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Application News

Liquid Chromatograph-Mass Spectrometer LCMS-8060NX
Inductively Coupled Plasma Mass Spectrometer ICPMS-2030

Culture Medium Analysis for a Metabolic Analysis of Antibody-Producing Cells Using LC-MS/MS and ICP-MS

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User Benefits

- Performs simultaneous measurement of organic components and inorganic components (separately) in culture medium and culture supernatant with simple pretreatment.
- Performs a metabolic analysis that includes both organic and inorganic components.

■ Introduction

Antibody drugs are produced by culturing antibody-producing cells. Developing these host cells is an active field of research and the Omasa Laboratory at Osaka University recently established a cell line derived from Chinese hamster lung cells (CHL-YN cells)[1]. CHL-YN cells have approximately 2-fold growth rate compared to CHO-K1 cells, a cell line that is frequently used in antibody production. Understanding the metabolism of host cells is important to realize industrial applications of these cells, but the metabolism of CHL-YN cells has not yet been fully studied. Recent studies have reported the effects of changes of organic/inorganic components in the culture medium on antibody production and quality. Organic and inorganic components are also known to interact with one another in culture medium over the course of antibody production[2]; hence an analysis of the culture medium must include both organic and inorganic components to be able to understand host cell metabolism. Furthermore, since organic/inorganic components are known to interact with each other in the culture medium[2]; we consider the analysis of both organic and inorganic components in the culture medium is important for understanding metabolism.

This Application News uses LC-MS/MS (LCMS-8060NX) to analyze 144 organic compounds and ICP-MS (ICPMS-2030) to analyze 9 inorganic elements in the culture medium and culture supernatant of a CHL-YN antibody producing cell line culture and describes using this data to identify which components and metabolic pathways are involved in antibody production.

■ CHL-YN Cell Culture

CHL-YN cells were cultured under the conditions shown in Table 1, and culture supernatant was collected every 24 hours (biological replicates n = 3).

Table 1 Culture Conditions

Culture Conditions	
Seeding Density	1.0×10^5 cells/mL
Agitation Rate	90 rpm
Temp. / Humidity / CO_2	37 °C / 80 % / 5 %

■ Preparing Culture Supernatant Samples

☐ Sample Preparation for LC-MS/MS Analysis

Samples for LC-MS/MS analysis were prepared to remove protein from the culture supernatant, as shown in Fig. 1.

☐ Sample Preparation for ICP-MS Analysis

Samples for ICP-MS analysis were prepared by dilution with 1 % aqueous nitric acid, as shown in Fig. 2.

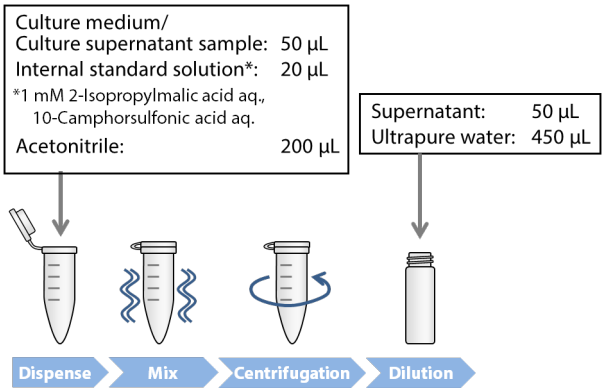

Fig. 1 Sample Preparation for LC-MS/MS Analysis

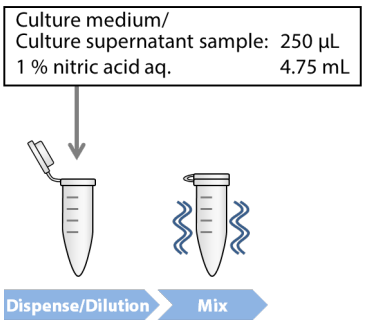

Fig. 2 Sample Preparation for ICP-MS Analysis

■ Analytical Conditions

☐ LC-MS/MS

Organic components in a culture medium were analyzed using the Nexera™X3 system and the LCMS-8060NX.

LC/MS/MS Method Package for Cell Culture Profiling Ver. 3 was used for the analytical method. This method enables simultaneous analysis of a total of 144 components* such as medium components and secreted metabolites.

* All 144 components are shown in Table 3 for reference.

