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Simultaneous analysis of supernatant components of cell culture using triple quadrupole LC/MS/MS

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Introduction

Fermentation for the production of biofuels, biopharmaceutics or other compounds requires routine monitoring of medium conditions such as pH, dissolved gas, carbon source and nitrogen source for optimization and control of the fermentation process. However, culture media also consist of various other biologically important compounds such as vitamins, nucleic acids and other primary metabolites, which would lead to more detailed understanding of the bioprocess if monitored altogether. To meet the demand for comprehensive analysis of medium components, we optimized analytical conditions and developed "Method Package for Cell Culture Profiling" for monitoring relative abundance of 95 compounds. Using this method package, we demonstrated the change in abundance of culture supernatant (bacteria and streptomyces cells) components for over the culture periods.

Table 1. List of 96 compounds

Internal Standard	**Amino Acid**	Ornithine	**Others**
2-Isopropylmalic acid	2-Aminoadipic acid	Oxidized glutathione	2-Aminoethanol
Sugars	4-Aminobutyric acid	Phenylalanine	2-Ketoisovaleric acid
Gluconic acid	4-Hydroxyproline	Pipecolic acid	3-Methyl-2-oxovaleric acid
Glucosamine	5-Glutamylcysteine	Proline	4-Hydroxyphenyllactic acid
Hexose (Glucose)	5-Oxoproline	Serine	Citric acid
Sucrose	Alanine	Threonine	Ethylenediamine
Threonic acid	Alanyl-glutamine	Tryptophan	Fumaric acid
Nucleic acid	Arginine	Tyrosine	Glyceric acid
Adenine	Asparagine	Valine	Histamine
Adenosine	Aspartic acid	**Vitamins**	Isocitric acid
Adenosine monophosphate	Citrulline	4-Aminobenzoic acid	Lactic acid
Cytidine	Cystathionine	Ascorbic acid	Malic acid
Cytidine monophosphate	Cysteine	Ascorbic acid 2-phosphate	O-Phosphoethanolamine
Deoxycytidine	Cystine	Biotin	Putrescine
Guanine	Glutamic acid	Choline	Pyruvic acid
Guanosine	Glutamine	Cyanocobalamin	Succinic acid
Guanosine monophosphate	Glutathione	Ergocalciferol	
Hypoxanthine	Glycine	Folic acid	
Inosine	Glycyl-glutamine	Folinic acid	
Thymidine	Histidine	Lipoic acid	
Thymine	Isoleucine	Niacinamide	
Uracil	Kynurenine	Nicotinic acid	
Uric acid	Leucine	Pantothenic acid	
Uridine	Lysine	Pyridoxal	
Xanthine	Methionine	Pyridoxine	
Xanthosine	Methionine sulfoxide	Riboflavin	
Antibiotics	N-Acetylaspartic acid	Tocopherol acetate	
Penicillin G	N-Acetylcysteine		

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Methods and Materials

Pretreatment: The culture solution was taken from a culture dish or flask, and then was separated by centrifugation. The culture supernatant was transferred to a new tube and then the internal standard solution was added into the tube. After that acetonitrile was added into the tube and the solution was thoroughly agitated. After centrifugal separation centrifuged supernatant was transfer to a new tube with water and then the solution was thoroughly agitated. and then analyzed by LC-MS/MS.
Instrument: As an LC-MS/MS system, UHPLC was coupled to triple quadrupole mass spectrometer (Nexera MP with LCMS-8050, Shimadzu Corporation, Kyoto, Japan).
LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode with ultra-fast polarity switching.

High Speed Mass Spectrometer

Ultra Fast Polarity Switching
- 5 msec

Ultra Fast MRM
- Max. 555 transition /sec

Figure 1. LCMS-8050 triple quadrupole mass spectrometer

Result

In order to achieve appropriate signals for 95 components in high concentration (glutamine, glucose, etc.) and in trace amounts(such as vitamins), ion source parameters, voltage, data acquisition time and LC condition were optimized. Under optimized condition, all the 95 compounds had good signals, and the analysis can be performed at a rate of 17 minutes per cycle. Bacteria and streptomyces samples were investigated.
Figure 2 showed that it was possible to measure a wide concentration range components in culture supernatant using single method.

