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Application News

SSI-BioTech-002

Liquid Chromatography Mass Spectrometry

A Novel Cell Culture Media Analysis Platform for Culture Process Development

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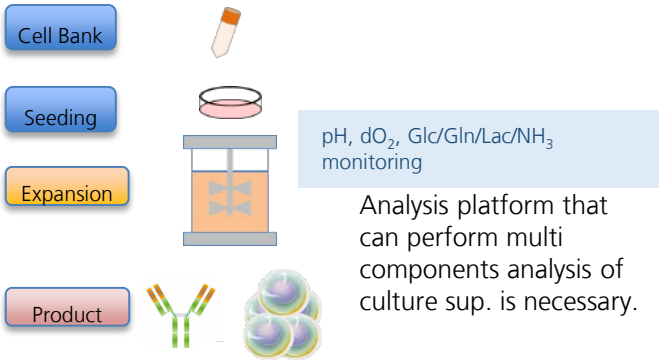
■ Introduction

Optimization and control of cell culture processes are essential to increase production efficiency of biopharmaceuticals. In the field of cell therapy including regenerative medicines, enhanced control of the culture process is also becoming important to reduce cell variability and improve consistency of mass production of the cells. Comprehensive monitoring of culture supernatant components gives researchers useful information for these purposes. However, current technologies for process monitoring are limited to measurement of pH, dissolving gases, and some small compounds such as glucose, glutamine, lactate, and ammonia in culture supernatant.



We have developed a "Cell Culture Media Analysis Platform, C2MAP system" that combines automated pretreatment module for culture supernatant samples with LC/MS/MS. This system can perform automated sample pretreatment and simultaneous analysis of up to 95 compounds including basal medium components and secreted metabolites (The list of target compounds is shown in the column to the right.). This system contains a software that can visualize temporal change in each culture supernatant components through the cell culture.

In this application, we present features of the C2MAP system and its applications.

■ List of Compounds

No.	Compound Name	Class.	No.	Compound Name	Class.	No.	Compound Name	Class.
1	2-Isopropylmalic acid	IS	33	N-Acetylaspartic acid	Amino acid	65	Cytidine	Nucleic acid
2	Gluconic acid	Carbohydrate	34	N-Acetylcysteine	Amino acid	66	Cytidine monophosphate	Nucleic acid
3	Glucosamine	Carbohydrate	35	Ornithine	Amino acid	67	Deoxycytidine	Nucleic acid
4	Hexose (Glucose)	Carbohydrate	36	Oxidized glutathione	Amino acid	68	Guanine	Nucleic acid
5	Sucrose	Carbohydrate	37	Phenylalanine	Amino acid	69	Guanosine	Nucleic acid
6	Threonic acid	Carbohydrate	38	Pipecolic acid	Amino acid	70	Guanosine monophosphate	Nucleic acid
7	2-Aminoadipic acid	Amino acid	39	Proline	Amino acid	71	Hypoxanthine	Nucleic acid
8	4-Aminobutyric acid	Amino acid	40	Serine	Amino acid	72	Inosine	Nucleic acid
9	4-Hydroxyproline	Amino acid	41	Threonine	Amino acid	73	Thymidine	Nucleic acid
10	5-Glutamylcysteine	Amino acid	42	Tryptophan	Amino acid	74	Thymine	Nucleic acid
11	5-Oxoproline	Amino acid	43	Tyrosine	Amino acid	75	Uracil	Nucleic acid
12	Alanine	Amino acid	44	Valine	Amino acid	76	Uric acid	Nucleic acid
13	Alanyl-glutamine	Amino acid	45	4-Aminobenzoic acid	Vitamin	77	Uridine	Nucleic acid
14	Arginine	Amino acid	46	Ascorbic acid	Vitamin	78	Xanthine	Nucleic acid
15	Asparagine	Amino acid	47	Ascorbic acid 2-phosphate	Vitamin	79	Xanthosine	Nucleic acid
16	Aspartic acid	Amino acid	48	Biotin	Vitamin	80	Penicillin G	Antibiotics
17	Citrulline	Amino acid	49	Choline	Vitamin	81	2-Aminoethanol	Other
18	Cystathionine	Amino acid	50	Cyanocobalamin	Vitamin	82	2-Ketoisovaleric acid	Other
19	Cysteine	Amino acid	51	Ergocalciferol	Vitamin	83	3-Methyl-2-oxovaleric acid	Other
20	Cystine	Amino acid	52	Folic acid	Vitamin	84	4-Hydroxyphenyllactic acid	Other
21	Glutamic acid	Amino acid	53	Folinic acid	Vitamin	85	Citric acid	Other
22	Glutamine	Amino acid	54	Lipoic acid	Vitamin	86	Ethylenediamine	Other
23	Glutathione	Amino acid	55	Niacinamide	Vitamin	87	Fumaric acid	Other
24	Glycine	Amino acid	56	Nicotinic acid	Vitamin	88	Glyceric acid	Other
25	Glycyl-glutamine	Amino acid	57	Pantothenic acid	Vitamin	89	Histamine	Other
26	Histidine	Amino acid	58	Pyridoxal	Vitamin	90	Isocitric acid	Other
27	Isoleucine	Amino acid	59	Pyridoxine	Vitamin	91	Lactic acid	Other
28	Kynurenine	Amino acid	60	Riboflavin	Vitamin	92	Malic acid	Other
29	Leucine	Amino acid	61	Tocopherol acetate	Vitamin	93	O-Phosphoethanolamine	Other
30	Lysine	Amino acid	62	Adenine	Nucleic acid	94	Putrescine	Other
31	Methionine	Amino acid	63	Adenosine	Nucleic acid	95	Pyruvic acid	Other
32	Methionine sulfoxide	Amino acid	64	Adenosine monophosphate	Nucleic acid	96	Succinic acid	Other

