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Application Note

Monitoring Nutrients and Metabolites in Spent Cell Culture Media for Bioprocess Development Using the BioAccord LC-MS System With ACQUITY Premier

Yun Wang Alelyunas, Mark D. Wrona

Waters Corporation

Abstract

In biotherapeutics process development, cell culture media solution provides essential building blocks and nutrients for cell health and biotherapeutics production. Monitoring the feed components and metabolites can provide information on cell growth, biotherapeutic titer, and product quality. The BioAccord LC-MS System has been used to support product quality analysis such as intact protein analysis, peptide MAM, released glycan, and oligonucleotide mass confirmation. In this application the BioAccord System is utilized for the monitoring of the nutrients and metabolites in cell culture media. The method package includes a comprehensive reversed-phase LC-MS method, a 200+ compound library; a simple, stepwise workflow for data review including trend plots; a suite of tools for unknown screening; multivariate data analysis tools; and reporting template. The method has been applied in spent media analysis for clone selection and process optimization.

Benefits

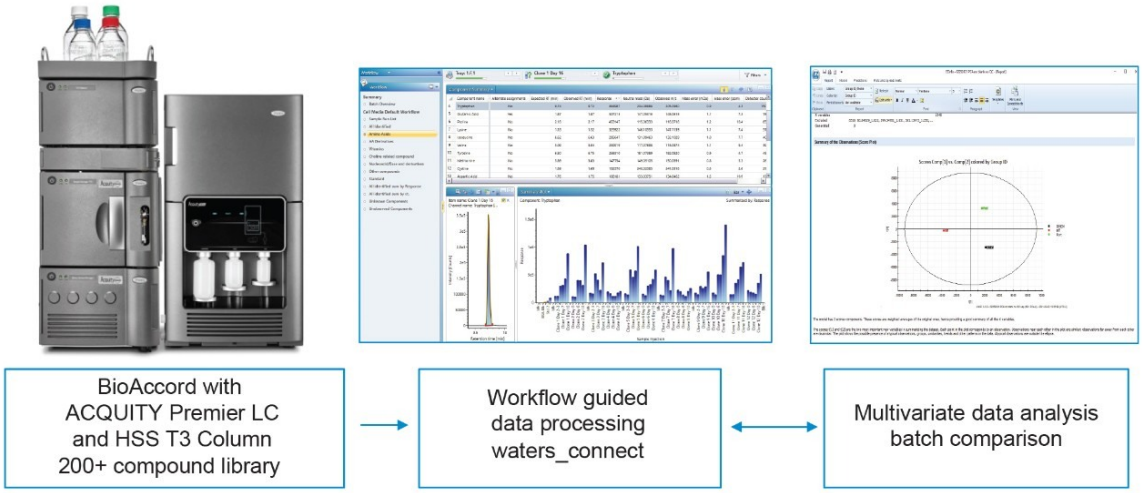
- Excellent chromatographic performance delivered by ACQUITY Premier Technology implemented for both the column and UPLC system hardware
- Rich full scan HRMS data using the simple-to-use BioAccord LC-MS System

- Inclusion of 200+ compound library, comprising amino acids, vitamins, nucleic acids/bases, nucleotides, metabolites, and others compounds of interest in bioprocessing development
- Streamlined function and class-based workflow to facilitate stepwise data review
- A single compliant-ready informatics package supporting data acquisition, data review, elucidation of unknowns, report template, and multivariate data analysis

Introduction

In biotherapeutics process development, cell culture media solution provides essential building blocks and nutrients for cell health and biotherapeutics production. The media solution is a complex mixture containing 100s of compounds, including starting chemicals and metabolites formed during the production cycle. Composition of the media component is constantly changing during the cell growth cycle. Monitoring the feed components and metabolites and ensuring they remain in optimal ranges have been shown to have impact on both cell growth, biotherapeutic titer, and product quality.[1] Routine monitoring of selective key feed components has been a part of bioprocess workflows. The desire to holistically monitor all feed components and metabolites present in the media is growing in the biomanufacturing industry for bioprocess development and optimization.

This application note describes a detailed reversed-phase LC-MS method and a comprehensive workflow for the monitoring of compounds in cell culture media (Scheme 1). The system is based on BioAccord with ACQUITY Premier Technology, a compact, self-optimizing LC-HRMS platform that broadens the accessibility to this technology and generates the same high-quality results whether you are an LC-MS expert or a new user. HRMS offers rich full scan datasets for analyte investigation and ease in method development since optimization of precursor to product transition needed in triple quadrupole MS[2] is not required. The ACQUITY Premier LC comprises low adsorption UPLC systems and columns based on MaxPeak High Performance Surfaces (HPS).[3] Recent literature has shown the ACQUITY Premier LC System is beneficial in improved peak symmetry and increased signal for many compound classes in the cell culture media such as TCA acids,[4] vitamins,[5] nucleic acids/bases, and nucleotides.[6] The current workflow includes 200+ compound library, a stepwise guide for data review, structural elucidation of unknowns, multivariate data analysis/batch comparison, and report. Detailed information for each of the workflow steps and examples of cell culture media analysis are described.



