcomes of product quality attributes. A novel analytical instrument has growing interest from biomanufacturers with the promise to speed up metabolomic and media nutrient studies: The REBEL Cell Culture Media Analyzer from 908 Devices.

Here we present the supporting advances in bioreactor media monitoring by studying the REBEL Cell Culture Media Analyzer against currently established LC-MS methodologies. Some major benefits of the REBEL spent media analysis are its ability to analyze neat media without any derivatization, fast analysis time, and simple operations. This analytical instrument is new to the market and the technology behind its analysis is not well described, therefore a better understanding of its capabilities and limitations is recommended. We chose an established method from Waters, also a non-derivatized and normal-phase chromatography method, using their BioAccord RDa time-of-flight (Tof) LC-MS to perform the comparative spent media analysis. We aim to show comparability between REBEL and RDa spent media analysis results and identify the costs and benefits between the two analytical instrument methods. Furthermore, we hope to establish these methods in our lab with both analytical instruments to later evaluate process changes, feeding strategies, and their product quality impacts in future metabolic studies. Thus, deepening our understanding of critical process parameters and their effects on critical quality attributes of drug products.

Results and Discussion

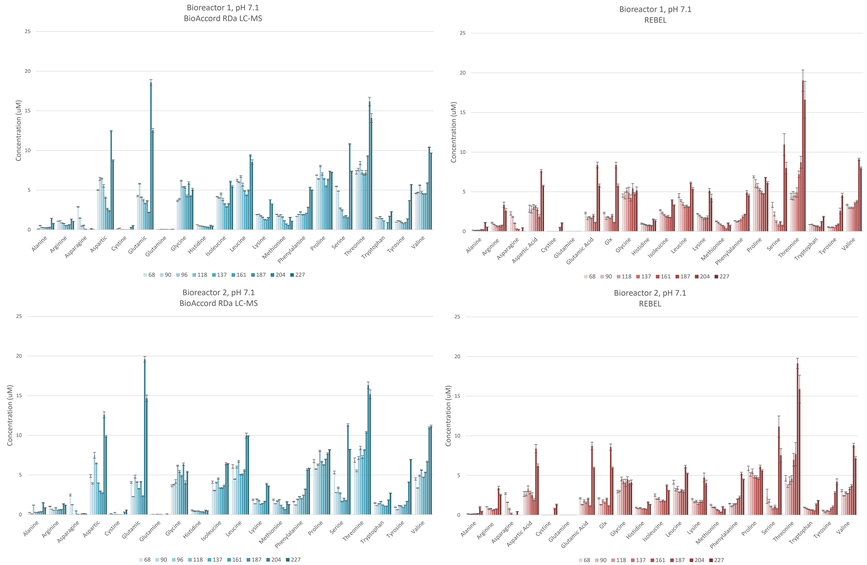

Figure 1. Amino acid concentration trends throughout culture from timepoints 68 to 227 hours of bioreactor 1 (top) and 2 (bottom) ran at pH 7.1 setpoint. Samples analyzed by Waters BioAccord RDa LC-MS (blue) and REBEL Cell Culture Media Analyzer (red).

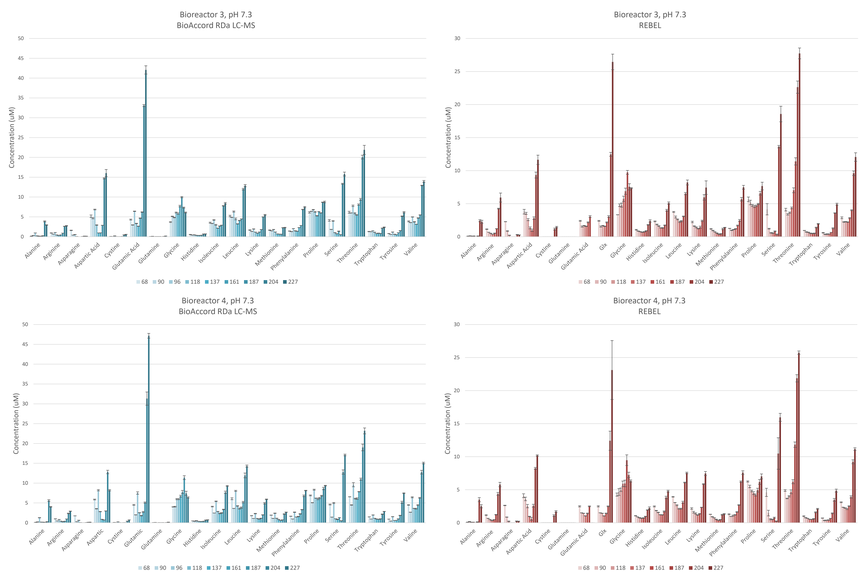

Figure 2. Amino acid concentration trends throughout culture from timepoints 68 to 227 hours of bioreactor 3 (top) and 4 (bottom) ran at pH 7.3 setpoint. Samples analyzed by Waters BioAccord RDa LC-MS (blue) and REBEL Cell Culture Media Analyzer (red).

