



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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Presentation outline

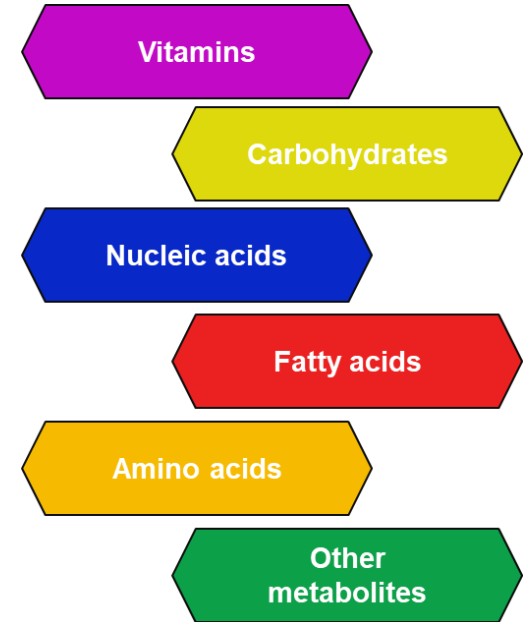
MAIN POINTS

- 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

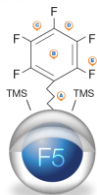
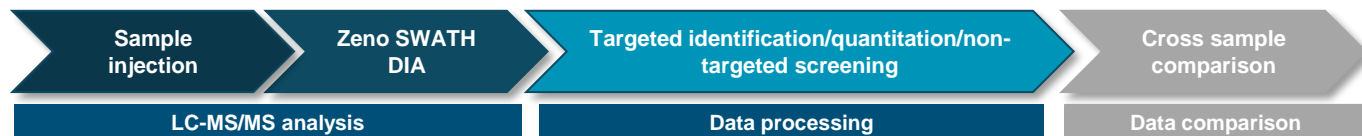
ANALYTICAL REQUIREMENTS