Scheme 1. A schematic illustration of BioAccord/waters_connect based workflow for cell culture media analysis.

Experimental

Sample Description

Commercial media solutions were prepared by diluting 1:100 (V/V) with H_2O. Spent media solutions were prepared by diluting 1:200 (V/V) with either H_2O or H_2O containing 0.1% formic acid.

LC Conditions

LC-MS system:	BioAccord LC-MS System with ACQUITY Premier BSM
Column(s):	ACQUITY Premier HSS T3 2.1 × 150 mm (P/N 186009469)
Column temp.:	40 °C
Sample temp.:	6 °C
Injection volume:	2 µL
Flow rate:	0.25 mL/min
Mobile phase A:	H_2O/0.1% FA
Mobile phase B:	90%ACN/10%IPA/0.1%FA

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.25	100	0	6
1.5	0.25	100	0	6
6	0.25	95	5	6
9	0.25	65	35	6
14	0.25	5	95	6
17	0.25	5	95	6
17.1	0.25	100	0	6
20	0.25	100	0	6

MS Conditions

LC-MS system:	BioAccord LC-MS System with ACQUITY Premier BSM	
Ionization mode:	Full scan or Full scan with fragmentation	
Acquisition range:	Low (50–2000 *m/z*)	
Polarity	Positive	
	Capillary voltage:	1 kV
	Cone voltage:	20 V
	Fragmentation cone voltage	40–60 V
	Negative	
	Capillary voltage:	0.8 kV
	Cone voltage:	15 V
	Fragmentation cone voltage	50–70 V
Scan rate	5 Hz	
Desolvation temperature	550 °C	
Intelligent data capture	On	
Dynamic lock mass correction	On	

Data Management

LC-MS software: waters_connect

Informatics: waters_connect base kit with UNIFI 1.9 SR13

Results and Discussion

General Method Discussion

The application on cell culture media compounds was developed using BioAccord LC-MS with ACQUITY

Premier LC and HSS T3 Column. The system has excellent chromatographic performance with narrow and symmetrical peaks across diverse classes of compounds. Figure 1 is an overlaid extracted ion chromatogram (XIC) of compounds in the method under positive ionization mode of data acquisition. XIC chromatograms of representative individual compounds are available in the Appendix. Figure 1 also shows excellent baseline resolution between isobaric compound pairs, leucine/isoleucine, and citric acid/isocitric acid. Samples were run with either full scan or full scan with fragmentation mode of data acquisition. Full scan was recommended for general monitoring, especially when a large number of samples are analyzed. Full scan with fragmentation is preferred to aid compound elucidation and reduce false positives in external ChemSpider database searches.

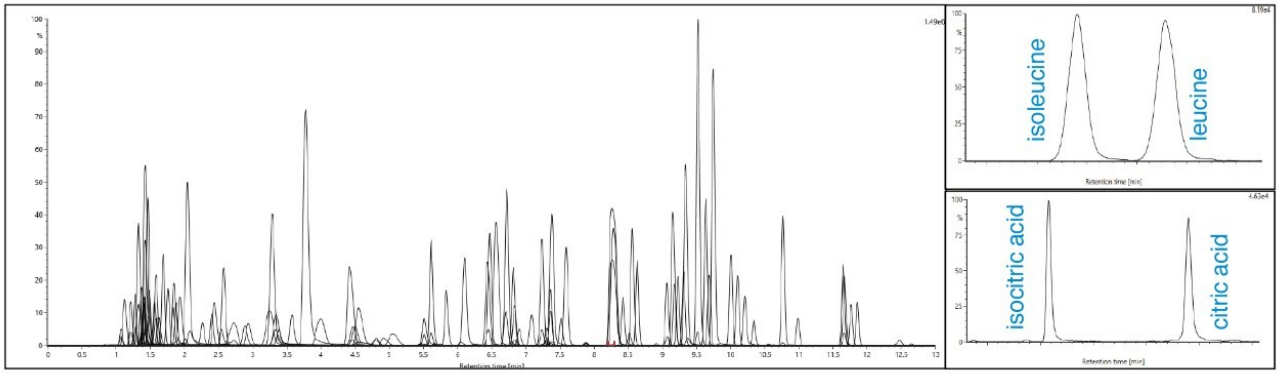


Figure 1. Overlaid extracted ion chromatogram (XIC) of general compound coverage, isobaric pair isoleucine/leucine, and isocritic acid/citric acid.

System performance was assessed based on 6 replicate injections of a basal cell culture media solution, IMDM (ext. Sigma Aldrich) at 1:100 diluted concentration. Reproducibility in retention time, mass error, and peak response are summarized for representative compounds across elution time window (Table 1). Results show excellent reproducibility in retention time (<0.02 min shift), mass error (<5 ppm), and response (<5%), all of which are independent of compound elution time. The system also produces excellent sensitivity ranging from sub ng/mL to ng/mL detection limit. The good sensitivity and reproducibility along with other attributes make the BioAccord System a suitable platform for the cell culture media compound monitoring.