☐ ICP-MS

Inorganic components were analyzed using the ICPMS-2030 with the conditions described in Application News 01-00372 (Using ICPMS-2030 to analyze metal elements in culture media). The analysis mainly focused on elements that are reported to affect antibody production: Co, Cu, Fe, Mg, Mn, Mo, Ni, Se, and Zn.

■ Results

□ LC-MS/MS Results

The results obtained with the LC/MS/MS Method Package for Cell Culture Profiling Ver. 3 were visualized using the Multi-omics Analysis Package, which is included in the package (Fig. 3). The time course of 74 components detected by culture supernatant analysis with Cell Culture Profiling Ver. 3 are shown in Fig. 4.

Of these, components that exhibited distinctive changes are shown in Figs. 5 and 6. The components shown in Fig. 5 became depleted in the later part of cell culture, providing information that can assist in an investigation of feed conditions.

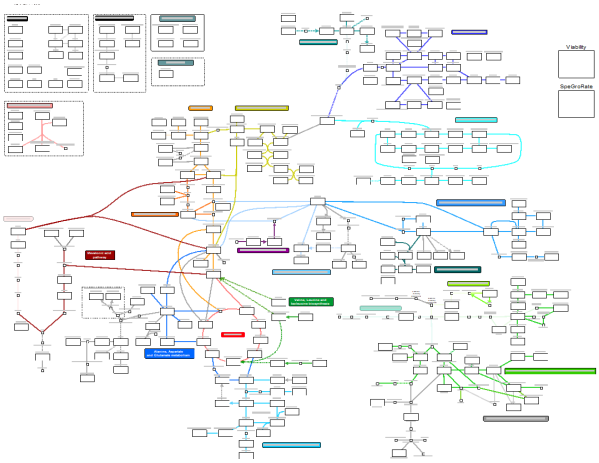

Fig. 3 Example Blank Map (Optimized for Metabolic Pathways of CHO Cells)
LC/MS/MS Method Package for Cell Culture Profiling Ver. 3 includes three templates for data visualization called "blank maps" that are designed around the components registered in the method package. The user selects the best blank map for their use case to create a simple means of viewing analytical results.

The time course of components shown in Fig. 6 seems to switch between consumption and secretion over time, a valuable finding that suggests a shift in metabolism.

□ ICP-MS Results

The results obtained by ICP-MS analysis are shown in Fig. 7. Data were obtained on the time course of all nine targeted elements. The results suggest only some elements were taken up by cells and show the timing of uptake differed between each element.

Fig. 4 Time Course of Components Detected in Culture Supernatant Using Cell Culture Profiling Ver. 3

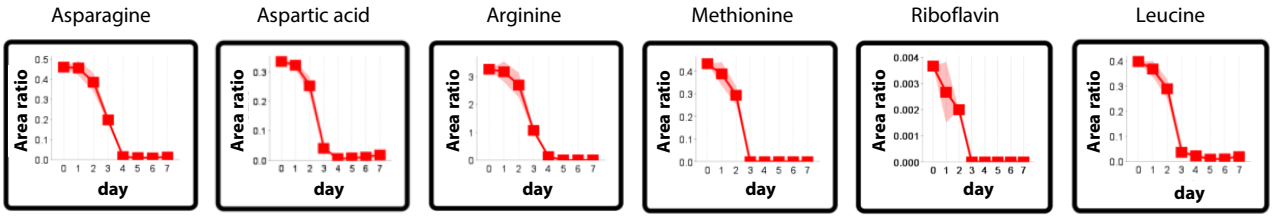

Fig. 5 Organic Components Depleted in Later Stage of Cell Culture
Area ratio (vertical axis): Peak area of measured component divided by peak area of internal standard.
Each data point represents the mean of n = 3 biological replicates; shaded areas represent the error range.

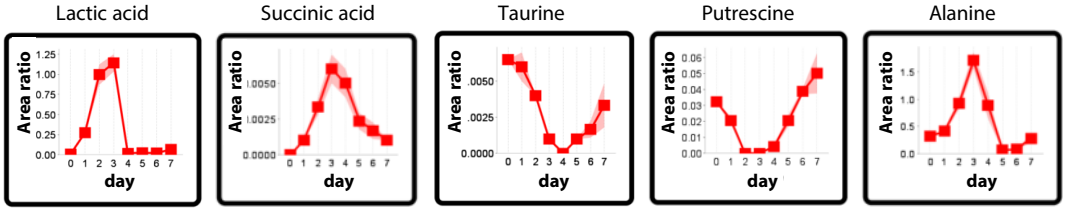

Fig. 6 Organic Components that Switched between Consumption and Secretion over Time
Area ratio (vertical axis): Peak area of measured component divided by peak area of internal standard.
Each data point represents the mean of n = 3 biological replicates; shaded areas represent the error range.

