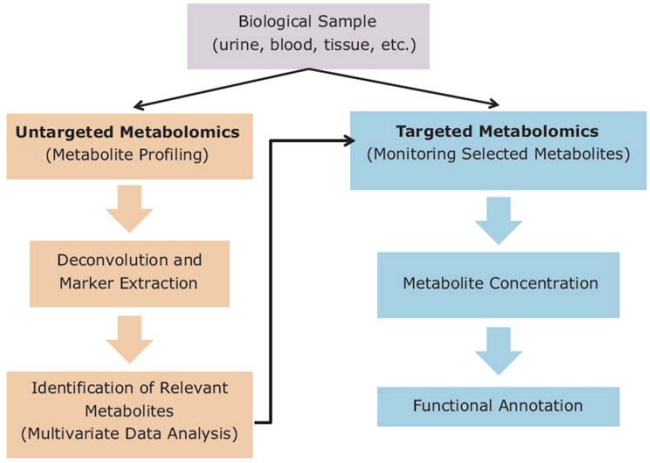
SWATH™ Acquisition;
Accelerating Quantitative Metabolomics

***Brigitte Simons*, Academic Business Market Development Manager**
AB SCIEX, Canada

Metabolomics Workflow Strategies



MS Accelerating the Future of Metabolomics = Optimizing and combining the best of targeted and non-targeted strategies for comprehensive data collection with scalability and throughput

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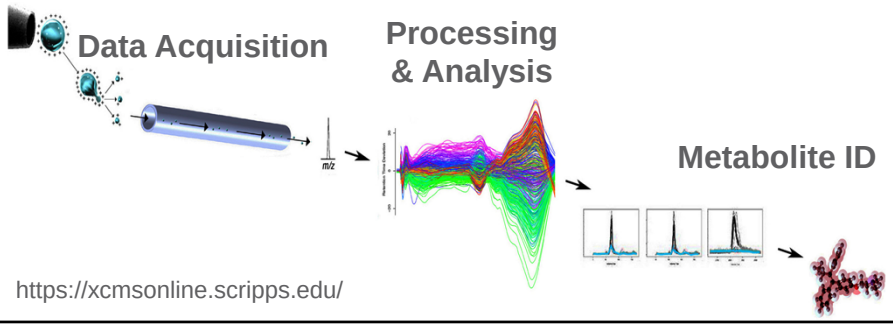
Pave the Way Forward…
Reducing Data Interpretation Bottlenecks…

PROTOCOL

Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database

Zheng-Jiang Zhu[1], Andrew W Schultz[1], Junhua Wang[1], Caroline H Johnson[1], Steven M Yannone[2], Gary J Patti[3–5] & Gary Siuzdak[1]



https://xcmsonline.scripps.edu/

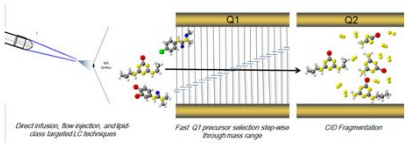
Pave the Way Forward…
Reducing Data Interpretation Bottlenecks…

LIPID Metabolites and Pathways Strategy (LIPID MAPS)
Lipidomics Gateway

Lipidomics reveals a remarkable diversity of lipids in human plasma[1]

Quehenberger *et al*, J Lipid Res. 2010 Nov;51(11):3299-305

SWATH MS for Lipidomics



Avanti Polar Lipids, Inc.

Design and Implementation of Quantitative Lipid Internal Standards

Pave the Way Forward…
Reducing Data Interpretation Bottlenecks…

Anal Bioanal Chem (2012) 402:2587–2596
DOI 10.1007/s00216-011-5641-8

ORIGINAL PAPER

High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices

Gérard Hopfgartner · David Tonoli · Emmanuel Varesio

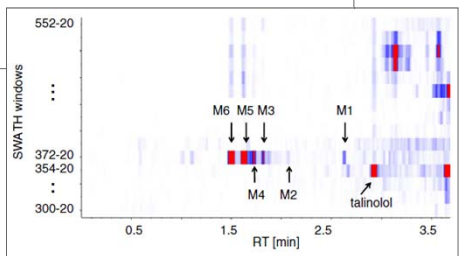
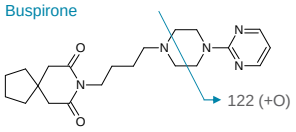