Compound	Approx. conc. (µg/mL)	Neutral mass	r.t. (min)		Mass error (ppm)		Response	
			Avg	Stdev	Avg	Stdev	Avg	%RSD
Arginine	0.84	174.112	1.31	0.00	-1.3	2.0	1.1E+05	1.5
Cystine	0.91	240.024	1.36	0.00	-2.1	2.7	5.5E+04	2.9
Threonine	0.95	119.058	1.46	0.00	-0.7	0.8	5.0E+04	2.1
Valine	0.94	117.079	2.41	0.01	-0.2	1.7	1.2E+05	1.4
Nicotinamide	0.04	122.048	3.66	0.02	-3.2	1.6	7.1E+03	4.6
Isoleucine	1.05	131.095	5.21	0.02	-1.1	0.7	2.6E+05	1.3
Phenylalanine	0.66	165.079	8.10	0.00	-0.6	1.7	2.5E+05	1.8
Pantothenic acid	0.04	219.111	8.74	0.00	0.2	2.3	7.9E+03	3.5
Tryptophan	0.16	204.090	9.08	0.01	-0.9	1.7	7.0E+04	2.4
Folic acid	0.04	441.140	9.10	0.00	-0.4	1.9	1.3E+04	0.7

Table 1. Summary of reproducibility data for representative compounds based on 6 replicate injections. Sample was prepared by diluting neat media with H_2O 1:100 (V/V). The concentration was approximate concentration derived from vendor's product brochure.

Compound library

A UNIFI library containing 200+ compounds was created to facilitate their identification and tracking in analyses. Retention time and fragmentation data for each compound were derived and confirmed with authentic compound standards. Each compound was tagged in the library according to the following subclasses: amino acid, amino acid derivatives, organic acids, vitamins, nucleic acids/bases and nucleotides, and others. Each compound was also tagged with preferred mode of ionization, ESI^+ or ESI^-. The tagging information can be used to retrieve the subclass of compounds during library searching, compound importing into analysis method, and/or creation of filters during workflow step constructions (see below). The distribution of compound classes and example of library entry is shown in Figure 2.

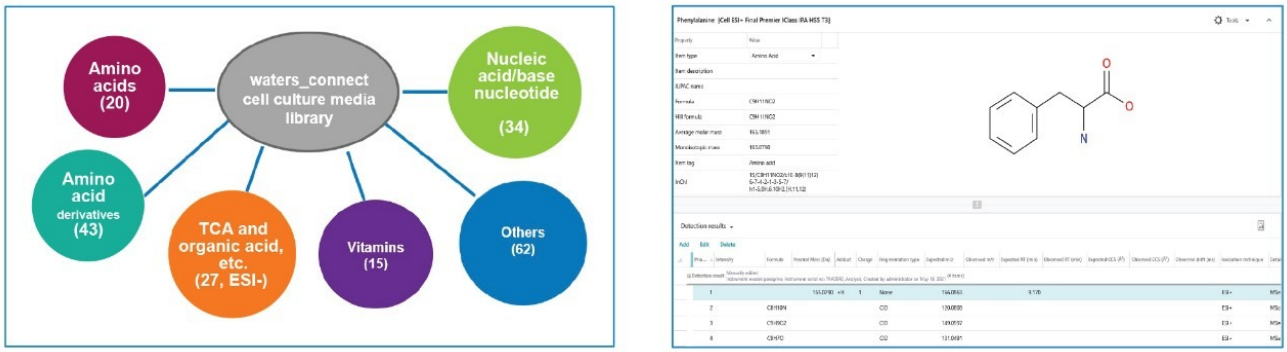


Figure 2. Compound classes included in the scientific library (left), and an example of compound display in the library (right).

Workflow guided data review

A bioprocess development project typically generates many cell culture media samples for analysis. In each sample, there are potentially greater than 100 compounds that bioprocessing engineers would like to acquire information for review. It is critical to have a streamlined data review process to readily extract the information needed and expedite the process. The workflow functionality available in UNIFI Informatics System is designed to provide a straightforward and stepwise review of the processed data based on function, class, and/or other criteria. A default data review workflow for cell culture media analysis is shown in Figure 3. Several key steps covering sample run list, compound classes, and cross sample comparisons were created to demonstrate how the information can be gathered and displayed in UNIFI. Compound review can be managed via trend plot across injections, graphic overlays, and/or tabulated information. The workflow can also be customized, and specific steps can be added easily to cover compounds of interest, critical pairs, and/or specific transformation pathways. In the default workflow, an example of choline pathway is included where three related compounds, choline, choline phosphate, and choline glycerophosphate are displayed. Changes of these compounds along with the incubation time and across bioreactors are readily displayed in the overlaid trend plot. The "unknown compound" step is a collection of all compounds that are not identified. It helps to aggregate data for compounds that merit further investigation/elucidation. The workflow can be exported or imported for sharing with other waters_connect platforms/data processing workstations.

