Upstream LC-MS Monitoring of Cell Culture and Microbial Growth Media Supporting Bioprocess Development

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INTRODUCTION

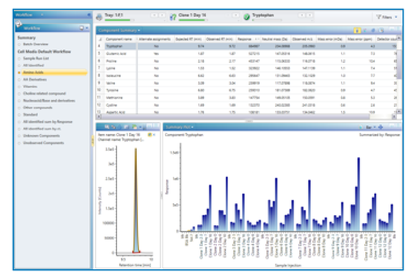
In bioprocess development, mammalian cell culture system and microbial based fermentation have been employed for protein expression and production. Media used in both systems provide nutrients and support the growth and maintenance of cells and microorganisms. There is increasing interest by bioprocess group to monitor raw media, feed supplements and spent media. Composition and concentration of these media components could have direct influence on process quality (cell density, viability) and product quality attributes. In this presentation, the LC-MS methodology and workflow for the media monitoring is described.

System / informatics overview

BioAccord™ System

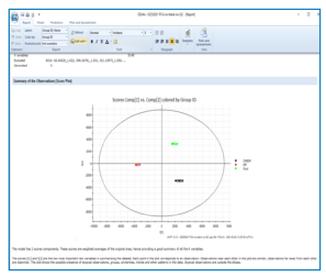
ACQUITY™ Premier LC
ACQUITY HSS T3 Column

waters_connect™
UNIFI™ screening Software



200+ compound library
Custom data review workflow
Report template

Umetrics™ EZinfo™
Software



Multivariate data analysis
Batch comparison

METHOD. The method includes a reversed-phase chromatography, LC-MS data acquisition using an easy-to-use BioAccord HRMS Platform and waters_connect Informatics package composed of a 200+ compound library (Figure 1), a guided workflow for ease of data review (Figure 2), compound elucidation tools for investigation of unknowns (Figure 3), and multivariate data analysis tools for batch analysis (Figure 5). Detailed LC-MS method parameters are described in reference 1.

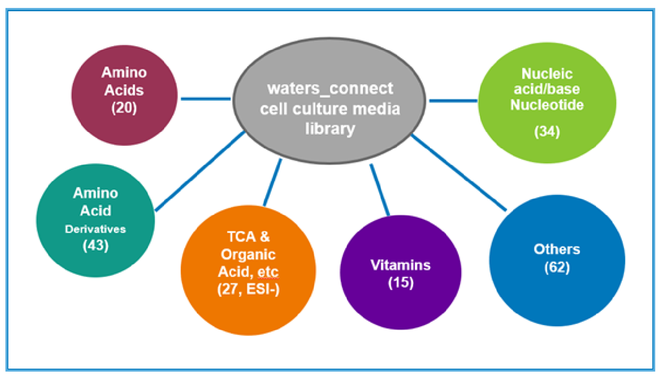


Figure 1. Compound classes included in the scientific library.

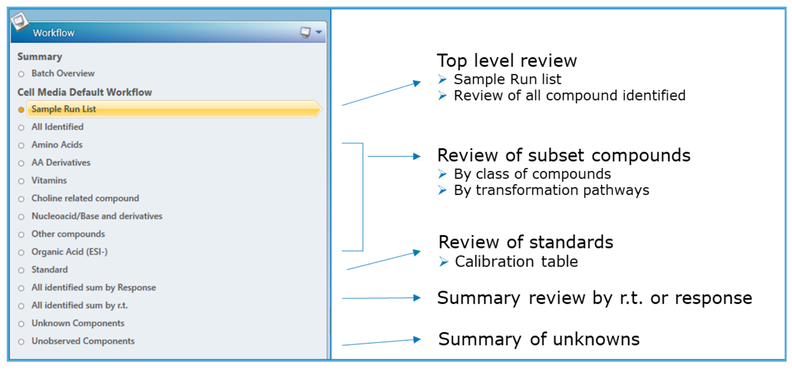


Figure 2. Workflow providing stepwise data view.

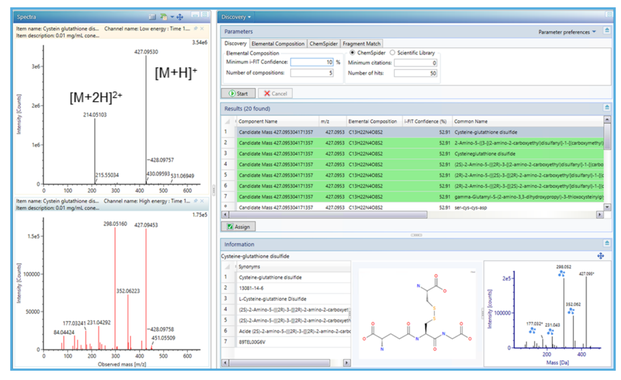


Figure 3. Investigation of unknowns. MSe spectra of an unknown compound was submitted to ChemSpider™ search. Cysteine glutathione disulfide was found and confirmed using authentic standard.

