A powerful single method for sensitive quantitation and targeted/non-targeted identification of cell culture media (CCM) components using accurate mass spectrometry

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The Power of Precision

Presentation outline

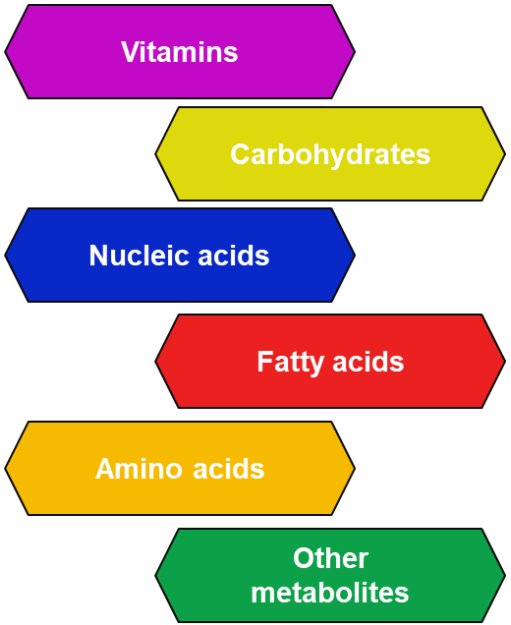
MAIN POINTS

- Introduction and background for CCM analysis
- Overview of the methodology and data analysis
- Results
  - Zeno SWATH data-independent acquisition (DIA) MS and MS/MS for quantitation
  - Zeno SWATH DIA MS and MS/MS for qualitative analysis of CCM components
- Conclusions

Introduction

WHY MONITOR COMPOSITION MEDIUM DURING BIOTHERAPEUTICS PRODUCTION?

- Biopharmaceuticals are produced by a wide range of media systems
- CCM components, levels and consumption can vary by product, cell type and cell line
- Qualitative understanding followed by quantitative tracking is important to meet quality requirements and reduce inefficient manufacturing



Analytical requirements and challenges for CCM analysis

ANALYTICAL REQUIREMENTS

- Monitor and identify high number of metabolites with diverse chemical properties
- Analyze complex matrices with wide natural abundance and chemical properties
- Sensitive quantitation and targeted/non-targeted ID in parallel

ANALYTICAL CHALLENGES

- Analysis of polar and non-polar metabolites in a single chromatographic run
  - *Retention of polar analytes (amino acids)*
  - *Separation of isomers*
- Cover a wide dynamic range
- Build a robust and comprehensive LC-MS method
- Ultra sensitive targeted quantitation
- Detection of low-level target analytes

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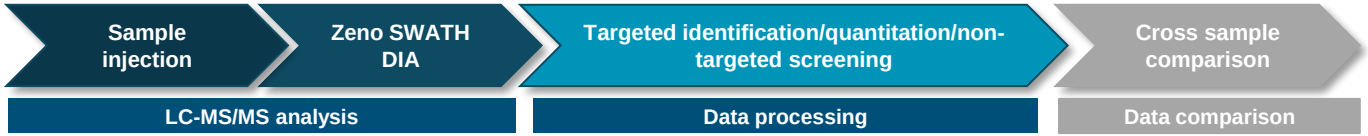
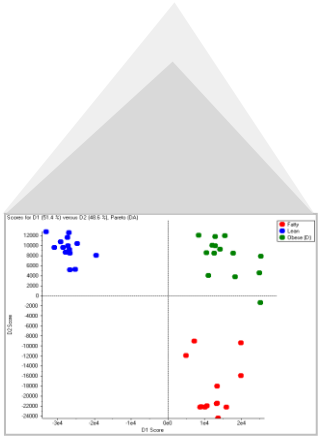
CCM analysis workflow



Metabolite identification and quantitation

Targeted data extraction using a library and quantitation

Statistical analysis

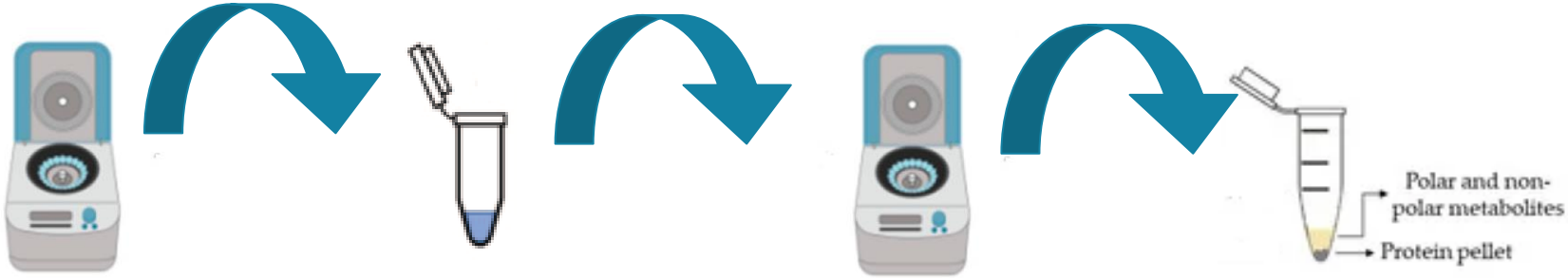


T-tests, PCA analysis and others

Overview of the methodology

Sample extraction

SAMPLES AND STANDARDS DILUTION SERIES*



1. Vortex and centrifuge samples for 10 sec

2. Dilute 25 µL of the sample with 100 µL of 50:50, acetonitrile/water with 0.1% formic acid

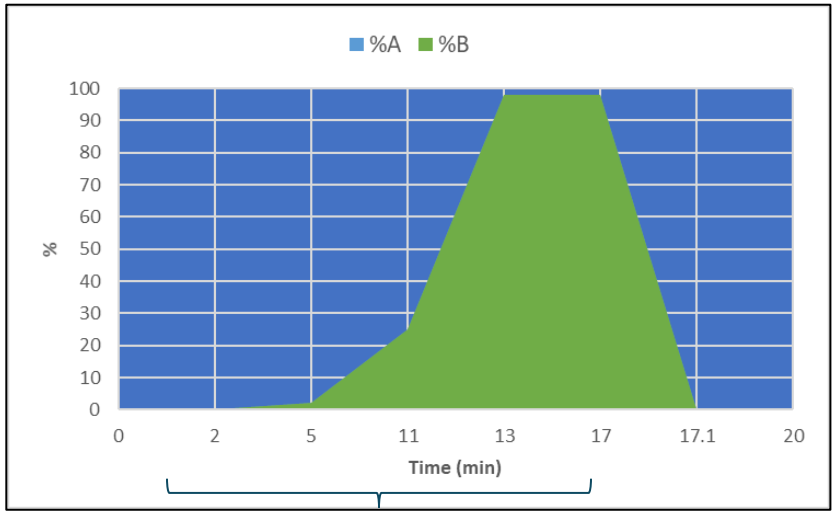
3. Vortex for 10 sec and centrifuge at 10,000 g for 10 min

