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A novel cell culture media analysis platform for culture process development

ASMS 2018 MP371

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Overview

We have developed a novel cell culture media analysis platform, C2MAP, that can carry out automated deproteinization of cell-free culture supernatant samples and simultaneous analysis of up to 95 compounds found in basal media components and secreted metabolites using triple quadrupole LC-MS/MS.

Introduction

Optimization and control of cell culture processes are essential to increase production efficiency of biopharmaceuticals. In the field of cell therapy, 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. We have developed a "Cell Culture Media Analysis Platform, C2MAP system" that can perform automated sample pretreatment and simultaneous analysis of up to 95 compounds listed below. We also developed a viewer software that can easily visualize temporal change in each measured compounds through the cell culture. In this poster, we present features of C2MAP system and its applications.

List of Compounds

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

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MAX 65 samples
28 min/ 1 sample
Required sample volume: 400-500 µL

Figure 1　Cell Culture Media Analysis Platform

C2MAP-2000

Autosampler
SIL-30AC

LCMS-8060

Filter aging
Addition of internal std. (Reagent probe)
Addition of culture sup. (Sample probe)
Addition of organic solvent (Reagent probe)
Stirring
Suction filtration
Delivery of filtrate to the HPLC autosampler

Dispensing into 96 well MTP
Dilution by pure water
Sample injection

Measurement of relative abundance of
95 compounds in 17 min

Fig.2　Pretreatment and measurement flow

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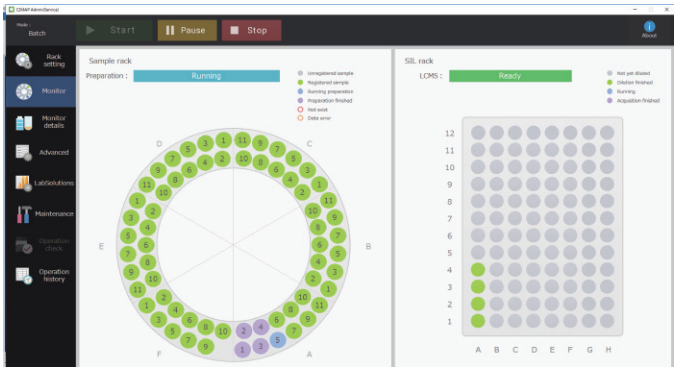

Fig.3 C2MAP software

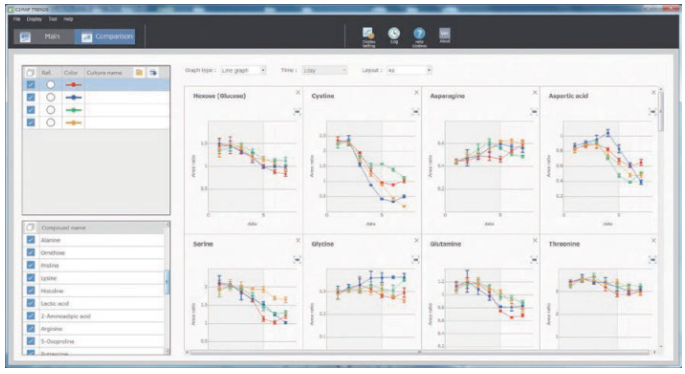

Fig.4 C2MAP TRENDS

A dedicated software, C2MAP software, can control both 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.

Tempral changes in each component can be graphed with the dedicated viewer software, C2MAP TRENDS, using LC/MS/MS data set. Users can monitor variations in basal media components and secreted metabolites during cultivation.

Materials and Methods

Various media analysis using C2MAP

Various kinds of mammalian cell culture media showed in Table I were analysed with C2MAP. IgG was added to the CHO cells media (final conc. at 10 mg/ mL) prior to analysis. Fetal bovine serum (FBS) often affects cell growth.

Detection of component amount variation among the product lots was also tested using three different lots of FBS.

Analysis for culture supernatant of human iPS cells

A spent medium of human iPS cells was collected every 24 hrs. The temporal changes in the components in the culture supernatant were monitored using the C2MAP system. Maintaining undifferentiated state is one of important characters of iPS cells. 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.

Results

Applicability of C2MAP for culture media analysis

The following cell culture media showed in Table I were analysed (six replicates each). Coefficient of variations for all detected compounds from tested media were less than 10% (data not shown).