CELL CULTURE MEDIA ANALYSIS EXAMPLE[1]

Spent cell culture media samples from a clone selection experiment were analyzed. The spent media were sampled from 12 bioreactors with each bioreactor sampled for 6 days during a 16-day cultivation period. Post data acquisition, the data are reviewed as trend plot shown for choline in Figure 4. Batch analyses using multivariate data analysis are displayed in Figure 5.

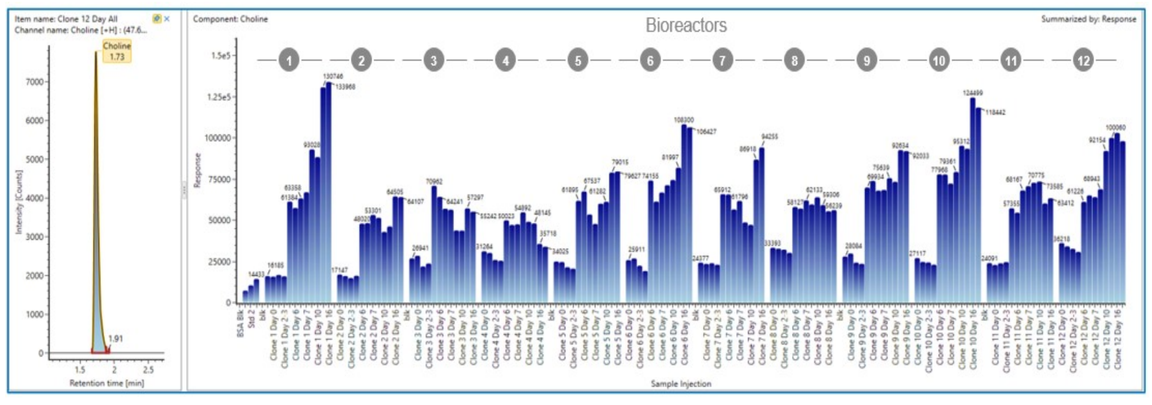


Figure 4. Abundance changes of choline in spent cell culture media is shown by the trending plot bar chart for 12 different bioreactors across multiple days. The bar chart of each grouped trend plot represents time course of one bioreactor. Using this view, changes of media components during the process development can be readily observed.

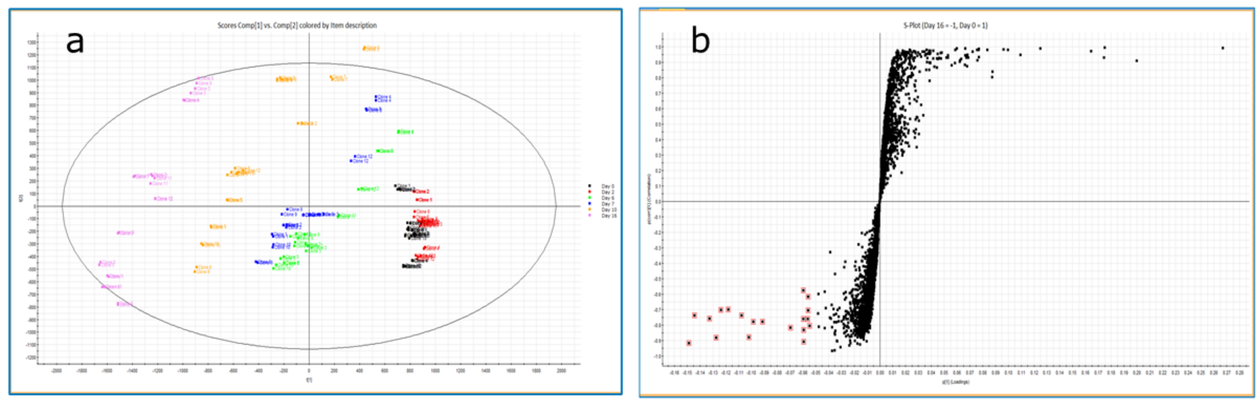


Figure 5. Batch analysis of the dataset, (a) PCA plot showing increasing differentiation of media composition. The plot is colored by date of sampling from different bioreactors and labeled by each bioreactor name (b) Group difference plot, S-Plot, of day 0 and day 16. Discriminatory compounds in day 16, highlighted in red, can be further investigated.

