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Application News

Cell culture profiling method package / LCMS-8050

Cell Culture Profiling Analysis with LC-MS/MS and Bioanalyzer for Antibody Production in Chinese Hamster Ovary (CHO) Cell System

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User Benefits

- LC-MS/MS cell culture profiling method package allows simultaneously analysis of up to 125 targeted nutrients and metabolites in cell culture medium.
- Graphical trend plots of detected targets enable to view the changes of metabolite concentrations with cell culture time directly.
- Trends of metabolite changes from LC-MS/MS are highly correlated with the trends observed from CEDEX Bio Analyzer.

■ Introduction

Chinese hamster ovary (CHO) cells are widely used in the biopharmaceutical industry as the workhorse to produce therapeutical recombinant protein including monoclonal antibody (mAb). Monitoring the abundance of nutrients and metabolites during CHO cell culture provides important insights into the process performance since cell growth and protein product quality can be impacted by the depletion of key nutrients or accumulation of cellular metabolism by-products. Thus, it is worthwhile to perform a comprehensive analysis on cell culture medium as such knowledge can aid in developing a well-balanced media formulation and an optimized feeding strategy for biopharmaceutical production [1, 2].

With conventional sensors on bioreactor or standalone bioanalyzers, no more than 10 metabolites can be monitored in a single run. Furthermore, expensive reagent kits may be needed and some of these kits have short shelf life once being opened. This puts additional stress in experiment planning and resource allocation. Herein, we monitored the cell culture process in mAb production by an LC-MS/MS approach using Shimadzu cell culture profiling method package [3] which enables analysis of 125 media components in a single run of LC-MS/MS analysis.

■ Experimental

CHO cell culture and Bio Analyzer monitoring

The CHO cell producing mAb process was maintained in both Gibco™ CD CHO medium and a mixture medium that combines HyClone™ PF CHO Multi-powder System containing 2g/L of sodium bicarbonate with CD CHO at a 1:1 ratio. Every 3 days, passaging of cells was carried out with cell viability and density being monitored by Vi-CELL XR Cell Viability Analyzer (Beckman Coulter). After a week, the cell culture was scaled up and then left to grow for 5 days in a shaking flask with sampling on a daily interval (Day 0 to Day 5) [2].

The cell culture sample was measured with CEDEX Bio Analyzer (Roche), which has been routinely used for checking important cell culture metabolites levels including glucose, lactate and glutamine using proprietary assay kits (Glucose Bio, Lactate Bio and Glutamine V2 Bio, respectively) [2].

Table 1 Analytical conditions for Cell Culture Profiling method package on LCMS-8050

LC Conditions (on Nexera UHPLC)	
Column	Shim-Pack™ GIST PFPP column (2.1 x 150 mm, 3 µm)
Flow Rate	0.3 mL/min
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile
LC program	Gradient elution, 20 minutes
Oven Temp.	40°C
Injection Vol.	1.0 µL
MS Conditions (on LCMS-8050)	
Interface	HESI
Interface Temp.	300 °C
DL Temp.	250 °C
Heat Block Temp.	400 °C
Nebulizing Gas	3 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Mode	MRM, positive and negative mode for 125 targets and IS (2-Isopropylmalic acid)

LC-MS/MS monitoring of CHO Cell culture

A cell culture profiling method package developed by Shimadzu (version 2) was used for monitoring of the culture medium components and metabolites [3]. The analytical conditions (Table 1) of this ready-to-use method are optimized with a Shim-Pack GIST PFPP column (2.1 x 150 mm, 3 µm). Two MRM transitions are included for each compound as quantifier ion and reference ion. An internal standard (2-Isopropylmalic acid) was added to every cultural sample collected and used for ensuring the analysis reliability and obtaining relative quantitation value (area ratios) of every detected component *vs.* cell culture time (Day 1 to Day 5).