Fig. 4 Heat map of SWATH experiments (15 product-ion experiments) of the LC–MS analysis after incubation of talinolol with rat liver microsomes for 2 h. Blue (zero), red max signal corresponding to 10% of base peak

Buspirone



SWATH MS for Metabolite Fishing

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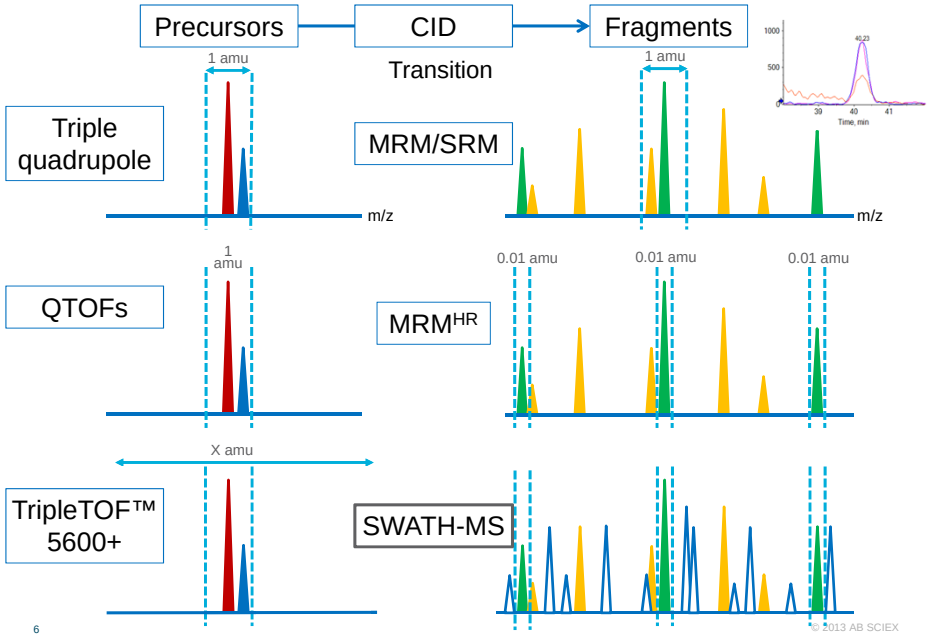
Quantitative LC-MS Techniques



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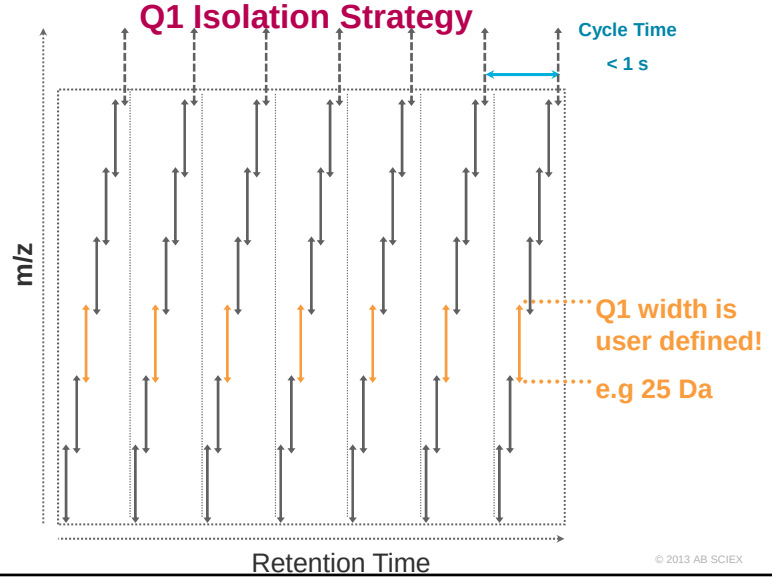
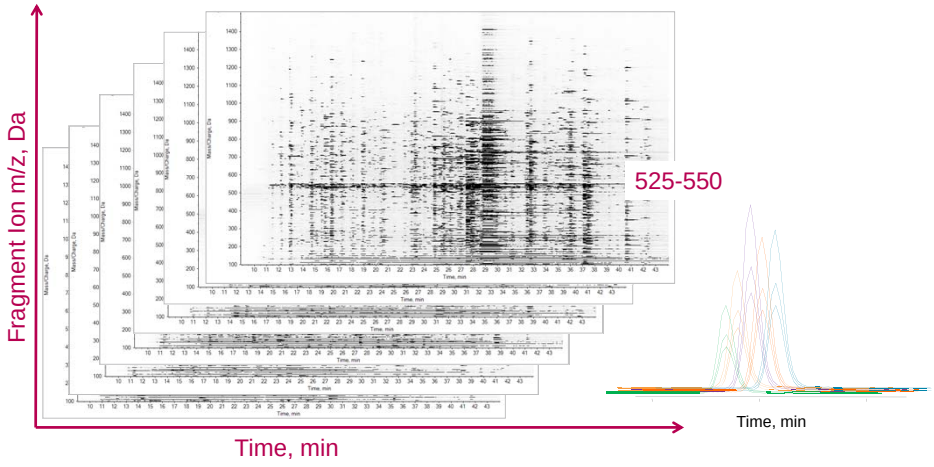
SWATH = Sequential Windowed Acquisition of All Theoretical MS
Q1 Isolation Strategy