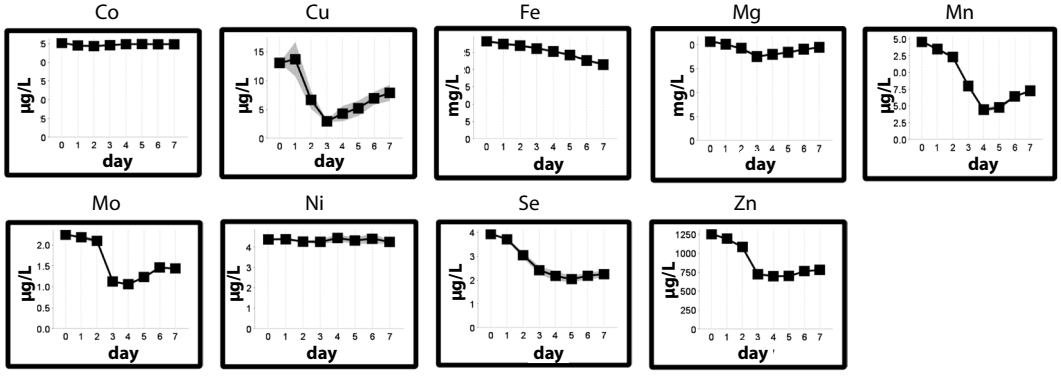

Fig. 7 Time Course of Metals in Culture Supernatant Measured by ICP-MS
Each data point represents the mean of n = 3 biological replicates; shaded areas represent the error range.

■ Data Analysis

□ Correlation Analysis

Components and metabolic pathways involved in antibody production were identified by performing a correlation analysis with the specific antibody production rate (the amount of antibodies produced by a single cell per unit time) as the objective variable and the measured amount of organic and inorganic components as the explanatory variable.

Components with an absolute correlation coefficient greater than 0.5 and a false discovery rate (FDR) less than 0.05 were identified as correlated with the specific antibody production rate. Both organic and inorganic components were identified that met these criteria. Table 2 shows some of the components identified as positively correlated with the specific antibody production rate (correlation coefficient > 0.5 and FDR < 0.05).

□ Enrichment Analysis

Enrichment analysis was also performed for a systematical understanding of the characteristics of each component that was correlated with antibody production (Fig. 8). Enrichment analysis is a method for selecting statistically significant metabolic pathways that compares a list of components correlated with antibody production against a list of components grouped by metabolic pathway. Enrichment analysis was performed using Metaboanalyst[3),4)].

The enrichment analysis revealed glutathione metabolism and the urea cycle as particularly significant metabolic pathways. Thus, a series of analyses using the results of analyses of organic and inorganic components in the culture medium led to the identification of components and characteristic metabolic pathways that correlated with the targeted antibody production.

Table 2 Components with Correlation Coefficient > 0.5 and FDR < 0.05

* Red boxes: Inorganic

	correlation	FDR		correlation	FDR
SPR	1.00000	9.631600e-125	**2-Aminoethanol**	0.92712	2.399500e-07
Arginine	0.98120	3.064900e-11	**Pyruvic acid**	0.92587	2.508600e-07
Asparagine	0.97464	2.197200e-10	**Mn-55**	0.92508	2.513700e-07
Isoleucine	0.95867	7.686900e-09	**Phenylalanine**	0.91185	8.215200e-07
Serine	0.95756	7.686900e-09	**Se-78**	0.90977	9.065400e-07
Aspartic acid	0.95487	1.038400e-08	**Pyridoxine**	0.90916	9.065400e-07
Cystine	0.95382	1.066700e-08	**Hexose (Glucose)**	0.90389	1.317700e-06
Deoxycytidine	0.94914	1.990900e-08	**Lysine**	0.90095	1.568300e-06
Valine	0.94451	3.498800e-08	**Zn-66**	0.89172	2.793800e-06
Leucine	0.94143	4.806500e-08	**Tyrosine**	0.88603	3.933600e-06
			Glutamic acid	0.86884	1.044700e-05

