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应用纪要

Targeted Quantification of Cell Culture Media Components by LC-MS

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

An LC-MS/MS method library was developed for the quantitative analysis of components in cell culture medium and spent cell culture medium. This method, which covers a wide range of compounds including amino acids, vitamins, nucleoside pyrimidines and purines, nucleotides, organic acids, polyamines, and carbohydrates, enables the analysis of cell culture medium and the supernatant from the spent cell culture medium in multiple aspects. Meanwhile, its excellent reproducibility renders relative quantification of spent cell culture medium even when standards are not available.

Benefits

- A fast and universal LC-MS/MS method for media component analysis of cell culture medium and spent cell culture samples.
- Relative quantification of metabolites in spent cell culture medium can be achieved by this method when standards are not available.
- No derivatization is required for sample preparation.

Introduction

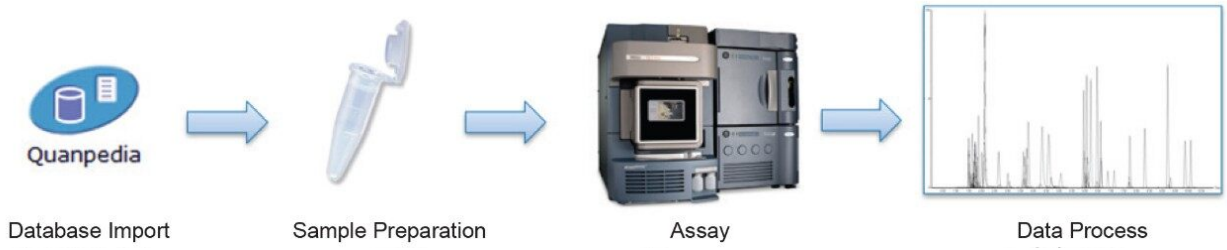
Cell culture medium components and feeding strategy are important variables in upstream bioprocess development that must be optimized due to their direct impact on the quality and titer of recombinant therapeutic products. A comprehensive and quantitative understanding of cellular metabolic components could facilitate more robust bioprocess development that ensures productivity and quality. Thus, it is of great significance for the establishment of an efficient analytical method to monitor the changes of various components in cell culture medium and in spent cell culture medium during the cell culture process.

In order to quickly and comprehensively analyze the components of raw and spent cell culture medium, and intracellular substance, we developed a method library for the analysis of raw and spent cell culture medium, which comprises two LC methods and covers hundreds of compounds. Method one is mainly used for amino acids, vitamins, nucleoside pyrimidines and purines, organic

acids, monophosphate nucleotides, polyamines, and carbohydrates, and method two is mainly used for nucleotides. Due to the diversity of these compounds and the large differences of their chemical properties, conventional assays are extremely complex requiring the use of different instruments and different sample preparation protocols, which makes it difficult to meet the throughput and standardization requirements of the biopharm industry. On the other hand, the ultra-high performance liquid chromatography-mass spectrometry system can provide a fast and highly specific method for the component analysis of cell culture medium, spent cell culture medium, and intracellular substance.

Experimental

EXPERIMENTAL PROCESS



Compound list

The compounds contained in the method library are listed in Table 1.