4. Collect the supernatant and dilute 60-fold with 0.1% formic acid in water

*Tested standard dilutions were in the range 0.0001-0.5 µmol/mL

LC conditions and MS parameters

Analyte	117 analytes
LC opt	20 min gradient Kinetex F5
Injections	5 µl of extracted sample
Flow rate	0.2 mL/min
Mobile phase	0.1% formic acid in water/acetonitrile

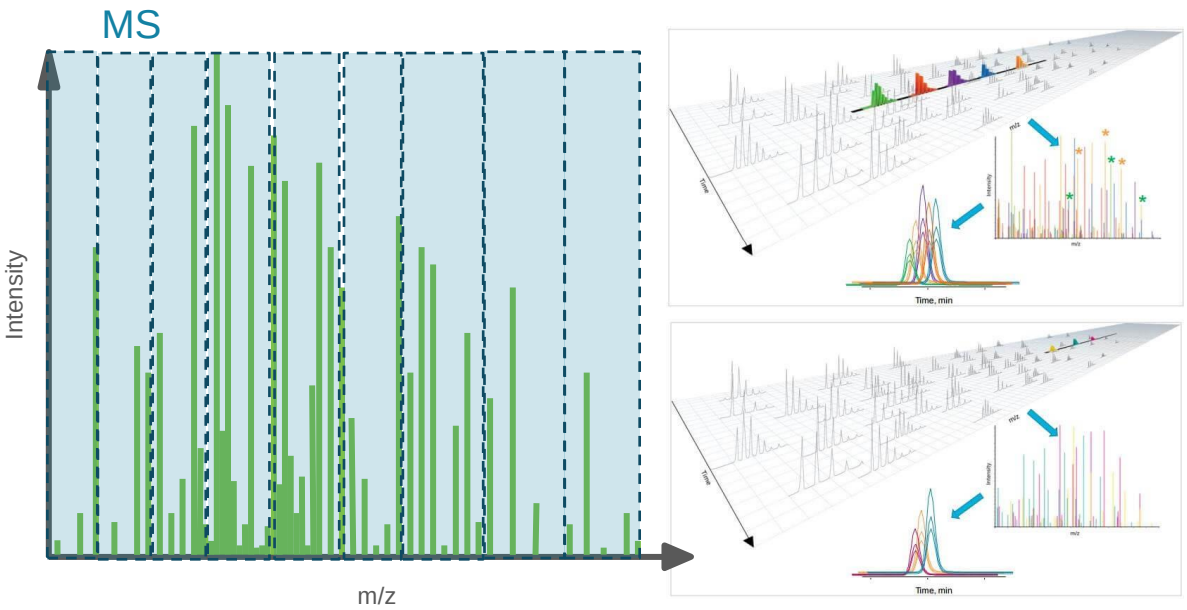


Compound elution time frame

Parameter	Value
Ion source gas 1	50 psi
Ion source gas 2	50 psi
Source temperature	400°C
Ion spray voltage	5500 V/-4500 V
TOF MS Accumulation time	50-700 m/z 0.1 sec
TOF MS/MS Accumulation time	25-700 m/z 0.015 sec
No. of variable windows	25
Total scan time	0.689 sec
Declustering potential	40 V
Collison energy	35 V

Zeno SWATH DIA MS and MS/MS for quantitation

SWATH DIA

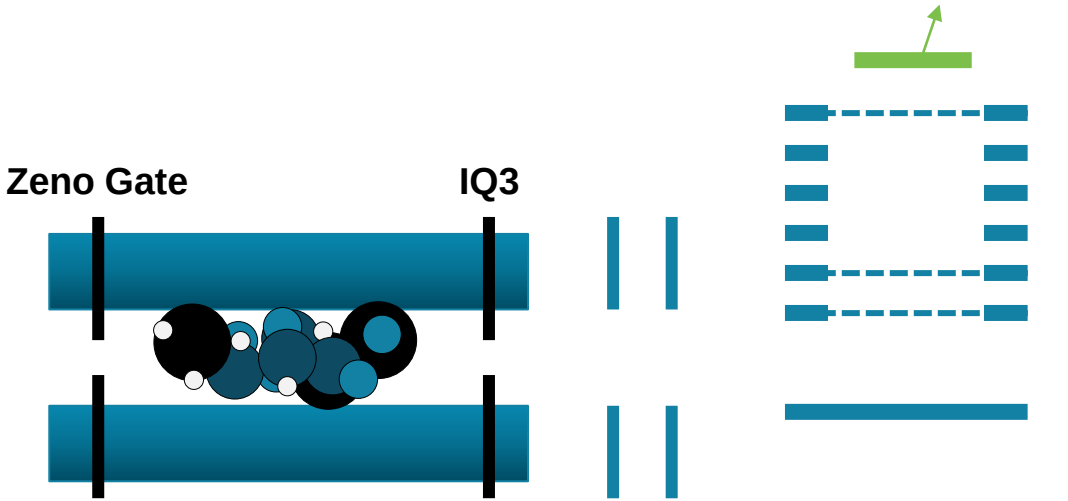


- Capture a complete digital archive of your sample
  - Comprehensive, acquires MS/MS of everything
  - Reduce the risk of missing any targets
- High-quality and sensitive quantitation, similar to the triple quadrupole
- Reduce method development
  - Single acquisition method
  - Re-analyze without re-acquisition

Zeno trap technology

FOR SENSITIVITY GAINS IN MS/MS

- The Zeno trap provides control of the ion beam from the collision cell into the accelerator
- Ions exit the Zeno trap in an ordered release based on potential energy
  - Ion are generally released from a high m/z to low m/z
  - All ions now arrive in the accelerator at the same time and location