■ Cell Culture Media Analysis Platform, C2MAP

Cell Culture Media Analysis Platform, C2MAP, is configured from pretreatment module (C2MAP-2000), UHPLC system (Nexea-X2), and triple quadrupole mass spectrometer LCMS-8060/-8050 (Fig.1).

Figure 1: Overview of C2MAP system

After removal of the cells from culture fluid, vials containing cell culture supernatant (400 to 500 mL) are set into the sample rack of C2MAP-2000 (Max. 65 samples). Pretreatment and measurement flow of C2MAP system are shown in Figure 2.

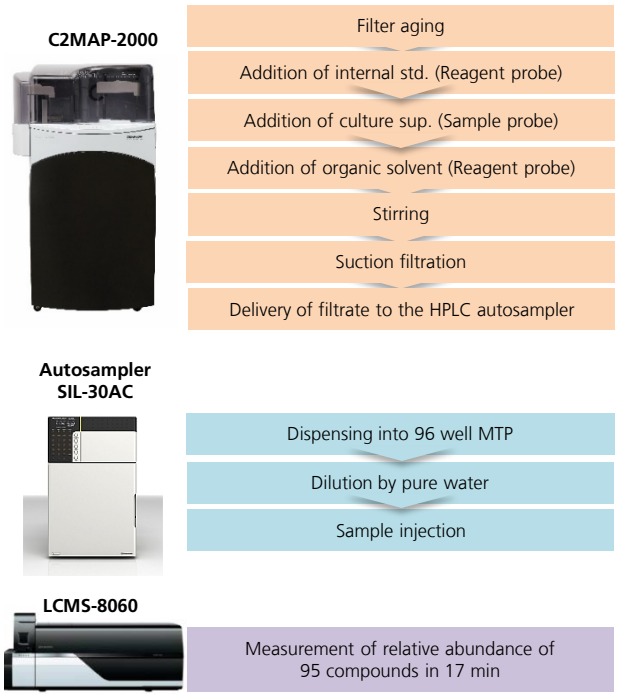


Figure 2: Pretreatment and measurement flow

A dedicated software, C2MAP software, can control both the pretreatment module and LC/MS/MS system, making it possible to carry out seamless analysis and to associate the treated sample and the measurement results easily because pretreatment and analysis are carried out with the common sample ID. The progress of pretreatment and analysis is easily confirmed (Figure 3).