Compound	Category	Compound	Category	Compound	Category	Compound	Category
L-Tyrosine	Amino acid	D-biotin	Vitamin	Inosine	Nucleoside pyrimidine/purine	CTP	Nucleotide
L-Aspartic Acid	Amino acid	Folic acid	Vitamin	Thymidine	Nucleoside pyrimidine/purine	CDP	Nucleotide
L-Glutamic Acid	Amino acid	Riboflavin	Vitamin	Uridine	Nucleoside pyrimidine/purine	CMP	Nucleotide
L-Alanine	Amino acid	Myo-inositol	Vitamin	Cytidine	Nucleoside pyrimidine/purine	UDP-glucose	Sugar nucleotide
L-Arginine	Amino acid	Niacinamide	Vitamin	Adenosine	Nucleoside pyrimidine/purine	UDP-galactose	Sugar nucleotide
Glycine	Amino acid	Calcium pantothenate	Vitamin	Guanosine	Nucleoside pyrimidine/purine	UDP-GlcNAc	Sugar nucleotide
L-Histidine	Amino acid	Pyridoxine	Vitamin	Uracil	Nucleoside pyrimidine/purine	UDP-GalNAc	Sugar nucleotide
L-Isoleucine	Amino acid	Thiamine	Vitamin	Cytosine	Nucleoside pyrimidine/purine	GDP-glucose	Sugar nucleotide
L-Leucine	Amino acid	Choline chloride	Vitamin	Thymine	Nucleoside pyrimidine/purine	CMP-sialic acid	Sugar nucleotide
L-Lysine	Amino acid	Vitamin B-12	Vitamin	Guanine	Nucleoside pyrimidine/purine	GSH	Other
L-Methionine	Amino acid	Ascorbic Acid	Vitamin	Adenine	Nucleoside pyrimidine/purine	GSSG	Other
L-Phenylalanine	Amino acid	Thioctic acid	Vitamin	Hypoxanthine	Nucleoside pyrimidine/purine	NAD+	Other
L-Proline	Amino acid	4-aminobenzoic acid	Vitamin	Xanthine	Nucleoside pyrimidine/purine	NADP+	Other
L-Serine	Amino acid	Sodium Pyruvate	Organic acid	Spermine	Polyamine		
L-Threonine	Amino acid	Citric acid	Organic acid	2-Aminoethanol	Polyamine		
L-Valine	Amino acid	Malic acid	Organic acid	Putrescine	Polyamine		
L-Cystine	Amino acid	α-ketoglutaric acid	Organic acid	ATP	Nucleotide		
Taurine	Amino acid	Fumarate	Organic acid	ADP	Nucleotide		
L-Asparagine	Amino acid	Lactic acid	Organic acid	AMP	Nucleotide		
L-Cysteine	Amino acid	Isocitrate	Organic acid	GTP	Nucleotide		
L-Glutamine	Amino acid	Succinic acid	Organic acid	GDP	Nucleotide		
L-Tryptophan	Amino acid	Glucosamine	Carbohydrate	GMP	Nucleotide		
N-Acetyl-L-cysteine	Amino acid	Sucrose	Carbohydrate	UTP	Nucleotide		
L-Ornithine	Amino acid	D-Gluconate	Carbohydrate	UDP	Nucleotide		
L-Citrulline	Amino acid	Mannitol	Carbohydrate	UMP	Nucleotide		

Table 1. Compounds contained in the method library.

Sample preparation protocol for cell culture medium: the culture medium sample was diluted by water (containing 0.1% acetic acid) before direct injection. The dilution factor was determined based on the concentration of the compound in the sample;

Sample preparation protocol for the supernatant of spent cell culture media: performing protein precipitation with 2 volumes of acetonitrile, centrifuge at 14000 rpm for 5 min, and take the supernatant and dilute 100 time with water (containing 0.1% acetic acid) before injection for analysis.

LC conditions

System: ACQUITY UPLC I-Class

LC method one:

Column:	ACQUITY UPLC HSS T3 (2.1 x 150 mm, 1.8 µm)
Column temperature:	40 °C
Flow rate:	0.2 mL/min
Injection volume:	1 µL
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B	Curve
0.0	100	0	
1.5	100	0	6
6.0	90	10	6
9.0	65	35	6
11.0	5	95	6
14.0	5	95	6
14.1	100	0	6
17.0	100	0	6

LC method two:

Column:	ACQUITY UPLC BEH Amide (2.1 x 100 mm, 1.7 µm)
Column temperature:	25 °C

Flow rate:	0.3 mL/min
Injection volume:	1 µL
Mobile phase A:	ACN/H_2O (v:v 95/5) with 10 mM Ammonium bicarbonate (pH=9)
Mobile phase B:	ACN/H_2O (v:v 5/59) with 10 mM Ammonium bicarbonate (pH=9)

Gradient:

Time	%A	%B	Curve
0.0	99	1	
0.1	99	1	6
6.0	30	70	6
7.0	30	70	6
7.01	99	1	6
10.0	99	1	6

MS conditions

Mass spectrometer:	Xevo TQ-S micro
Acquisition mode:	ESI Positive/Negative switching
Capillary voltage:	2.5 kV
Ion source temperature:	150 °C
Nebulizer gas temperature:	500 °C

Nebulizer gas flow rate:	1000 L/h
Data processing software:	MassLynx v4.2 with TargetLynx

Results and Discussion

Figure 1 is the ion chromatograms of the compounds contained in the method library.