UHPLC conditions (Nexera MP system)	
Column	: see "Method Package for Cell Culture Profiling"
Mobile phase A	: 0.1% formic acid in H_2O
B	: 0.1% formic acid in acetonitrile
Flow rate	: 0.35 mL/min
Time program	: see "Method Package for Cell Culture Profiling"
Injection vol.	: 1 µL
Column temperature	: 40 °C
MS conditions (LCMS-8050)	
Ionization	: ESI, Positive/Negative MRM mode
MRM parameters	: see "Method Package for Cell Culture Profiling"

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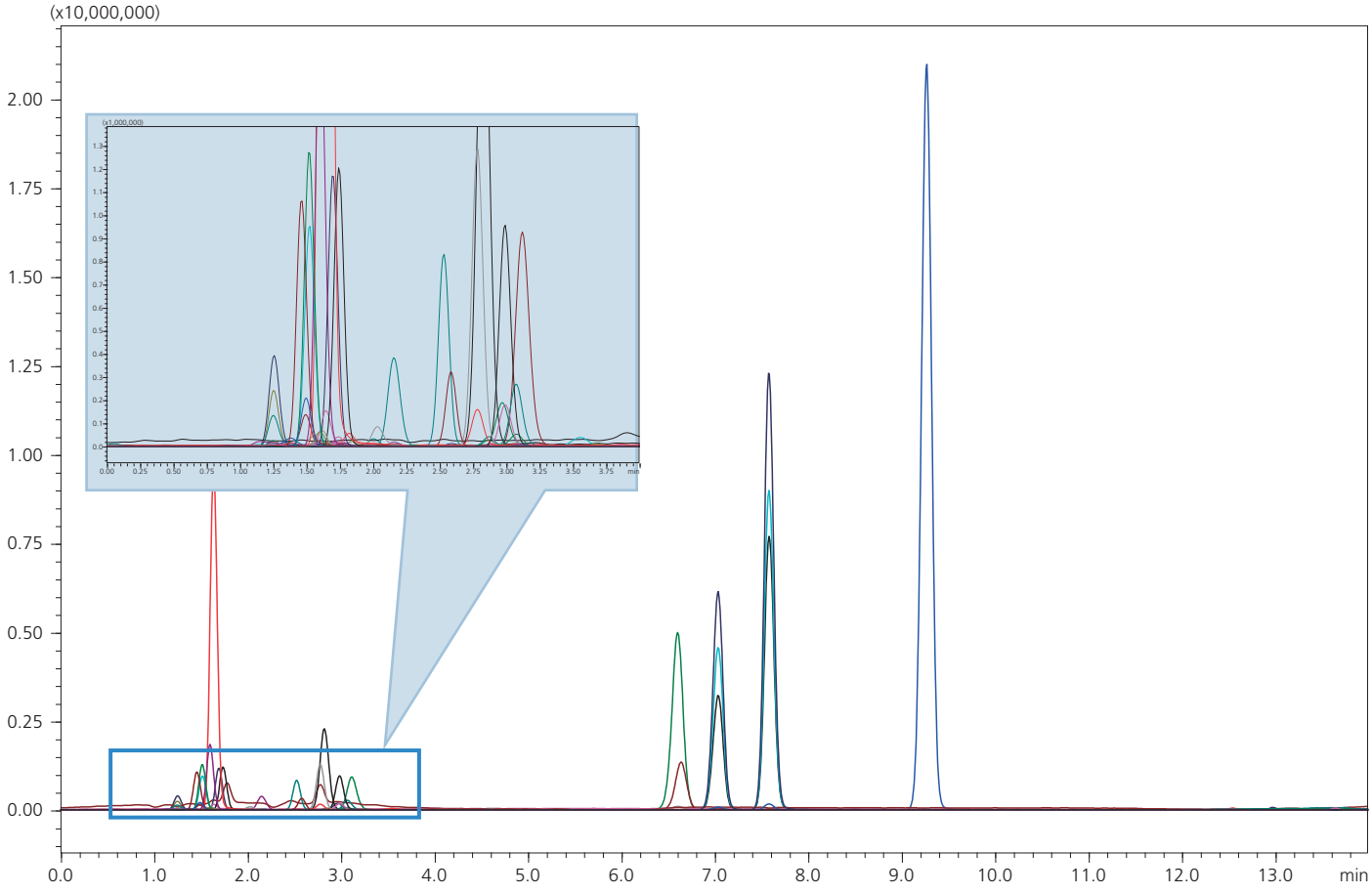

