Profiling Cell Culture Media via LC/MS and Multivariate Statistical Analysis

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INTRODUCTION

The development and optimization of cell culture media for the production of therapeutic proteins is fundamental to the expanding biopharmaceutical industry. Cell culture media contains a precise blend of nutrients (amino acids, carbohydrates, vitamins, lipids, growth factors, trace elements, minerals) and their stoichiometric balance can have a significant and irremediable impact on the growth, function and relative phenotype of cells responsible for expressing the protein target. There is a vital need to optimize cell culture media formulations in order to maximize cell growth and to increase productivity of biopharmaceutical proteins.

One of the challenges encountered in the analysis of multi-component cell culture media lies in the chromatographic separation of a mixture of very polar analytes in a single LC/MS run with little or no sample preparation. Another significant challenge for the analysis of cell culture media is posed by the difficulty to perform fast and reliable comparisons between multiple complex datasets containing several hundred components.

Previous reports described the successful use of perfluorinated carboxylic acids as ion pairing reagents for reversed phase separation of polar analytes [1-4]. In this study, we investigated three ion pairing reagents and developed an efficient and robust LC/MS method for profiling biopharmaceutical grade cell culture media. A high-resolution (10,000), high-mass accuracy (<10 ppm) quadrupole time-of-flight mass spectrometer (QTOF Premier) was used for analyte detection. The list of "components" (RT, *m/z* pairs) identified in each LC/MS run were extracted using MarkerLynx data processing software and exported to Easy Info (Waters) for multivariate statistical analysis. Statistically significant compounds, related to the minor differences in sample composition, were identified using PCA (Principal Component Analysis) and OPLS-DA (Orthogonal Partial Least-Squares to Latent Structures Discriminate Analysis).

METHODS

UPLC System and Mass Spectrometry

LC Conditions:

UPLC System: Waters ACQUITY™ UPLC
Column: 2.1 x 150 mm BEH, 1.7 µm C_{18} column
Mobile Phases:
Mobile Phase A: Water with 0.025, 0.05 or 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA)
Mobile Phase B: 80% ACN/20% H_2O w/ 0.025, 0.05 or 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA)
Flow Rate: 0.5 mL/min
Column Temp: 45 °C
Gradient: 1% -20% B in 10 minutes
Sample Volume: 5 µL

	Chemical	Elemental			Average MW	Density	Molarity (mM)	
Symbol	Name	Formula	[M+H] +	pKa	(g/mole)	(g/mL)	0.1% sol	0.05% sol
HFBA	heptafluoro butyric acid	C4HF7O2	214.9938	0.4	214.04	1.65	7.7	3.9
NFPA	nonafluoro pentanoic acid	C5HF9O2	264.9906	0.8	264.05	1.7	6.4	3.2
PDFDA	pentadecafluoro octanoic acid	C8HF15O2	414.9810	2.5	414.06	solid	2.4	1.2

Table 1. *Ion pairing reagents used in this study.*

Mass spectrometry:

Instrument: Waters QTOF Premier™
Capillary Voltage 3.6 KV
Nebulization gas: 800L/min
Desolvation Temperature: 400 °C
Source Temperature: 150 °C
Cone Voltage: 15 V
Collision energy: 2 eV
Lock-spray: Leucine-enkephalin (*m/z*= 556.2766, 0.5 µM).

Samples

Cell culture media samples were diluted 1:20 in Mobile Phase A and injected directly in the LC/MS system.

QTOF Premier mass spectrometer coupled to the ACQUITY™ UPLC system.