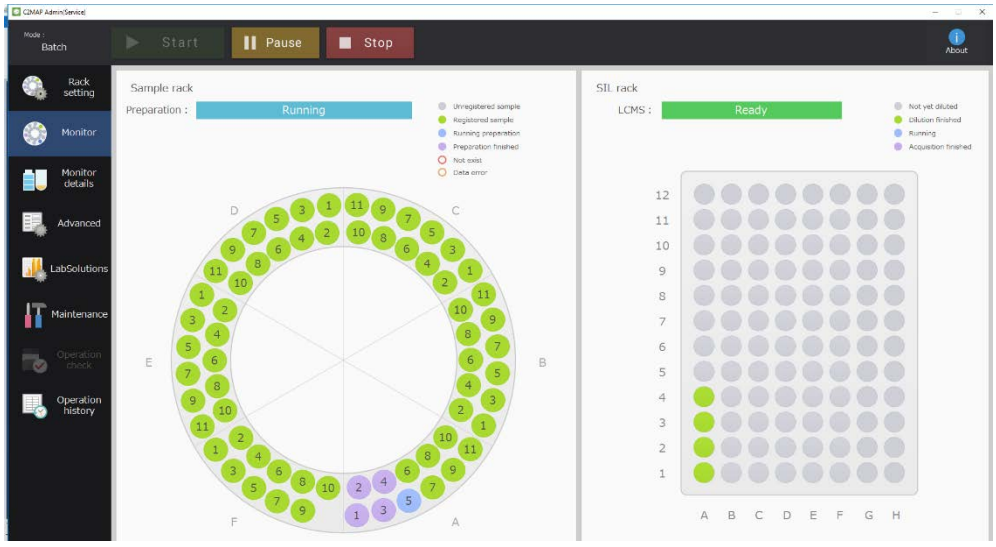


Figure 3: C2MAP software

Temporal changes in each component can be graphed with the dedicated viewer software, C2MAP TRENDS, using LC/MS/MS data set. Analysts can monitor variations in basal media components and secreted metabolites during cultivation, as well as display graphs of component comparisons with samples from different culture series. These observations can provide useful insights into considerations of the optimal culture conditions and the culture process.

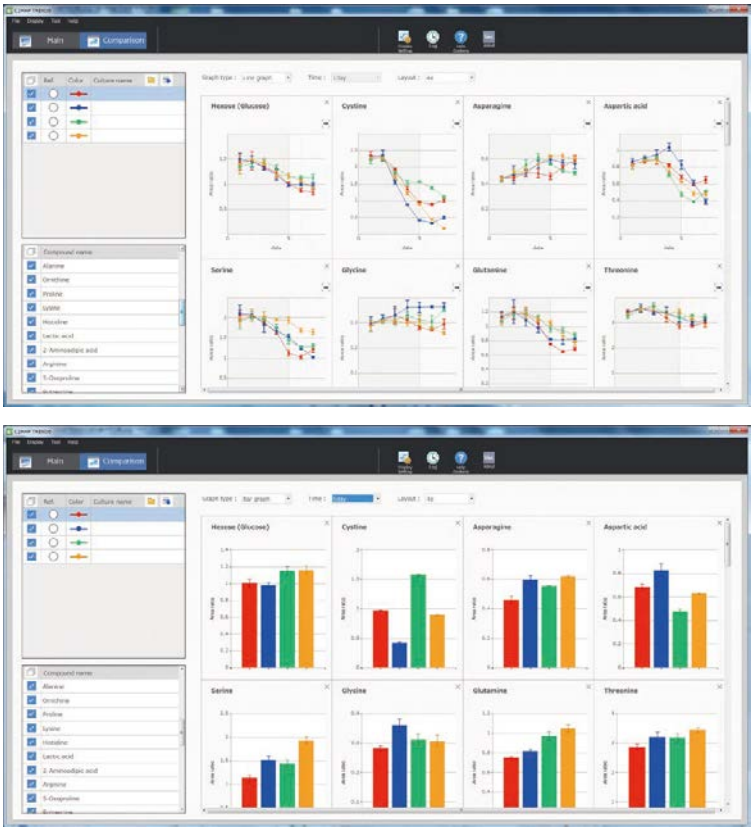


Figure 4: C2MAP TRENDS

■ Results and Discussion

Pluripotent stem cells (PSCs) have a feature maintaining undifferentiated state. In this experiment, C2MAP system was used to compare the temporal changes in the culture supernatant components in undifferentiated human iPS cells and its differentiated counterparts. As a result, significant difference could be found in the time course of some compounds (Fig.5). We think these compounds can be marker candidates for culture process management.

Fetal bovine serum (FBS) often affect cell growth. In this experiment, detection of component amount variation among the product lots was tested. Three different lots of FBS were analyzed by C2MAP system. We could detect 56 compounds from FBS sample. Overall pattern of mass chromatogram from each lot was similar, whereas significant differences were detected in some compounds (Fig.6).