Sample pretreatment before LC-MS/MS analysis

Following the instruction manual of cell culture profiling method package [3], aliquots of culture medium samples collected across 5 different days were pre-treated as the procedure illustrated in Figure 1. The collected culture medium sample was spin down at 3,000 rpm for 5 min under room temperature. Supernatant was transferred into a new 1.5 mL tube and stored at -20 °C until analysis time. After collecting all samples, the samples were further pre-treated following step 3 to step 8 (Figure 1) for LC-MS/MS batch analysis.

1) Aliquot 500 µL of cell culture medium in a 1.5 mL centrifuge tube and centrifuge at 3,000 rpm for 5 min under room temp
2) Transfer supernatant into a new 1.5 mL tube and stored at -20°C until LC-MS/MS analysis was performed
3) Aliquot 50 µL of above supernatant into a new 1.5 mL centrifuge tube and diluted with 150 µL of Milli-Q water
4) Add 20 µL of 0.5 mM 2-Isopropylmalic acid (ISTD)
5) Add 200 µL of acetonitrile to the mixture, vortex for 1 min
6) Centrifuge sample at 15,000 rpm for 15 min under room temp.
7) Transfer 100 µL of sample supernatant to a 1.5 mL sample vial, dilute with 900 uL of Milli-Q water
8) Inject to LC-MS/MS for analysis

Figure 1 Sample collection and pre-treatment procedure of cell culture medium for LC-MS/MS analysis.

■ Results and Discussion

Detection of components by CCP method package

Two duplicated sets of cell culture in medium CD CHO and medium CD/PF CHO (1:1) were maintained for 5 days. The cell growth curve of cell density cross 6 days in medium CD/PF CHO (1:1) is shown in Figure 2 [2]. The samples collected on Day 0 (before cell culture was started) and Day 5 of both sets were analyzed first with the CCP method on LCMS-8050. This is because the Day 0 culture medium have the highest concentrations of the nutrients (glucose, pyridoxine and glutamate, etc.). Whereas, Day 5 culture medium may have the highest concentrations of metabolites produced (deoxycytidine, methionine sulfoxide and malic acid, etc.). Screening against the 125 target analytes provided in the CCP method package, a total of 52 components was detected in CD/PF CHO medium sample, while 48 were found in CD CHO sample. The four missing components in the latter are Taurine, Aconitic acid, Citric acid and 2-Aminobutyric acid. Figure 3 shows representative chromatograms of the Day 4 CD CHO medium. Note that peak verification and retention time adjustment were performed carefully when sample was analyzed for first time on the LC-MS/MS system.

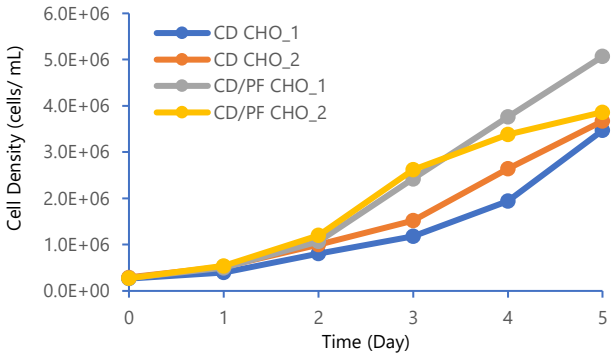

Figure 2: CHO cell growth curve of viable cell density against 5 days in cell culture medium CD CHO and CD/PF CHO (1:1).

Trends of detected analytes over cell culture period

Both sets of analysis results (CD and CD/PF CHO (1:1)) were exported and plotted as bar chart using Shimadzu's Multi-omics Analysis Packages on GARUDA Platform [4]. Three different trends of the detected analytes was observed, i.e., (a) analyte concentration decreasing with culture time, (b) analyte concentration increasing with culture time and (c) analyte concentration fluctuating over the whole period. For convenience, the results are displayed according to the trends observed in CD CHO (red trendline) in Figures 4a~4c.