MICROBIAL MEDIA ANALYSIS EXAMPLE[2]

Microbial media, Terrific Broth (ext. Millipore Sigma), was analyzed. Results showed 90+ compounds are detected. They include all compound classes in the library such as amino acid and its derivatives, vitamins, nucleobase, nucleoside, nucleotide, organic acid, peptide fragments and many other compounds. The most abundant compounds are a panel of amino acids and organic acids as exhibited in the overlaid chromatogram in Figure 6.

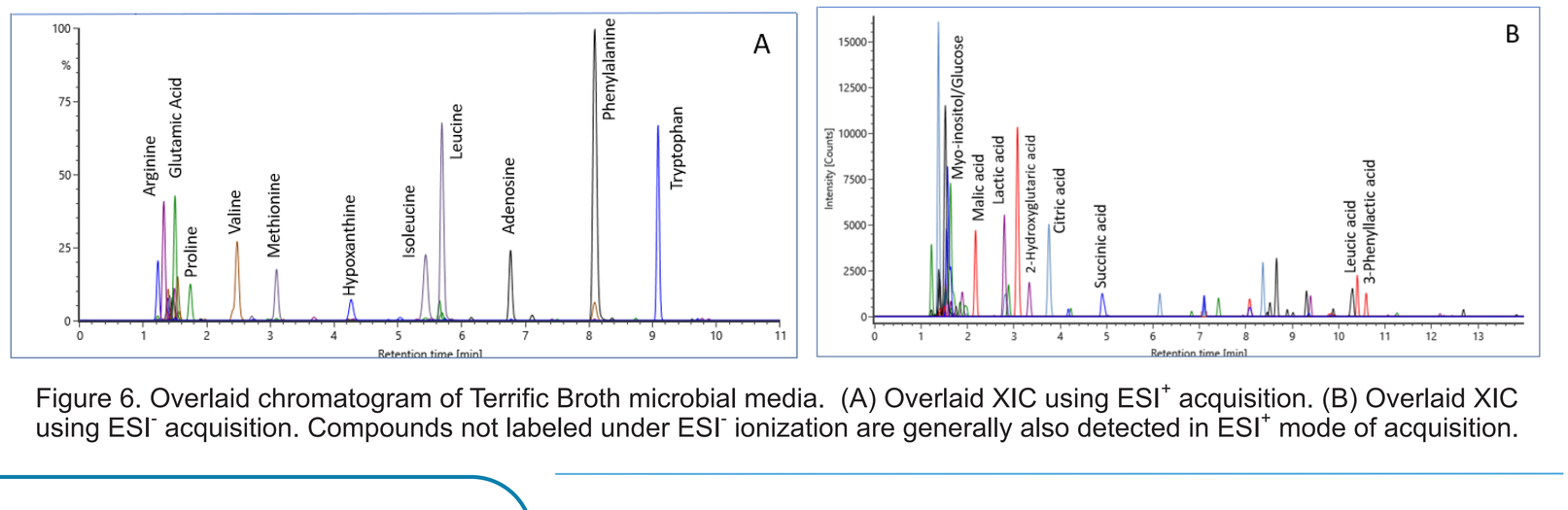


Figure 6. Overlaid chromatogram of Terrific Broth microbial media. (A) Overlaid XIC using ESI^+ acquisition. (B) Overlaid XIC using ESI^- acquisition. Compounds not labeled under ESI^- ionization are generally also detected in ESI^+ mode of acquisition.

SUMMARY

A comprehensive LC-MS methodology and workflow for the analysis of cell culture and microbial media samples are described. The simplicity of BioAccord LC-MS system in terms of ease of setup and long-term performance stability will enable bioprocessing engineers with limited LC-MS experience to quickly and easily run and process large number of samples, thus enabling bioprocess groups to quickly gain insight during bioprocess development and optimization.

References

1. YW Alelyunas, MD Wrona, “Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System With ACQUITY Premier” Waters Application Note P/N 720007359.
2. YW. Alelyunas, MD Wrona, YQ Yu, “Monitoring Nutrients and Metabolites in Microbial Culture Media using the BioAccord LC-MS System with ACQUITY Premier”, Waters Application Brief P/N 720007485.

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