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Development of cell culture supernatant analysis using LC-MS/MS and their application for Chinese hamster ovary cell

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1. Introduction

Optimization of cell culture process is crucial to improve the productivity and quality of biopharmaceuticals. Infrared spectroscopy and biosensor are used for analyzing cell culture fluid during culture process. While these methods are simple and short in measurement time, the measurement items are limited to pH, dissolving gases, and major small compounds such as glucose, glutamine, and lactic acid. Therefore, a simultaneous analysis method including trace components such as vitamins in the culture medium and secreted metabolites has been required for examining culture process in detail. We have developed simultaneous analysis method up to 125 compounds in 17 minutes using LC-MS/MS. In this poster, we present features of our developed method and its applications for Chinese hamster ovary cell.

2. Methods

CHO cell culture fluid were collected every 24 hours. The collected culture fluid was centrifuged to remove cell debris, and the supernatant was used as a sample. The supernatant samples were deproteinized by the addition of acetonitrile and centrifugation. The supernatant after centrifugation was collected and diluted 10 fold with ultrapure water to serve as an analytical sample (Fig.1).

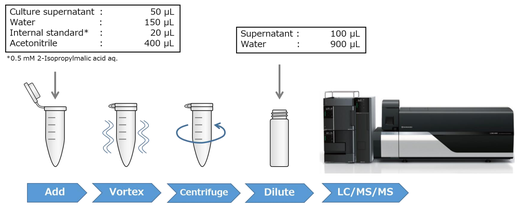

Figure 1 Sample preparation

We have developed the LC-MS/MS Method Package for Cell Culture Profiling Ver.2 (Shimadzu Corp, Japan). This product enables us to analyze simultaneously 125 compounds including basal medium components and secreted metabolites. The list of target compounds were shown in Table I. In this research, We used Nexera X3 with LCMS-8060.

Table 1 Target compounds

Amino acids and their metabolites		Nucleic acids and their metabolites
5-Oxoproline	Glycyl-glutamine	Adenine
1-Methylhistidine	Histidine	Adenosine
2-Aminoadipic acid	Homocysteine	Adenosine monophosphate
2-Aminobutyric acid	Hydroxykynurenine	Deoxyadenosine
3-Hydroxyanthranilic acid	Hydroxylysine	Deoxyadenosine monophosphate
3-Hydroxyisobutyric acid	Indole-3-acetic acid	Deoxyguanosine
3-Methyl-2-oxovaleric acid	Isoleucine	Deoxyguanosine monophosphate
3-Methylhistidine	Kynurenic acid	Guanine
4-Aminobutyric acid	Kynurenine	Guanosine
4-Hydroxyphenyllactic acid	Leucine	Guanosine monophosphate
4-Hydroxyproline	Lysine	Hypoxanthine
5-Glutamylcysteine	Methionine	Inosine
5-Hydroxytryptophan	Methionine sulfoxide	Inosine monophosphate
5'-Methylthioadenosine	N-Acetylaspartic acid	Uric acid
Alanine	N-Acetylcysteine	Xanthine
Alanyl-glutamine	Ornithine	Xanthosine
Anthranilic acid	Oxidized glutathione	Xanthosine monophosphate
Arginine	Phenylalanine	3-Aminoisobutyric acid
Argininosuccinic acid	Pipecolic acid	3-Aminopropanoic acid
Asparagine	Proline	Cytidine
Aspartic acid	Putrescine	Cytidine monophosphate
Citrulline	Saccharopine	Cytosine
Cystathionine	S-Adenosylhomocysteine	Deoxycytidine
Cysteine	Serine	Deoxycytidine monophosphate
Cystine	Serotonin	Orotic acid
Formylkynurenine	Threonine	Thymidine
Glutamic acid	Tryptophan	Thymidine monophosphate
Glutamine	Tyrosine	Thymine
Glutathione	Urocanic acid	Uracil
Glycine	Valine	Uridine
		Uridine monophosphate

Sugars	Others
Gluconic acid	2-ketoglutaric acid
Hexose (Glucose)	Acotinic acid
Sucrose	Citric acid
Threonic acid	Fumaric acid
Vitamins	Isocitric acid
Riboflavin	Lactic acid
Niacinamide	Malic acid
Nicotinic acid	Pyruvic acid
Pantothenic acid	Succinic acid
4-Pyridoxic acid	Penicillin G
Pyridoxal	2-Aminoethanol
Pyridoxalphosphate	Glyceric acid
Pyridoxine	NAD
Biotin	O-Phosphoethanolamine
4-Aminobenzoic acid	Taurine
Folic acid	**Internal Standard**
Choline	2-Isopropylmalic acid
Ascorbic acid	
Cyanocobalamin	
Lipoic acid	

3. Results

3-1. General performance

The linearity of the major compounds in cell culture supernatant was evaluated to examine the quantification performance of the developed method. We confirmed that the quantitative ranges of the components contained in the culture supernatant were present in the high concentration range, and those of the trace components were present in the low concentration range(Fig.2).