For instance, alanine and lactic acid exhibit increasing trend over time (Fig. 4a). Glutamine and hexose show decreasing trend (Fig. 4b). Proline and 2-aminobutyric acid were fluctuating for instance (Fig. 4c), while pyridoxine and 2-aminoethanol had depleted entirely over time (Fig. 4b). Generally, the trends for metabolites are similar in the two different media, but there were some exception such as lactic acid and putrescine.

Correlation between LC-MS/MS and Bio Analyzer measurements

The LC-MS/MS based Cell Culture Profiling method is a novel approach aimed at measuring the trend of change for the nutrients and metabolites in the cell culture process simultaneously. Glucose (hexose), lactate and glutamine levels were also checked from CEDEX Bio Analyzer using proprietary assay kits (Glucose Bio, Lactate Bio and Glutamine V2 Bio, respectively). Although there is not practical purpose to compare the value of measurement directly due to detection principal difference, it is very important to verify that the trend reported from both methods resemble each other in an almost perfect manner for the cell culture samples that were tested.

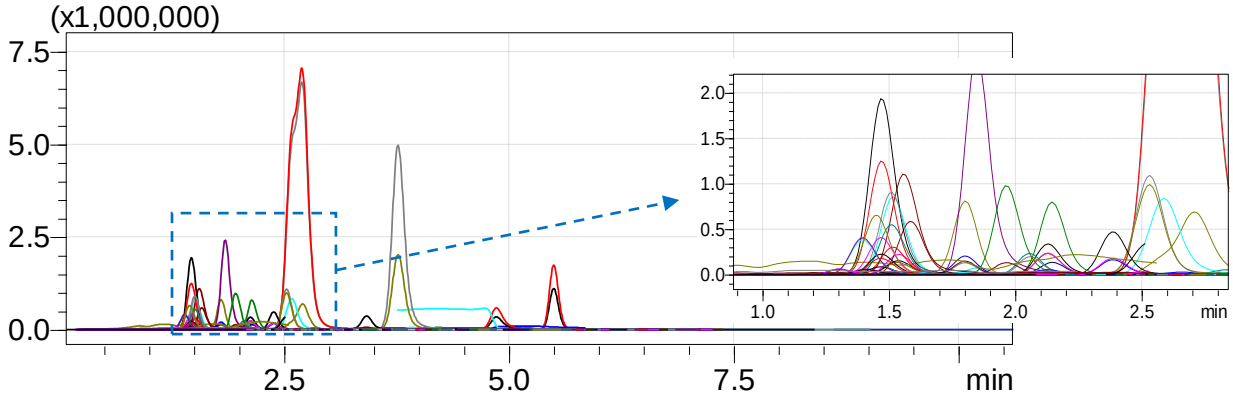

Figure 3: MRM chromatograms of 48 components detected in Day 4 cell culture medium (CD CHO) sample

