Qualitative and targeted analysis of cell culture media (CCM) components using accurate mass spectrometry

Marialuce Maldini, PhD | Sr. Applications Support Specialist, LSR EMEAI

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Presentation outline

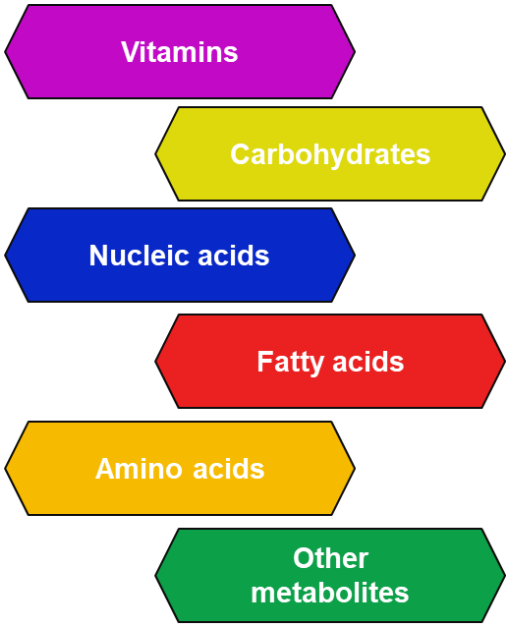
MAIN POINTS

- Introduction and background for CCM analysis
- Overview of the methodology and data analysis
- Results
  - Targeted identification and quantification of cell culture components
  - Non-targeted analysis and putative identification of components present in media
- Conclusions

Introduction

WHY MONITOR COMPOSITION MEDIUM DURING BIOTHERAPEUTICS PRODUCTION?

- Biopharmaceuticals are produced by wide range of media systems
- Cell culture medium components, levels, and consumption can vary by product, cell type and cell line
- Qualitative understanding followed by quantitative tracking, is critical in meeting quality requirements and 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
- Targeted quantification and unknown compound 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-MRM method
- Ultra sensitive targeted quantification
- Detection of low-level target analytes

CCM analysis workflow

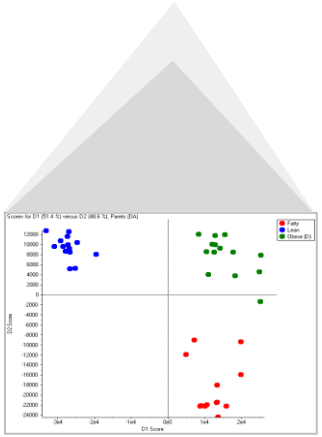
Sample injection → SWATH → Targeted identification/quantification/ non-targeted screening → Cross sample comparison

LC-MS/MS analysis | Data processing | Data comparison

Metabolite identification and quantification

Targeted data extraction using a library and quantification

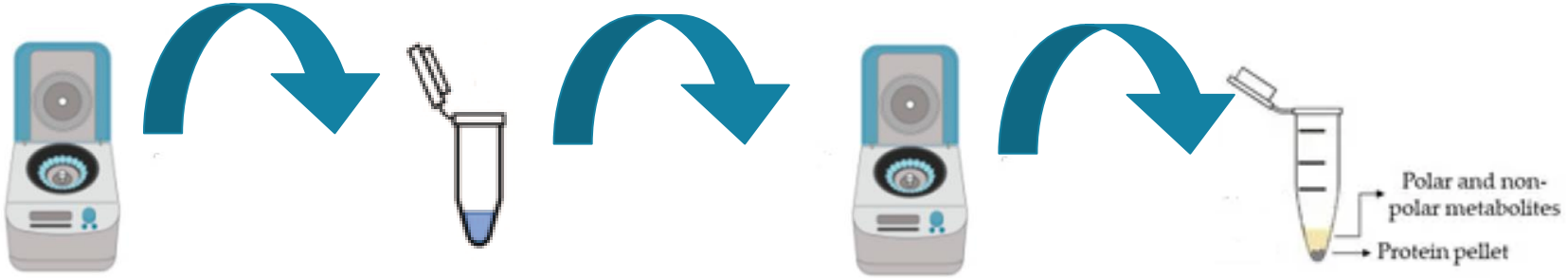
Statistical analysis



T-tests, PCA analysis and others

Overview of the methodology

Sample preparation



1. Vortex and centrifuge samples for 10 sec

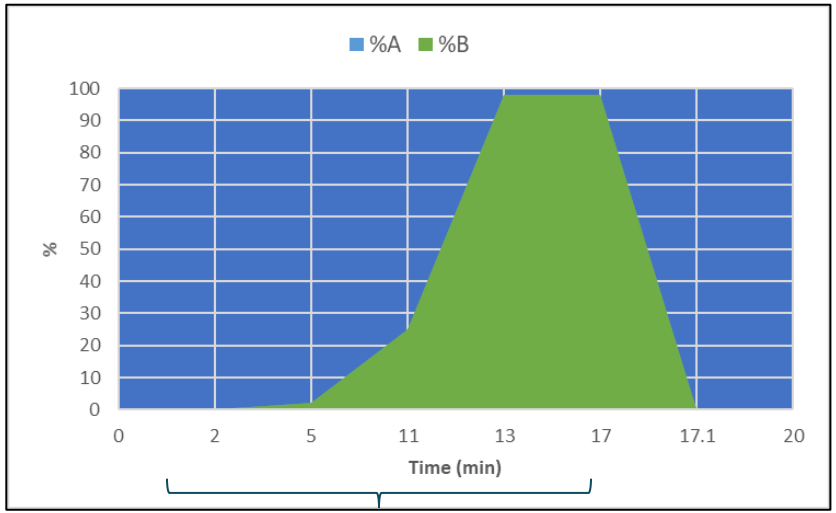
2. Dilute 25 µL of the sample with 100 µL of 50:50 (ACN/0.1% FA) (*aq*)