7

Easy Method Development and Data Mining is Carried Out Retrospectively

24 LCMS maps of fragment ions



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Why SWATH Analysis for Metabolomics?

- Data Independent Acquisition – no prioritization or bias in precursor data collection given LC separations are not always ideal
- Best Quant and Best Qual metabolite ID all in one run
  - Quant is MS/MS for best selectivity and signal to noise
  - No pre-designed inclusion lists
  - No pre-set compound specific information
- All the information is extracted post LC-MS acquisition
- Designing a balance between Specificity vs Sensitivity
  - Dependence on separation of isomers
  - Association of Precursor and putative Products signals is critical
  - Managing LC eluted m/z in dense regions of the chromatography
- A Permanent Digital Record of MS/MS fragment of your samples
  - Come back to it for hypothesis-driven discovery
  - Build an MRM method to transfer for targeted analysis easily.

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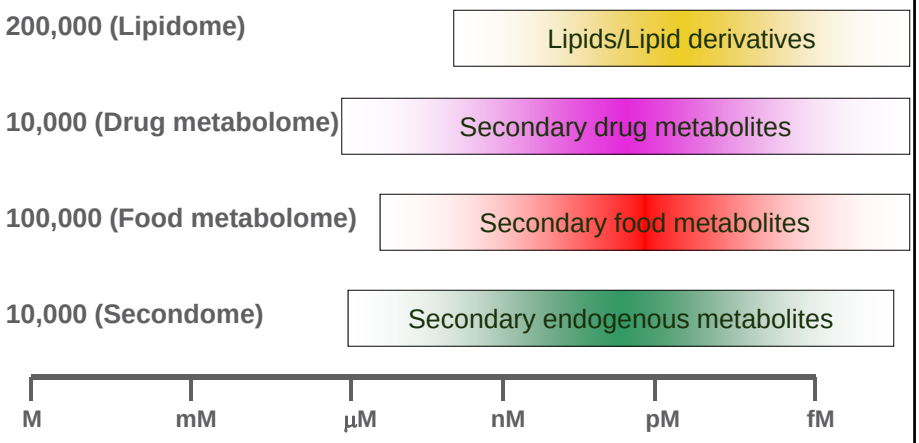
Theoretical Human Metabolomes
How Important is Relative Quantitation?



