LC/MS PROFILING OF CELL CULTURE MEDIA

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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 and polar synthetic mixtures. 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 Simca-P (Umetrics AB, Umea, Sweden) for multivariate statistical analysis. Statistically significant compounds, related to the minor differences in sample composition, were identified using PCA (Principal Component Analysis).

METHODS

UPLC System and Mass Spectrometry

LC Conditions:

UPLC System: Waters ACQUITY™ UPLC
Column: 2.1 x 150 mm T3 HSS (high-strength silica), 1.8 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 Temperature: 45 °C
Gradient: 1% -20% B in 10 minutes
Sample Volume: 5 µL

Symbol	Chemical name	pKa	Elemental Formula	[M+H] +
HFBA	heptafluoro butyric acid	0.4	C4HF7O2	214.9938
NFPA	nonafluoro pentanoic acid	0.8	C5HF9O2	264.9906
PDFOA	pentadecafluoro octanoic acid	2.5	C8HF15O2	414.9810

Table I. *The 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

Three cell media components (Q, dipeptide AG and HEPES) were spiked at a concentration of 1-10 µg/mL in a 10 µM amino acid test mixture. Cell culture media samples, with slightly different chemical composition, were diluted 1:50 and injected directly in the LC/MS system.

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

RESULTS

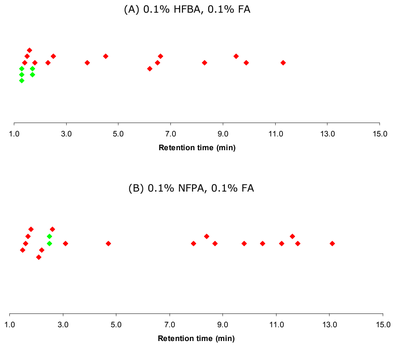

Figure 1. *Amino acid distribution (red dots) within a 15 min UPLC run. Mobile phase composition was either (A) 0.1% HFBA and 0.1% FA in water or (B) 0.1% NFPA and 0.1% FA in water. The green dots indicate co-eluting amino-acids.*

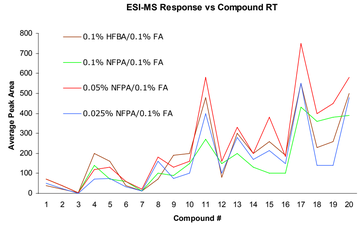

Figure 2. *ESI-MS response of 20 common amino acids in the presence of NFPA at 3 different mobile phase concentrations and in the presence of 0.1% (v/v) HFBA. All mobile phases also contained 0.1% (v/v) FA.*

Concentration of Ion Pairing Reagent	Average PW at Half-Height	Peak Width Range
0.025% NFPA / 0.1% FA	5.1	2.8 - 11
0.5% NFPA / 0.1% FA	4.7	2.6 - 7.8
0.1% NFPA / 0.1% FA	4.3	2.9 - 7.2
0.1% HFBA / 0.1% FA	3.5	1.7 - 6.0

Figure 3. *Chromatographic peak widths measured at half height for 20 common amino acids when HFBA and NFPA were used as ion pairing reagents. Chromatographic peaks get broader as the ion pairing concentration decreases.*

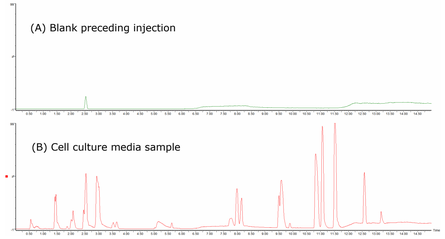

Figure 4. *Typical LC/MS base-peak chromatogram of a cell culture media sample. The sample was diluted 1:50 with Solvent A before injection.*

Ret. Time	Mass	070116_H...	070116_H...	070116_H...	070116_H...	070116_H...	070116_H...	070116_H...	070116_H...	070116_H...
2.9731	70.0607	33	50	3	16	0	51	49	0	33
7.7061	72.0842	164	157	162	148	161	171	143	70	153
2.8119	74.0637	32	33	31	31	37	37	31	36	34
5.5902	84.0954	41	41	41	34	41	13	15	31	41
1.4241	85.0641	9	9	10	0	11	11	9	9	10
11.5212	86.1001	1057	1128	1094	1763	1132	1087	1024	1224	1151
11.0711	86.1002	955	891	930	821	940	960	893	1136	946
1.4099	87.0553	31	31	33	28	33	33	27	28	32
2.0127	102.0596	47	48	52	45	55	55	46	49	52
8.1482	104.0584	57	56	58	52	61	58	53	60	59
5.2041	104.1111	195	91	77	71	220	226	217	109	46
1.5269	106.0545	58	59	61	54	65	66	52	53	61
8.0001	110.0769	45	46	44	38	46	46	40	49	47
2.9781	116.0765	653	696	694	751	717	720	798	746	784
7.7091	118.0909	222	232	235	276	244	248	208	209	119
2.0143	120.0892	34	77	36	81	95	96	77	86	99
12.5779	126.0871	218	233	183	134	159	159	237	263	295
3.8537	134.0663	0	0	0	0	0	0	0	0	0
2.0847	136.0555	59	60	62	55	66	66	54	64	63
1.7424	138.0574	0	0	0	0	0	0	0	0	0
5.6879	138.0905	198	266	262	174	207	204	178	114	202

Figure 5. *Portion of the component table obtained after processing the LC/MS data with Marker Lynx.*

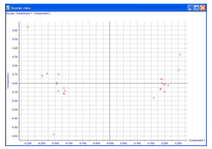

Figure 6. *PCA (Principal Component Analysis) of the AA mixture (10 µM) and of the Mix 3 (1 µg/mL Q, AQ, HEPES) spiked in the AA Mix. Each sample was injected 12 times. All non-spiked samples occupied the left area of the plot, while all the spiked samples clustered to the right.*

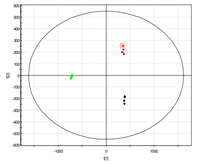

Figure 7. *PCA reduces the complexity of the LC/MS data to a single plot showing the relationship between different cell culture media samples. In this example, three cell culture media samples, containing minor modifications in their chemical composition, can be clearly differentiated.*

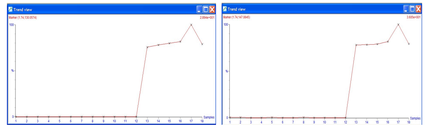

Figure 8. *Marker Lynx trend plots indicating the presence of unique components in certain cell culture media samples belonging to the same production lot. In this example six replicate injections from three different batches of the same lot, were performed.*

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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