Cell Culture Media Profiling and Unknown Identification by Liquid Chromatography and Accurate Mass High Resolution Mass Spectrometry

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Introduction

The depletion of nutritional components and the formation of new metabolites in media during cell culture may influence cell growth, function, final protein yield and quality. There is an increased need to profile and identify unknown components in cell culture media and to investigate their correlation with monoclonal antibody (mAb) product quality. Comprehensive analysis of cell culture media at 4 different time points from 3 different cell culture media conditions was conducted. Non-targeted metabolomics approaches were used to discover differences between the samples. Major unknown components were identified and the correlation between culture media and its final product quality were evaluated.

Methods

Twelve media samples were prepared by centrifuging and filtering (0.2µm) for cell removal. The media samples were collected at 4 different time points from 3 different cell culture media conditions. About 100 µL of media, without further dilution, was transferred into HPLC vial inserts, and 2 µL sample was injected and analyzed (n=6). LC-MS runs were carried out on an UHPLC (Elute, Bruker) interfaced with an ultra-high resolution QTOF MS (maXis II, Bruker). LC separation was performed by a C18 reversed phase column (2.2µm, 120A, 2.1x100mm) in a 20-min run including both mobile phase and flow-rate gradient elution in positive and negative ESI modes.
The comprehensive data sets were processed and statistically evaluated with MetaboScape 4.0 (Bruker) -Figure 1- in combination with Data Analysis (Bruker) for profiling and identification of the unknown components in cell culture media

Results and Discussion

The nutritional components depletion and the formation of new metabolites during cell culture process can influence cell growth, cell function and protein productivity yield and quality.

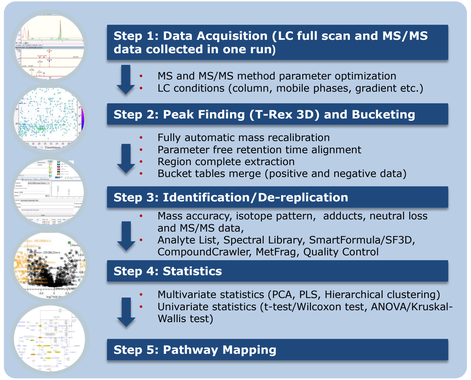

Figure 1. A fully integrated metabolomics workflow of MetaboScape 4.0

Cell Culture Media

No significant differences were observed between the three media chromatograms (Figure 2); Similar separation patterns were observed at different time points for the same cell culture with some variation in peak intensities (Figure 3); The largest difference in intensities was observed at Day 20 (Figure 4). These LC-MS chromatograms could be used as fingerprint to monitor batch-to-batch variation and protein quality.

Unknown ID and Cell Culture Control Media

Many features are observed in the LC-MS analysis (Figure 2) of all three media, BAK004-040, BAK012-010, and BAK012-023. After MetaboScape 4.0 data processing (Figure 1), 4342 features were detected (3159 having MSMS spectra) and were screened against an analyte list containing 100 common nutritional compounds including amino acids, monosaccharides, vitamins, nucleic acids, antibiotics and other. These compounds were verified with further searching against major public available database like PubChem, ChemSpider, ChEBI, and MetaboBASE Personal Library, HMDB Metabolite Library, MSDIAL – Lipid DB-VS34, MetFrag and in-silico MetaboBASE Personal Library. Arginine level was similar in the three cell culture media (Figure 5A) but tyrosine shows significant variation (Figure 5B).

Profiling in Growth Cell Culture Media

About ~50% arginine was depleted but it plateaus at the same level in three cell culture media after 20 days incubation (Figure 5C). However, 100% depletion of tyrosine was observed in BAK004-040, ~40% in BAK010-10 and ~30% in BAK010-23 (Figure 5D). Some components decrease with increasing incubation time like vitamin B3 Niacinamide (Figure 6A) which is an essential nutritional compound for cell culture growth. Others like cytidine monophosphate metabolite increases with incubation time (Figure 6B). These profiling patterns could quickly help to understand the relationship of nutrients and its metabolism process, and help to optimize cell culture bioprocess for better protein production and quality.

Statistical Analysis

PCA statistical analysis of cell culture media BAK012-010 at different time points were conducted based on a 3-dimensional feature finder algorithm implemented into MetaboScape 4.0 that allows feature complete extraction. Based on PCA score and loading plots, choline, arginine, HEPES, lactic acid, citric, tyrosine, leucine, proline, pantothenic acid demonstrate a clear separation between cell culture media at different time points with a high degree of reproducibility (Figure 7), whereas choline was completely depleted (Figure 7B).

