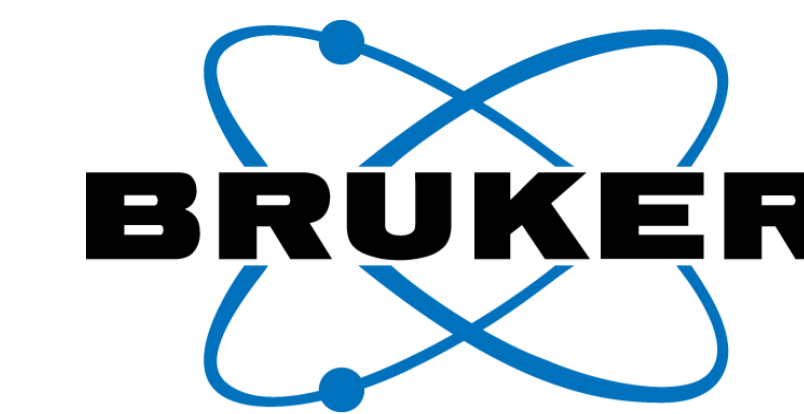


Metabolomics Characterization of Cell Culture Media by Ultra High Resolution LC-QTOF-MS Analysis



ASMS 2019, MP576

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Introduction

Metabolomics characterization and identification of cell culture media is of increasing interest. Comprehensively profiling up to 95 compounds in culture media including amino acids, monosaccharides, vitamins, nucleic acids, antibiotics, and other primary metabolites was carried out by targeted and non-targeted metabolomics simultaneously, hence establishing a media profile that can be correlated to cell growth and product quality.

Methods

A polar-embedded reversed-phase column (3 μ , 120A⁰, 3.0x100mm) was used in a 20-min UHPLC separation before detection with an ultrahigh resolution QTOF (impact II, Bruker) in both positive and negative modes (Figure 1). Commercial cell culture media NCTC-109, HAM's F-10, HAM's F-12, McCoy's 5A, and six lots of in-house CHO cell culture supernatant (2 different media) using the same cell line were prepared by adding acetonitrile, mix, and centrifugation to separate the cells, followed by preparative IEX and SEC, and sterile filtering (0.2 μ m). 100 μ L sample without further dilution was transferred into HPLC vials. 2 μ L sample was injected and analyzed (n=6). All data were processed using MetaboScape software (Bruker).



Figure 1. Elute UHPLC and QTOF mass spectrometer

Data Analysis

The comprehensive data sets were processed and statistically evaluated with MetaboScape 4.0 program (Figure 2) in combination with DataAnalysis software for profiling and identification of the components in cell culture media.

Results and Discussion

Profiling of Cell Culture Media

To improve process knowledge, it is essential to profile the ingredients of starting cell culture media, nutrients depletion during the fermentation and the formation of new metabolites. The nutritional components in cell culture media promote cell growth, maintain cell function and influence the final protein production in terms of yield and quality. About 95 commonly used and important biological nutrients in cell culture media, including amino acids, monosaccharides, vitamins, nucleic acids, antibiotics and others, were screened against commercially available cell culture media. The LC-MS separation chromatogram (Figure 3) could be used as fingerprint to monitor batch-to-batch variation and quality of cell culture media.

