High-throughput Metabolite Profiling of Cell Media for Improved Antibody Production Utilizing a Dual Separation/Mass Spectrometry System with Intelligent MS^n Acquisition

Ioanna Ntai[1], Anson Pierce[2], Paul Gulde[2], Martin Samonig[3], John Brann[4], Christopher Elicone[4], Amanda Souza[1], Ralf Tautenhahn[1], Daniel Lopez Ferrer[1], and Andreas Huhmer[1]
[1]Thermo Fisher Scientific, San Jose, CA, [2]Thermo Fisher Scientific, Grand Island, NY, [3]Thermo Fisher Scientific, Germering, Germany, [4]Thermo Fisher Scientific, Franklin, MA

ABSTRACT

Purpose: Measure nutrient limitations or metabolite buildup during cell culture to determine optimized cell media for improved antibody production.

Methods: Separate cultures of CHO cells expressing the same recombinant antibody were supplemented with 6 different feeds, in 3 bioreactors per feed. A Thermo Scientific™ Transcend™ Duo LX-2 UHPLC system was coupled to a Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer and extracellular metabolites were analyzed in both positive and negative mode. Data were processed using Thermo Scientific™ Compound Discoverer™ software suite for unknown identification and differential analysis.

Results: This dual LC/MS system was successfully applied in the differential analysis of cell media with increased throughput, without compromising reproducibility or compound detection.

INTRODUCTION

Chinese hamster ovary (CHO) cells are often used for commercial production of recombinant therapeutic proteins (antibodies). Optimization of cell culture medium and feeds is required to obtain maximum product yield. Metabolomics analysis allows for quick determination of nutrient limitations or metabolite buildup during the cell culture. A semi-targeted workflow was designed to confidently measure critical nutrients, such as amino acids, while allowing for the discovery of previously unidentified metabolites that affect growth and antibody yield. A dual UHPLC separation system coupled to an Orbitrap mass spectrometer was used to increase analysis throughput of a time-course study of 6 different feeds, while also maximizing metabolome coverage. This high-throughput metabolomic workflow provided greater understanding in the molecular rationale behind improved antibody production.

MATERIALS AND METHODS

Sample Preparation

Separate cultures of CHO cells expressing the same recombinant antibody were supplemented with 6 different feeds, in 3 bioreactors per feed. Cell media were collected at 7 time points and extracellular metabolites were extracted with an excess of cold methanol (3x), containing 4 internal standards ($^{13}C_9$-tyrosine and $^{13}C_9$-Phenylalanine, D_4-succinic acid, $^{13}C_6$-adipic acid). After centrifugation, samples were dried and resuspended in water for reverse phase (RP) and 9:1 acetonitrile/water for HILIC separation. Authentic standards were obtained from MetaSci.

Test Methods

A Transcend Duo LX-2 UHPLC system equipped with an autosampler with two injection units, two binary pumps and two column compartments, was coupled to a Orbitrap ID-X Tribrid mass spectrometer and data were collected in both positive and negative mode for all samples and authentic standards. For the RP separation, a Thermo Scientific™ Hypersil GOLD™ column (15cm × 2.1mm ID, 1.9µm particle size) was used and the mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The RP gradient is described in Table 1. For the HILIC separation, Thermo Scientific™ Accucore™ 150 Amide HILIC column (10cm × 2.1mm ID, 2.6µm particle size) was used and the mobile phase consisted of solvent A (95% acetonitrile/ 5% water with 10mM ammonium acetate and 0.1% acetic acid) and solvent B (50% acetonitrile/ 50% water with 10mM ammonium acetate and 0.1% acetic acid). The HILIC gradient is described in Table 2.

300 standards were analyzed first with RP and HILIC separation collecting Full MS and MS/MS. A pooled sample was generated from all the study samples and it was used as a QC every 11 injections and to collect MS/MS data with AcquireX for compound identification.

Data Analysis

Data from the authentic standards were processed with Compound Discoverer software to generate an in-house spectral library, complete with structures and retention time information. Cell media data were processed using Compound Discoverer software suite for unknown identification against this in-house library and the online mzCloud™ library, differential analysis and pathway mapping.

RESULTS

Independent Fluidic Paths for Increased Throughput

We developed a dual liquid chromatography / mass spectrometry (LC/MS) system that combines RP and HILIC separation and we evaluated it for its reproducibility and separation capabilities of complex samples. Two independent UHPLC pumps were incorporated into the system to allow independent control of the two columns (RP and HILIC). This enabled the use of different solvents, additives and pH ranges and, as a result, a broader metabolite coverage. Having two independent fluidic paths, resulted in a 30% decrease in analysis time and increased the overall throughput of the method. This was accomplished by equilibrating one column, while separation was carried out on the other.

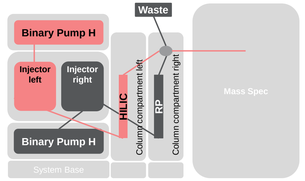


Figure 1. Schematic representation of the Transcend Duo LX-2 system used in this study illustrating the two separate fluidic paths.

Having two separate flow paths can allow for longer re-equilibration times and by overlapping the two gradients, the analysis time can be decreased and sample throughput is expedited (Figure 2)

Figure 2. Illustration of the overlap of two separations (HILIC and RP) resulting in a 30% decrease in analysis time and increased throughput. Flow is diverted to waste during injection and the re-equilibration step (grey boxes).