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

4. Collect the supernatant and dilute 10-fold with 0.1% FA (*aq*)

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 H_2O/ACN

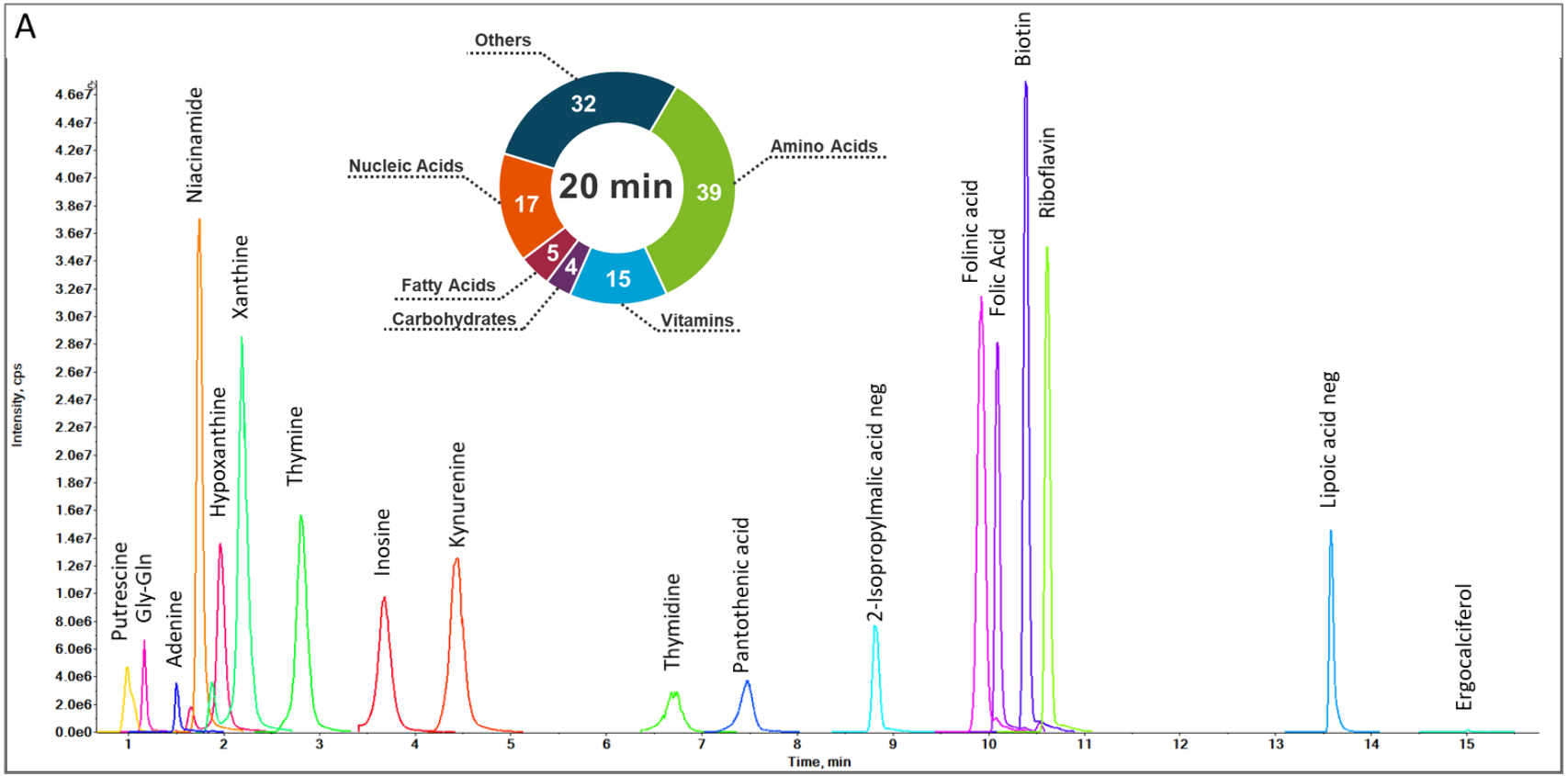


Compound elution time frame

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* 0.100 sec
TOF MS/MS Accumulation time	25-700 *m/z** 0.025 sec
No. of variable windows	25
Total scan time	0.860 sec

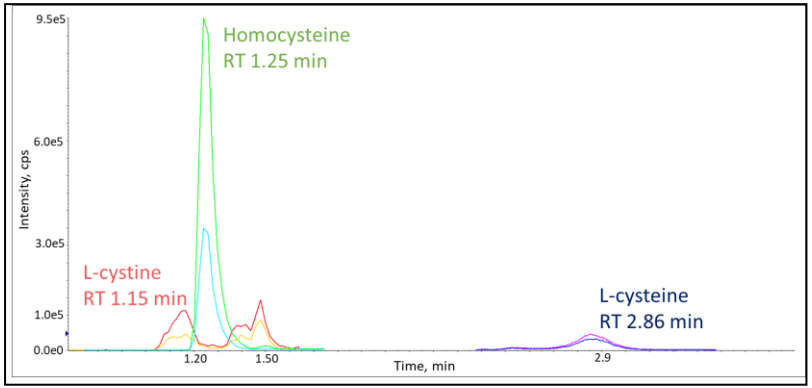
*All CE and CXP parameters were optimized per compound.