- 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

CCM analysis workflow

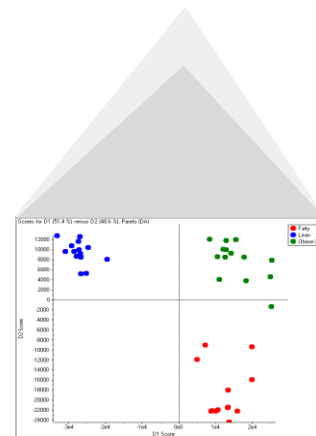


Metabolite identification and quantitation



Targeted data extraction using a library and quantitation

Statistical analysis



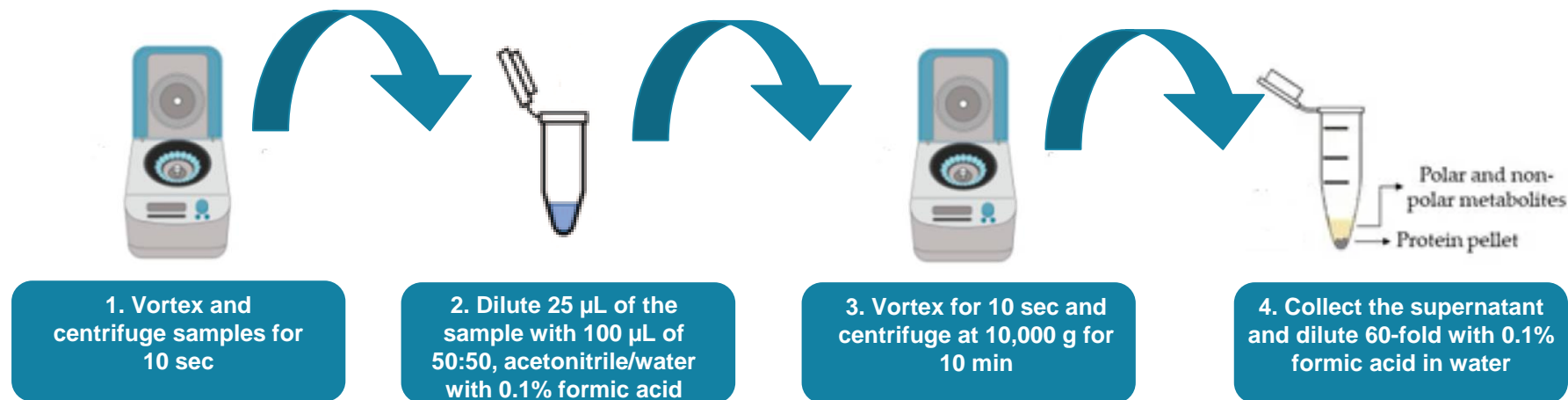
T-tests, PCA analysis and others

Overview of the methodology



Sample extraction

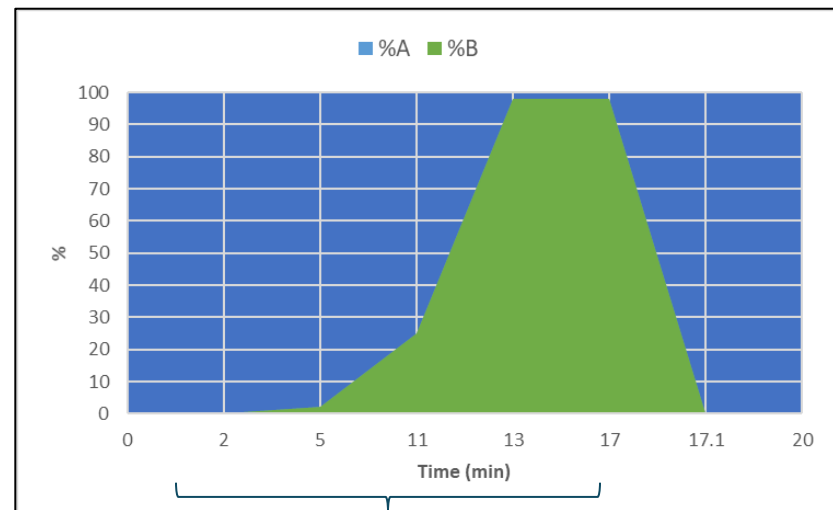
SAMPLES AND STANDARDS DILUTION SERIES*



*Tested standard dilutions were in the range 0.0001-0.5 $\mu\text{mol/mL}$


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



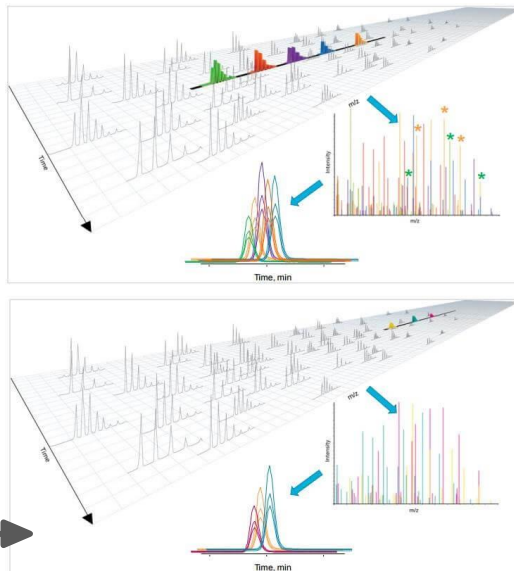
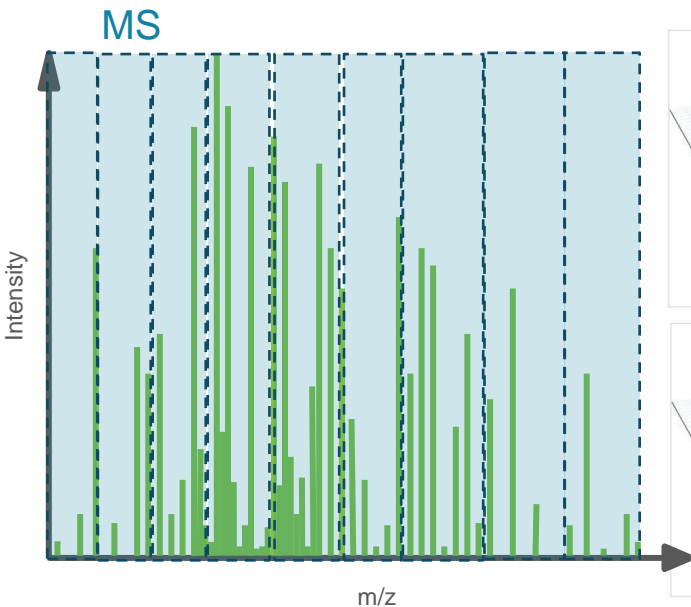
Compound elution time frame

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	50-700 m/z
Accumulation time	0.1 sec
TOF MS/MS	25-700 m/z
Accumulation time	0.015 sec
No. of variable windows	25
Total scan time	0.689 sec
Declustering potential	40 V
Collision energy	35 V