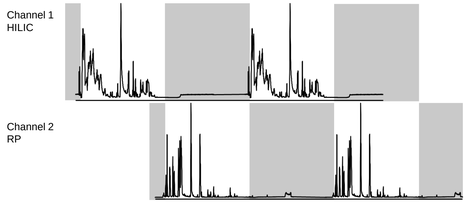


Table 1. RP gradient

Time (min)	% B	Flow rate (mL/min)
0	0	0.3
5	50	0.3
6	98	0.3
10	98	0.3
10.1	0	0.3
15	0	0.3

Table 2. HILIC gradient

Time (min)	% B	Flow rate (mL/min)
0	1	0.5
1	1	0.5
9	95	0.5
10	95	0.5
10.5	1	0.5
15	1	0.5

UNCOMPROMISED REPRODUCIBILITY

Increased Throughput Does not Compromise Reproducibility

The increased throughput afforded by this setup did not compromise reproducibility. In figures 3 and 4, the retention times of the internal standards spiked into all samples were monitored over 260 injections, highlighting the robustness of the Transcend Duo LX-2 system.

Figure 3. The retention times of internal standards remain unchanged during a 260-injection sequence on the HILIC column

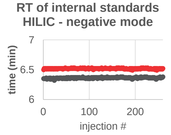
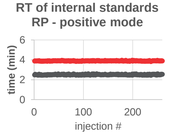


Figure 4. The retention times of internal standards remain unchanged during a 260-injection sequence on the RP column



Mass accuracy (Figures 5 and 6) and signal response (Figures 7 and 8) are also highly reproducible for both separations and do not deteriorate during this 4 day experiment.

Figure 5. The mass accuracy of internal standards remain unchanged during a 260-injection sequence on the HILIC column

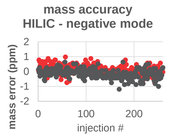


Figure 6. The mass accuracy of internal standards remain unchanged during a 260-injection sequence on the RP column

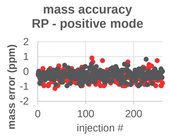


Figure 7. The signal response of internal standards remain unchanged during a 260-injection sequence on the HILIC column

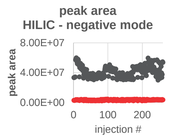
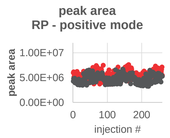


Figure 8. The signal response of internal standards remain unchanged during a 260-injection sequence on the RP column



DIFFERENTIAL ANALYSIS OF CELL MEDIA

Metabolic profiles of cell media change over time and are dependent on feed nutrients

Media and feed development for commercial biopharmaceutical production has been largely accomplished via empirical screening, and thus the understanding of CHO cell metabolism, as it relates to product titer and quality, remains limited. To better understand the metabolic events that affect cell biomass and antibody titer, we designed a metabolomics workflow that enables the efficient screening of multiple growth conditions, simultaneously.

Metabolomic profiles of cell cultures supplemented with different feeds were used to detect compounds important in antibody production. Principal component analysis (PCA) of different cell cultures over several time points showed clear differentiation as growth duration increased (Figure 9).

Figure 9. PCA plot illustrating the metabolic changes that happen during CHO cell culture. During early days of culture , all feeds cluster together. As the days of culture progress, the different feeds result in different metabolic profiles.

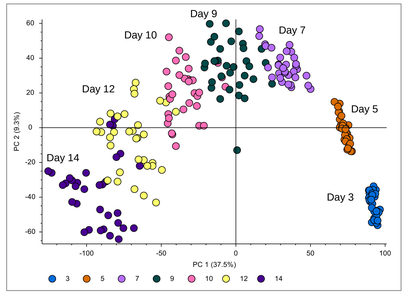
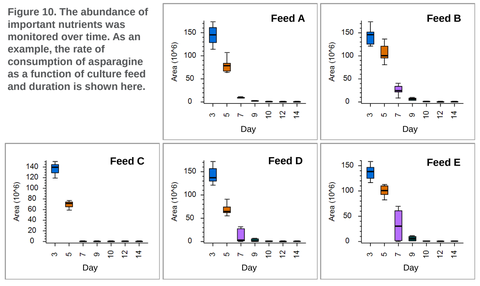


Figure 10. The abundance of important nutrients was monitored over time. As an example, the rate of consumption of asparagine as a function of culture feed and duration is shown here.



COMPREHENSIVE METABOLOME COVERAGE

Complementary separations for improved metabolome coverage

By analyzing the samples using both RP and HILIC separation, a greater number of compounds with more diverse structures and chemical properties can be identified. The numbers of compounds detected and identified using the two different separations are listed in table 3.

Table 3. Numbers of compounds detected and identified at different confidence levels using RP and HILIC separation.

Compounds	HILIC - negative	RP - positive
After background removal	971	4786
With predicted chemical compositions	899	3866
With MS/MS spectra	883	2851
With spectral match against mzCloud	616	1844
Confirmed against authentic standard (mzVault)	112	158

Figure 11. Venn diagram illustrating the overlap of metabolites identified against authentic standards using RP (158 compounds – blue circle) and HILIC (112 compounds – grey circle). 35 metabolites were identified using both technologies.

CONCLUSIONS

- The Transcend Duo LX-2 system was successfully applied in the differential analysis of cell media and provided robust metabolic indicators of cell growth and antibody yield and possible optimization strategies.
- Two independent UHPLC pumps were incorporated into the system to allow independent control of the two columns (RP and HILIC), resulting in a 30% decrease in analysis time and increasing the overall throughput of the method.
- This increased throughput did not compromise reproducibility or compound detection. Internal standards during the analysis exhibited highly reproducible retention times (%CV < 1%) and peak areas (%CV < 20%), while the mass accuracy of the Orbitrap measurements remained well below 1ppm for this 4 day experiment.
- One can envision extending utilization of this metabolomic workflow to the optimization of other bioproduction processes or any metabolomics application, where increased throughput is needed.

TRADEMARKS/LICENSING

PO65549-EN0519S

ThermoFisher SCIENTIFIC