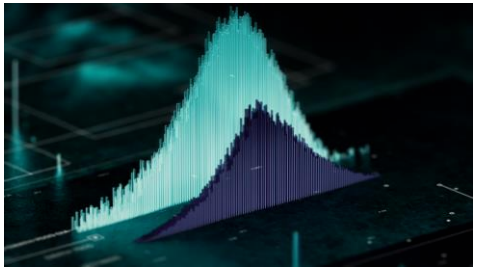
Quadrupole region
Continuous ion beam

TOF accelerator
Ion pulsing

Benefits of Zeno SWATH DIA

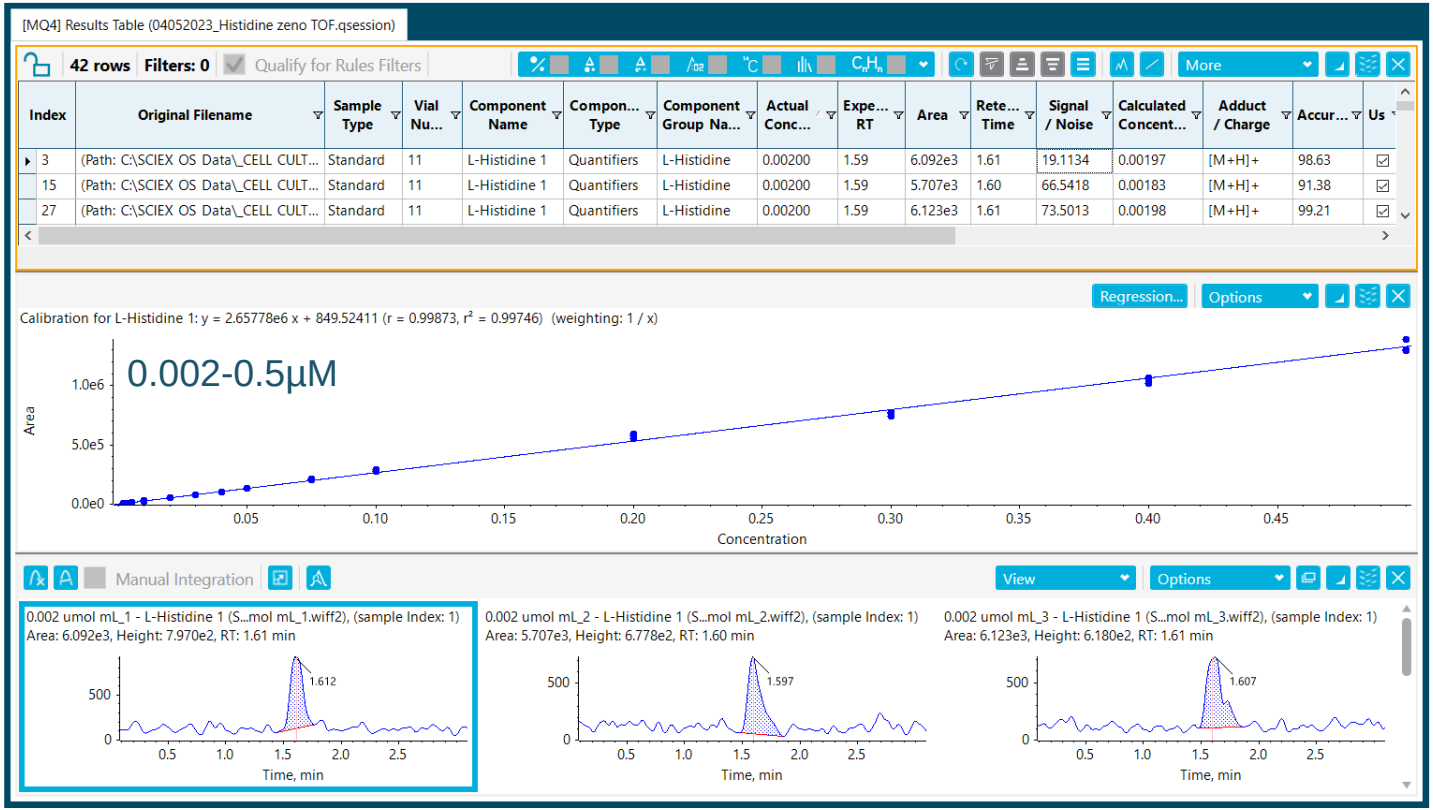
ACHIEVE MAXIMUM INFORMATION FROM EVERY SAMPLE

- Combines the benefits of SWATH DIA with the boost in MS/MS sensitivity from the Zeno trap
  - Higher quality data
  - Identify and quantify more analytes at lower levels or from low sample loads
  - Deeper sample coverage
- Robust and reproducible