Profiling of Cell Culture Media

Because cell culture media greatly influences the cell

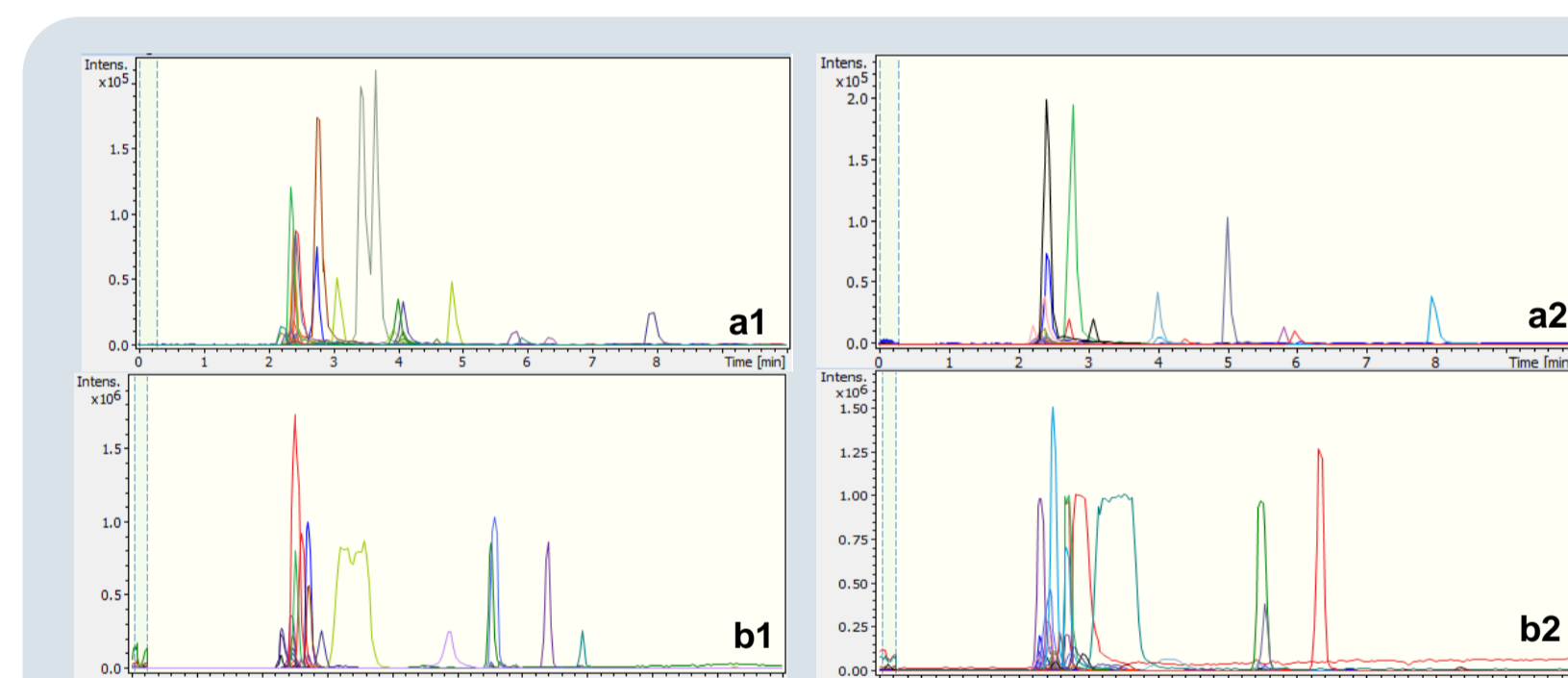


Figure 3. Cell Culture Media Profiling
Cell culture media MCCOYS: (a1) ESI_Pos; (a2) ESI_Neg
Spent media from Cell growth supernatant RP_SZ_648_02B: (b1) ESI_Pos; (b2) ESI_Neg

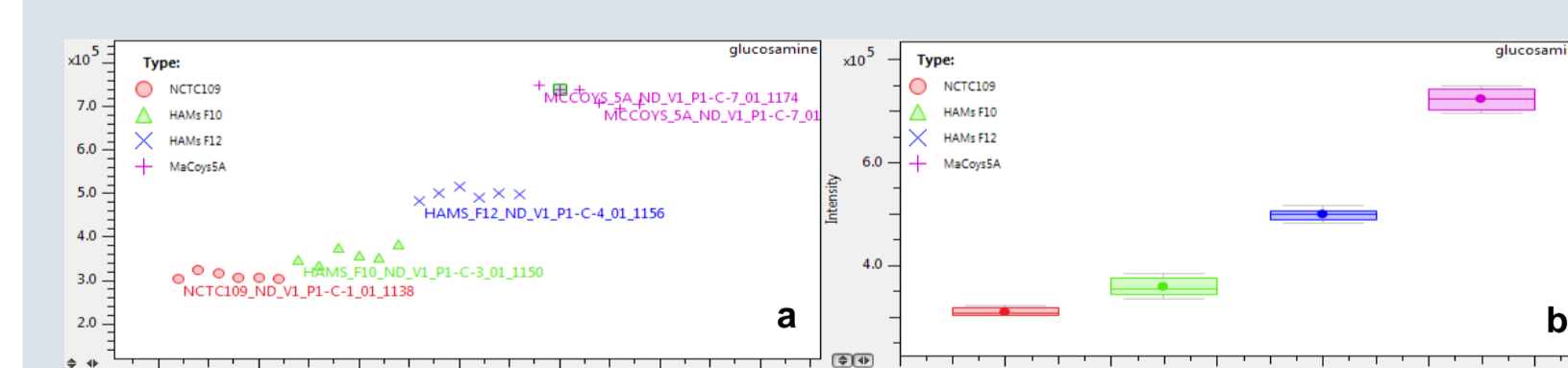


Figure 4. Cell Culture Media Selection
Example: Glucosamine, C₆H₁₃NO₅ m/z 180.0866. (a) bucket statistics; (b) box plot