Zeno SWATH DIA MS and MS/MS for quantitation

SWATH DIA

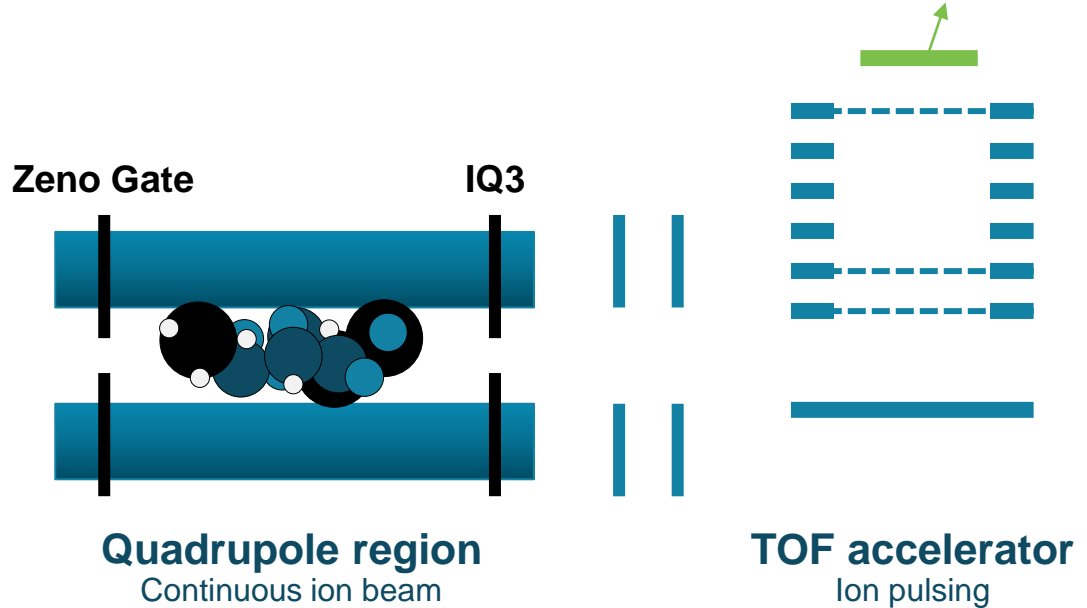


- Capture a complete digital archive of your sample
 - Comprehensive, acquires MS/MS of everything
 - Reduce the risk of missing any targets
- High-quality and sensitive quantitation, similar to the triple quadrupole
- Reduce method development
 - Single acquisition method
 - Re-analyze without re-acquisition

Zeno trap technology

FOR SENSITIVITY GAINS IN MS/MS

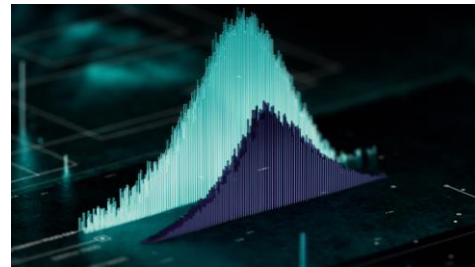
- The Zeno trap provides control of the ion beam from the collision cell into the accelerator
- Ions exit the Zeno trap in an ordered release based on potential energy
 - Ion are generally released from a high m/z to low m/z
 - All ions now arrive in the accelerator at the same time and location



Benefits of Zeno SWATH DIA

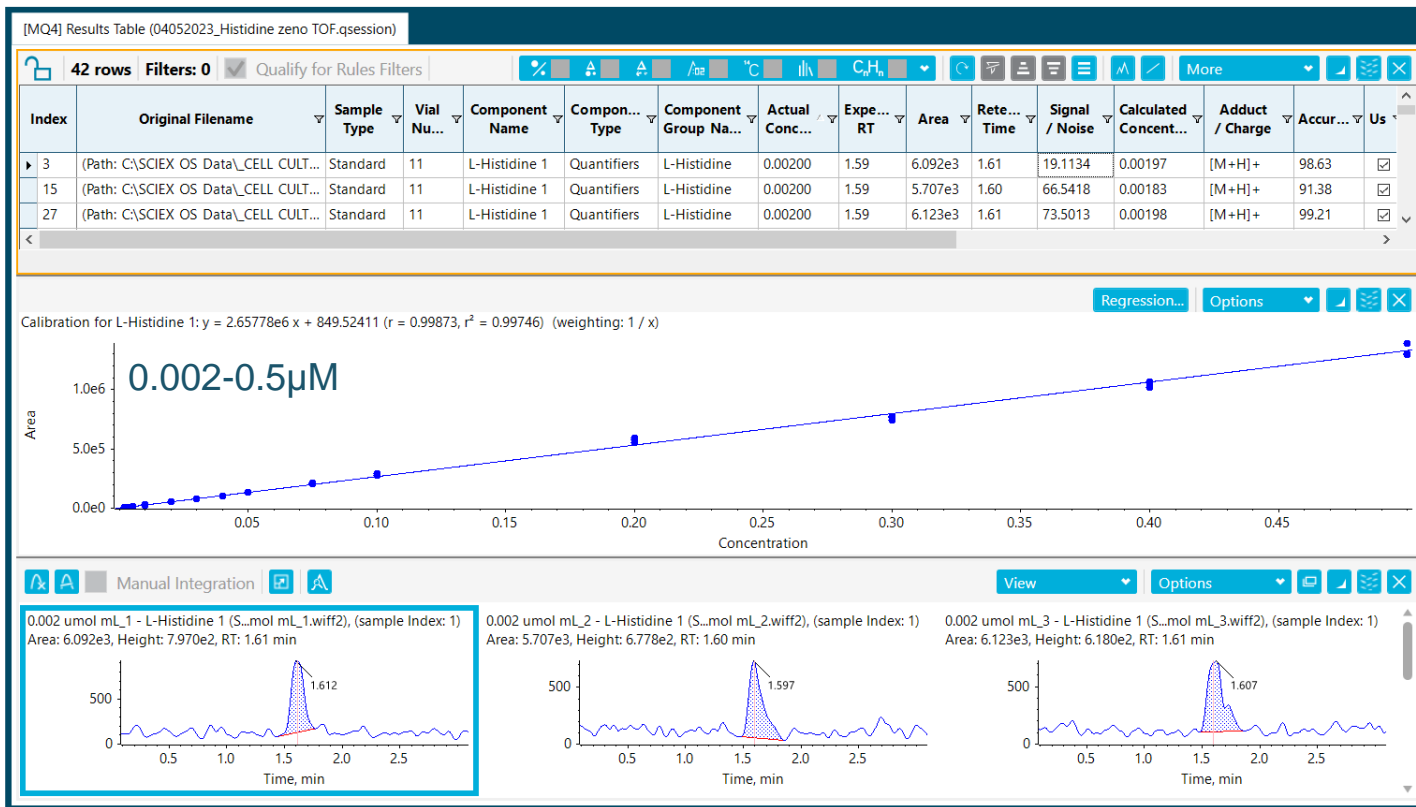
ACHIEVE MAXIMUM INFORMATION FROM EVERY SAMPLE