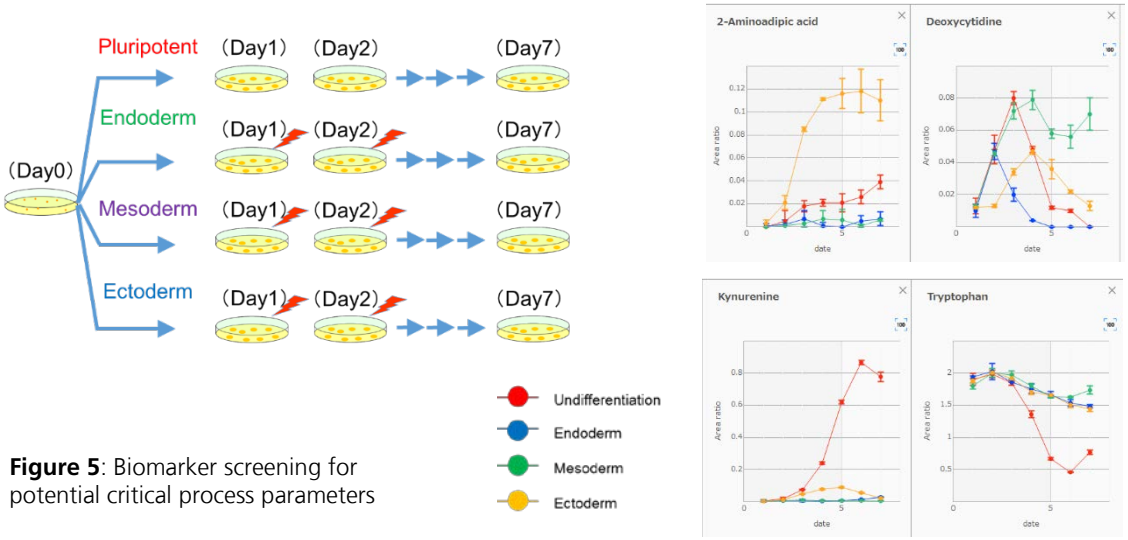


Figure 5: Biomarker screening for potential critical process parameters

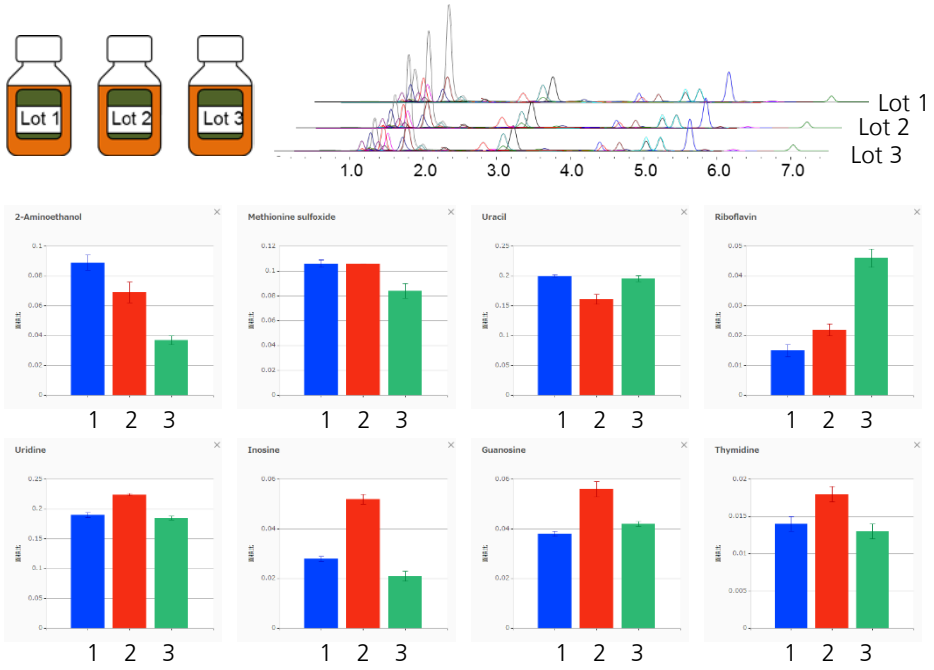


Figure 6: Evaluation of lot to lot variation of FBS

■ Conclusion

Through multicomponent monitoring of the culture supernatant using C2MAP system, various useful information can be obtained. This information provides useful insights into optimization of the culture media composition and the culture process.

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