L-histidine

m/z 156.07 → 110.071



r^2: 0.998

Lower limit of quantitation (LLOQ) 0.002 µmol/mL

Quantitative performance for L-histidine

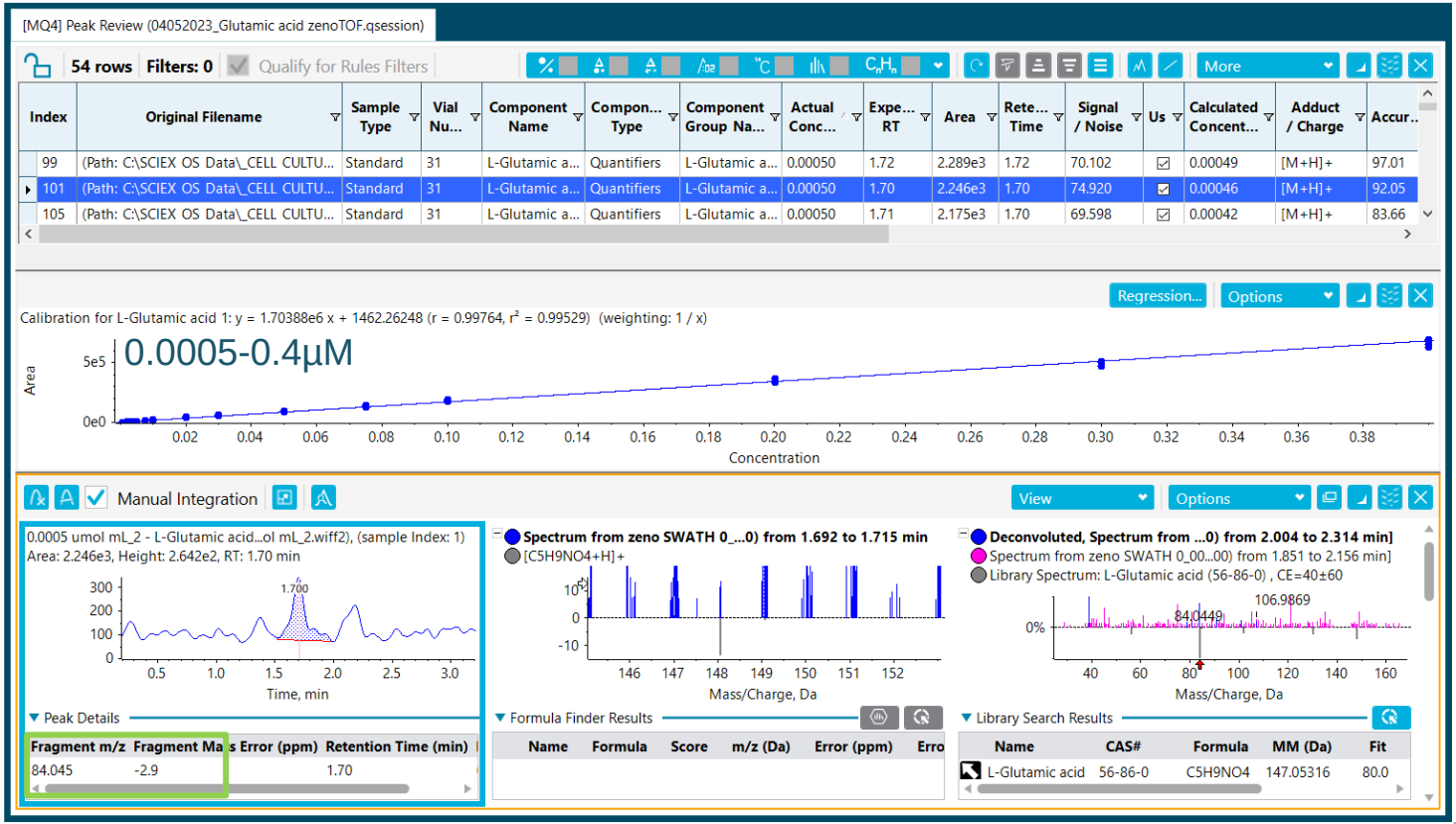
%CV, STANDARD DEVIATION AND MEAN

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Avera...	Value #1	Value #2	Value #3
1	L-Histidine 1	0.00200	3 of 3	0.00193	0.00009	4.52	96.41	0.00197	0.00183	0.00198
2	L-Histidine 1	0.00300	3 of 3	0.00318	0.00017	5.40	106.07	0.00315	0.00337	0.00303
3	L-Histidine 1	0.00500	3 of 3	0.00468	0.00017	3.59	93.60	0.00475	0.00449	0.00481
4	L-Histidine 1	0.01000	3 of 3	0.01053	0.00068	6.45	105.28	0.01003	0.01130	0.01025
5	L-Histidine 1	0.02000	3 of 3	0.01929	0.00037	1.94	96.47	0.01972	0.01912	0.01904
6	L-Histidine 1	0.03000	3 of 3	0.02882	0.00084	2.91	96.07	0.02977	0.02819	0.02850
7	L-Histidine 1	0.04000	3 of 3	0.03756	0.00095	2.53	93.90	0.03686	0.03718	0.03864
8	L-Histidine 1	0.05000	3 of 3	0.04994	0.00119	2.39	99.89	0.04890	0.04969	0.05124
9	L-Histidine 1	0.07500	3 of 3	0.07926	0.00312	3.94	105.68	0.08134	0.07567	0.08077
10	L-Histidine 1	0.10000	3 of 3	0.10542	0.00437	4.14	105.42	0.10220	0.11039	0.10367
11	L-Histidine 1	0.20000	3 of 3	0.21531	0.00835	3.88	107.66	0.21560	0.20682	0.22351
12	L-Histidine 1	0.30000	3 of 3	0.28582	0.00491	1.72	95.27	0.28029	0.28755	0.28964
13	L-Histidine 1	0.40000	3 of 3	0.39286	0.00972	2.48	98.22	0.38178	0.39995	0.39687
14	L-Histidine 1	0.50000	3 of 3	0.50039	0.01916	3.83	100.08	0.52245	0.49073	0.48797

The accuracy was less than ±6% of the nominal concentration for all concentrations measured