Chromatography: representative components

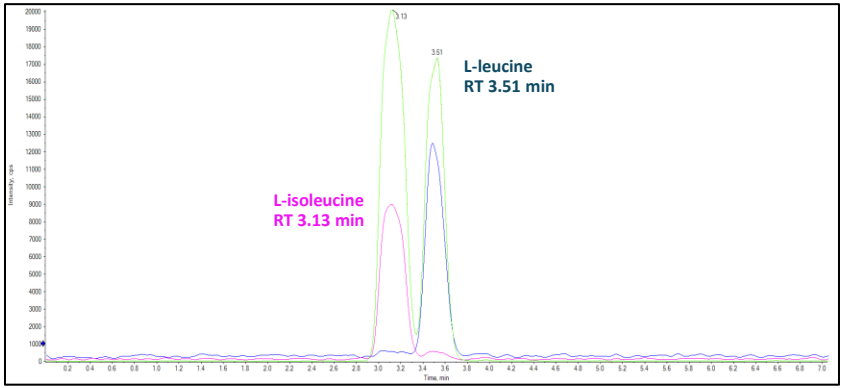


Chromatographic separation of closely related compounds

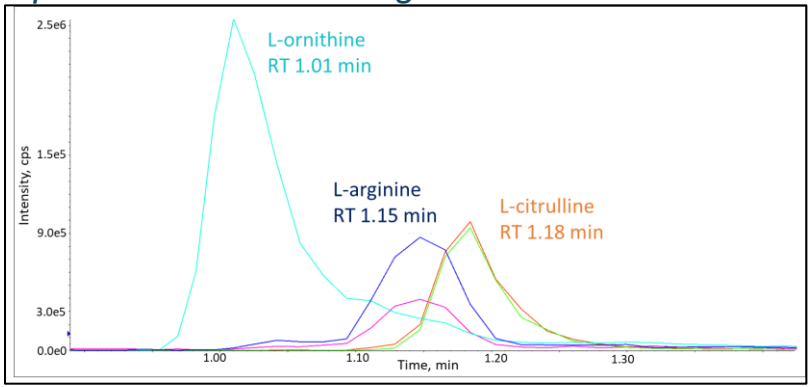
Separation between L-cystine and its dimers



Separation between isoleucine and leucine

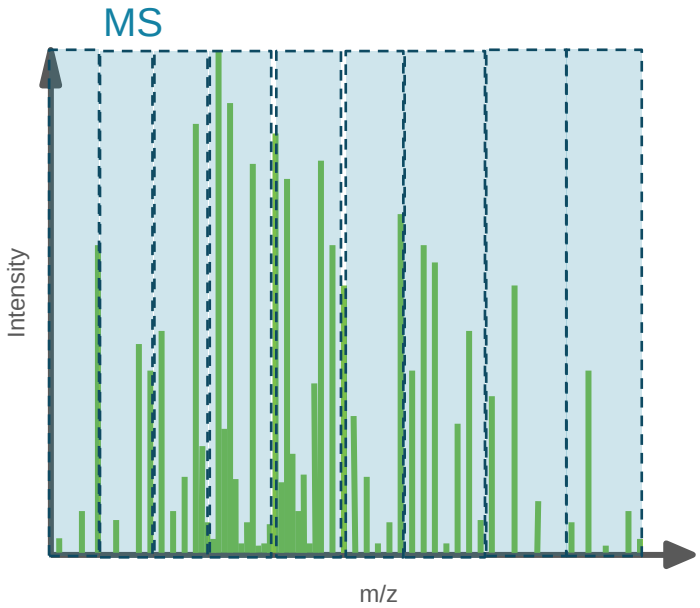


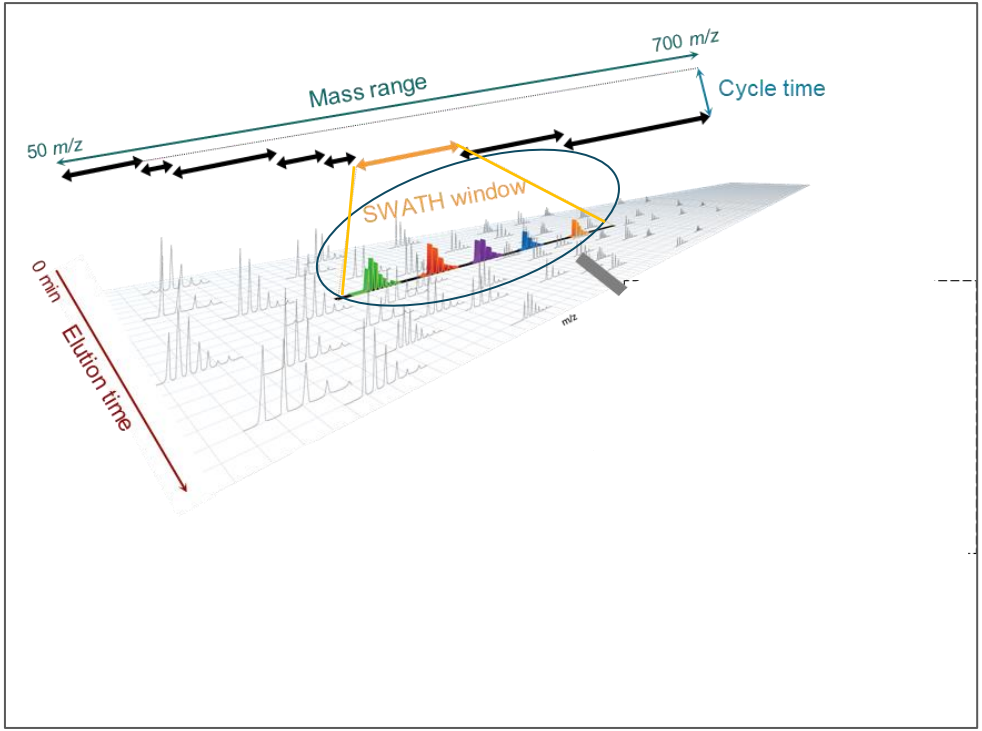
Separation between L-arginine and its metabolites



