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Application News

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Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of Culture Supernatant of Mammalian Cells Using Triple Quadrupole LC/MS/MS

Industrial fermentation for the production of biofuels or biopharmaceutics requires routine monitoring of medium conditions such as pH, dissolved gas, carbon source (glucose) and nitrogen source (glutamine) 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 component, we optimized the analytical conditions and developed this "Method Package for Cell Culture Profiling" that can monitor relative abundance of 95 compounds listed herein. Using this Method Package, we demonstrated the change in abundance of culture medium components associated with hybridoma growth over a period of 5 days.

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

HPLC Conditions

Column	: Discovery HS F5-3 (150 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phase A	: 0.1 % Formic Acid aq.
Mobile Phase B	: 0.1 % Formic Acid in Acetonitrile
Time Program	: 0 %B. (0-1.4 min) → 25 %B. (3.5 min) → 35 %B. (7.5 min) → 95 %B. (10.3-13.7 min) → 0 %B. (13.8-17.0 min)
Flowrate	: 0.35 mL/min.
Injection Volume	: 1 µL
Column Oven Temp.	: 40 °C

MS Conditions (LCMS-8050)

Ionization	: ESI (Positive / Negative)
Nebulizer Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C
Interface Temp.	: 300 °C

A murine hybridoma cell line was cultured in DMEM (see Table 1 for conditions) and its culture supernatant was sampled every 24 hours for 5 days after inoculation. LCMS sample was prepared by adding an internal standard to the sample and then removing proteins by taking supernatant after mixing with acetonitrile, which was further diluted with ultrapure water prior to injection. 1 µL was injected to LCMS for simultaneous MRM quantitation of all 96 compounds. Fig. 1 shows a growth curve and viability plot of the cell line, and Fig. 2 shows the quantitative value (ratio of peak area with respect to internal standard) of representative compounds over 5 days.

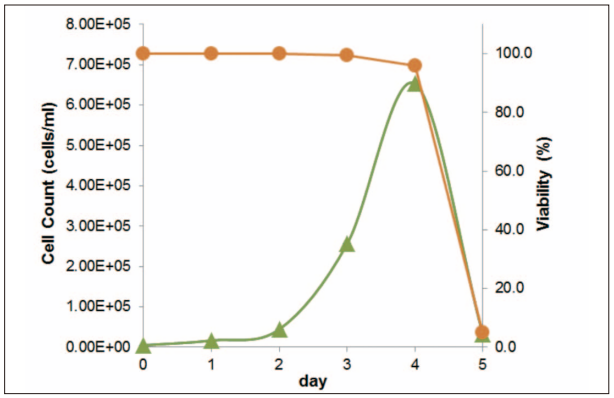

Fig. 1 Growth Curve and Viability of Cell Culture

Table 1 Cell Culture Conditions

Cell line	: SJK-287-38 (ATCC® CRL-1644™)
Medium	: DMEM (Low Glucose) + 10 % FBS + Gln, $NaHCO_3$)
Condition	: 37 °C, 5 % CO_2, cells pelleted at 120 rpm
Scale	: 24 mL (n = 4)

The culture supernatant sample and Fig. 1 was courteously provided by Kyokuto Pharmaceutical Industrial Co., Ltd.

Representative results are shown below. (A) Glucose, glutamine and few other amino acids, which are the primary sources of carbon and nitrogen, have decreased in abundance with growing cell number. (B) In contrast, lactic acid increased in abundance over time as a result of glucose consumption for anaerobic respiration. Similar pattern of increase was observed for a few other compounds. (C) No change in relative abundance was observed for essential amino acids and some vitamins.

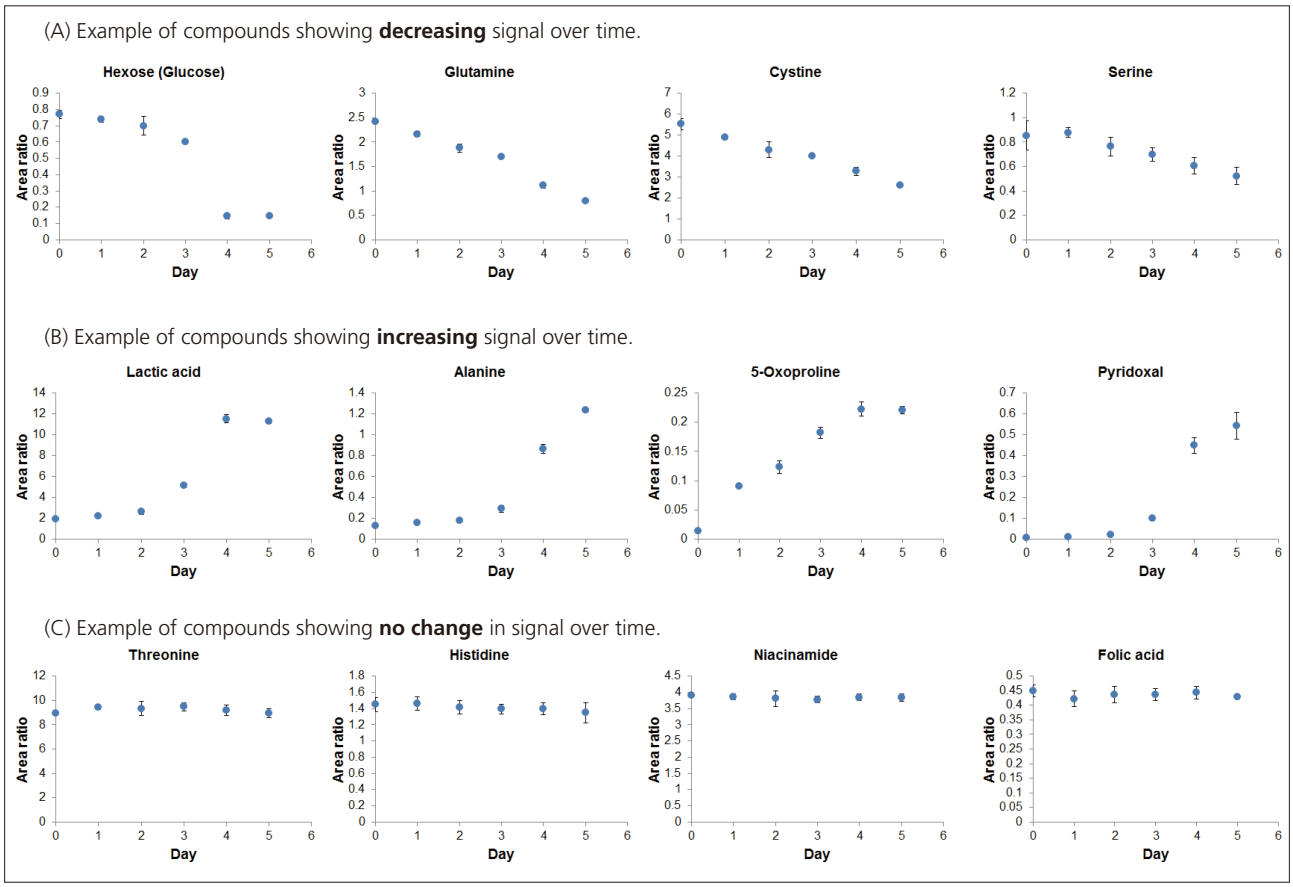

Fig. 2 Monitoring the Change in Culture Supernatant Components with Culture Time

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