L-glutamic acid

m/z 148.06 → 84.045



r^2: 0.995

LLOQ
0.0005 µmol/mL

Good MS/MS mass accuracy

Quantitative performance for L-glutamic acid

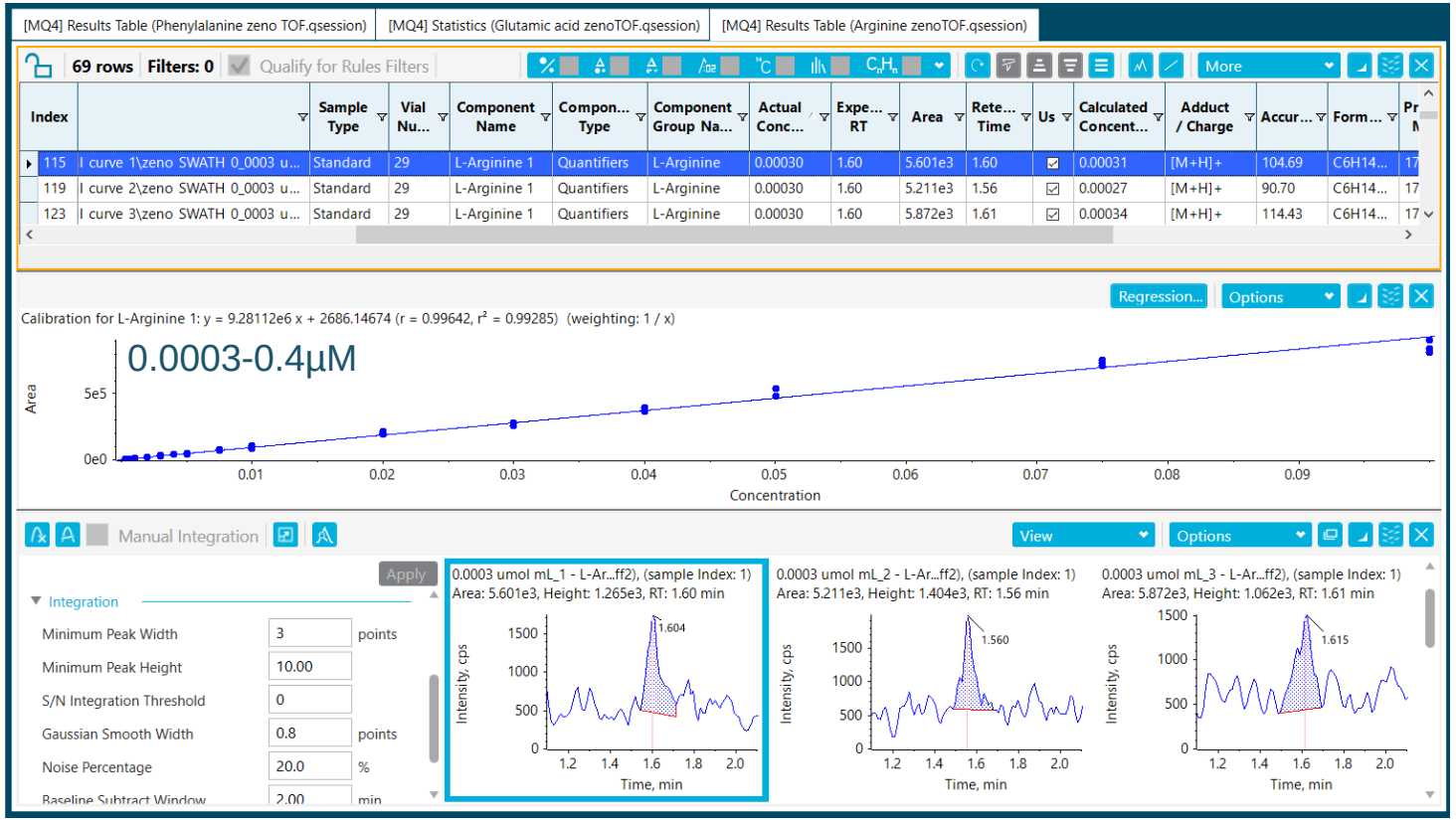
%CV, STANDARD DEVIATION AND MEAN

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Avera...	Value #1	Value #2	Value #3
1	L-Glutamic acid 1	0.00050	3 of 3	0.00045	0.00003	7.42	90.90	0.00049	0.00046	0.00042
2	L-Glutamic acid 1	0.00075	3 of 3	0.00058	0.00005	9.07	77.43	0.00062	0.00052	0.00060
3	L-Glutamic acid 1	0.00100	3 of 3	0.00084	0.00007	8.85	83.82	0.00081	0.00092	0.00078
4	L-Glutamic acid 1	0.00200	3 of 3	0.00184	0.00012	6.58	91.97	0.00192	0.00170	0.00190
5	L-Glutamic acid 1	0.00300	3 of 3	0.00313	0.00004	1.42	104.49	0.00318	0.00309	0.00313
6	L-Glutamic acid 1	0.00400	3 of 3	0.00394	0.00009	2.36	98.59	0.00385	0.00404	0.00395
7	L-Glutamic acid 1	0.00500	3 of 3	0.00514	0.00012	2.29	102.74	0.00527	0.00510	0.00504
8	L-Glutamic acid 1	0.00750	3 of 3	0.00702	0.00016	2.29	93.63	0.00684	0.00712	0.00711
9	L-Glutamic acid 1	0.01000	3 of 3	0.01089	0.00043	3.96	108.93	0.01123	0.01041	0.01104
10	L-Glutamic acid 1	0.02000	3 of 3	0.02459	0.00253	10.27	122.97	0.02200	0.02473	0.02705
11	L-Glutamic acid 1	0.03000	3 of 3	0.03336	0.00127	3.82	111.22	0.03331	0.03467	0.03212
12	L-Glutamic acid 1	0.05000	3 of 3	0.05374	0.00113	2.10	107.49	0.05502	0.05335	0.05286
13	L-Glutamic acid 1	0.07500	3 of 3	0.07849	0.00425	5.41	104.65	0.07755	0.07479	0.08313
14	L-Glutamic acid 1	0.10000	3 of 3	0.10661	0.00349	3.28	106.61	0.10570	0.11047	0.10366
15	L-Glutamic acid 1	0.20000	3 of 3	0.20374	0.01082	5.31	101.87	0.21623	0.19781	0.19717
16	L-Glutamic acid 1	0.30000	3 of 3	0.28923	0.01309	4.53	96.41	0.29872	0.27429	0.29468
17	L-Glutamic acid 1	0.40000	3 of 3	0.38514	0.01990	5.17	96.28	0.38356	0.40577	0.36607

The accuracy was less than ±10% of the nominal concentration for all concentrations measured