- 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 \rightarrow 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 $\pm 6\%$ of the nominal concentration for all concentrations measured

L-glutamic acid

m/z 148.06 \rightarrow 84.045



r^2 : 0.995

LLOQ
0.0005 μ mol/mL

Good MS/MS
mass accuracy

Quantitative performance for L-glutamic acid

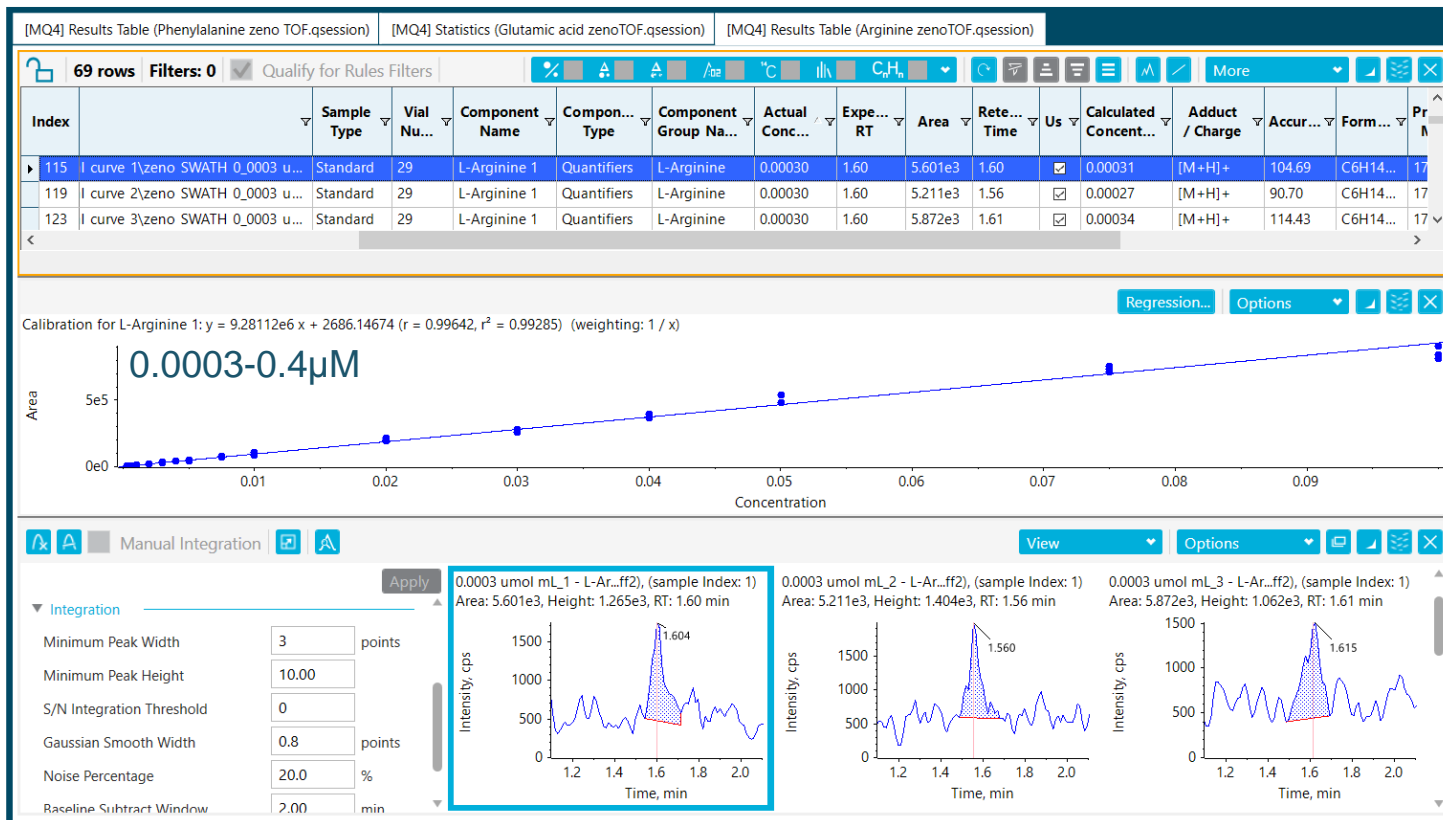
%CV, STANDARD DEVIATION AND MEAN

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 $\pm 10\%$ of the nominal concentration for all concentrations measured