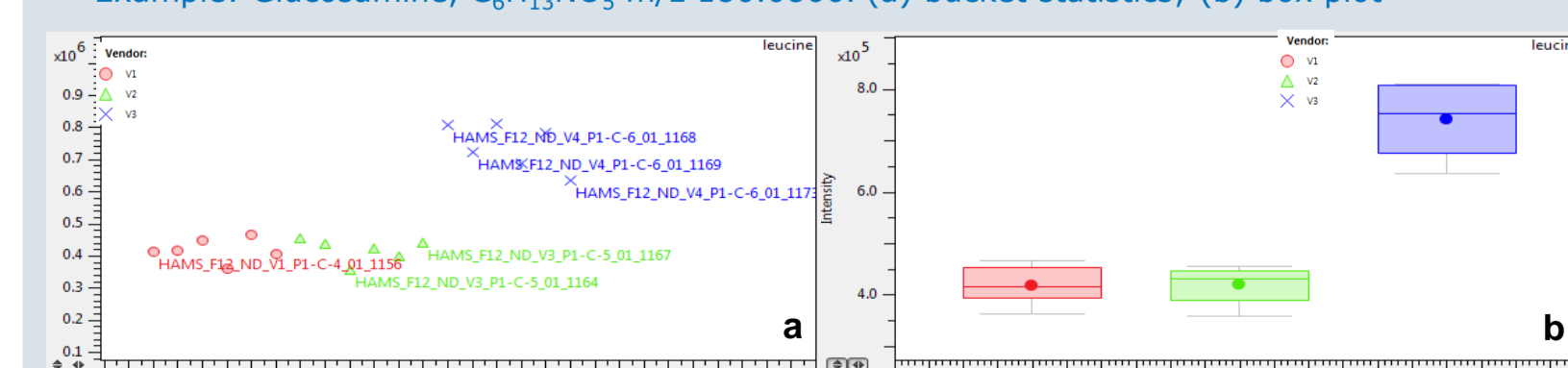


Figure 5. Cell Culture Media Vendor Selection
Example: Leucine, C₆H₁₃NO₂ m/z 132.1019. (a) bucket statistics; (b) box plot

growth process, finding an appropriate starting cell culture medium is critical to process performance. For example the amino acid composition and concentration in the media are critical since amino acids make up the primary structure of a therapeutic protein in cell culture, and support cell growth and viability at increasing cell densities. It was observed (Figure 4) that glucosamine concentration in NCTC-109, HAM-F10, HAM-F12 and McCoy-5A from same vendor are different.

Evaluation of Raw Cell Culture Media

It was noticed (Figure 5) HAM's F12 cell culture media is formulated with different leucine levels depending on the vendor. In order to maintain consistent cell growth, it is essential to establish a screening analytical method and monitor the nutritional components in starting cell culture media, even it was obtained from same vendor in order to avoid lot to lot variability and maintain reproducible cell growth process.