L-arginine

m/z 175.118 → 70.0659

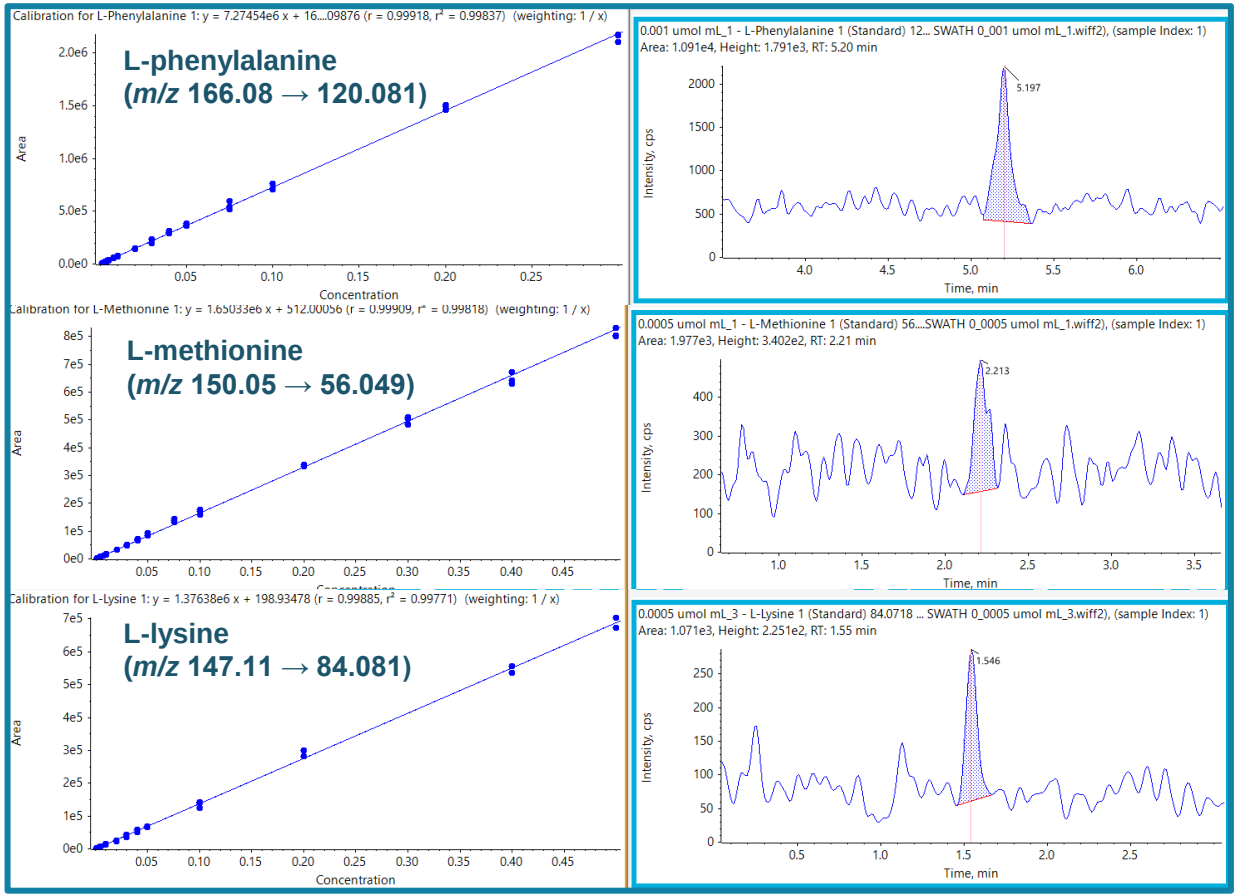


r^2: 0.993

LLOQ
0.0003 µmol/mL

Quantitative analysis of CCM components

CALIBRATION CURVES AND EVALUATION OF AMINO ACID CONCENTRATION IN CCM



r²: 0.998

r²: 0.998

r²: 0.997

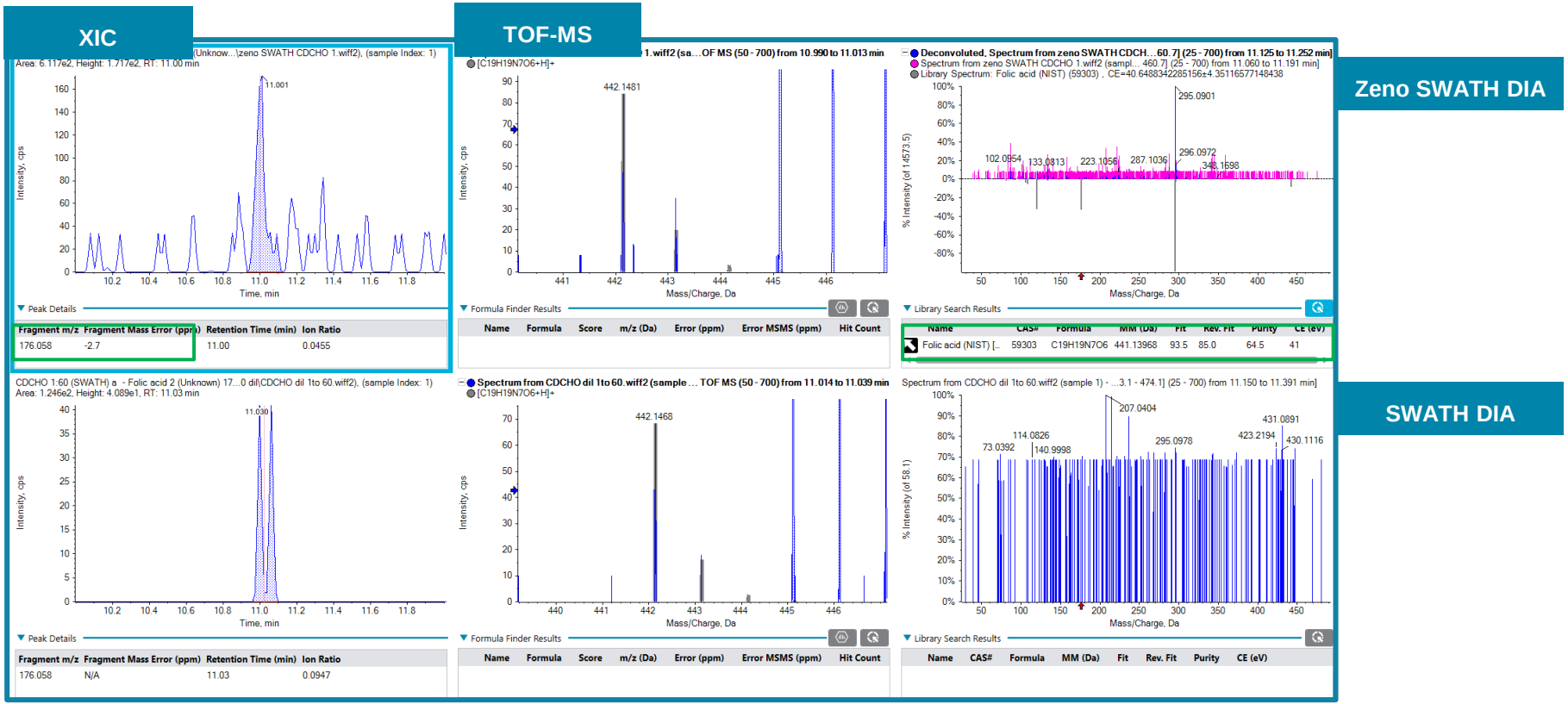
LLOQ of amino acids

Compounds	µmol/mL
L-aspartic acid	0.001
Glycine	0.01
L-threonine	0.002
L-isoleucine	0.001
L-alanine	0.03
L-arginine	0.0003
L-cystine	0.001
L-glutamic acid	0.0005
L-histidine	0.002
L-leucine	0.002
L-lysine	0.0005
L-methionine	0.0005
L-phenylalanine	0.001
L-proline	0.001
L-serine	0.004
L-tyrosine	0.002
L-valine	0.003