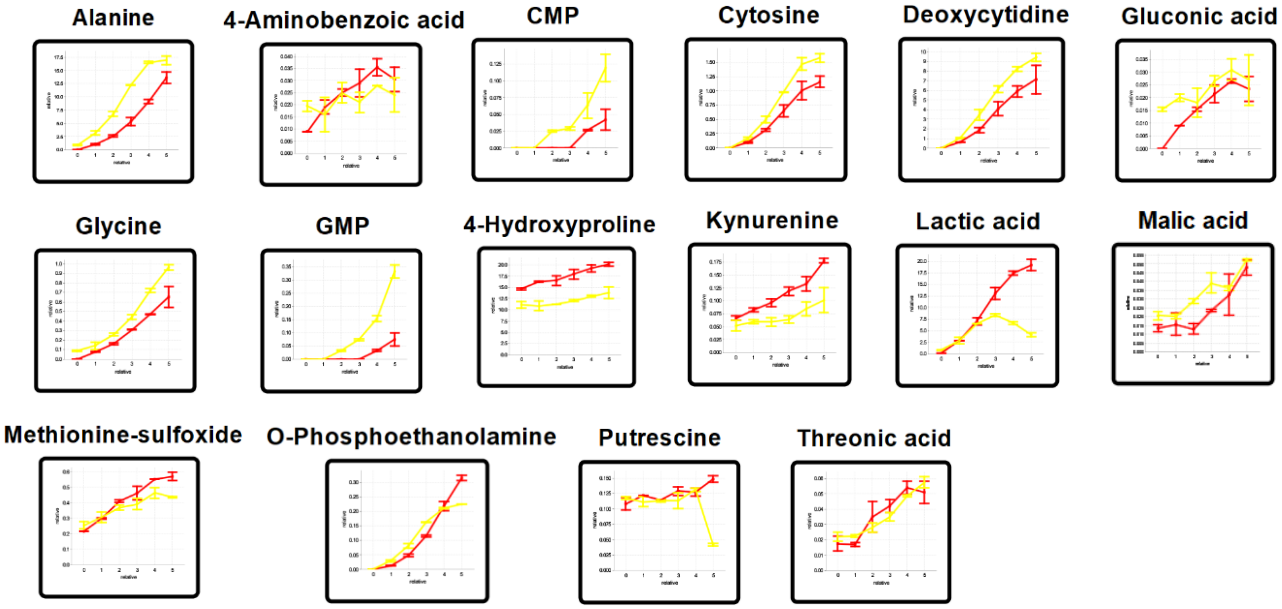

Figure 4a: 16 analytes show increasing trend with time in CD CHO medium (red trendline). The yellow trendline represents concentration change of analytes in CD/PF CHO medium.

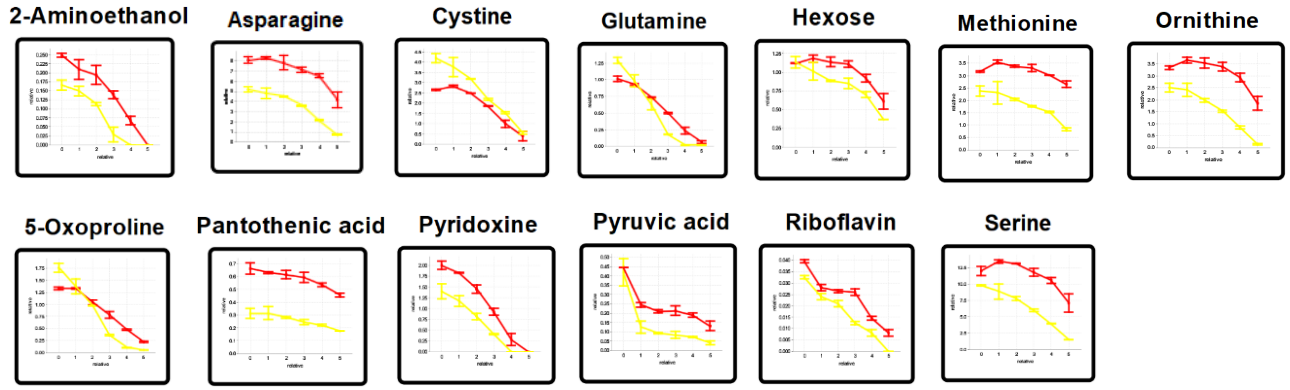

Figure 4b: 13 analytes shows decreasing trend with time in CD CHO medium (red trendline). The yellow trendline represents concentration change of analytes in CD/PF CHO medium.