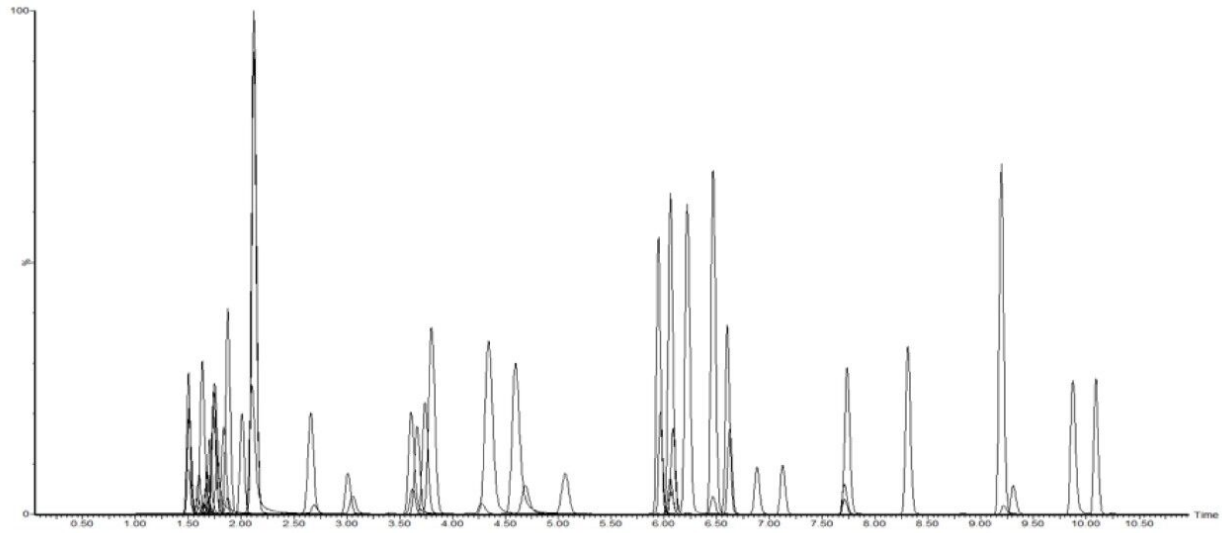


Figure 1. Chromatograms of the compounds contained in the method library.

Chromatograms of two pairs of isomers contained in the analysis package, i.e., citric acid/isocitric acid and leucine/isoleucine, are shown in Figure 2.

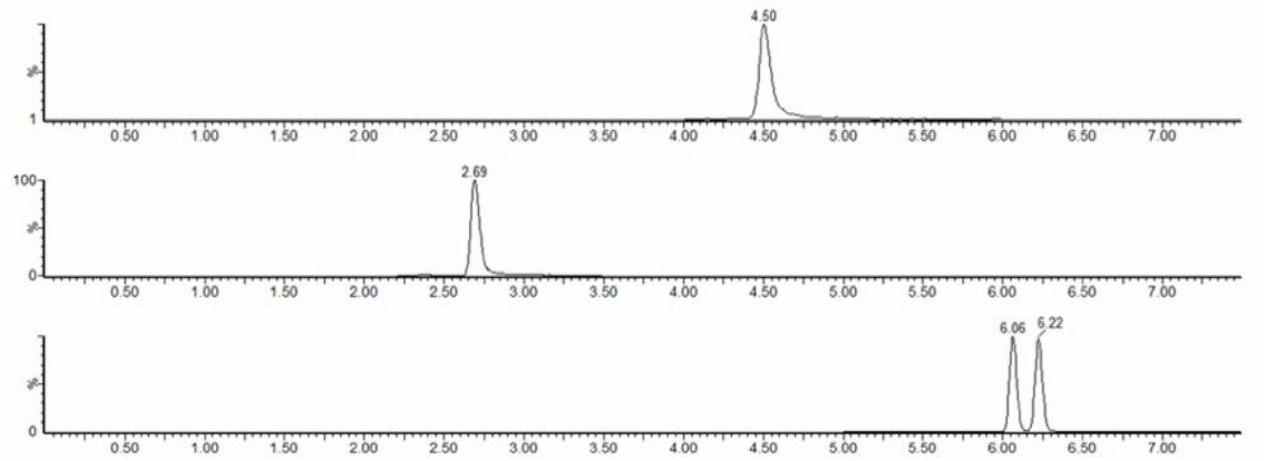


Figure 2. Chromatograms of two pairs of isomers contained in the analysis package, i.e., citric acid/isocitric acid and leucine/isoleucine.

Linear Result

Calibration curves were constructed for compounds that are commonly used in culture medium. The linear range, and reproducibility of retention time and peak area obtained from 6 consecutive injections are shown in Table 2 below.