Zeno SWATH DIA MS and MS/MS for qualitative analysis of CCM components

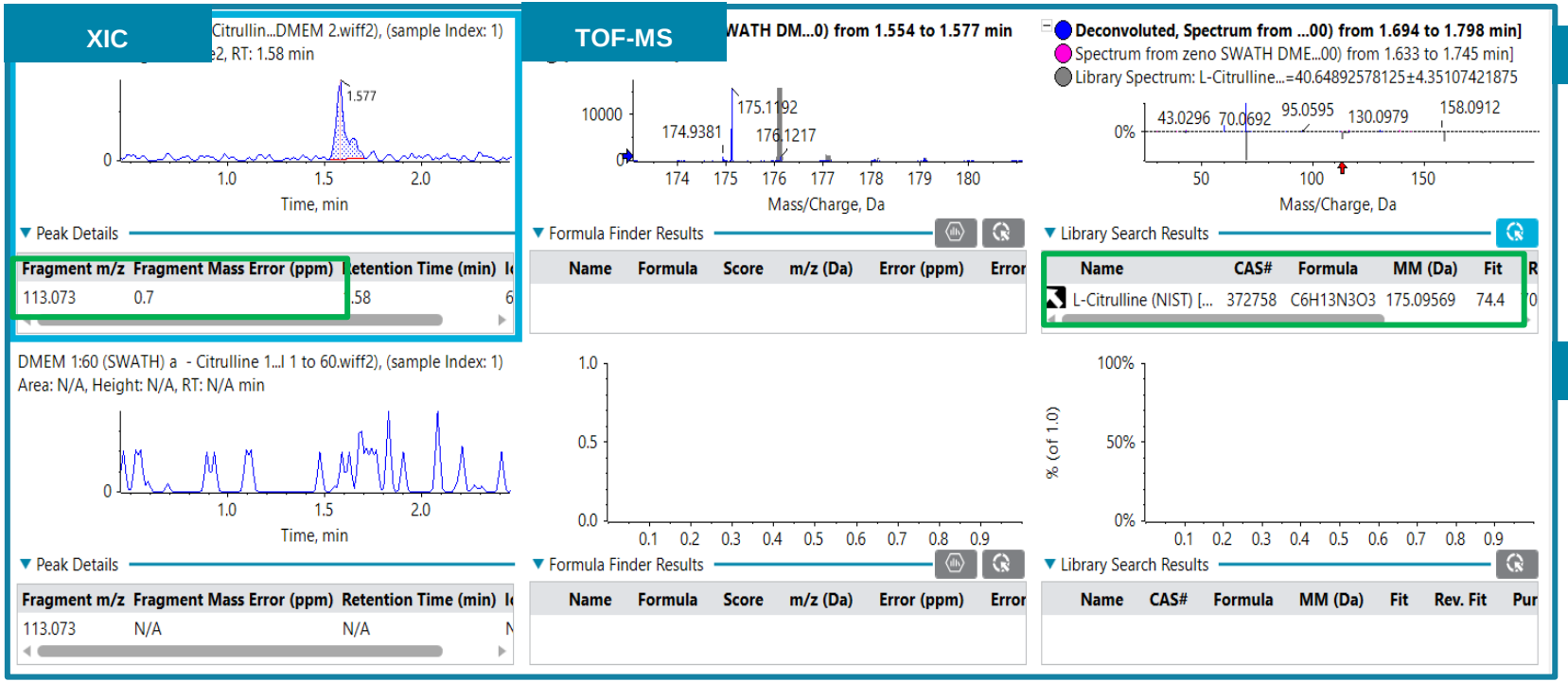
Qualitative analysis of CCM components

FOLIC ACID - HIGH SENSITIVITY WITH ZENO SWATH DIA



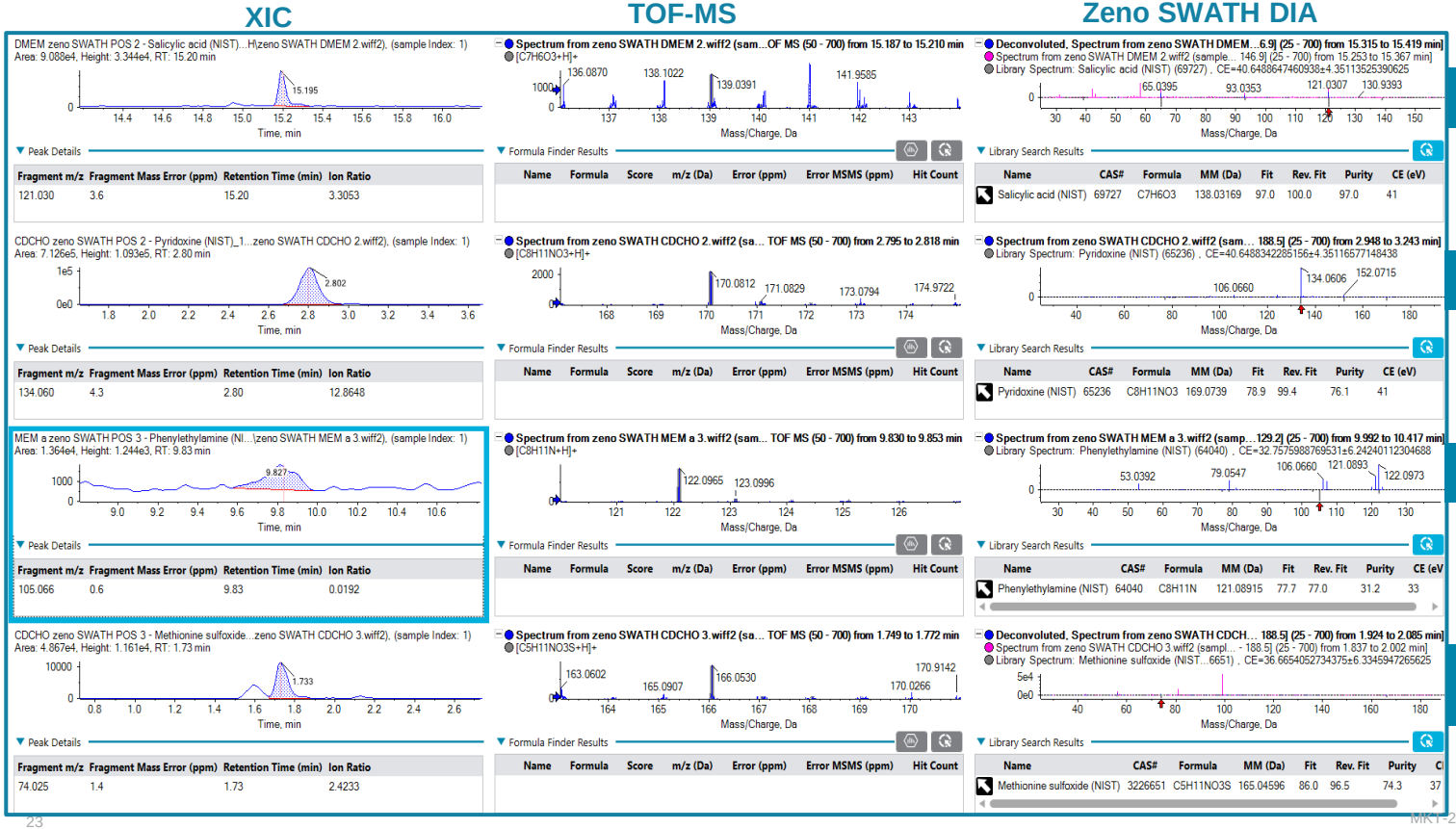
Qualitative analysis of CCM components

L-CITRULLINE - HIGH SENSITIVITY WITH ZENO SWATH DIA



Putative identification of CCM components

LIBRARY MATCHING



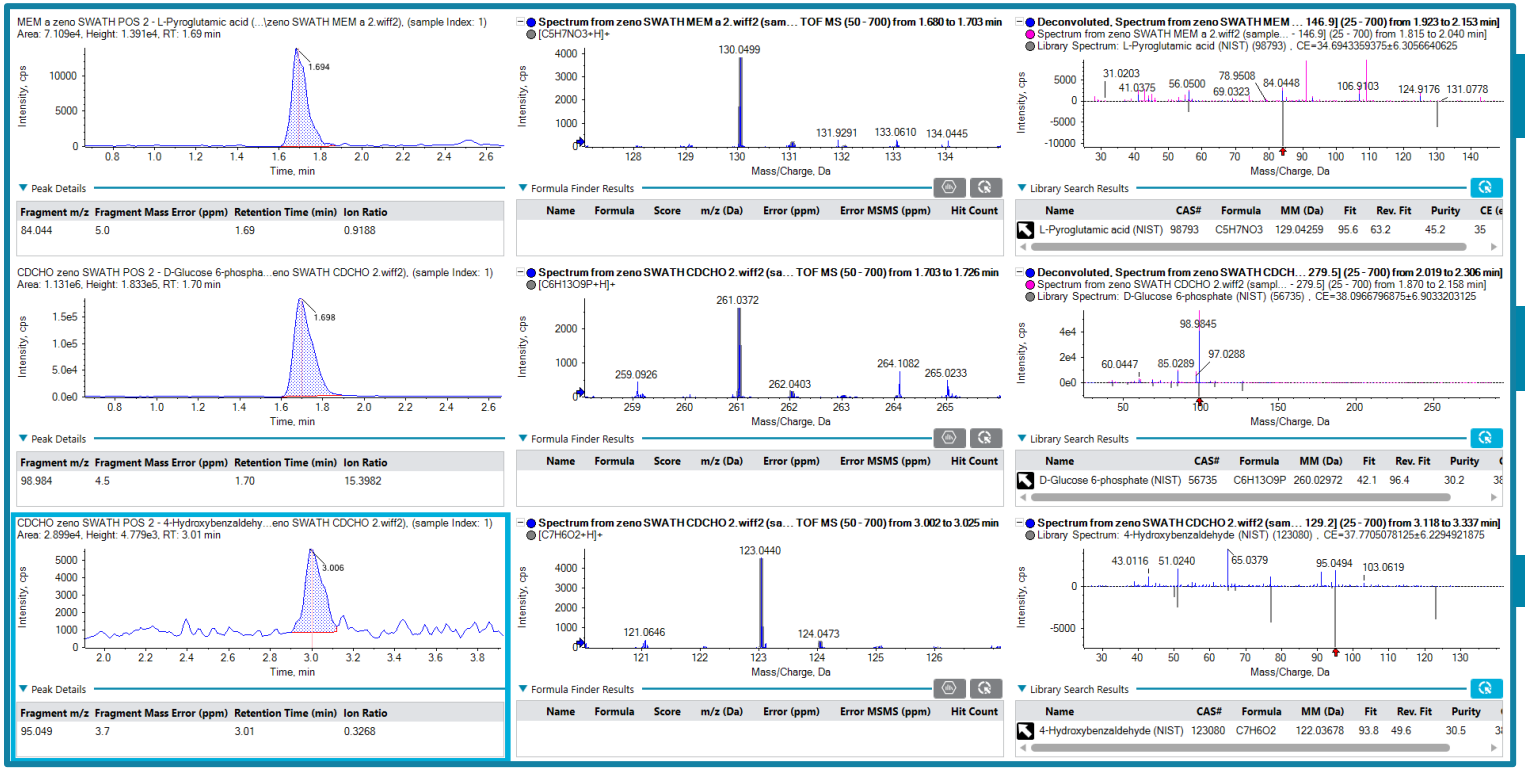
Putative identification of CCM components

LIBRARY MATCHING

XIC

TOF-MS

Zeno SWATH DIA



L-pyroglutamic acid

D-glucose-6-phosphate

4-hydroxybenzaldehyde

Conclusion

- **Highly sensitive LC-MS/MS for accurate mass** has been developed and optimized
- **The combination of DIA and the Zeno trap** enabled very low levels of quantitation with low %CVs for all concentrations
- **Zeno SWATH DIA vs. SWATH DIA approaches** allowed the detection of very low abundant targeted metabolites
  - In addition, **the showed high mass accuracy** (at MS and MS/MS levels) allowed a confident putative identifications (vs. CCM library)

Acknowledgments

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- Eshani Nandita

Questions and answers

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