Table I. Tested cell culture media

Cell Type	CHO cells	iPS/ES cells	T cells	Mesenchymal stem cells
Culture Media	BalanCD® CHO	AK03N	X-VIVO™ 10	MSCBM
	1×CD CHO	Essential-8™	X-VIVO™ 15	MesenPRO™
	EX-CELL® CHO	mTeSR™1 / TeSR™-E8™	TexMACS™	Stempro®

Fifty-six compounds were detected from FBS. Overall pattern of mass chromatogram from each lot was similar, whereas significant differences were detected in some compounds.

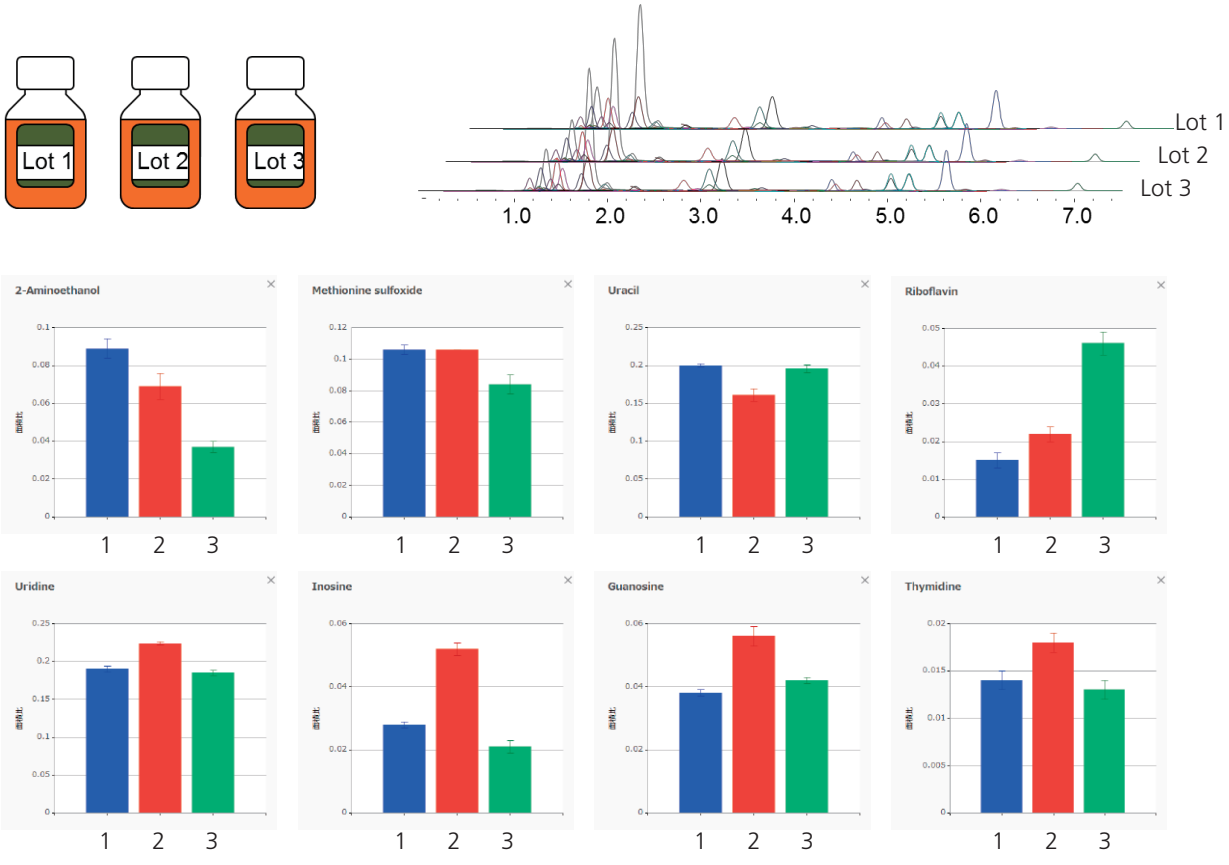

Fig.5 Evaluation of lot to lot variation of FBS

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Spent media analysis of human iPS cells

Spent media analysis showed which medium components were favoured and consumed by cells, and which metabolites were secreted by cells. This information provides useful insights into optimization of the culture media composition and the culture process.