Targeted identification and quantification of cell culture components

SWATH data acquisition

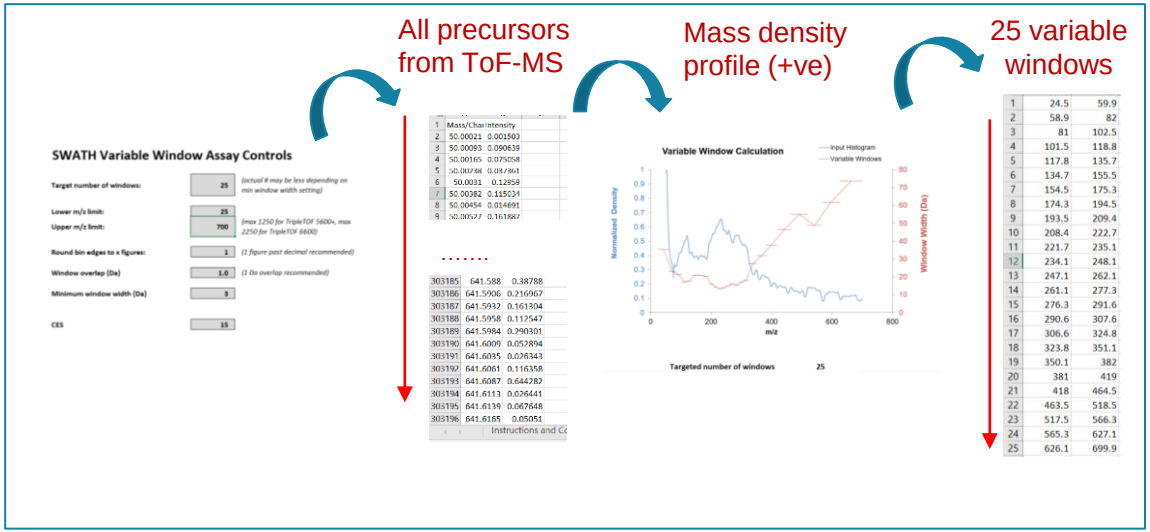




- HRMS SWATH acquisition provides complete qualitative and quantitative data
- High resolution MS/MS data for compound identification and confirmation
- Superior sensitivity and reproducibility

SWATH acquisition with variable windows

IMPROVE SELECTIVITY BY VARY WINDOWS SIZE

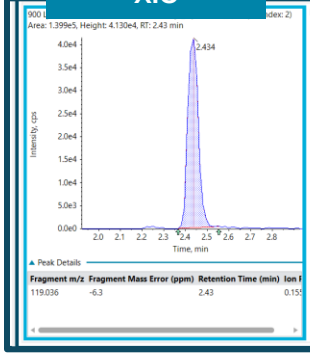


- Adjusts Q1 selection window to maintain a roughly constant number of analytes in each window
  - Narrower window in *m/z* dense regions
  - Optimal cycle time maintained by adjusting accumulation time and number of windows
- Reduce number of precursors in dense windows for increased specificity

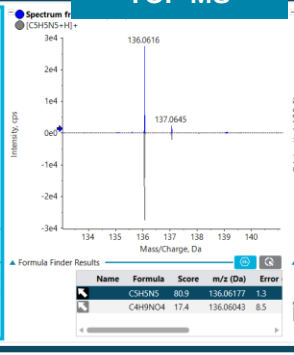
Targeted analysis of CCM analytes

ADENINE POSITIVE ION MODE

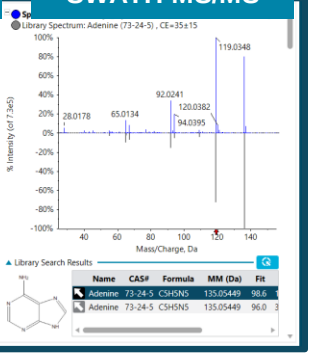
XIC



TOF-MS



SWATH MS/MS



CITRULLINE POSITIVE ION MODE

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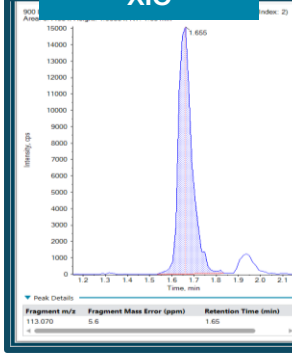
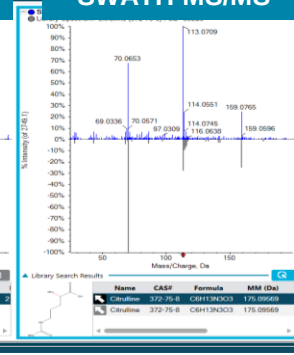


