A High Throughput LC-MS Method for Cell Culture Media Nutrient and Metabolite Analysis Supporting Upstream Bioprocessing

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INTRODUCTION

A 9-minute rapid and direct liquid chromatography and mass spectrometry (LC-MS) analysis method for cell culture media (CCM) nutrients and metabolites covering 220+ compounds is described. The method is based on reversed phase chromatography where amino acids are directly detected without derivatization. This rapid method reduces turn-around time for monitoring and decision making using previously established user-friendly data review workflows and access to multivariate data analytics (MVDA). The method has been applied for the qualitative and quantitative determination of cell culture media nutrient and metabolite analysis in commercial cell culture media and spent media from process optimization in cNISTmAb production using NISTCHO cells. Monitoring of glucose directly from media using this method was also investigated and described.

METHODS

Spent media samples were generated by Waters Immerse Delaware center where a fed-batch bioprocess experiment was carried out using NISTCHO cell line to produce cNISTmAb product in CHO Fed-batch medium. NISTCHO cell line (RGTM 10971 NISTCHO Test material) was obtained from nist.gov (https://www.nist.gov/programs-projects/nistcho).

All sample preparation from clarified media solution and standards were performed using the Andrew+™ Pipetting Robot. Spent media samples were diluted with 1:400 (V:V) using 0.1% formic containing 0.1 µM stable isotope labeled Tyrosine as the internal standard. Standard calibration solutions were prepared by serial dilution of Waters™ Amino Acid Cell Culture Standards. Analysis was performed on the BioAccord™ LC-MS System (Waters Corporation, USA) using waters_ connect™ Informatics System (ref. 1 and 2).

References

1. Introducing a Rapid Throughput LC-MS Method for Cell Culture Media Nutrient and Metabolite Analysis Supporting Upstream Bioprocessing. Waters application note: P/N **720008170**

2. Simplifying Bioreactor In-Process Monitoring with Waters Bioprocess Walk-Up Solutions, Waters application note: P/N **720008062**

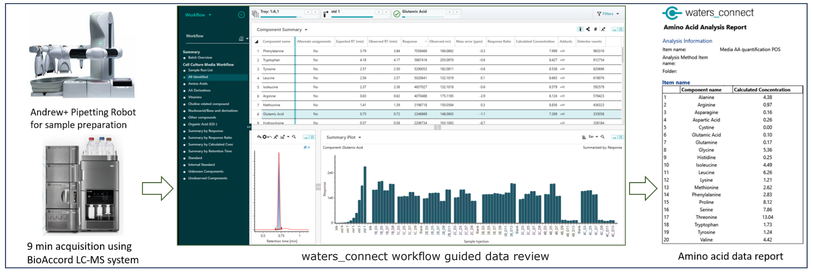

Figure 1. Schematic overview of sample preparation, LC-MS analysis and report for cell culture media nutrient and metabolite analysis based on Andrew+ Pipette Robot and BioAccord LC-MS platform.

RESULTS

The method performance was determined using Waters amino acid cell culture kit which contains 26 amino acids (Figure 2 and Table 1).

For spent media analysis, media from a CHO cell cultivation to produce cNISTmAb. were used. Media samples were analyzed using both a 9 min fast method and a 20 min method (Figure 3). Results showed excellent correlation where same trends were obtained using either method, indicating high quality data obtained in the 20-min method is maintained using the rapid 9 min method. There is also good correlation of glucose between data obtained using either LC-MS method with the BioProfile™ FLEX2 Analyzer (Nova Biomedical Corporation, Figure 4).

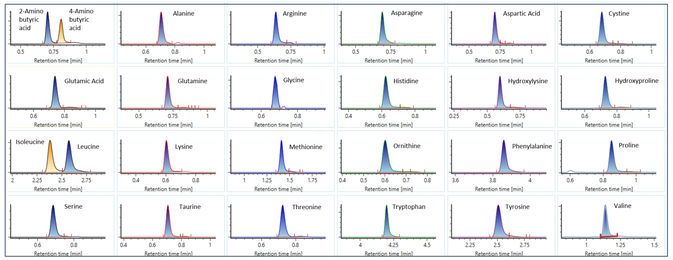

Figure 2. Extracted ion chromatogram (XIC) of 26 compounds in Waters amino acid cell culture standard kits. Two isobaric compound pairs, isoleucine/leucine and 2-amino/4-amino butyric acid are baseline separated.