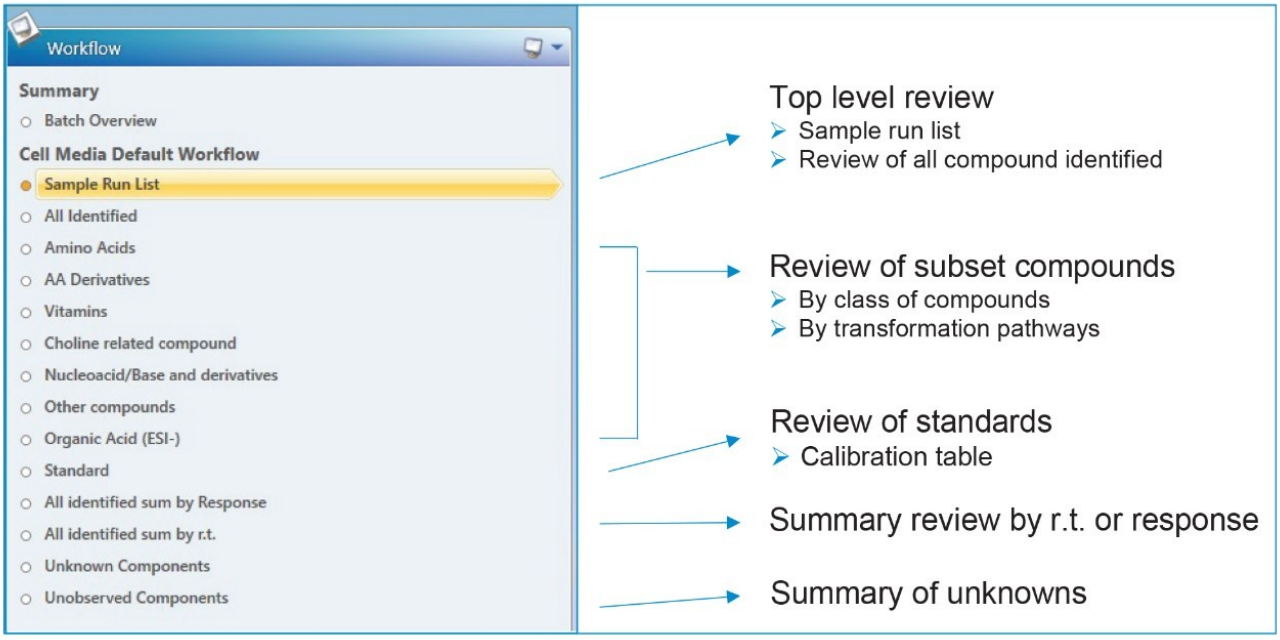


Figure 3. Default workflow for cell culture media data review.

Graphic Display showing the results from cell culture media analysis

Figure 4 is an overlaid chromatogram of commercially available basal cell culture media solution, DMEM (ext. Sigma Aldrich). DMEM contains amino acid and vitamins at concentration ranging from µg/mL to 100's µg/mL. For this analysis the media sample was diluted 100x with H_2O and 2 µL was injected. Excellent chromatographic performance was obtained on the system where all compounds were detected. This sample is very useful for the optimization of LC-MS separation conditions.

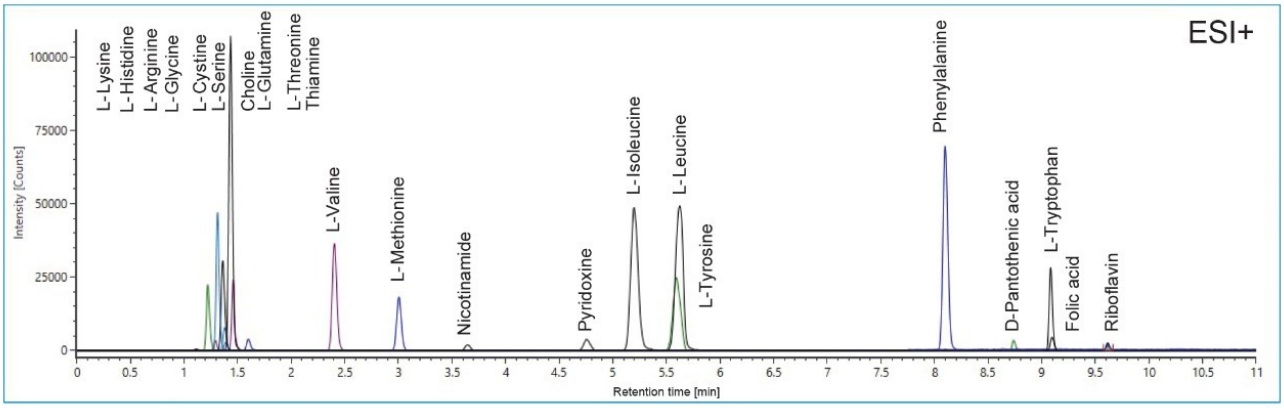


Figure 4. Overlaid chromatogram of basal cell culture media DMEM under ESI^+ ionization conditions.

For the analysis of spent media solutions from bioreactors in upstream process development, it is useful to track the abundance changes of specific component(s) in the media over incubation time and/or different bioreactors. A sample sets from 12 bioreactors sampled at 6 time points up to 12 days were analyzed by the present method. Figure 5 shows the overall trending plot of choline from all bioreactors and all sampling dates. The bar chart of each grouped trend plot below represents time course of one bioreactor. Using this view, changes of cell culture media components during the process development can be readily observed.