Compound	Linear range (ng/mL)	R^2	Retention Time RSD%	Peak Area RSD%
Glycine	10.0–1000	0.9977	0.0	2.0
L-Alanine	3.0–300	0.9945	0.2	3.4
L-Serine	1.0–300	0.9951	0.0	2.5
L-Proline	0.3–300	0.9969	0.0	1.4
L-Valine	0.3–300	0.9971	0.0	1.9
L-Threonine	0.3–300	0.9923	0.2	2.3
Taurine	3.0–300	0.9953	0.0	1.4
L-Isoleucine	0.3–300	0.9942	0.0	1.9
L-Leucine	1.0–300	0.9978	0.0	1.9
L-Ornithine	0.3–300	0.9964	0.0	1.7
L-Asparagine	1.0–300	0.9927	0.0	1.8
L-Aspartic acid	0.3–300	0.9993	0.0	2.4
L-Glutamine	0.3–300	0.9976	0.0	2.2
L-Lysine	0.3–300	0.9918	0.0	2.6
L-Methionine	0.1–1000	0.9952	0.0	2.4
L-Histidine	1.0–1000	0.9973	0.0	1.5
N-Acetyl-L-Cysteine	1.0–3000	0.9979	0.0	1.4
L-Phenylalanine	0.3–1000	0.9945	0.1	1.8
L-Arginine	0.1–1000	0.9961	0.0	1.2
L-Citrulline	0.1–300	0.9936	0.0	4.0
L-Tyrosine	1.0–1000	0.9979	0.1	1.7
L-Tryptophan	0.3–3000	0.9951	0.0	1.9
L-Glutamic acid	10.0–3000	0.9964	0.0	2.3
L-Cystine	1.0–300	0.9960	0.0	4.5
L-Cysteine	1.0–300	0.9950	0.0	3.5
D-biotin	0.1–1000	0.9969	0.0	1.9
Folic acid	0.3–100	0.9916	0.0	1.3
Riboflavin	0.3–3000	0.9981	0.1	1.5
Myo-inositol	3.0–3000	0.9927	0.0	4.9
Niacinamide	0.3–1000	0.9959	0.1	1.6
Calcium pantothenate	0.1–3000	0.9980	0.0	2.0
pyridoxine	0.1–100	0.9949	0.1	1.8
Pyridoxal	0.3–300	0.9964	0.0	2.1
Thiamine	0.3–300	0.9977	0.0	1.7
Choline chloride	0.3–300	0.9900	0.0	2.4
Vitamin B-12	3.0–1000	0.9938	0.0	1.8
Thioctic acid	0.3–3000	0.9953	0.0	1.2
PABA	0.1–1000	0.9950	0.0	3.7
Inosine	0.1–300	0.9962	0.0	0.9
Thymidine	3.0–1000	0.9978	0.0	1.5
Uridine	0.3-100	0.9953	0.1	2.7
Guanosine	0.1–300	0.9946	0.1	1.3
Uracil	0.1–300	0.9954	0.0	2.9
Cytosine	0.1–1000	0.9954	0.0	2.3
Thymine	0.3–3000	0.9950	0.0	2.0
Guanine	0.3–1000	0.9959	0.1	0.9
Adenine	0.1–300	0.9944	0.0	1.1
Hypoxanthine	0.1–1000	0.9925	0.1	1.9
Xanthine	0.3–1000	0.9933	0.1	1.9
Sodium Pyruvate	30.0–3000	0.9947	0.2	1.9
Citric acid	500.0–30000	0.9901	0.1	2.6
Malic acid	30.0–3000	0.9902	0.2	3.7
α-ketoglutaric acid	5.0–3000	0.9971	0.2	3.1

- The method library is imported by using Quanpedia database which contains information about compound retention time, MRM, mobile phase, elution gradient, and column. When performing method import, the LC gradient method, MS method, and quantitation method can be imported simultaneously in just three steps. The Quanpedia database is an open database that can be expanded at any time according to customer needs.

References

1. Giuseppe Paglia[1], James Langridge[2], and Giuseppe Astarita[3], Development of a Metabolomic Assay for the Analysis of Polar Metabolites Using HILIC UPLC/QTof MS. [1]Center for Systems Biology, University of Iceland, Iceland; [2–3]Waters Corporation, Manchester, UK, and Milford, MA, USA.

2. Sunil U. Bajada, Wenyun Lua, Elizabeth H. Kimballa, Jie Yuana, Celeste Peterson[b], Joshua D. Rabinowitz[a], Separation and Quantitation of Water Soluble Cellular Metabolites by Hydrophilic Interaction Chromatography- Tandem Mass Spectrometry. [a]Lewis-Sigler Institute for Integrative Genomics and Department of Chemistry, Princeton University, Princeton University, Princeton, NJ 08544, USA; [b]Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA. Received 13 January 2006; received in revised form 8 May 2006; accepted 10 May 2006; available online 6 June 2006.

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