Entry	Component name	Expected RT min	Neutral mass Da	Range (uM)	R2	QC conc = 0.5 uM (n=6) %Accuracy	QC conc = 0.5 uM (n=6) %Precision	QC conc = 2.5 uM (n=6) %Accuracy	QC conc = 2.5 uM (n=6) %Precision
1	4-Aminobutyric acid	0.62	103.0633	0.025-10	0.99	94	3.6	98	1.7
2	Alanine	0.67	89.0477	0.05-10	0.99	108	4.3	103	2.3
3	Arginine	0.63	174.1117	0.01-5	0.99	92	5.6	107	1.4
4	Asparagine	0.68	132.0535	0.025-10	0.99	98	3.3	97	2
5	Aspartic Acid	0.66	133.0375	0.25-10	0.98	101	5.6	108	5.1
6	Cystine	0.64	240.0239	0.01-5	0.99	92	5.3	96	1.7
7	Glutamic Acid	0.75	147.0532	0.01-10	0.99	97	1.6	103	2.8
8	Glutamine	0.72	146.0691	0.01-10	0.99	98	3.6	105	1.4
9	Glycine	0.64	75.032	0.5-10	0.99	113	5.5	108	4
10	Histidine	0.62	155.0695	0.1-10	0.99	96	5.9	102	1.6
11	Hydroxylysine	0.57	162.1004	0.25-10	0.99	107	3.1	102	2.6
12	Hydroxyproline	0.62	131.0582	0.025-10	0.99	96	4.4	104	2.5
13	Isoleucine	2.37	131.0946	0.01-10	0.99	90	3.7	104	2
14	Leucine	2.56	131.0946	0.025-5	0.99	89	3.8	108	3.9
15	Lysine	0.58	146.1055	0.025-10	0.99	95	3	106	2.5
16	Methionine	1.41	149.0511	0.01-5	0.99	91	3.5	107	2.7
17	Ornithine	0.58	132.0899	0.025-10	0.99	99	5.1	104	2.8
18	Phenylalanine	3.79	165.079	0.01-10	0.99	91	5.4	103	3.3
19	Proline	0.83	115.0633	0.05-10	0.99	97	3.6	111	2.8
20	Serine	0.65	105.0426	0.05-10	0.99	98	4.3	100	2
21	Taurine	0.66	125.0147	0.05-2.5	0.99	116	9	95	3.6
22	Threonine	0.69	119.05824	0.025-10	0.99	100	3.6	100	2.3
23	Tryptophan	4.18	204.08988	0.01-10	0.99	88	2.8	103	2.3
24	Tyrosine	2.57	181.07389	0.025-10	0.99	92	3.9	103	2.1
25	Valine	1.12	117.07898	0.025-10	0.99	93	2.7	110	11.8
26	Glucose	0.76	215.0323 [+Cl]	0.1-5	0.99	118	2.3	105	2.3

Table 1. Summary of linear range, accuracy, and precision of amino acids and glucose quantification using the 9 min method. The linear range is derived from linear regression with $1/x^2$ fitting, 20% deviation was used as the exclusion criteria.

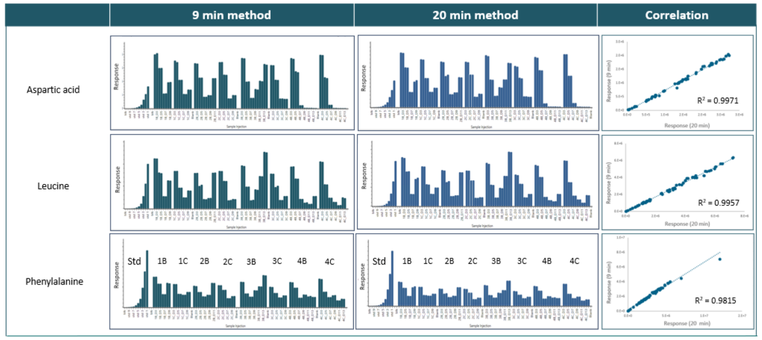

Figure 3. Trending plot of compound showing observed response as function of incubation condition and sampling time. Green graphs on the left are data from 9 min method, blue graphs in the middle are from 20 min method. On the right is correlation plot of response obtained from 9 min method vs 20 min method. All spent media samples were injected in duplicate.

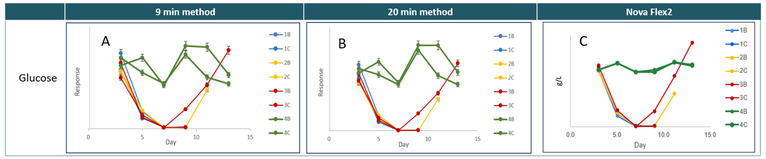

Figure 4. Plots of glucose response versus incubation time overlaying all feeding conditions. (A) data from 9 min method, (B) data from 20 min method, and (C) data from Bioprofile FLEX2 Analyzer. Four glucose feeding conditions, represented by different colors, were used using four separate flasks in duplicate. Error bars in the graph represent duplicate measurements.

CONCLUSION

- A rapid throughput method based on 9 min data acquisition is described for cell culture media nutrient and metabolite monitoring.
- The method uses less than 10 µL of spent media sample and has coverage of 220 compounds.
- Comparison with the previous 20 min method suggests the same high-quality data and method robustness, even when the analysis time is shortened by 50%.
- Combination of the rapid throughput method with Waters bioprocess walk-up solution[2] can enable analytical scientist and bioprocess engineers to obtain high quality data easily and routinely for upstream bioprocess optimization.

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