TOF-MS

SWATH MS/MS



SUCCINIC ACID NEGATIVE ION MODE

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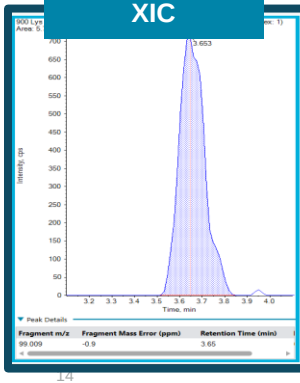
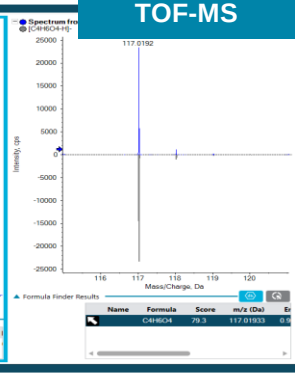
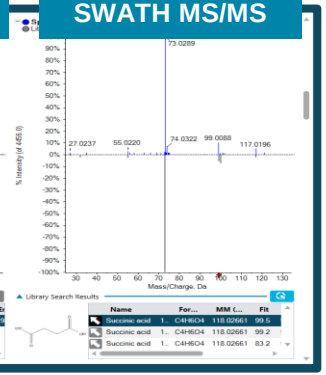


TOF-MS

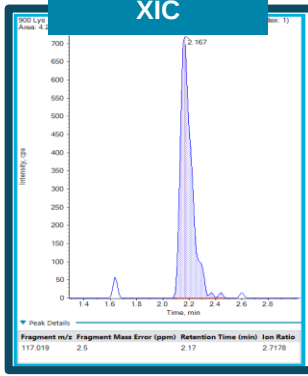


SWATH MS/MS

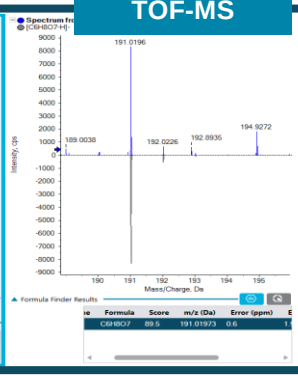


ISOCITRIC ACID NEGATIVE ION MODE

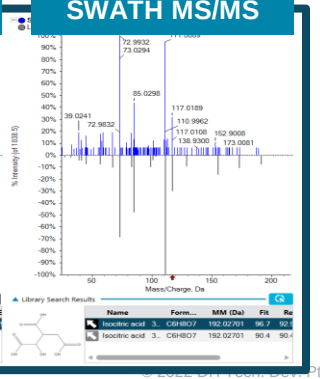
XIC



TOF-MS



SWATH MS/MS



Library matching

ADENINE (POSITIVE ION MODE)

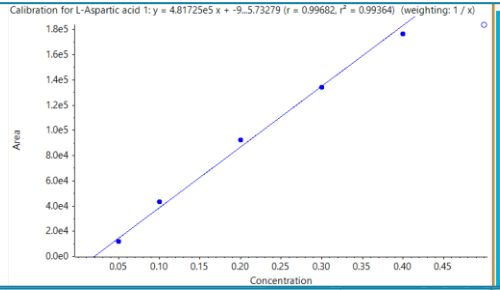
Acquired MS/MS spectrum

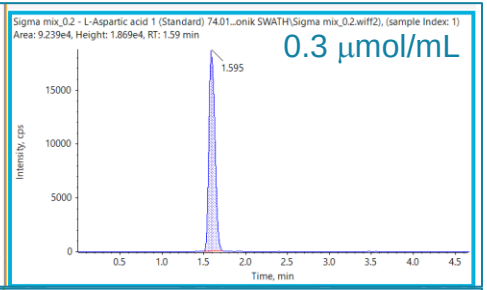
Library MS/MS spectrum

Quantitative analysis of CCM components

CALIBRATION CURVES AND EVALUATION OF AMINO ACID CONTCENTRATION IN CELL CULTURE MEDIA

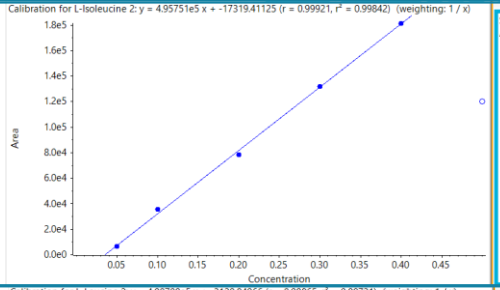
L-ASPARTIC ACID (134.04 -> 74.024)