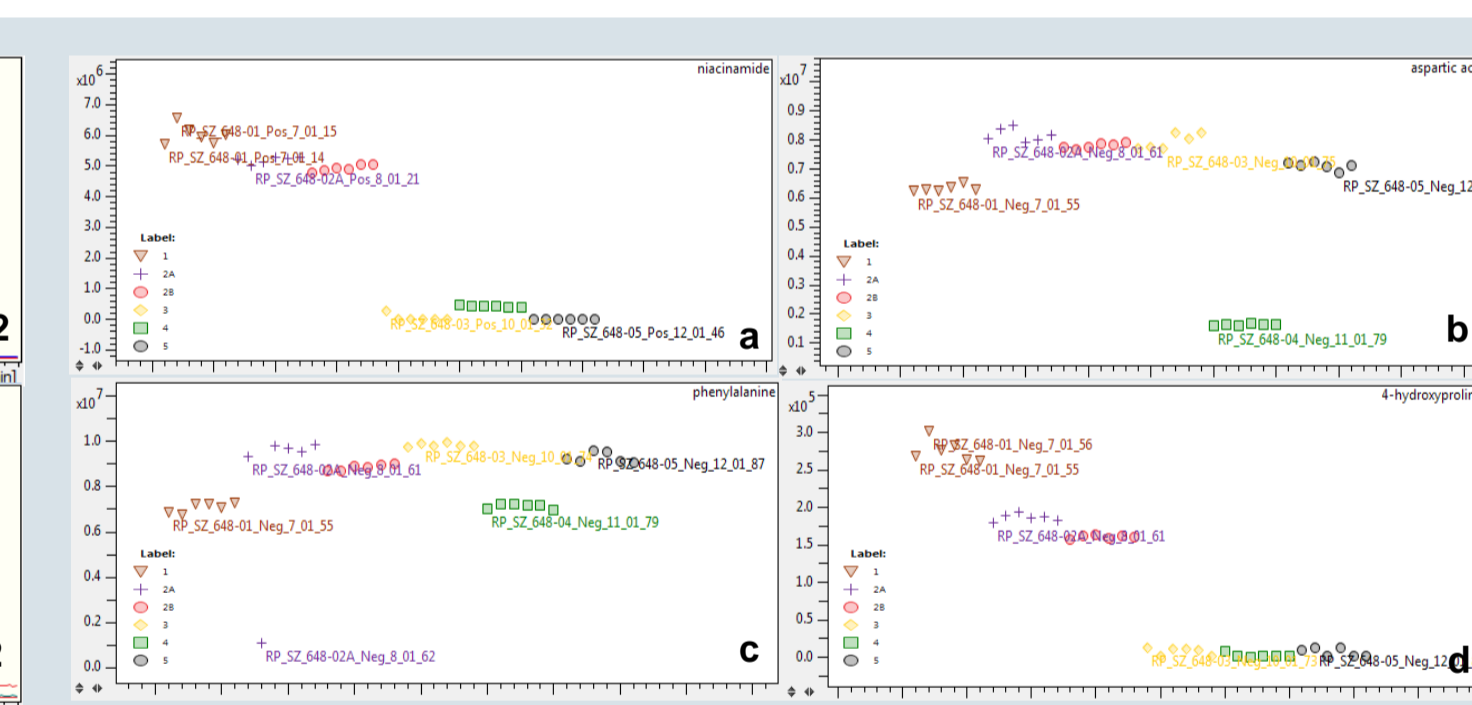


Figure 6. Relative Abundance of Components in Spent Media
(a) niacinamide; (b) phenylalanine; (c) aspartic acid; (d) 4-hydroxyproline