Figure 2. Mass chromatogram of bacteria culture supernatant

Simultaneous analysis of supernatant components of cell culture using triple quadrupole LC/MS/MS

The change trend of compounds in the bacteria culture supernatant over time

The results of culture supernatant of the bacteria showed the change trend of compounds in the supernatant during the culture period of 12 hours. Such as the amount of succinic acid decreased with time; the amount of 2-aminoethanol increased with time, the amount of deoxycytidine increased from 0 hour to 10 hours, and began to decrease after 10 hours; the amount of biotin showed no change (Fig.3-6).

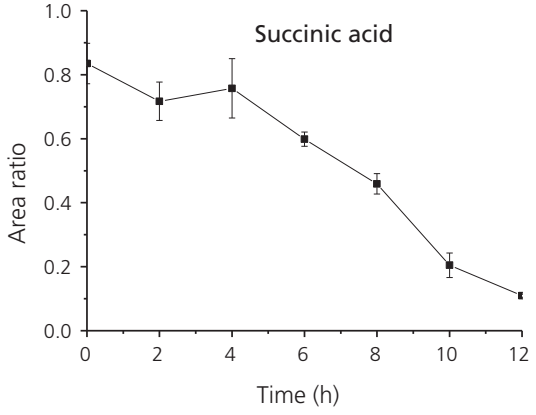

Figure 3. Area ratio of succinic acid change trend

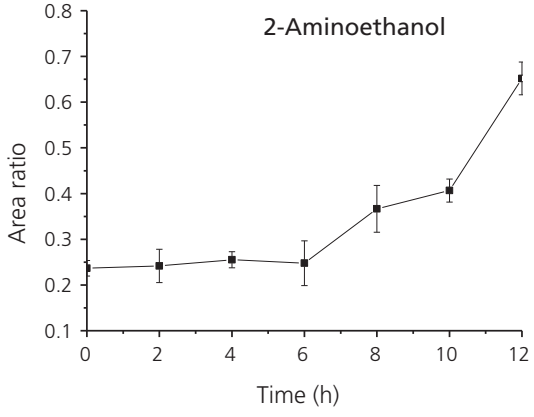

Figure 4. Area ratio of 2-Aminoethanol change trend

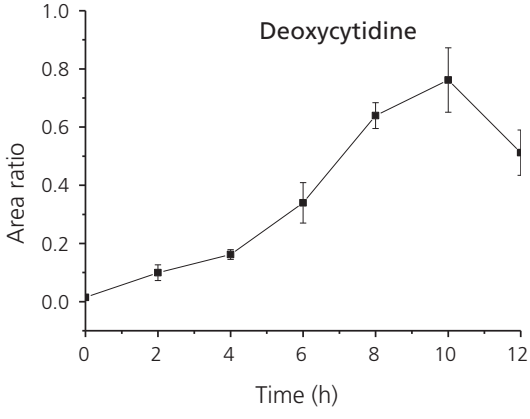

Figure 5. Area ratio of Deoxycytidine change trend

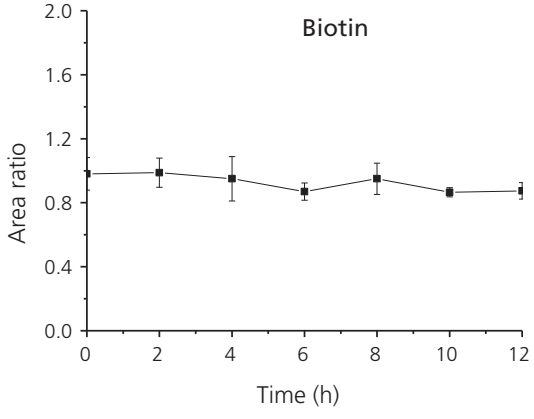

Figure 6. Area ratio of Biotin change trend

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The change trend of compounds in the streptomyces culture supernatant over time

The results of culture supernatant of Streptomyces showed the change trend of compounds in the supernatant during the culture period of 120 hours. Such as the amount of alanine sharply decreased from 0 hour to 72 hours, and stayed almost the same after 72 hours; the amount of citrulline sharply decreased from 0 hour to 72 hours, and began to increase after 72 hours; the amount of cytidine increased from 0 hour to 86 hours, and began to decrease after 86 hours; the amount of gluconic acid increased with time; (Fig.7-10).