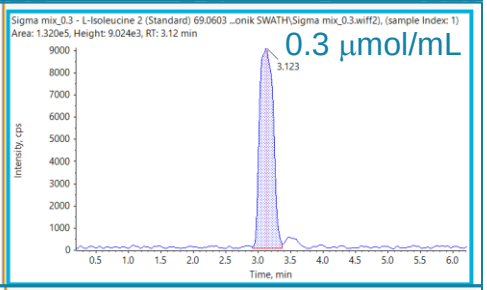




r^2: 0.993

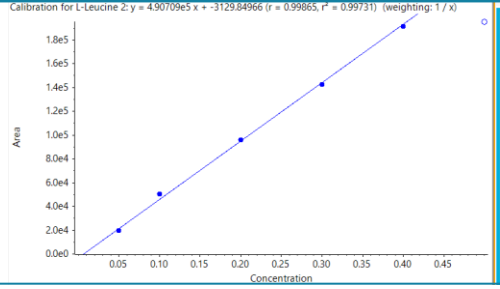
L-ISOLEUCINE (132.02 -> 69.070)

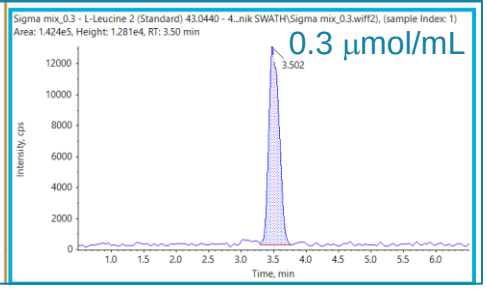




r^2: 0.998

L-LEUCINE (132.02 -> 43.050)





r^2: 0.997

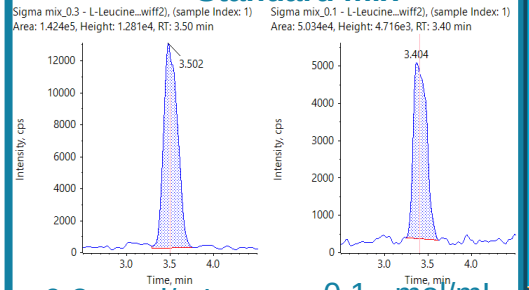
The accuracy was less than ±10% of the nominal concentration for all concentrations measured.

Quantitative analysis of CCM components

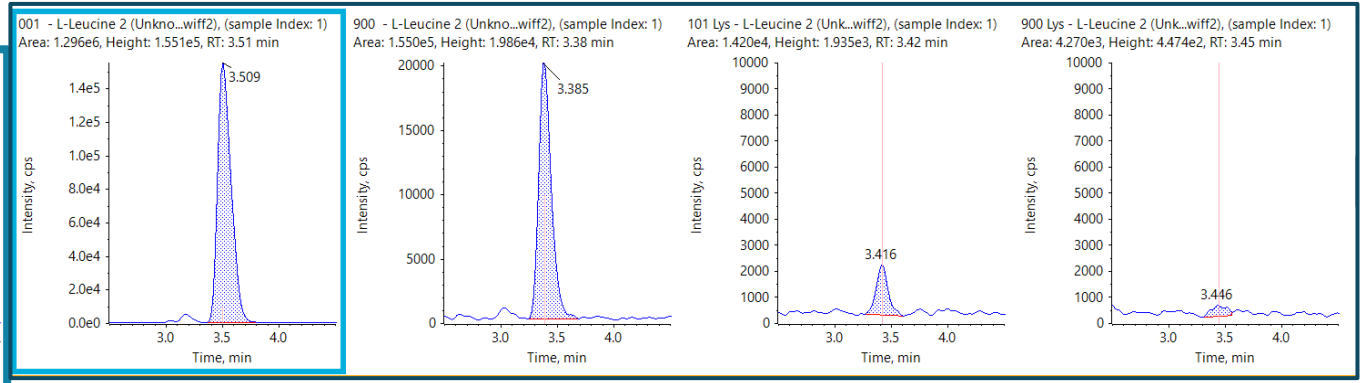
QUANTITATIVE RESULTS IN SAMPLES

LEUCINE

Standard mix



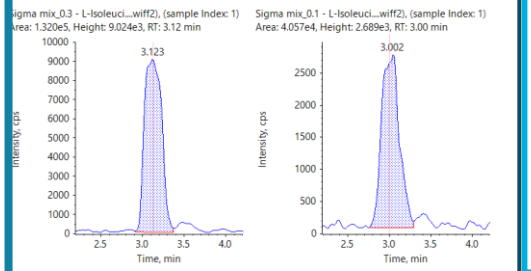
0.3 µmol/mL | 0.1 µmol/mL



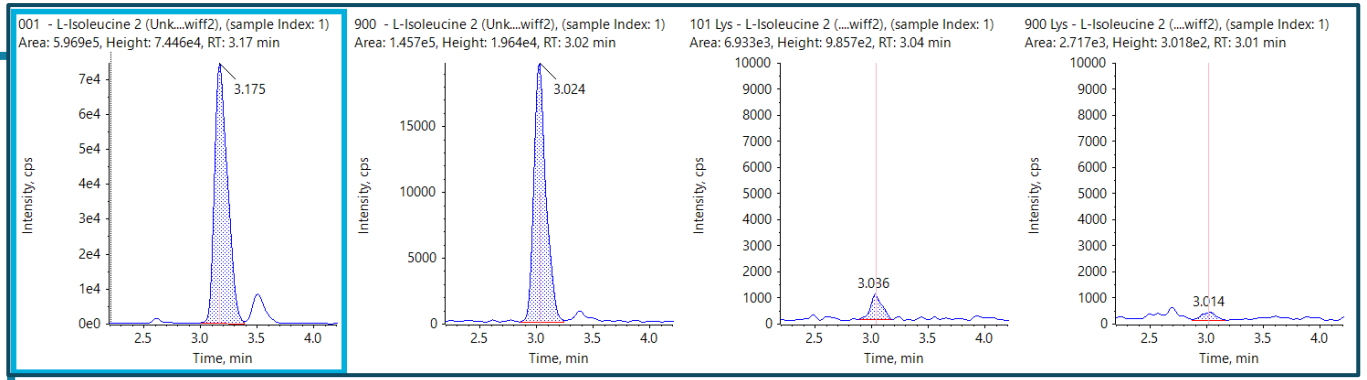
2.65 µmol/mL | 0.32 µmol/mL | 0.03 µmol/mL | 0.01 µmol/mL