L-arginine

m/z 175.118 \rightarrow 70.0659

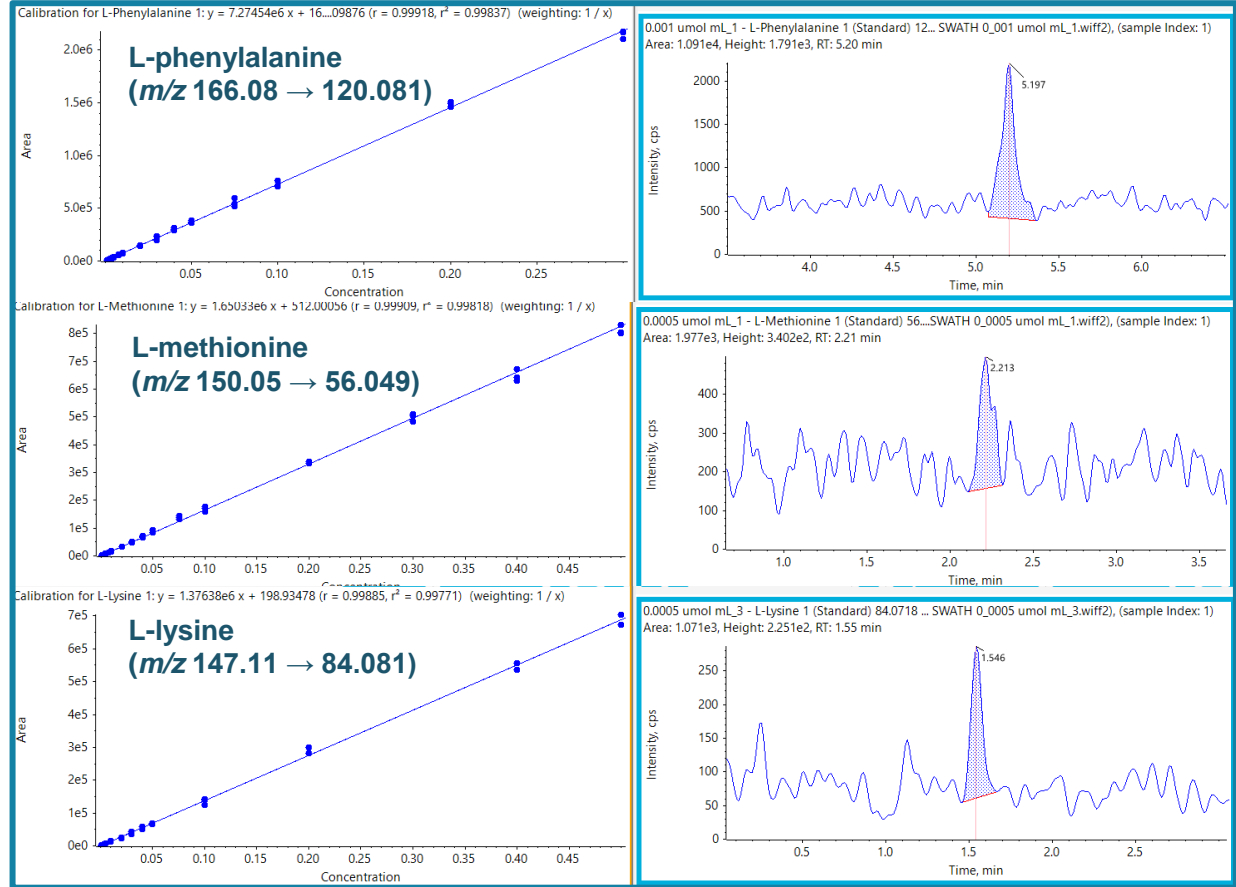


r^2 : 0.993

LLOQ
0.0003 μ mol/mL

Quantitative analysis of CCM components

CALIBRATION CURVES AND EVALUATION OF AMINO ACID CONCENTRATION IN CCM



$r^2: 0.998$

$r^2: 0.998$

$r^2: 0.997$

LLOQ of amino acids

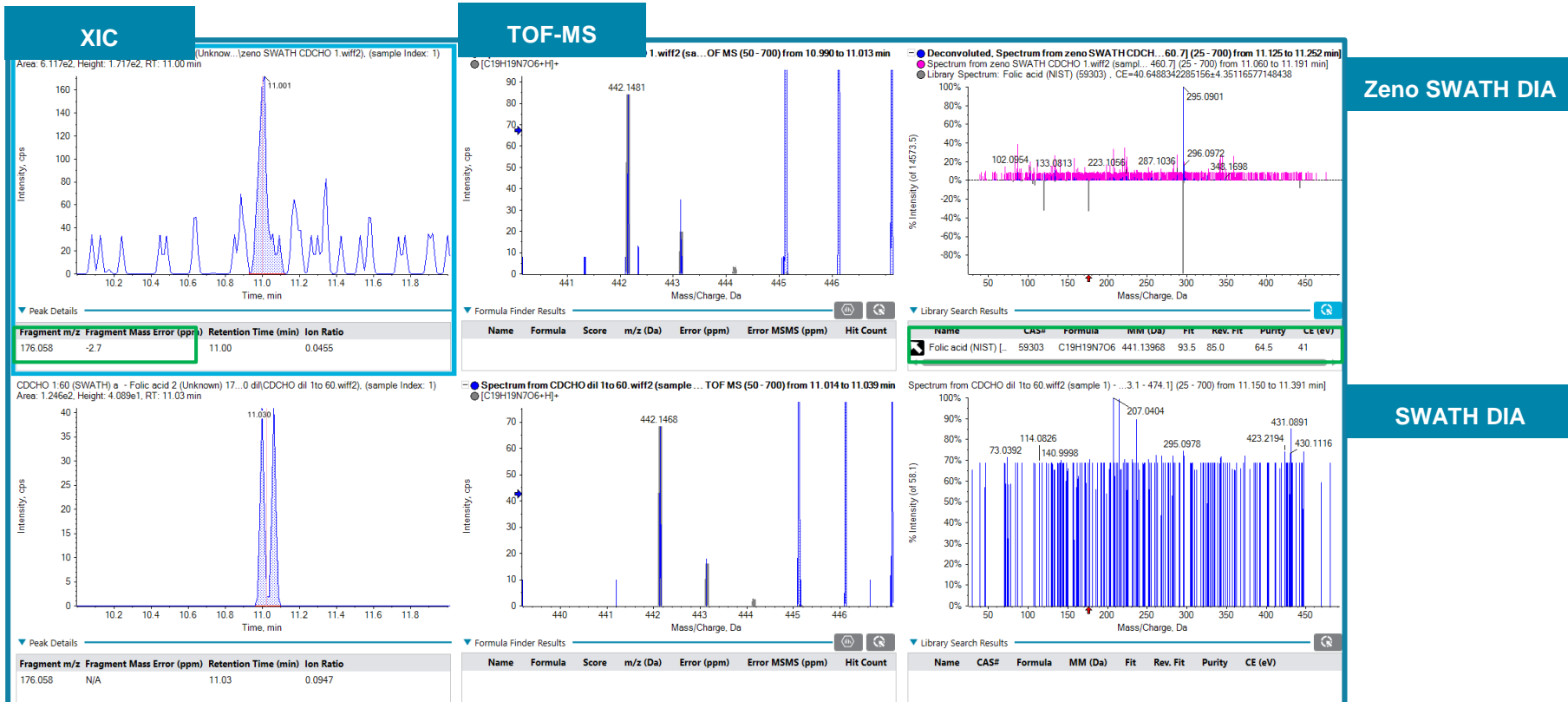
Compounds	μmol/mL
L-aspartic acid	0.001
Glycine	0.01
L-threonine	0.002
L-isoleucine	0.001
L-alanine	0.03
L-arginine	0.0003
L-cystine	0.001
L-glutamic acid	0.0005
L-histidine	0.002
L-leucine	0.002
L-lysine	0.0005
L-methionine	0.0005
L-phenylalanine	0.001
L-proline	0.001
L-serine	0.004
L-tyrosine	0.002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