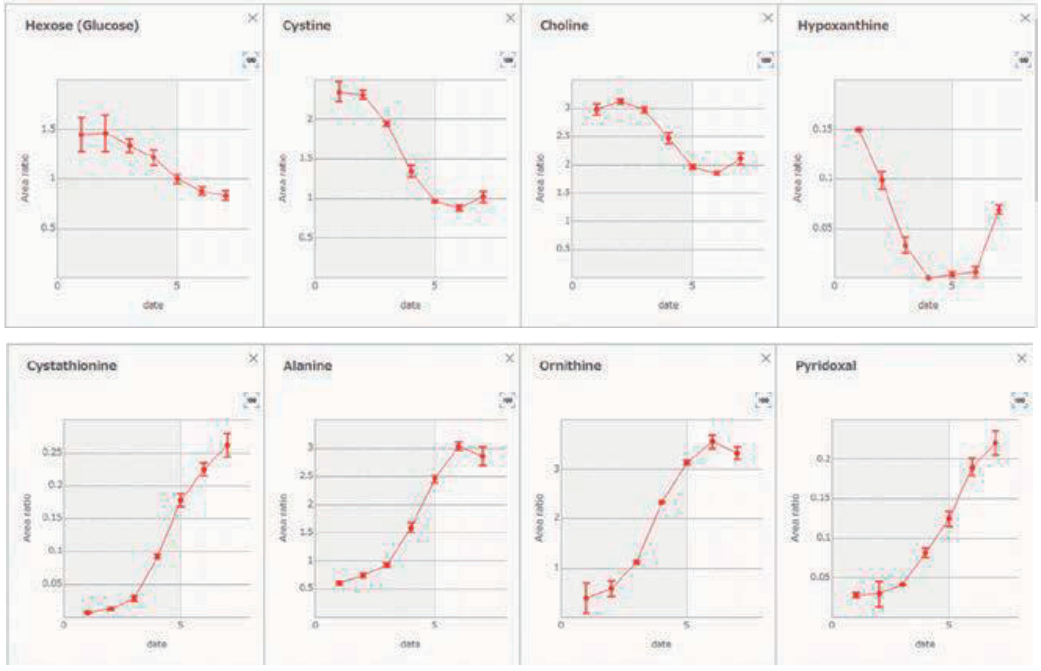

Fig.6 Time course of components in culture supernatant

C2MAP system was used to find biomarker candidates that can evaluate cell differentiation state using cell culture supernatant as the sample. Three germ layers differentiation was induced by the addition of appropriate cytokines and chemicals. Spent media was collected every 24 hours and analyzed with C2MAP. As a result, significant difference could be found in the time course of some compounds.

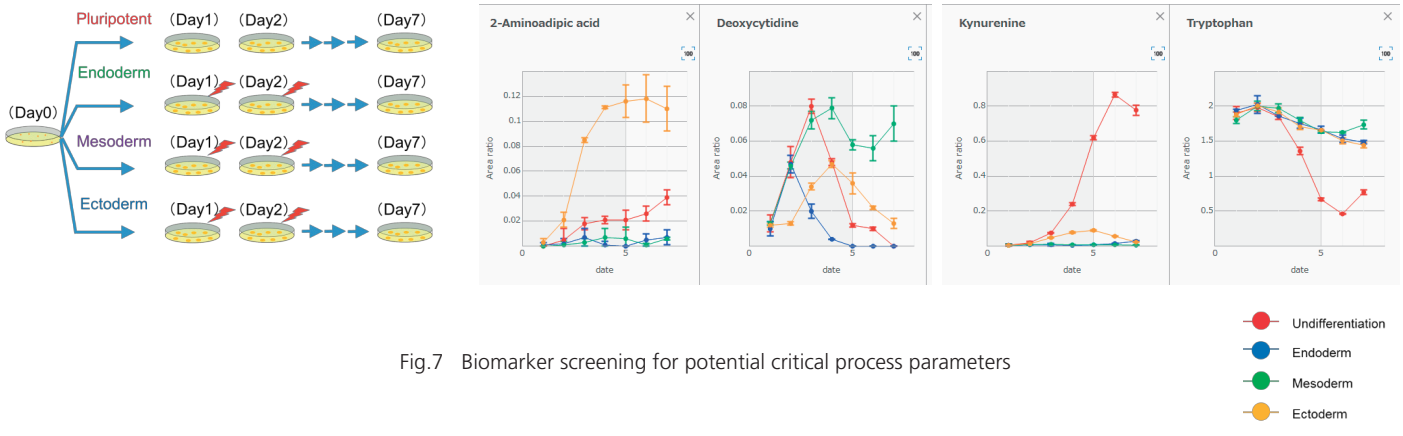

Fig.7 Biomarker screening for potential critical process parameters

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Conclusion

- C2MAP is applicable for analysis of wide range of mammalian cell culture media.
- C2MAP can give useful insights into optimization of the culture media composition and the culture process.
- Multi components analysis using C2MAP is also useful for finding for potential critical process parameters.

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First Edition: June, 2018

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