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 biopharmaceuticals.

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 agents for reversed phase LC/MS profiling of cell culture media as well as spent media samples obtained during protein production.

RESULTS

Table I. The ion pairing reagents used in this study.

<table>
<thead>
<tr>
<th>Ion Pairing Agent</th>
<th>Mobile Phase Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% NFPA/0.1% FA</td>
<td>1% - 20% B in 10 minutes</td>
</tr>
<tr>
<td>0.1% HFBA/0.1% FA</td>
<td>1% - 20% B in 10 minutes</td>
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Sample preparation

Three cell media components (Q, dipeptide AG and HEPES) were spiked at a concentration of 1000 ppm in 200 µL of media. Samples were then diluted 1:50 with solvent A before injection.

UPLC Conditions:

- 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% H2O with 0.025, 0.05 or 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA)
- Gradient: 1% - 20% B in 10 minutes
- Flow Rate: 0.5 mL/min
- Sample Volume: 5 µL
- Temperature: 45 ºC
- Mobile Phases: Kratos Acquity UPLC HSS T3 (1.8 µm) C18 column
- Mobile Phase Compositions

Sample analysis

Figure 1. Amino acid distribution (red dots) within a 15 min UPLC run. Mobile phase composition: 1% - 20% B in 10 minutes. Mobile phase B: 80% ACN/20% H2O with 0.1% (v/v) ion-pairing reagent and 0.1% formic acid (FA). Sample volume: 5 µL. Gradient: 1% - 20% B in 10 minutes.

Figure 2. PCA (Principal Component Analysis) of the AA mixture (10 µM) and of the neutral component table obtained after processing the LC/MS data. Samples were extracted using MarkerLynx data processing software and exported to Simca-P (Umetrics AB, Umeå, Sweden) for multivariate statistical analysis. Statistically significant differences were identified using PCA (Principal Component Analysis).

Figure 3. ESI-MS Response vs Compound RT. The ESI-MS response of a 209 amino acids mixture was measured at a pressure of 4000 mTorr in 3 different mobile phases. The mobile phases were the same as those used for the separation of the AA mixture, but with a 20 µm column and without ion-pairing agents. The mass spectrometric parameters were: spray voltage, 3.6 KV; source temperature, 150 ºC; collision energy, 2 eV; peptide mass fingerprint (PMF) range, 100-2000 Da; minimum ionization current (MINC), 10 µA; number of scans, 100; number of ions, 50; number of spectra, 100; number of ESI-MS spectra, 1.

Figure 4. Comparison of the AA mixture at the LC/MS run for different mobile phases. The AA mixture was prepared in 200 µL of media containing 0.1% (v/v) FA. The mobile phases were 1% - 20% B in 10 minutes. The mobile phase B was the same as that used for the separation of the AA mixture, but with a 20 µm column and without ion-pairing agents. The mass spectrometric parameters were: spray voltage, 3.6 KV; source temperature, 150 ºC; collision energy, 2 eV; peptide mass fingerprint (PMF) range, 100-2000 Da; minimum ionization current (MINC), 10 µA; number of scans, 100; number of ions, 50; number of spectra, 100; number of ESI-MS spectra, 1.

Figure 5. PCA (Principal Component Analysis) of the AA mixture at the LC/MS run for different mobile phases. The AA mixture was prepared in 200 µL of media containing 0.1% (v/v) FA. The mobile phases were 1% - 20% B in 10 minutes. The mobile phase B was the same as that used for the separation of the AA mixture, but with a 20 µm column and without ion-pairing agents. The mass spectrometric parameters were: spray voltage, 3.6 KV; source temperature, 150 ºC; collision energy, 2 eV; peptide mass fingerprint (PMF) range, 100-2000 Da; minimum ionization current (MINC), 10 µA; number of scans, 100; number of ions, 50; number of spectra, 100; number of ESI-MS spectra, 1.

Figure 6. PCA (Principal Component Analysis) of the AA mixture at the LC/MS run for different mobile phases. The AA mixture was prepared in 200 µL of media containing 0.1% (v/v) FA. The mobile phases were 1% - 20% B in 10 minutes. The mobile phase B was the same as that used for the separation of the AA mixture, but with a 20 µm column and without ion-pairing agents. The mass spectrometric parameters were: spray voltage, 3.6 KV; source temperature, 150 ºC; collision energy, 2 eV; peptide mass fingerprint (PMF) range, 100-2000 Da; minimum ionization current (MINC), 10 µA; number of scans, 100; number of ions, 50; number of spectra, 100; number of ESI-MS spectra, 1.

Figure 7. PCA (Principal Component Analysis) of the AA mixture at the LC/MS run for different mobile phases. The AA mixture was prepared in 200 µL of media containing 0.1% (v/v) FA. The mobile phases were 1% - 20% B in 10 minutes. The mobile phase B was the same as that used for the separation of the AA mixture, but with a 20 µm column and without ion-pairing agents. The mass spectrometric parameters were: spray voltage, 3.6 KV; source temperature, 150 ºC; collision energy, 2 eV; peptide mass fingerprint (PMF) range, 100-2000 Da; minimum ionization current (MINC), 10 µA; number of scans, 100; number of ions, 50; number of spectra, 100; number of ESI-MS spectra, 1.

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 spectrometry 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