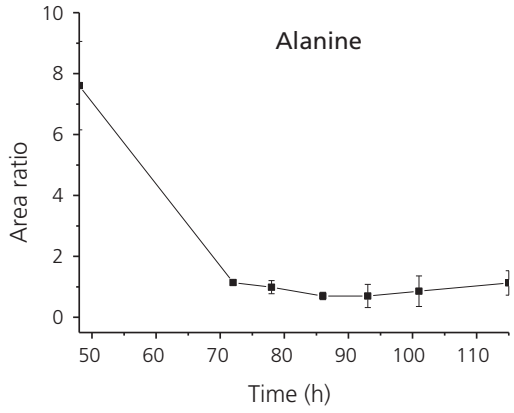

Figure 7. Area ratio of Alanine change trend

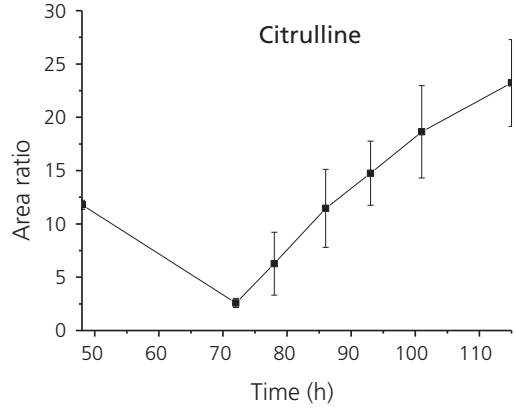

Figure 8. Area ratio of Citrulline change trend

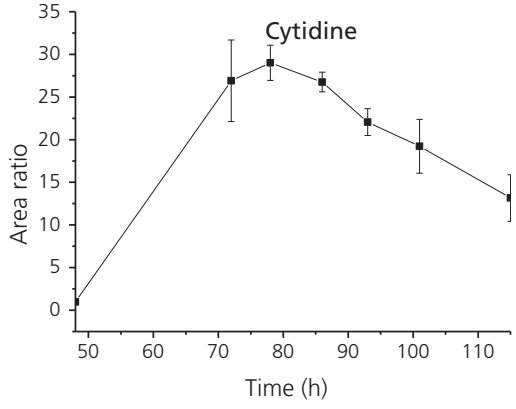

Figure 9. Area ratio of Cytidine change trend

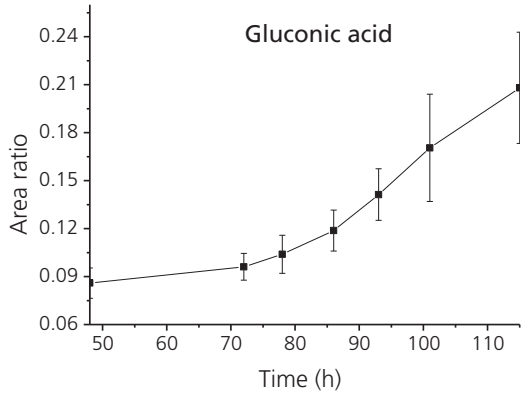

Figure 10. Area ratio of Gluconic acid change trend

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Conclusions

"Method Package for Cell Culture Profiling" enables users to analyze simultaneously multicomponents of culture supernatant using UHPLC coupled with triple quadrupole mass spectrometry.
This package covers analysis method not only for basal medium of cell culture but also metabolites secreted by cells. And the optimized method realizes simultaneous analysis in 17 minutes that include chromatographic time and column equilibration time. This package supports simultaneous analysis of high concentration components and trace components in single analysis.

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