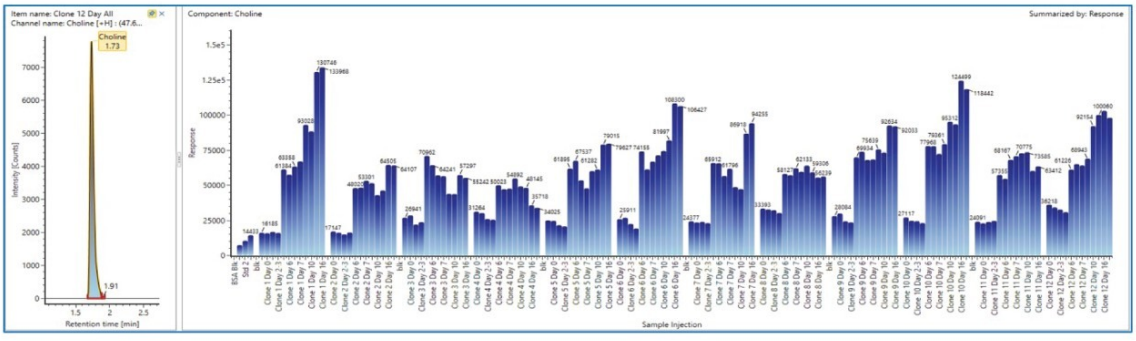


Figure 5. An example of abundance changes of choline in spent cell culture media is shown by the trending plot bar chart for 12 different bioreactors across multiple days.

Elucidation of unknowns

Cell culture nutrient metabolism during bioprocessing is a complex process. Metabolites formed during biotherapeutics production but not identified by the library match may still be of interest, especially if they correlate with titer, product quality attributes or processing parameters. An example of structural elucidation for an unknown compound in spent cell culture media is shown in Figure 6. For this example, the most abundant unknown compound was detected at *m/z* of 427.0950. A search of the ChemSpider database returned several hits, including a compound of interest, cysteine-glutathione disulfide (Figure 6). Identity of the compound was subsequently confirmed using an authentic standard of cysteine-glutathione disulfide. This compound was then added to the component table in the analysis method for future application. Upon data reprocessing after inclusion of the compound, the results revealed its abundance changes in different bioreactors throughout the time course. Lastly, it is recommended that acquisition with "full scan with fragmentation" should be performed to collect both precursor as well as fragmentation data. Fragmentation data collected this way will aid in identifying and reducing false positives in database searching.

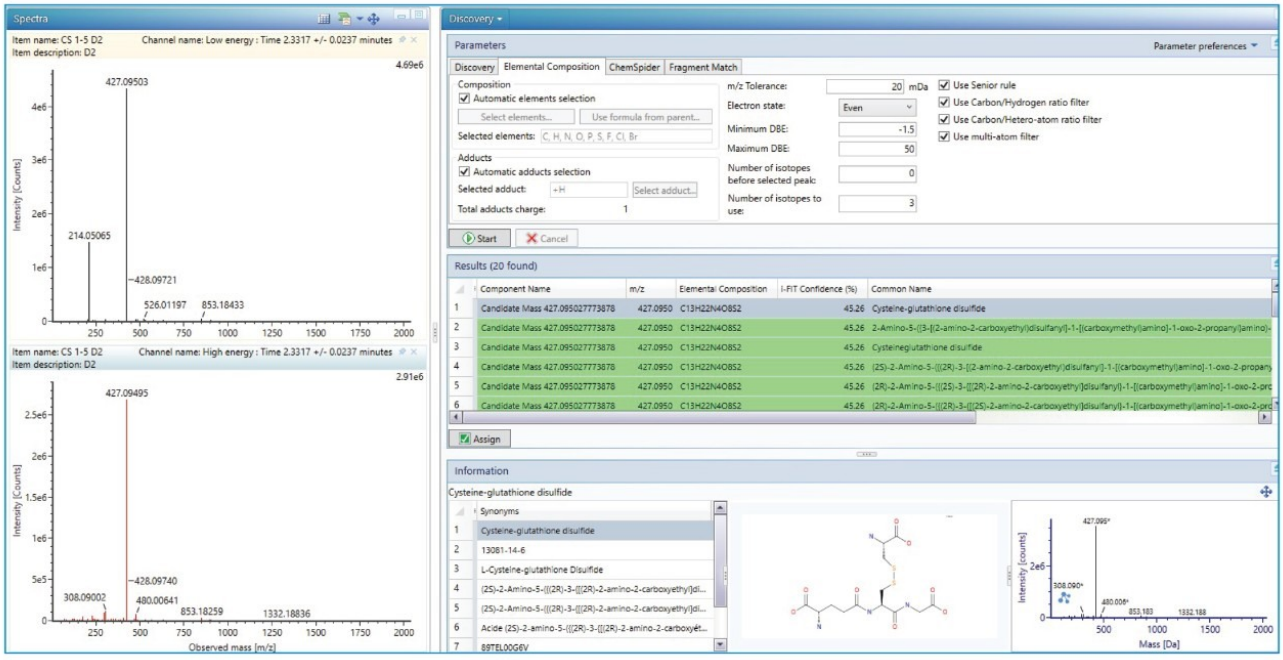


Figure 6. A screen capture of compound elucidation method setup displaying low and high energy fragmentation spectra of 427.0950 *m/z* unknown, and returns from ChemSpider database search.