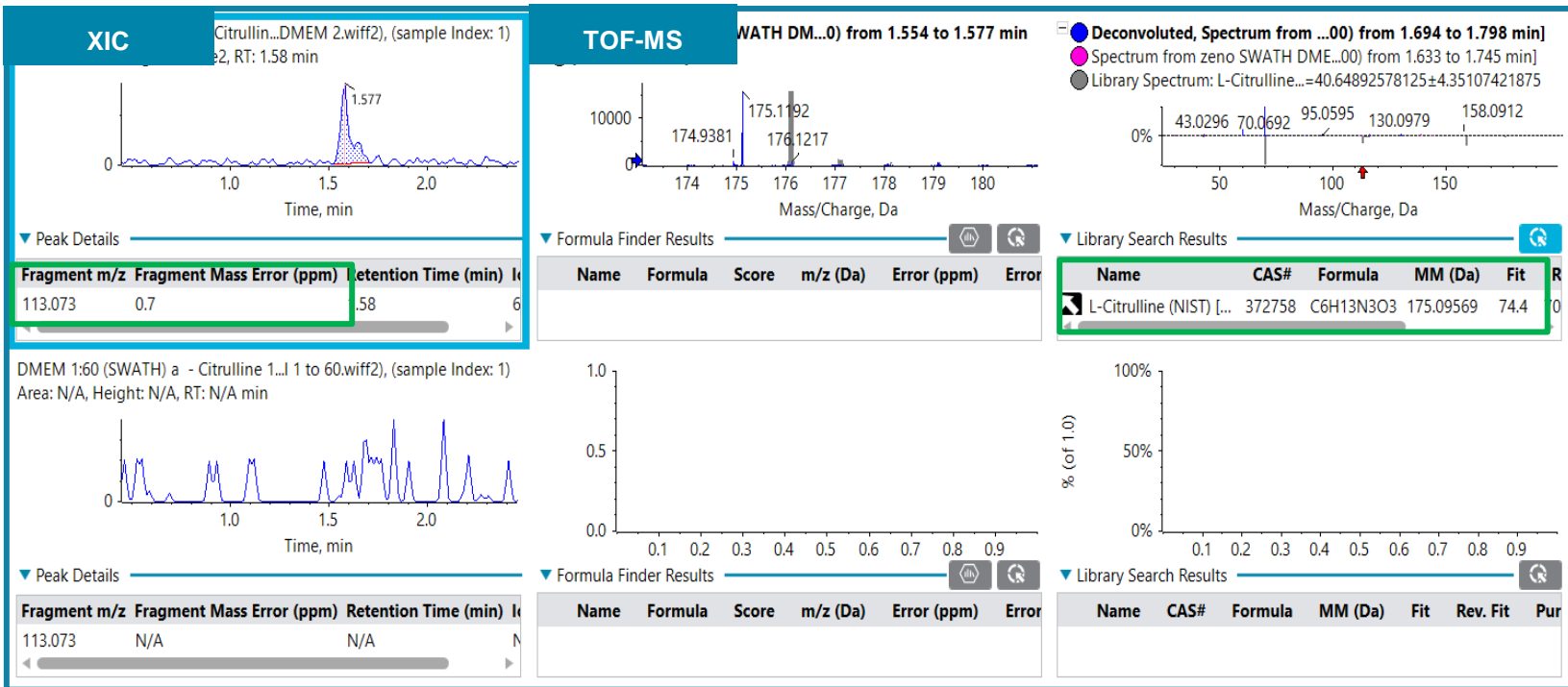


Qualitative analysis of CCM components

L-CITRULLINE - HIGH SENSITIVITY WITH ZENO SWATH DIA

Zeno SWATH DIA

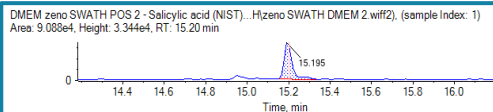
SWATH DIA



Putative identification of CCM components

LIBRARY MATCHING

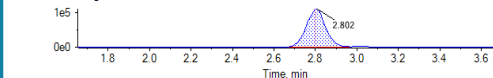
XIC



Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
121.030	3.6	15.20	3.3053

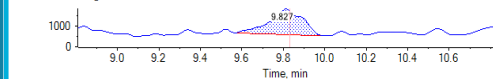
CDCHO zeno SWATH POS 2 - Pyridoxine (NIST)...H.zeno SWATH CDCHO 2.wiff2), (sample Index: 1)
Area: 7.126e5, Height: 1.093e5, RT: 2.80 min



Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
134.060	4.3	2.80	12.8648

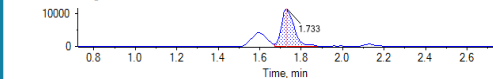
MEM a zeno SWATH POS 3 - Phenylethylamine (NIST)...H.zeno SWATH MEM a 3.wiff2), (sample Index: 1)
Area: 1.364e4, Height: 1.244e3, RT: 9.83 min



Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
105.066	0.6	9.83	0.0192

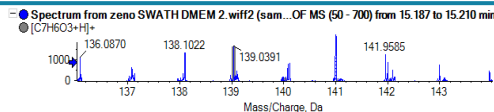
CDCHO zeno SWATH POS 3 - Methionine sulfoxide...H.zeno SWATH CDCHO 3.wiff2), (sample Index: 1)
Area: 4.867e4, Height: 1.161e4, RT: 1.73 min



Peak Details

Fragment m/z	Fragment Mass Error (ppm)	Retention Time (min)	Ion Ratio
74.025	1.4	1.73	2.4233

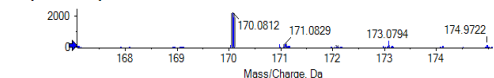
TOF-MS



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

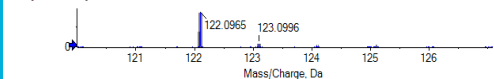
● Spectrum from zeno SWATH CDCHO 2.wiff2 (sa... OF MS (50 - 700) from 2.795 to 2.818 min
● [C8H11NO3+H]⁺



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

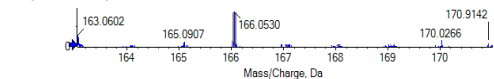