ISOLEUCINE

Standard mix



0.3 µmol/mL | 0.1 µmol/mL

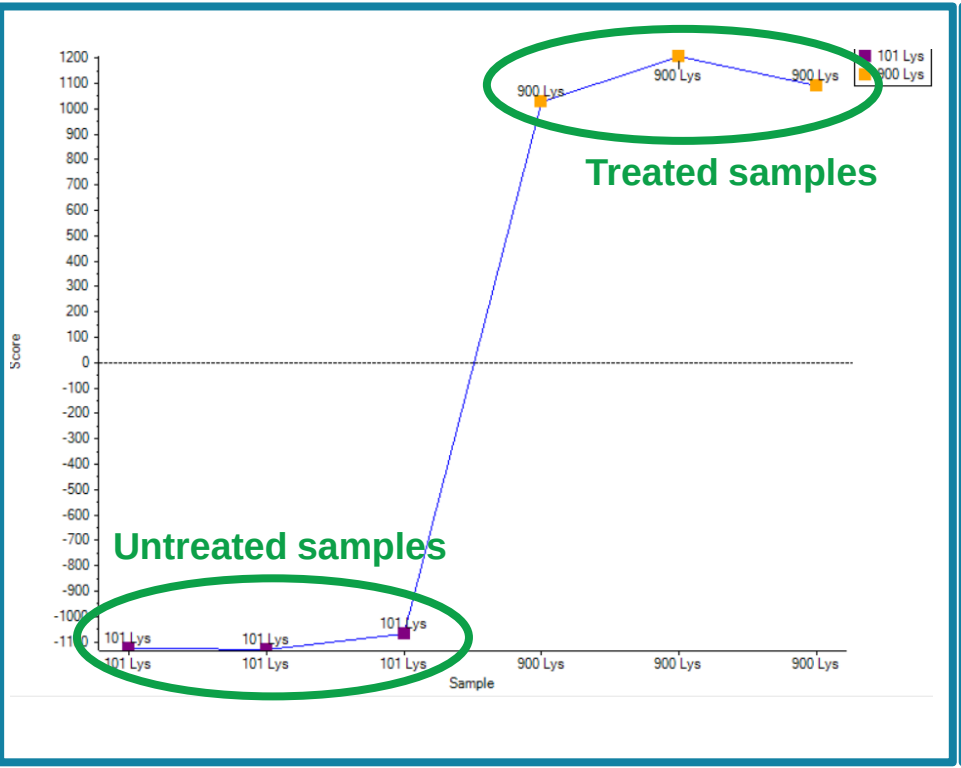


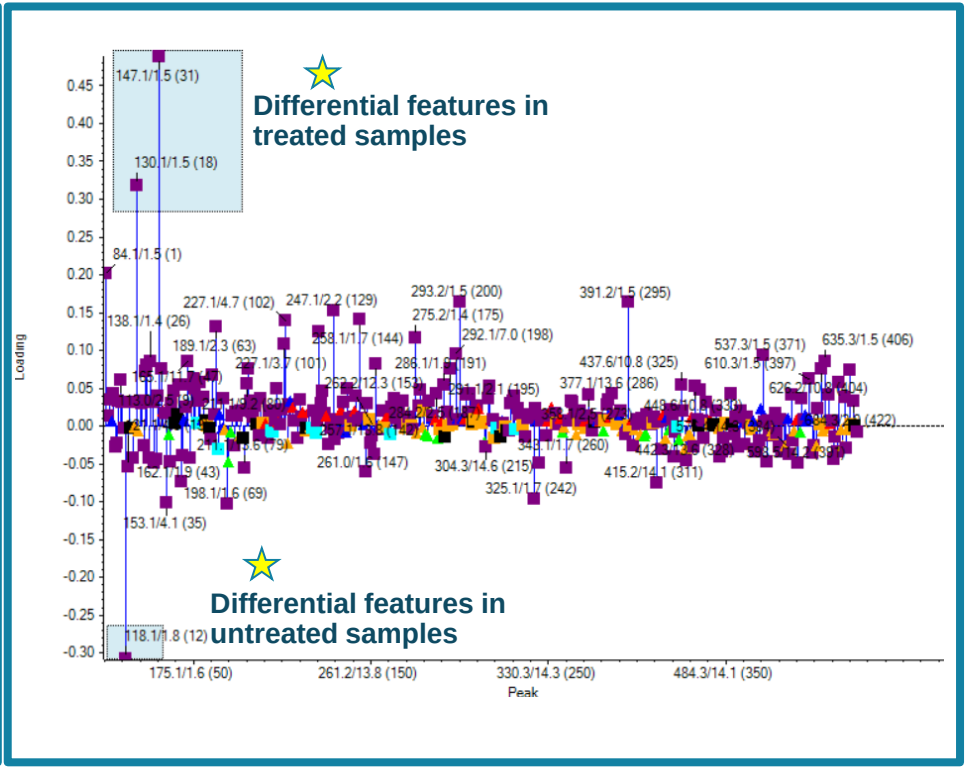
1.24 µmol/mL | 0.33 µmol/mL | 0.04 µmol/mL | 0.03 µmol/mL

