Developing a high-resolution LC-MS method for spent media analysis in biopharm upstream process development

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Introduction

- Cell culture media optimization plays an important role in biopharmaceutical upstream process development. A chemically defined media contains a variety of nutrients such as amino acids (AAs), carbohydrates, vitamins, lipids, trace elements, and many other compounds. The composition and concentration of components are directly related to the quality and yields of therapeutic proteins produced in the process. In addition, these nutrients are consumed and fed as needed to ensure continuous cell growth and protein production.
- An efficient and accurate detection method is thus needed for monitoring media components. In this presentation, we describe a high-resolution mass spectrometry (HRMS) based method for analysis of water-soluble compounds in cell culture media using the BioAccord™ LC-MS system and waters_connect™ informatics platform.
- The optimized method includes a 20 min reversed-phase liquid chromatography (LC) separation, MS detection in full scan mode with ESI+/ESI- ionization, a large compound library for media component identification, and a guided workflow for ease of data review. Application of the method for feed stock amino acid quantification and spent media monitoring are exemplified.

Method

- Spent media samples were prepared by 1:500 dilution using 0.1% formic acid containing 0.1 uM 3-chlorotyrosine as internal standard (IS). Amino acid standard solutions were prepared by diluting amino acid standard solution (ex. Sigma) using 0.1% formic acid (FA) with IS. Quantitation of amino acid concentration in raw media was carried out by diluting the media 1:2000 using 0.1% FA.
- LC and MS parameters are displayed in Table 1. Mobile phase A was H_2O/0.1%FA. Mobile phase B was 90%ACN/10%IPA/0.1%FA. Column was Acquity Premier HSS T3 2.1 x 150 mm. Data acquisition and processing were carried out using waters_connect informatics package.

Time (min)	Flow Rate (ml/min)	%A	%B
0	0.25	100	0
1.5	0.25	100	0
6	0.25	95	5
9	0.25	60	40
14	0.25	5	95
17	0.25	5	95
17.1	0.25	100	0
20	0.25	100	0

MS Parameters	
Mode	full Scan
Mass range	small molecules (50-800 m/z)
Polarity	Positive
Scan rate	5 Hz
Cone voltage	20 V
Capillary voltage	1 kV
Desolvation temperature	550 °C
Lockmass correction	Dynamix

Table 1. LC and MS parameters using BioAccord system

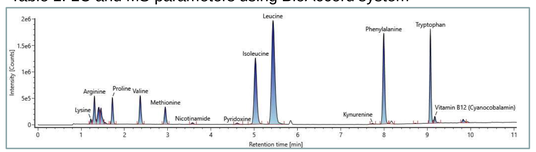
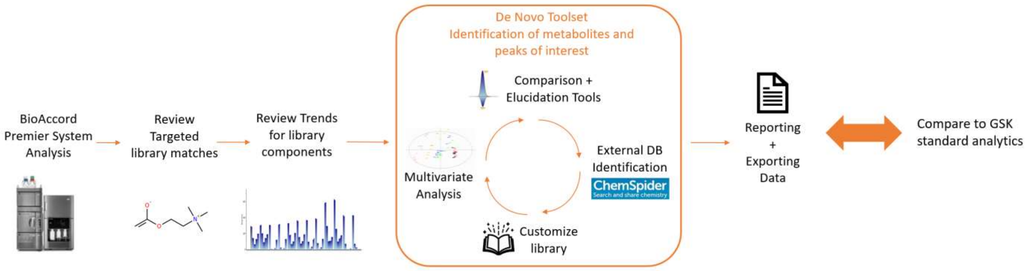


Figure 1. Extracted ion chromatogram of observed compounds in media of parental cell line in day 3.

Scheme 1. Proposed workflow applying LC-MS analytics for bioprocess monitoring



Results

Part 1. Quantification of amino acids in raw media

Amino acid concentration in raw media was quantified using the described LC-MS method. All amino acids showed acceptable reproducibility and accuracy, except glycine having slightly lower accuracy, due to it being near the detection limit.(Table 2).