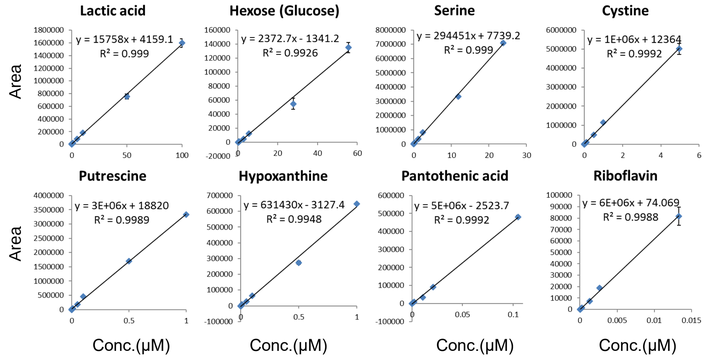

Figure 2 Linearity of the major target compounds

We analyzed 3 kinds of CHO cell culture media. good peak shapes were observed from high concentration components to trace components in the culture media(Fig.3), and analysis reproducibility with an area CV value of 10% or less was observed(Table. 2).

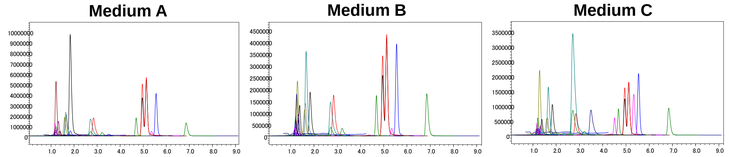

Figure 3 Chromatogram of 3 kinds of CHO cell culture media.

Table 2 Analysis reproducibility of 3 kinds of CHO cell culture media (n = 8)

	Average CV value	Max CV value
Medium A	2.71%	7.5% (Pantothenic acid)
Medium B	2.61%	7.86% (Pantothenic acid)
Medium C	3.5%	9.98% (Glutamic acid)

※Average CV value was calculated from the detected compounds above 100000 area.

3-2. Time course of culture supernatant of CHO cell

The culture supernatants of CHO cells were analyzed and the temporal change in signal intensity of the components was graphed. the signal intensity of sugars and amino acids decreased and that the signal intensity of metabolites such as lactic acid increased during cell culture process. For some amino acids and vitamins, the signal intensity did not increase or decrease during culture process. These results suggest that there are components that are preferentially consumed during the growth phase of CHO cells and components that are not involved in growth.

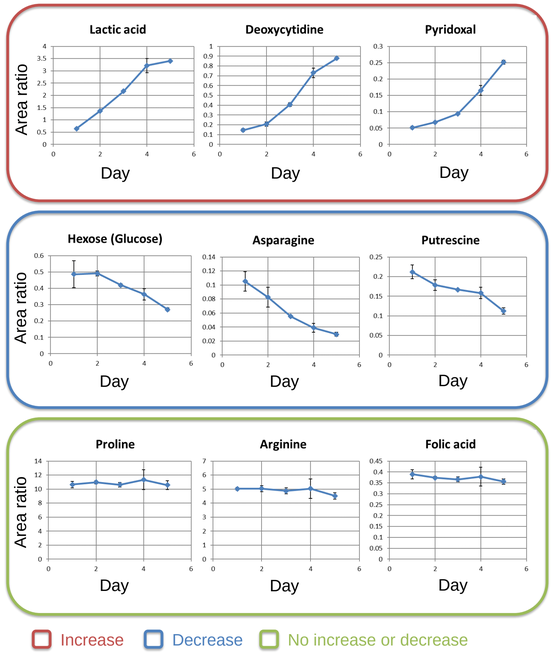

Figure 4 Time course of the major target compounds in CHO cell culture supernatant

4. Conclusions

•Our method has the potential to be an effective means to examine the detailed culture process of CHO cells.