We were able to successfully run all samples with the REBEL and a preliminary method on BioAccord RDa LC-MS to create trends for all amino acids in all four bioreactors. We were able to create a processing method on UNIFI to identify, mass confirm, and quantify all amino acids from standard calibration curves on our RDa LC-MS analysis. REBEL exported calculated concentrations for all amino acids and manual pivot tables were created to illustrate the results demonstrated to the left. Bioreactor replicates 1 & 2 ran at pH 7.1 setpoint and demonstrated similar amino acid trends throughout the culture (Figure 1). Similarly, bioreactor replicates 3 & 4 shared trends with each other ran at pH 7.3 setpoint (Figure 2). Amino acid trends for bioreactors 1-4 are illustrated in blue for the BioAccord RDa LC-MS analysis and in red for the REBEL analysis. Both REBEL and RDa LC-MS data show comparable amino acid trends for all reactors. For sample preparation, samples were diluted 1:100 for the REBEL method compared to 1:500 for RDa LC-MS, revealing that the RDa LC-MS is at least fives times more sensitive than the REBEL. However, we can see that the later timepoints at 204 of its scope and and 227 hours amino acid concentrations are much higher in RDa LC-MS than in REBEL. This is most likely because these values were found outside of the standard curve, therefore, the concentrations are extrapolated leading to error in calculation. REBEL can only analyze 33 analytes that include 22 amino acids, 5 vitamins, and 6 biogenic amines, where as the RDa is a Tof LC-MS that can analyze these plus other unknown analytes. Table 1 lists a comparison of REBEL vs traditional LC-MS where the REBEL is easy to use and does not require much time or effort to operate, but sacrifices it ability to analyze outside of the REBEL. require much time or effort to operate, but sacrifices it ability to analyze outside of the REBEL. Traditional LC-MS methods can be specifically optimized with better sensitivity, but this will require a much higher level of technical skill. REBEL provides all inclusive kits for the automated analysis, but the kits are much more costly to analyze per sample than the materials needed for the LC-MS methods. Overall, REBEL performs comparably well within its scope of analytes at a higher monetary cost, but the ease of use and fast analysis is a major benefit to advance real-time process optimization.

	REBEL	Traditional LC-MS
Overall Expertise Required	+	+++
Ease of Use	+	++
Supply Cost	+++	+
Analysis Run Time	+	++
Data Processing	+	++++
Daily Operations	+	+++
Maintenance & Troubleshooting	+	+++
Method Development	+	+++
Sensitivity	+	+++++
Additional Unknown Analytes		+
User Mass Confirmation		+

Table 1. List of instrument process and upkeep as a comparison between REBEL and LC-MS where + represents technical expertise required or respective amount.

Materials and Methods

Daily samples were collected from a fed-batch experiment of VRC01 CHO cell culture using AMBR250 bioreactors (Sartorius) that ran for ten days. Duplicate bioreactors 1 & 2 ran at a pH 7.1, and bioreactors 3 & 4 ran at a pH 7.3, and otherwise kept in the same conditions. Samples were spun down and filtered to analyze amino acid concentration of the cell-free spent media.
REBEL's spent media analysis kit (908devices) provides Spent Media Analysis BGE Solution and Diluent, as well as prepared standards. Samples were diluted 1:100 with the Spent Media Analysis Diluent. All results, including calculated concentrations, were exported as a .csv file. LC-MS methods for the BioAccord RDa (Waters) were based off the Waters Spent Media Analysis application note, and then modified to fit our unique set up with Acquity Patrols as our LC system. Amino Acid Cell Culture Standards (Waters) were prepared fresh according to instructions to a 6 point calibration curve of 5uM-0.5uM for each amino acid, except Cystine at 2.5uM-0.25uM. Samples were prepared fresh and diluted 1:500 with 0.1% formic acid. Acquired LC-MS data was processed with UNIFI (Waters) to create standard curves, identify and quantify amino acid concentrations. All samples were done in triplicate injections in both REBEL and BioAccord RDa methods.

Conclusion

Our preliminary RDa LC-MS method demonstrates that the data produced by the REBEL Cell Culture Media Analyzer is comparable for CHO cell culture bioreactors. Currently we are finalizing the RDa LC-MS method with an eight point calibration curve 30uM-0.1uM to capture all amino acid concentrations based on what we found from REBEL results. Further method comparison with statistical analysis will be performed once all samples are analyzed with the finalized RDa LC-MS method. We will assess methods with a null hypothesis that measurements made with REBEL are different than measurements made by RDa LC-MS and then look for data-based evidence that the measurements are the same. If the data supports the rejection of the null hypothesis, then we can conclude that the instruments have equivalent performance for each compared metabolite measurements. Future studies will further test REBEL's capacity to evaluate metabolic impacts on product quality, such as the impact of a temperature shift on metabolite utilization and its effect on glycan profiles in microbioreactors. Since cell metabolism can create collinearity in metabolite utilization and concentration, we will use multivariate data analysis (MVDA) to evaluate cellular metabolism and its impact on glycan profiles. The benefits of REBELs ease of use and fast analysis allows larger sample sets while retaining similar quality of data as a traditional LC-MS method to quickly advance our understanding of impacts on critical quality attributes, and thus critical process parameters.