● Spectrum from zeno SWATH MEM a 3.wiff2 (sam... OF MS (50 - 700) from 9.830 to 9.853 min
● [C8H11N+H]⁺



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

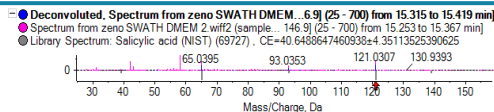
● Spectrum from zeno SWATH CDCHO 3.wiff2 (sa... OF MS (50 - 700) from 1.749 to 1.772 min
● [C5H11NO3S+H]⁺



Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS (ppm)	Hit Count

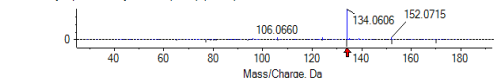
Zeno SWATH DIA



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Salicylic acid (NIST)	69727	C7H6O3	138.03169	97.0	100.0	97.0	41

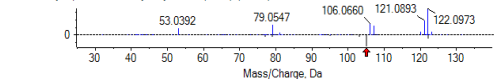
● Spectrum from zeno SWATH CDCHO 2.wiff2 (sam... 188.5] (25 - 700) from 2.948 to 3.243 min
● Library Spectrum: Pyridoxine (NIST) (65236), CE=40.6488342286156+4.35116577148438



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Pyridoxine (NIST)	65236	C8H11NO3	169.0739	78.9	99.4	76.1	41

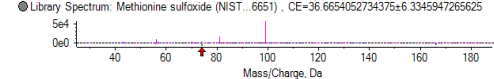
● Spectrum from zeno SWATH MEM a 3.wiff2 (sam... 129.2] (25 - 700) from 9.992 to 10.417 min
● Library Spectrum: Phenylethylamine (NIST) (64040), CE=32.7575988769531+5.24240112304688



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Phenylethylamine (NIST)	64040	C8H11N	121.08915	77.7	77.0	31.2	33

● Deconvoluted, Spectrum from zeno SWATH CDCHO... 188.5] (25 - 700) from 1.924 to 2.085 min
● Spectrum from zeno SWATH CDCHO 3.wiff2 (sam... 188.5] (25 - 700) from 1.837 to 2.002 min
● Library Spectrum: Methionine sulfoxide (NIST... 6651), CE=36.6654052734375+6.3345947265625



Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Methionine sulfoxide (NIST)	3226651	C5H11NO3S	165.04596	86.0	96.5	74.3	37