Multivariate data analysis to reveal the difference among cell culture media samples

Additional multivariate data analysis tools based on Umetrics EZ-info software are available in UNIFI Informatics System for data analysis. EZ-info offers a powerful way to analyze and perform batch comparison of large

number of datasets. For example, datasets for comparing multiple bioreactors with same/different cell lines, for comparing media components at the early incubation time to those in late incubation time in the same bioreactor, or for comparing different bioreactors at the same incubation timepoint, and many other comparisons. Figure 7 shows two example outputs after multivariate data analysis of samples from 12 bioreactors sampled for many days. After initial compound selection according to mass range, retention time and response, the selected compounds were automatically transferred to EZ-info. The PCA plot provided an overall view of differences between different bioreactors and different date of media sampling. The score plot or S-plot can be generated to visualize the differences between a pair of datasets, in this case, day 0 and day 16 from all bioreactors. The down regulated compounds on day 16 can then be selected and sent back to UNIFI for compound identification. This data processing approach allows users to quickly locate the biomarkers that are associated with the difference between samples.

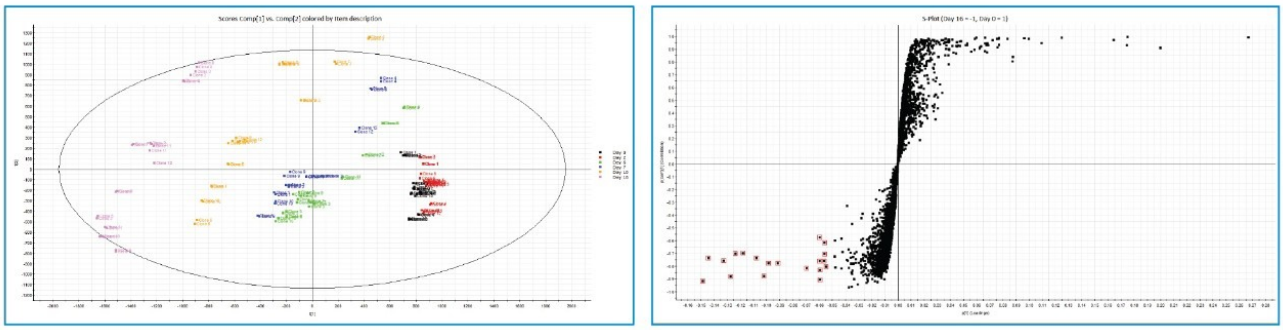


Figure 7. Example outputs of multivariate data analysis. (left) a PCA plot, colored by date of sampling from different bioreactors. (right) S-Plot of day 0 and day 16 cell culture media. Down regulated compounds (markers) in Day 16 are highlighed in red.

Reporting

A default report template is provided to offer a convenient way to summarize and present the analysis results of the cell culture media analysis. The report templates dramatically reduce the time needed to communicate the analytical results with collaborators or samples sources. The report consists of a data export in tabulated (.xls) format and a text/graphic export in PDF/XPS format. The text/graphic report contains information including filename, LC-MS method details, sample run list, bar or line chart plot either as single compound or overlay of multiple compounds, and others. Company logo image can also be added. The report can be customized or edited via user-friendly report template tools. A new report template can be generated through modification of existing report or adding additional report object.

Conclusion

A comprehensive LC-MS workflow based on the BioAccord System and UNIFI Informatics Platform has been developed for non-targeted cell culture media analysis. The BioAccord LC-MS System offers the benefits of easy setup, long-term performance stability and simplicity of operation. This platform allows bioprocessing engineers with limited LC-MS experience to quickly and easily run and process large numbers of cell culture media samples and provide them with rich, insightful information on both feed additives and metabolites formed during the bioprocess development. The described methodology is developed based on reversed-phase separation principles to cover the majority of components encountered in cell culture media. The MaxPeak HPS Technology, which is implemented on Waters ACQUITY Premier Products (LC systems and columns), has provided improved chromatographic performance for a range of compound classes. In addition, the inclusion of Premier technologies and the compound library into the analysis method results in a high-performance platform and an end-to-end workflow to cover the key steps required in cell culture media analysis, facilitating the adoption of LC-MS technologies for bioprocessing development. The method was successfully demonstrated for the analysis of both starting media solutions and spent media samples from bioreactors in upstream process development and optimization.