Non-targeted analysis and putative identification of components present in media

Non-targeted analysis on CCM samples

IDENTIFY DIFFERENTIAL FEATURES THROUGH MULTIVARIATE ANALYSIS





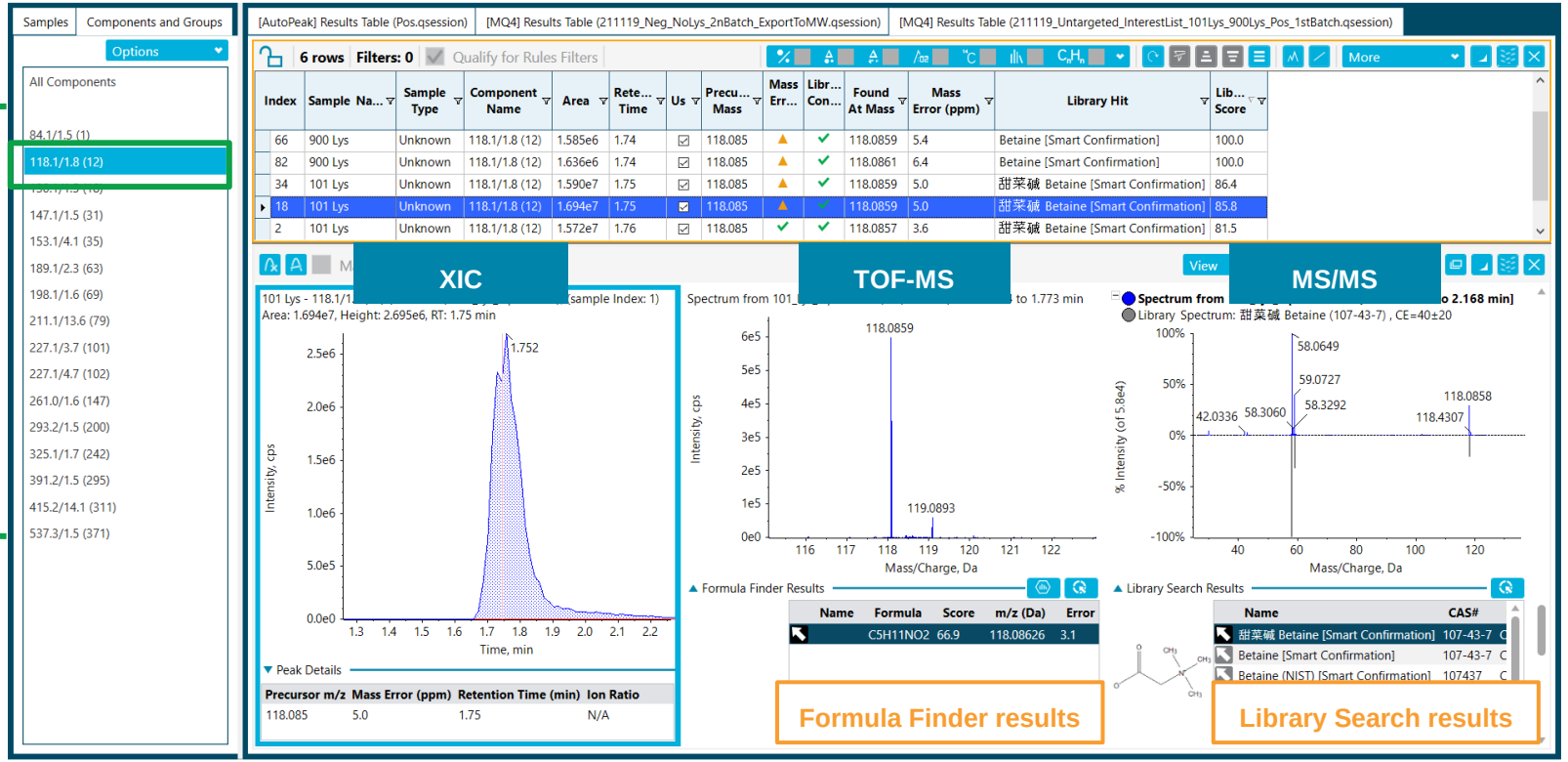
101 Lys: medium
900 Lys: medium fermented with lysine

Compound interest list

Non-targeted analysis on CCM samples

PUTATIVE IDENTIFICATION OF DIFFERENTIAL FEATURES USING FORMULA FINDER AND LIBRARY SEARCH

Compound interest list



Conclusion

- **A superior separation** of critical CCM components over a broad range of chemistries was performed
- **A highly sensitive** workflow for monitoring >110 analytes target analytes was developed
- **The developed Data Independent Acquisition (DIA) workflow** allowed for enhanced qualitative analysis and most comprehensive quantification method
- **Quick putative identification of differential features** is possible by combining the formula finder and library matching tools

Acknowledgements

- Antonella Chiapparino
- Eshani Nandita

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Speaker contact information:
Mariateresa.Maldini@sciex.com

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