

# Application News

## No. C106

### 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 biopharmaceuticals 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.	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	Threonine 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

### HPLC Conditions

Column	: Discovery HS F5-3 (150 mm L. x 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.

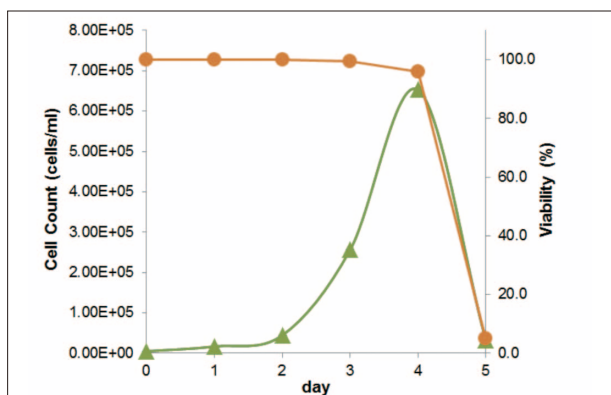


Fig. 1 Growth Curve and Viability of Cell Culture

Table 1 Cell Culture Conditions

Cell line	: SJK-287-38 (ATCC® CRL-1644™)
Medium	: DMEM (Low Glucose) + 10 % FBS + Gln, NaHCO <sub>3</sub>
Condition	: 37 °C, 5 % CO <sub>2</sub> , 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.

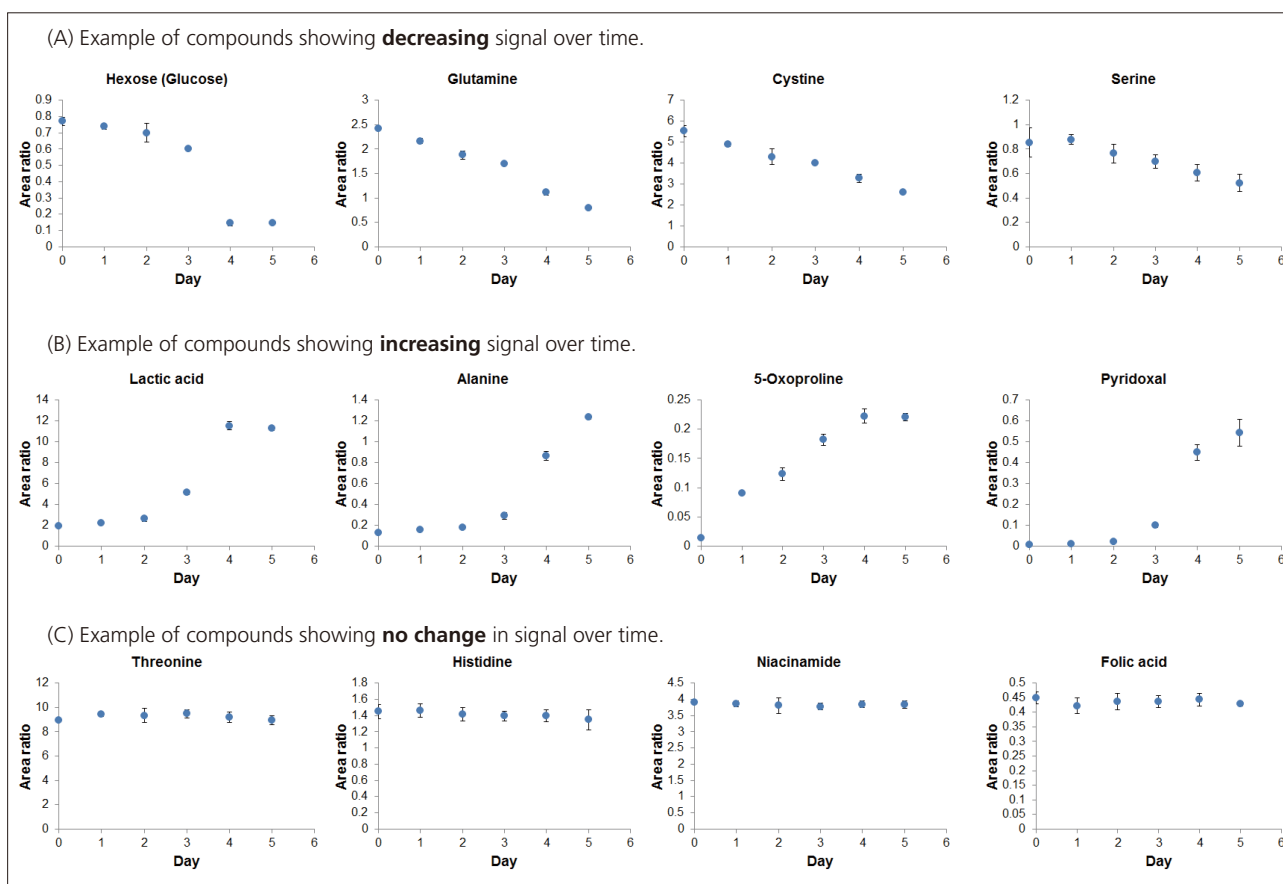


Fig. 2 Monitoring the Change in Culture Supernatant Components with Culture Time