References

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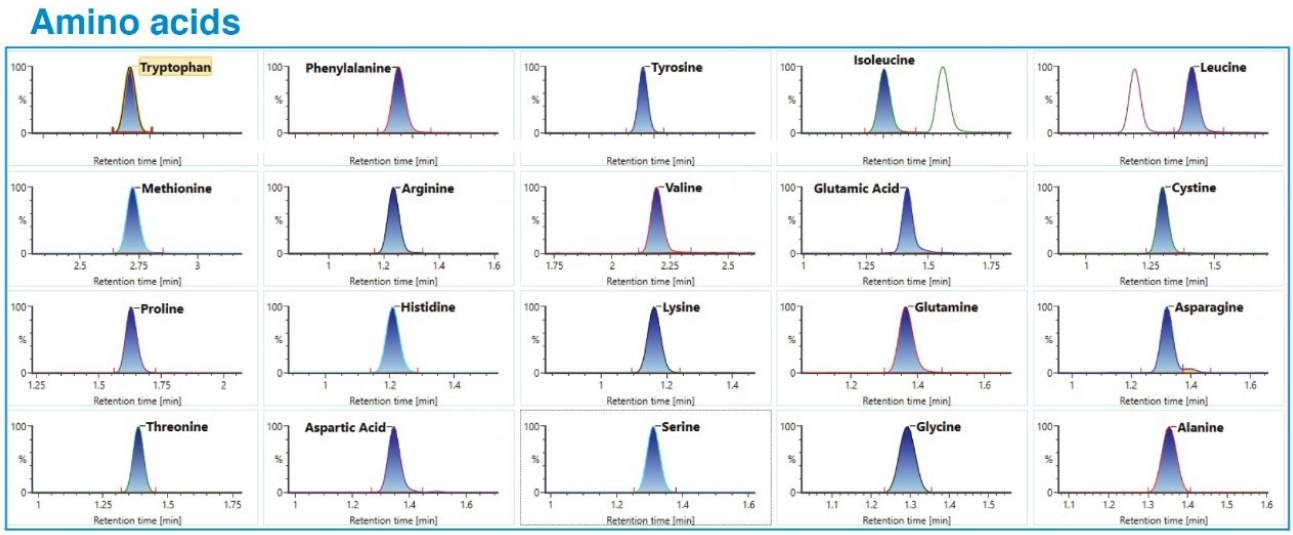
Acknowledgements

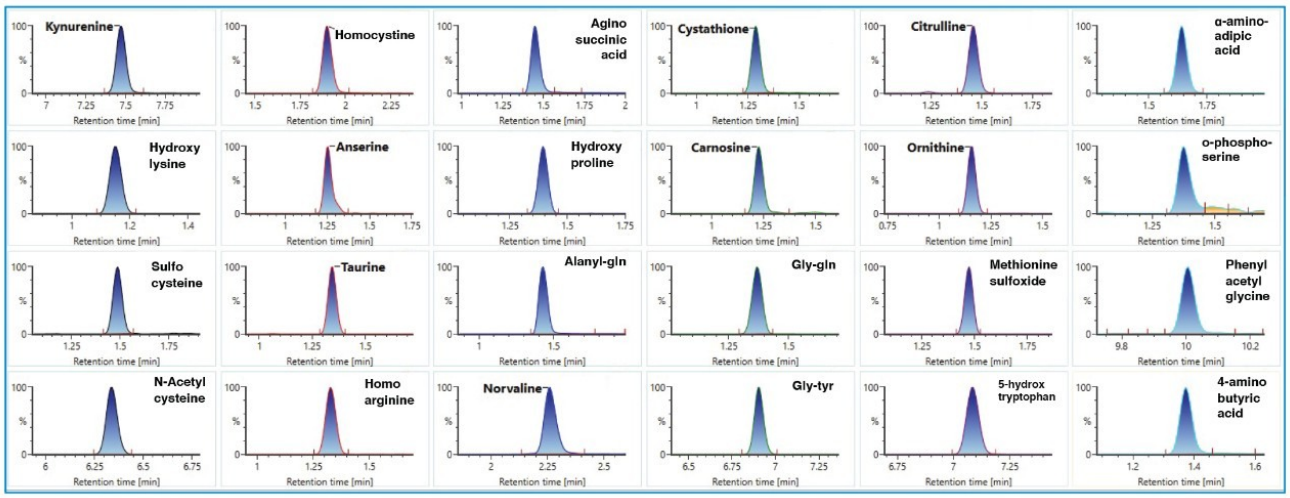
The authors would like to acknowledge our Waters colleagues for their contribution during development and preparation of this appnote including: XiaoXia Wang, Kerry Smith, Giorgis Isaac, Jinchuan Yang, Jeff Goshawk, Andy Baker, Steven Lai, Gitte Barknowitz, Steve Preece, Guillaume Bechade, Xiao Dong, Heidi Gastall, and many more.

Appendix

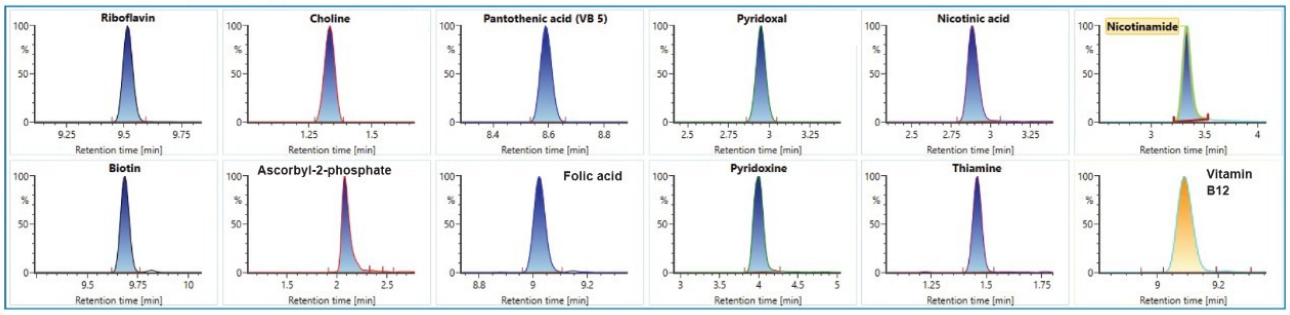
Example extracted ion chromatograms (XIC) of compounds in the library.