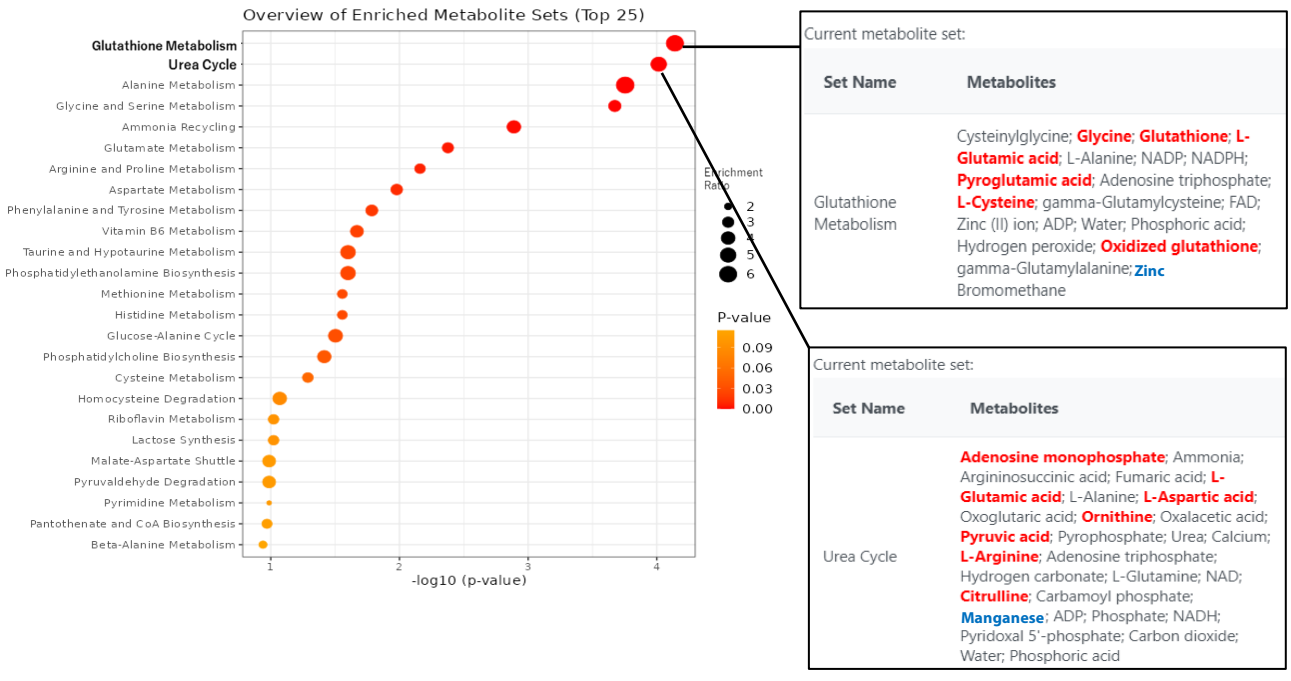

Fig. 8 Results of Enrichment Analysis

$p < 0.0001$ for glutathione metabolism and the urea cycle. Components identified by correlation analysis are shown in red in the reference metabolite list for each metabolic pathway.

■ Conclusion

- ✓ The simultaneous analysis of organic components and inorganic components (separately) in culture medium and culture supernatant was undertaken with simple pretreatment and provided data on the change in each component over time.
- ✓ Components that are depleted or suggest a metabolic shift were found.
- ✓ A correlation analysis performed using the specific antibody production rate as the objective variable identified components that correlated with antibody production and metabolic pathways that were linked to antibody production.

Compounds and metabolic pathways involved in antibody production can be identified using data obtained from the analysis of components in cell culture supernatant with Shimadzu's LC-MS/MS and ICP-MS analytical systems (LCMS-8060NX* and ICPMS-2030, respectively).

* LC/MS/MS Method Package for Cell Culture Profiling Ver. 3 is compatible with LCMS-8045, LCMS-8050, and LCMS-8060(NX).