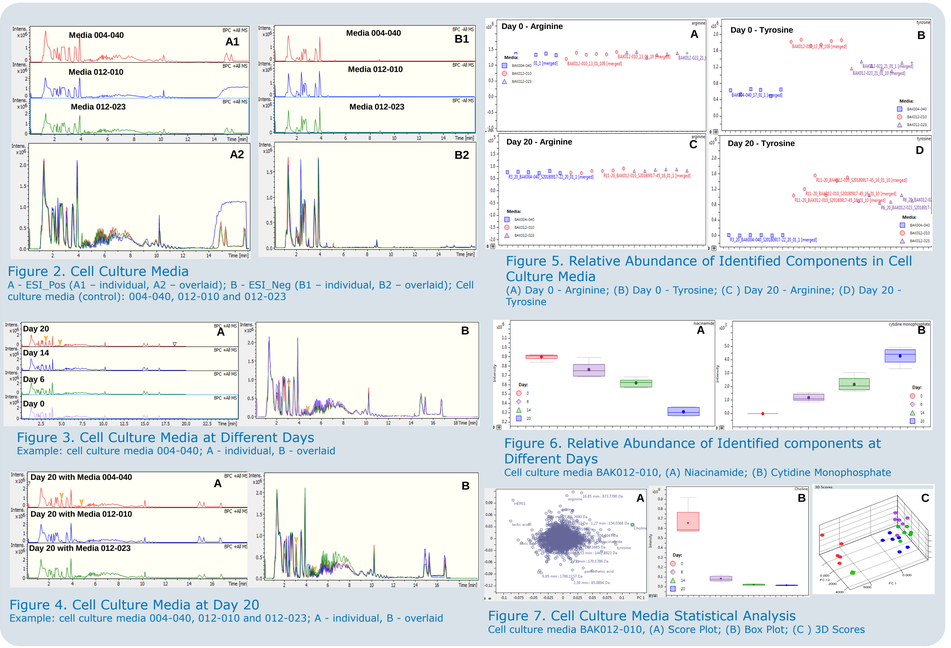

Figure 2. Cell Culture Media
A - ESI_Pos (A1 – individual, A2 – overlaid); B - ESI_Neg (B1 – individual, B2 – overlaid); Cell culture media (control): 004-040, 012-010 and 012-023

Figure 5. Relative Abundance of Identified Components in Cell Culture Media
(A) Day 0 - Arginine; (B) Day 0 - Tyrosine; (C) Day 20 - Arginine; (D) Day 20 - Tyrosine

Figure 3. Cell Culture Media at Different Days
Example: cell culture media 004-040; A - individual, B - overlaid

Figure 6. Relative Abundance of Identified components at Different Days
Cell culture media BAK012-010, (A) Niacinamide; (B) Cytidine Monophosphate

Figure 4. Cell Culture Media at Day 20
Example: cell culture media 004-040, 012-010 and 012-023; A - individual, B - overlaid

Figure 7. Cell Culture Media Statistical Analysis
Cell culture media BAK012-010, (A) Score Plot; (B) Box Plot; (C) 3D Scores

Unknown Identification and Verification

m/z 165.0545 (RT 2.44 min) was not annotated automatically. Manual identification was performed using isotopic pattern and MS/MS spectrum allowed the identification of phenylpyruvic acid ($C_9H_8O_3$). In-silico MetFrag searching achieves a score 1.0 with two fragments ions $[C_7H_7]^+$ and $[C_8H_6O]^{+\cdot}$ (Figure 8)

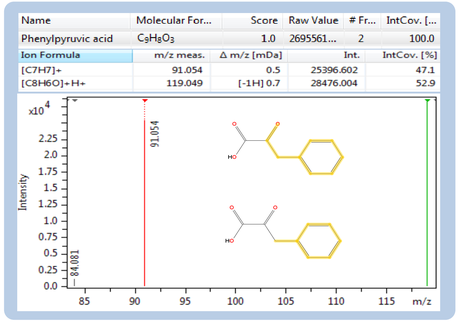

Figure 8. m/z 165.0545 Identification

Conclusions

- A robust, sensitive and reproducible analytical method was established to profile cell culture media which could help understanding the correlation between cell culture media nutrients and protein production and quality
- Data statistical analysis enables the identification of analytes responsible for variation in between cell cultures
- MetaboScape 4.0 provides all-in-one comprehensive and high throughput data process workflow for enabling LC-QTOF-MS based cell culture samples analysis

Cell Culture Media/ MetaboScape 4.0