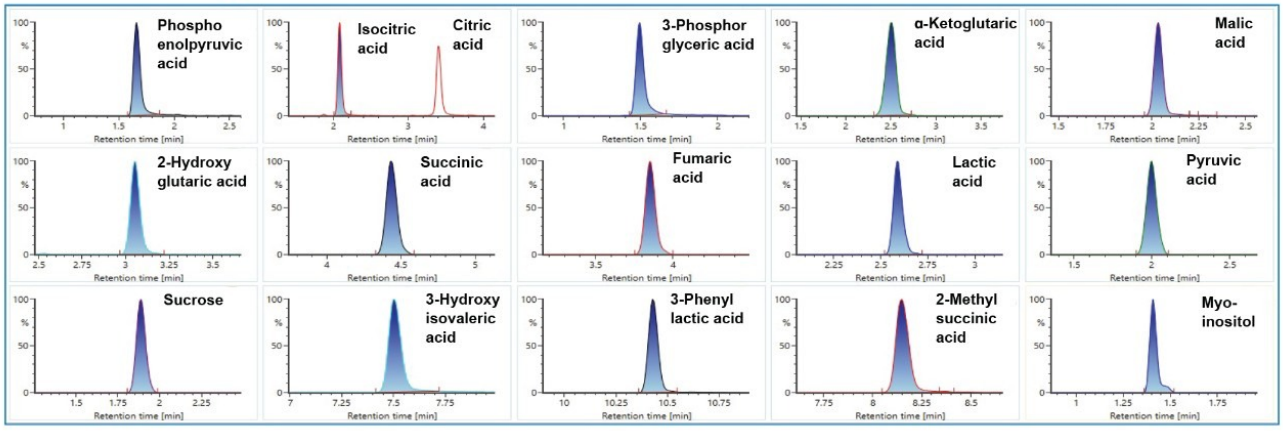
Amino acid derivatives

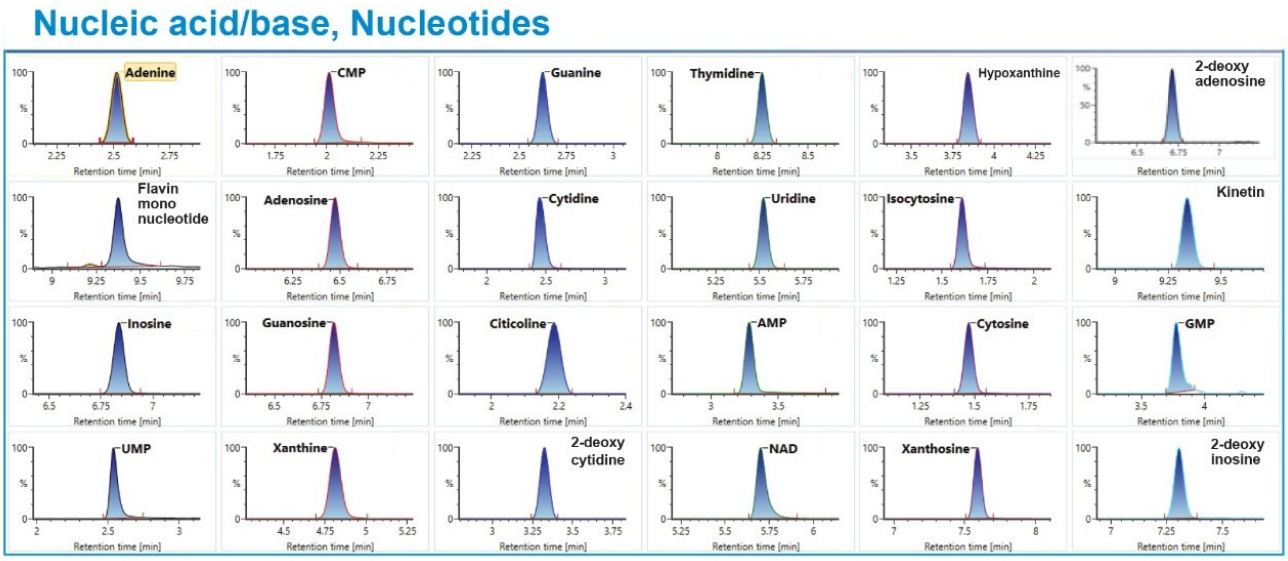


Vitamins (ESI+)



TCA and Organic acids





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- UNIFI Scientific Information System <https://www.waters.com/134801648>
- waters_connect <https://www.waters.com/waters/nav.htm?cid=135040165>

720007359, September 2021