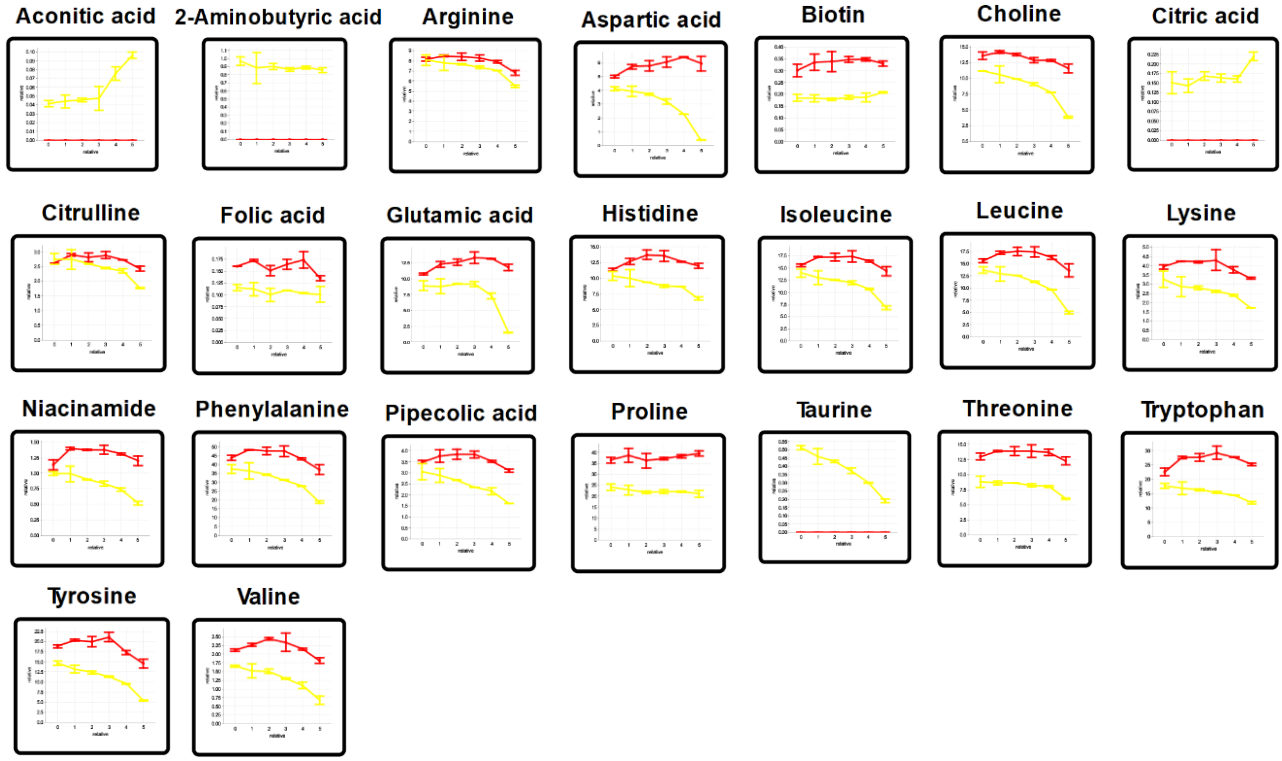

Figure 4c: 12 analytes show fluctuating trend and 4 analytes were not detected in CD CHO medium (red trendline). The yellow trendline represents concentration change of analytes in CD/PF CHO medium.

Correlation coefficient between two sets of data independently acquired using LC-MS/MS and CEDEX-Bio Analyzer was computed and reported in the Table 2. The high correlation obtained for glucose, glutamine and lactic acid gives high confidence in the trends measured on LC-MS/MS using Cell Culture Profiling method package.

Table 2 Correlation coefficient for glucose, glutamine and lactic acid measurement (Day 0 to Day 5) from LC-MS/MS and CEDEX-Bio Analyzer for 2 cell culture processes (duplicates)

Cell culture medium	Glucose (Hexose)	Glutamine	Lactic Acid
CD CHO_1	0.95	1.00	1.00
CD CHO_2	0.97	1.00	1.00
CD/PF CHO_1	0.99	1.00	1.00
CD/PF CHO_2	0.98	1.00	1.00