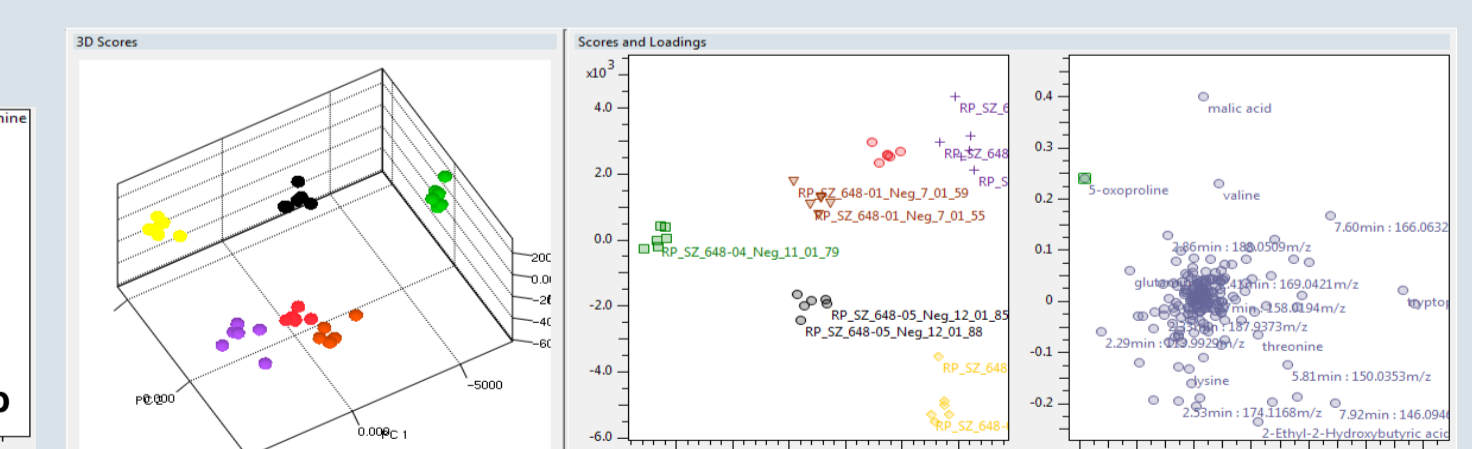


Figure 7. Statistical Analysis of Spent Media

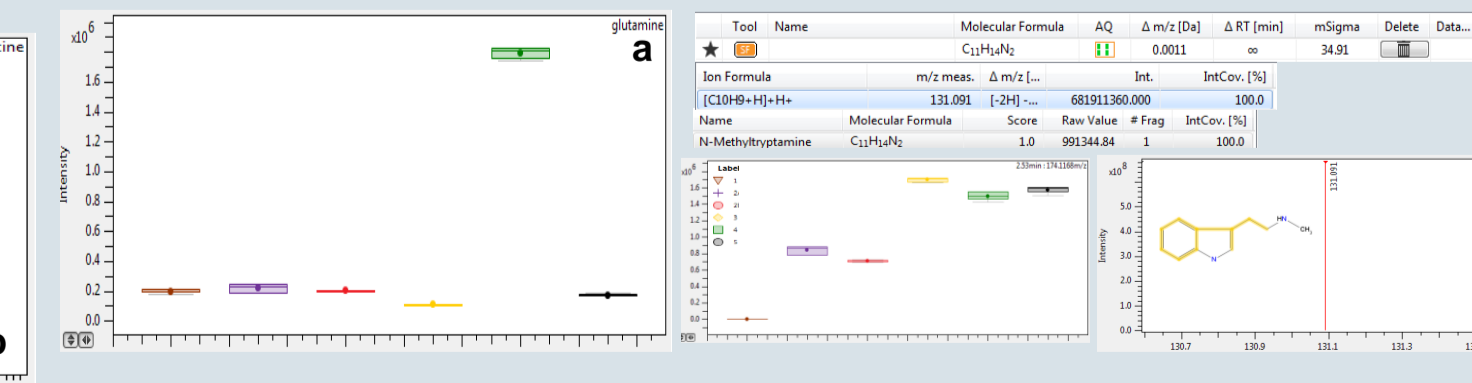


Figure 8. Spent Media Box Plot of Statistical Analysis
Example: (a) glutamine

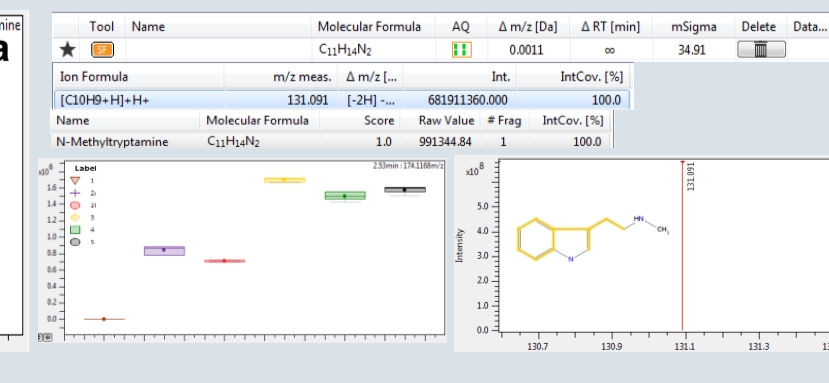


Figure 9. Unknown Identification and Verification

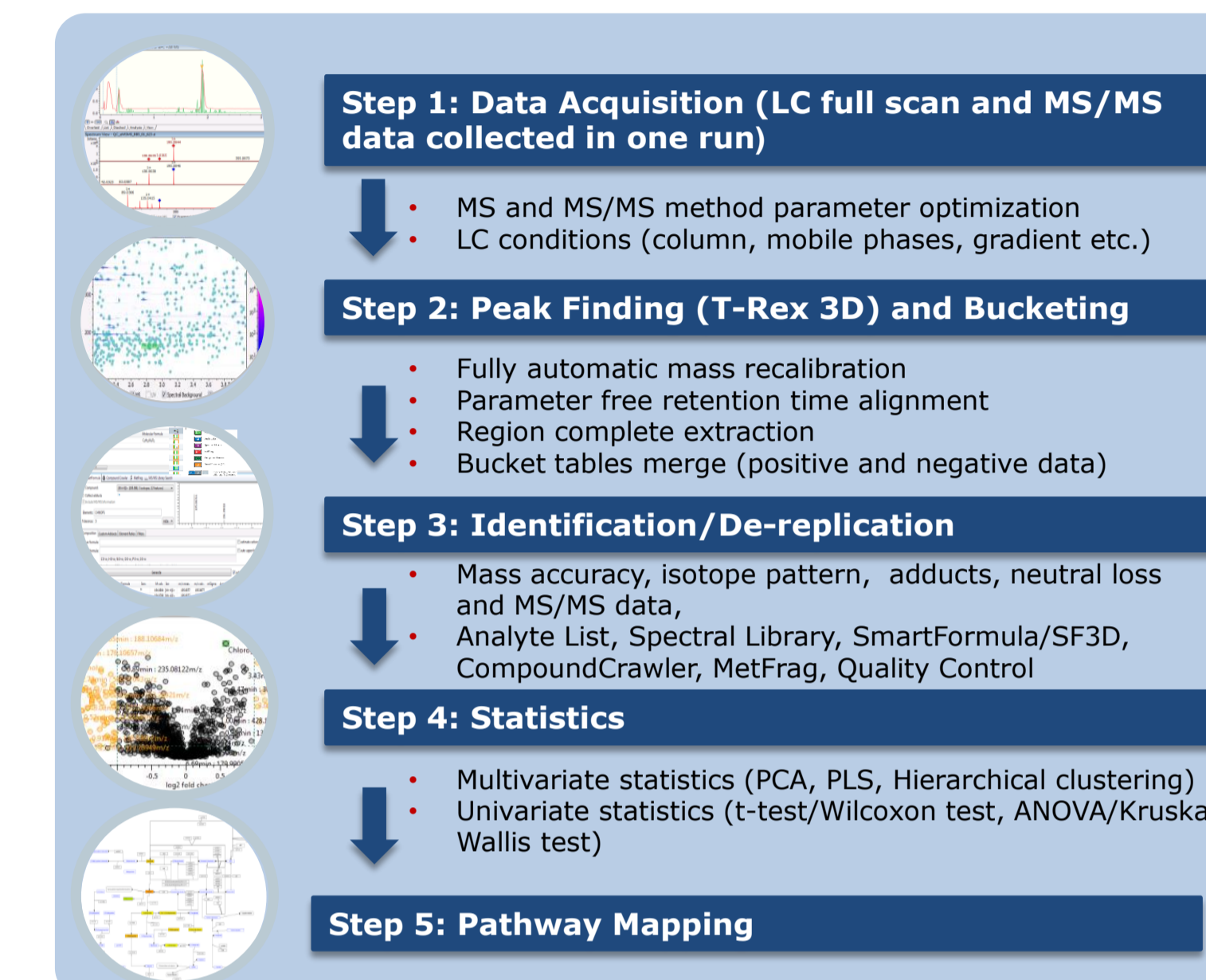


Figure 2. A fully integrated metabolomics workflow of MetaboScape 4.0

Spent Media Analysis

Based on the profile of different spent media [Figure 3 (b1, b2)], nutrients variation and metabolic byproducts were rapidly observed. That information could help improving the fermentation process through correlation with protein product yield and quality. The intensity differences of some components observed between spent media might indicate different cell growth bioprocess or different cultivation conditions (Figure 6).

Statistical Analysis of Spent Media

PCA statistical analysis of six different spent media batches was conducted based on a 3-dimensional feature finder algorithm available in MetaboScape 4.0. The algorithm (T-ReX3D) enables robust detection of features including recursive extraction. It automatically assigns available MSMS spectra to the found MS1 features. In this evaluation, 56 out of 95 screening compounds were identified in the spent media. Based on the scores and loading results of PCA statistical analysis, aspartic acid, malic acid, valine, lysine, serine, pantothenic acid, phenylalanine, methionine, and glutamine presented a clear separation between spent media samples with a high degree of reproducibility (Figure 7). A strong difference was observed for glutamine in particular, despite having identical concentrations in the media at the start of the fermentation process (Figure 8).