Salicylic acid

Pyridoxine

Phenylethylamine

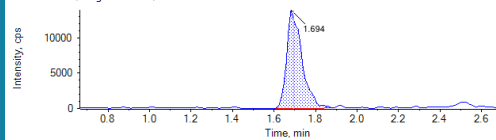
Methionine sulfoxide

Putative identification of CCM components

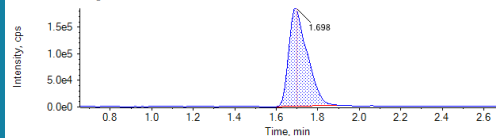
LIBRARY MATCHING

XIC

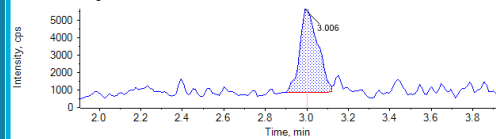
MEM a zeno SWATH POS 2 - L-Pyrogutamic acid (.zeno SWATH MEM a 2.wiff2). (sample Index: 1)
Area: 7.109e4, Height: 1.391e4, RT: 1.69 min



CDCHO zeno SWATH POS 2 - D-Glucose 6-phospha...eno SWATH CDCHO 2.wiff2). (sample Index: 1)
Area: 1.131e6, Height: 1.833e5, RT: 1.70 min

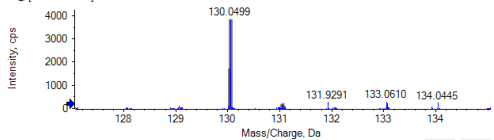


CDCHO zeno SWATH POS 2 - 4-Hydroxybenzaldehy...eno SWATH CDCHO 2.wiff2). (sample Index: 1)
Area: 2.899e4, Height: 4.779e3, RT: 3.01 min

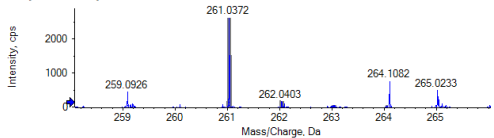


TOF-MS

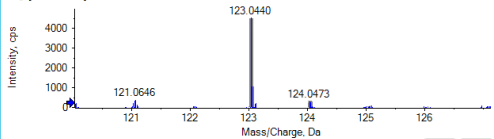
Spectrum from zeno SWATH MEM a 2.wiff2 (sam... TOF MS (50 - 700) from 1.680 to 1.703 min
● [C5H7NO3+H]⁺



Spectrum from zeno SWATH CDCHO 2.wiff2 (sa... TOF MS (50 - 700) from 1.703 to 1.726 min
● [C6H13O9P+H]⁺

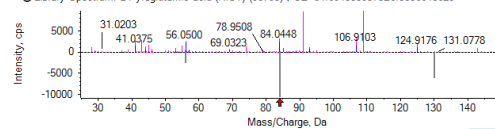


Spectrum from zeno SWATH CDCHO 2.wiff2 (sa... TOF MS (50 - 700) from 3.002 to 3.025 min
● [C7H6O2+H]⁺



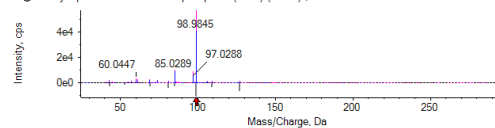
Zeno SWATH DIA

Deconvoluted, Spectrum from zeno SWATH MEM ... 146.9] (25 - 700) from 1.923 to 2.153 min
Spectrum from zeno SWATH MEM a 2.wiff2 (sample... - 146.9] (25 - 700) from 1.815 to 2.040 min
Library Spectrum: L-Pyrogutamic acid (NIST) (98793) . CE=34.6943359375±6.3056640625



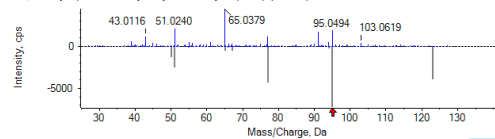
Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE
L-Pyrogutamic acid (NIST)	98793	C5H7NO3	129.04259	95.6	63.2	45.2	35

Deconvoluted, Spectrum from zeno SWATH CDCHO ... 279.5] (25 - 700) from 2.019 to 2.306 min
Spectrum from zeno SWATH CDCHO 2.wiff2 (sampl... - 279.5] (25 - 700) from 1.870 to 2.158 min
Library Spectrum: D-Glucose 6-phosphate (NIST) (56735) . CE=38.0966796875±6.9033203125



Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE
D-Glucose 6-phosphate (NIST)	56735	C6H13O9P	260.02972	42.1	96.4	30.2	36

Spectrum from zeno SWATH CDCHO 2.wiff2 (sam... 129.2] (25 - 700) from 3.118 to 3.337 min
Library Spectrum: 4-Hydroxybenzaldehyde (NIST) (123080) . CE=37.7705078125±6.2294921875



Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE
4-Hydroxybenzaldehyde (NIST)	123080	C7H6O2	122.03678	93.8	49.6	30.5	31

L-pyrogutamic 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

SCIEX

- Antonella Chiapparino
- Eshani Nandita

A woman with short, grey hair and a bright smile stands in a laboratory. She is wearing a white lab coat over a pink collared shirt. Her arms are crossed. The background is a blurred laboratory with various glassware and equipment, creating a bokeh effect.

Questions and answers

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