RESULTS

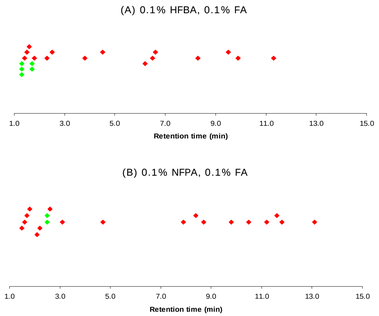

Figure 1. *Amino acid distribution (red dots) within a 15 min UPLC run. Mobile phase composition was either (A) 0.1% HFBA and 0.1% FA in water or (B) 0.1% NFPA and 0.1% FA in water. The green dots indicate co-eluting amino-acids.*

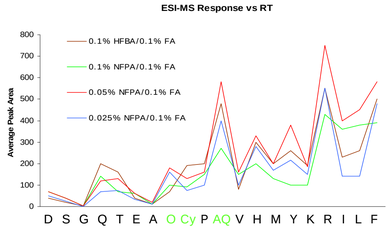

Figure 2. *ESI-MS response of 17 common amino acids in the presence of NFPA at 3 different mobile phase concentrations and in the presence of 0.1% (v/v) HFBA. All mobile phases also contained 0.1% (v/v) FA.*

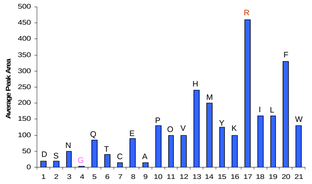

Figure 3. *Comparison of ESI-MS response of 21 common amino acids. The mobile phase contained 0.05% NFPA and 0.1% FA.*

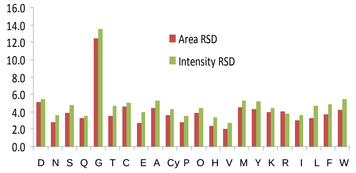

Figure 4. Reproducibility of peak area and intensity for 22 common amino acids. Concentration of the AA solution was 50 µM.

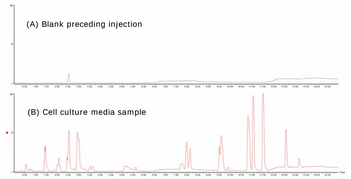

Figure 5. *Typical LC/MS base-peak chromatogram of a cell culture media sample. The sample was diluted 1:20 with Mobile Phase A before injection.*

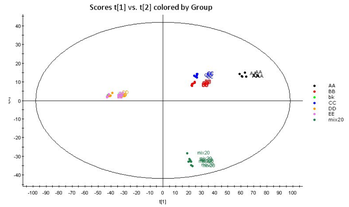

Figure 6. *PCA (Principal Component Analysis) reduces the complexity of the LC/MS data to a single plot showing the relationship between different cell culture media. In this example, three media samples (labeled AA, BB and CC), having minor modifications in their chemical composition, can be clearly differentiated.*

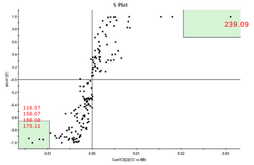

Figure 7. *Statistically significant components related to the differences between samples BB and CC can be easily found from the S-plot, the result of the OPLS discriminate analysis. In this case, one marker was found only in sample BB (m/z=239.09), while 4 other markers were present at different concentration levels in both samples.*

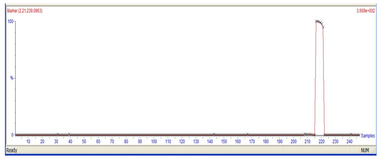

Figure 8. *MarkerLynx trend plot confirms the presence of an unique component (m/z = 239.09) in the cell culture media sample labeled BB. In this experiment six replicate injections from 31 different samples were performed.*

CONCLUSIONS

- Reversed-phase UPLC using various perfluorinated carboxylic acids as ion-pairing agents has been found suitable for the separation and MS detection of complex mixtures of very polar analytes. Best chromatographic results were obtained for the mobile phase containing 0.05% NFPA and 0.1% FA.
- High-resolution/high-mass accuracy mass spectrometer greatly enhances the ability to identify components of cell culture media.
- PCA analysis was successfully used to identify minor changes in the chemical composition of cell culture media.
- LC/MS profiling shows great potential for fast analysis of biopharmaceutical grade cell culture media as well as spent media samples obtained during protein production.

References

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