[Acknowledgments]

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<References>

1) Yamano-Adachi, Noriko, Rintaro Arishima, Sukwattananipaat Puriwat, and Takeshi Omasa. 2020. "Establishment of Fast-Growing Serum-Free Immortalised Cells from Chinese Hamster Lung Tissues for Biopharmaceutical Production." Scientific Reports 10 (1): 17612.
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3) Pang, Zhiqiang, Jasmine Chong, Guangyan Zhou, David Anderson de Lima Morais, Le Chang, Michel Barrette, Carol Gauthier, Pierre-Étienne Jacques, Shuzhao Li, and Jianguo Xia. 2021. "MetaboAnalyst 5.0: Narrowing the Gap between Raw Spectra and Functional Insights." Nucleic Acids Research 49 (W1): W388–96.
4) https://www.metaboanalyst.ca/MetaboAnalyst/

Table 3 List of Components Registered in LC/MS/MS Method Package for Cell Culture Profiling Ver. 3
Red Character: Components added in Ver. 3

Amino acids and their metabolites			Nucleic acids and their metabolites	Sugars
1-Methylhistidine	Glutamic acid	Serine	3-Aminoisobutyric acid	Gluconic acid
2-Aminoadipic acid	Glutamine	Serotonin	3-Aminopropanoic acid	Hexose (Glucose)
2-Aminobutyric acid	Glutathione	Symmetric dimetyhlarginine	Adenine	Sucrose
2-Aminoethanol	Glycine	Threonine	Adenosine	Threonic acid
3-Hydroxyanthranilic acid	Glycyl-glutamine	Tryptophan	Adenosine monophosphate	
3-Hydroxyisobutyric acid	Histidine	Tyrosine	Cytidine	**Others**
3-Methyl-2-oxovaleric acid	Homocysteine	Urocanic acid	Cytidine 3',5'-cyclic monophosphate	2-Ketoglutaric acid
3-Methylhistidine	Homocystine	Valine	Cytidine monophosphate	Acotinic acid
4-Aminobutyric acid	Hydroxykynurenine		Cytosine	Citric acid
4-Hydroxyphenyllactic acid	Hydroxylysine	**Vitamins**	Deoxyadenosine	Fumaric acid
4-Hydroxyproline	Indole-3-acetic acid	4-Aminobenzoic acid	Deoxycytidine	Glyceric acid
5-Glutamylcysteine	Isoleucine	4-Pyridoxic acid	Deoxycytidine monophosphate	Glycolic acid
5-Hydroxytryptophan	Kynurenic acid	Acetylcholine	Deoxyguanosine	Glyoxylic acid
5'-Methylthioadenosine	Kynurenine	Ascorbic acid	Deoxyguanosine monophosphate	Isocitric acid
5-Oxoproline	Leucine	Biotin	Guanine	Lactic acid
Acetylcarnitine	Lysine	Choline	Guanosine	Malic acid
Alanine	Methionine	Citicoline	Guanosine 3',5'-cyclic monophosphate	Mevalonic acid
Alanyl-glutamine	Methionine sulfoxide	Cyanocobalamin	Guanosine monophosphate	MVA-P
Anthranilic acid	N-Acetylaspartic acid	Folic acid	Hypoxanthine	Penicillin G
Arginine	N-Acetylcysteine	Lipoic acid	Inosine	Pyruvic acid
Argininosuccinic acid	Norepinephrine	NAD	Inosine monophosphate	Resveratrol
Asparagine	O-Phosphoethanolamine	Niacinamide	Orotic acid	Shikimic acid 3-phosphate
Aspartic acid	Ophthalmic acid	Nicotinic acid	Thymidine	Succinic acid
Asymmetric dimetyhlarginine	Ornithine	Pantothenic acid	Thymidine monophosphate	Taurine
Carnitine	Oxidized glutathione	Pyridoxal	Thymine	
Citrulline	Phenylalanine	Pyridoxalphosphate	Uracil	**Internal standard**
Creatine	Phenyllactic acid	Pyridoxine	Uric acid	2-Isopropylmalic acid
Cystathionine	Pipecolic acid	Riboflavin	Uridine	10-Camphorsulfonic acid
Cysteine	Proline		Uridine monophosphate	
Cystine	Putrescine		Xanthine	
Dopa	Saccharopine		Xanthosine	
Formylkynurenine	S-Adenosylhomocysteine		Xanthosine monophosphate	

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