Component name		Neutral mass (Da)	Expected RT (min)	Linear Range (uM)	R2	%Accuracy	Stdev	%RSD (n=3)
Alanine	Ala	89.0477	1.42	0.025 - 10	0.996	86	0.01	3.2
Arginine	Arg	174.1117	1.31	0.01 - 10	0.999	101	0.07	1.2
Aspartic Acid	Asp	133.0375	1.42	0.01 - 10	0.999	92	0.15	2
Cystine	Cys	240.0239	1.37	0.005 - 5	0.998	80	0.01	0.6
Glycine	Gly	75.0320	1.36	0.5 - 10	0.988	77	0.02	2.9
Histidine	His	155.0695	1.28	0.01 - 10	0.998	97	0.01	0.8
Isoleucine	Ile	131.0946	5.22	0.01 - 10	0.999	98	0.01	0.2
Leucine	Leu	131.0946	5.64	0.01 - 10	0.999	101	0.05	0.9
Lysine	Lys	146.1055	1.23	0.01 - 10	0.999	102	0.04	0.8
Methionine	Met	149.0511	3.01	0.01 - 10	0.998	99	0.07	5.1
Phenylalanine	Phe	165.0790	8.11	0.01 - 10	0.999	100	0.01	0.3
Proline	Pro	115.0633	1.73	0.01 - 10	0.999	102	0.05	1.2
Serine	Ser	105.0426	1.38	0.01 - 10	0.999	90	0.07	1
Tyrosine	Tyr	181.0739	5.64	0.01 - 10	0.999	89	0.01	0.8
Valine	Val	117.0790	2.39	0.01 - 10	0.997	104	0.05	1

Table 2. Summary of standard calibration characteristics and quantitation results in terms of accuracy and reproducibility of raw media.

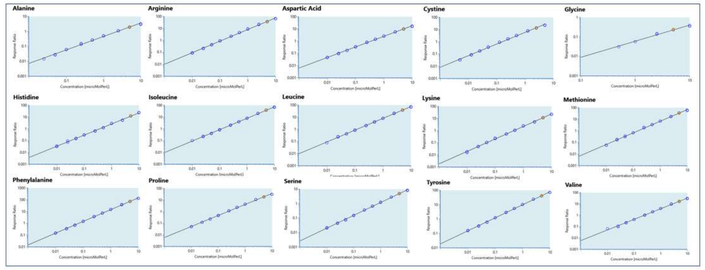
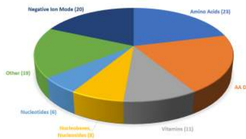


Figure 1. Standard calibration curve of amino acids based on Log-Log linear curve fitting.

Part 2. Spent media monitoring

LC-MS analysis of spent media samples from a clone selection experiment detected 100+ compounds based on the standard library included in the method package. Distribution of these detected compounds based on compound classes is shown on the right.



Results

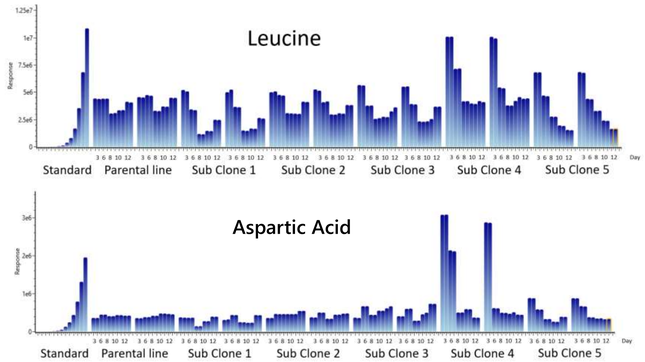


Figure 3. Spent media data for leucine and aspartic acid from a clone selection experiment comparing 5 subclones to a parental line. Media were sampled from bioreactors on days 3, 6, 8, 10, and 12. Each media solution was injected in duplicate. The data suggests that subclone 2 demonstrated the most similarity to the parental line while subclone 4 demonstrated significant difference in terms of amino acids consumption.

Conclusions

- A comprehensive LC-MS methodology and workflow for cell culture media samples is described.
- This platform technology has been applied successfully in one clone selection experiment in process development of protein therapeutics.
- When analytical standards are available, acceptable accuracy and reproducibility were observed, making the method potentially suitable for QC of raw media.
- In the absence of analytical standard, monitoring changes based on absolute response in spent media provides valuable information.
- The method could be applicable to the routine operation in upstream process for process characterization and development.