Biostatistical analysis by Volcano plot

A biostatistical analysis was performed for concentration changes between Day 0 and Day 4 using the Multi-omics Analysis Packages. The Volcano plots [4, 5] with $p < 0.05$ and 2 folds of change are shown in Figure 5. For CD CHO medium, 3 compounds (5-oxoproline, riboflavin, glutamine) were identified to be significant decrease, while four compound (kynurenine, methionine sulfoxide, alanine and lactic acid) were significant increase. These results are essentially in consistency with the trendlines in Figure 4. For the CD/PF CHO medium as shown in Figure 5(b), the compounds of significant decrease include 5-oxoproline, glutamine and serine etc.. The compounds of significant increase are alanine, lactic acid and glycine. Furthermore, 5-oxoproline and glutamine concentrations decreased significantly in both media, indicating that they might be important nutrients in the cell growth. Lactic acid and alanine concentrations increased significantly in both medium and they are likely secreted products.

■ Conclusion

The LC-MS/MS cell culture profiling method package was used in the analysis of nutrients and metabolites in CHO cell media for mAb production over a period of 5 days. A total of 48 and 52 components were detected and their relative concentrations were quantified in CD CHO medium and CD/RF CHO medium, respectively. Different trends of their concentrations with time were observed using the Multi-omics analysis program, which allows user to visualize the changes of components directly. The LC-MS/MS results are highly correlated with that were measured by CEDEX Bio Analyzer for glucose, glutamine and lactic acid. In addition, Volcano plots were constructed between Day 0 and Day 4 for the detected compounds in CD CHO and CD/PF CHO media.

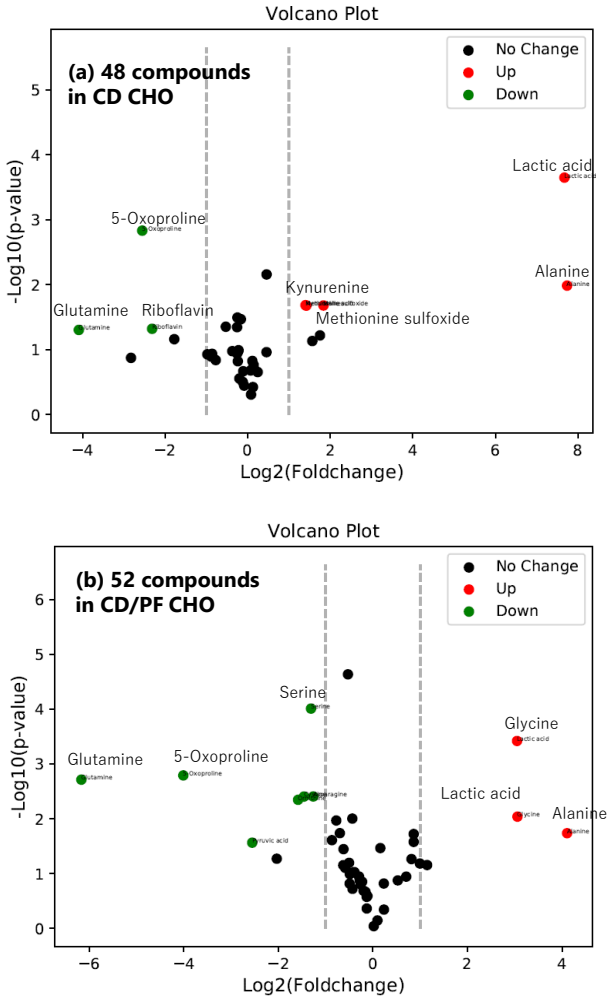

Figure 5: Biostatical analysis Volcano plots for detected compounds in (a) CD CHO medium and (b) CD/PF CHO with 95% confidence interval vs 2 fold in concentration change.

The results revealed that 5-oxoproline and glutamine have significantly decreased over time; while lactic acid and alanine concentration built up significantly in both media during cell culture process.

■ References

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04-AD-0267-EN First Edition: Dec 2022

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