Unknowns Identification and Verification

Although cytosine was not included in the initial screening list, it was identified by annotating the features listed in the bucket table after searching MetaboBASE Personal Library (Bruker). Other unknowns identified were Acetyl-DL-leucine; the dipeptide gamma-glu-leu which is a proteolytic breakdown product of larger proteins; and indolelactic acid which is a tryptophan metabolite. An unknown component at m/z 174.1168 (RT 2.53min) that showed a statistically relevant difference between spent media samples, was identified as N-methyltryptamine and verified by MetFrag in-silico fragmentation information (Figure 9).

Stability Evaluation of Cell Culture Media

Stability evaluation of HAM's F-12 cell culture media at different conditions (control, room temperature for 10 days and 37°C for 4 hrs.) was conducted, and found most of the components level did not change (Figure 10).

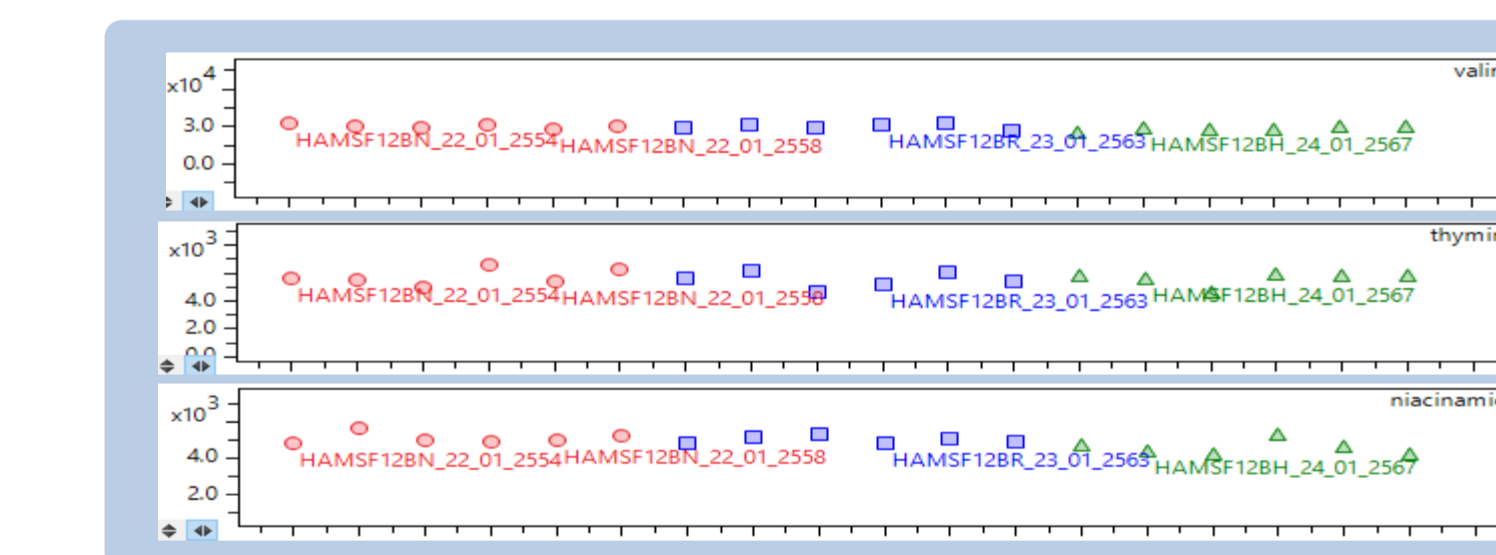


Figure 10. Stability evaluation of cell culture media
(a) Valine; (b) Thymine; (c) Niacinamide

Conclusions

- A robust, sensitive and reproducible analytical method was established to fingerprint and profile culture media quality
- Nutrients depletion between spent media were observed
- Unknown identification increases the understanding of the fermentation process
- MetaboScape 4.0 provides all-in-one comprehensive and fast data process workflow for enabling LC-QTOF-MS based cell culture samples analysis

Cell Culture Media