Wishart Bioinformatics Workshop; www.bioinformatics.ca

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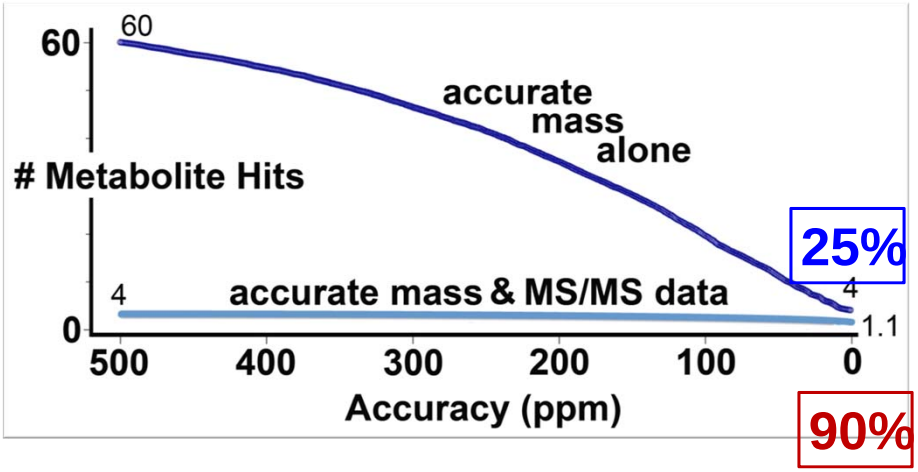
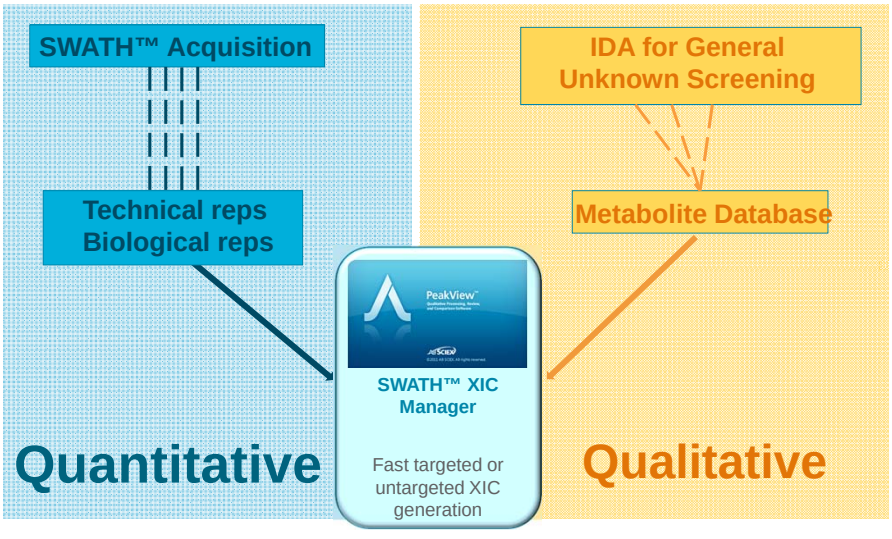
The Importance of MS/MS for Metabolite ID

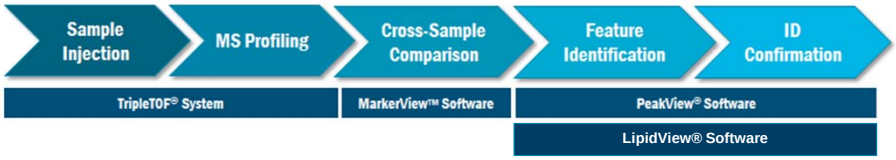


Slide courtesy of Jiang Zhu; Analytical Chemistry, **2013**, 85, 798-804

SWATH MS and Data Independent Acquisitions



AB SCIEX SWATH Quant/Qual Experimental Workflow



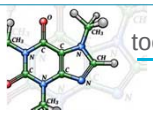
MS and SWATH MS/MS

Multivariate Statistical Analysis
– Find the differential features
– Link to raw data

Identify and confirm metabolite/lipid formula or structure

FormulaFinder™ predicts elemental composition and MS/MS fragment assignment

toolsets



toolsets

ChemSpider for Structural information if not in your in-house library

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Metabolomics Study Design

- 12 plasma samples were consensually collected.
- 6 male  and 6 female  samples.
- Female samples were split into 2 groups, pre-menopausal (4) and post menopausal (2).
- Urine was diluted 1:4 with distilled water and centrifuged.
- A pooled sample was prepared using an aliquot from all samples.

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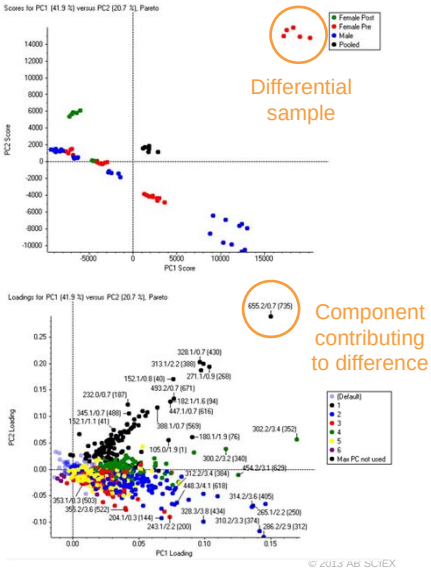
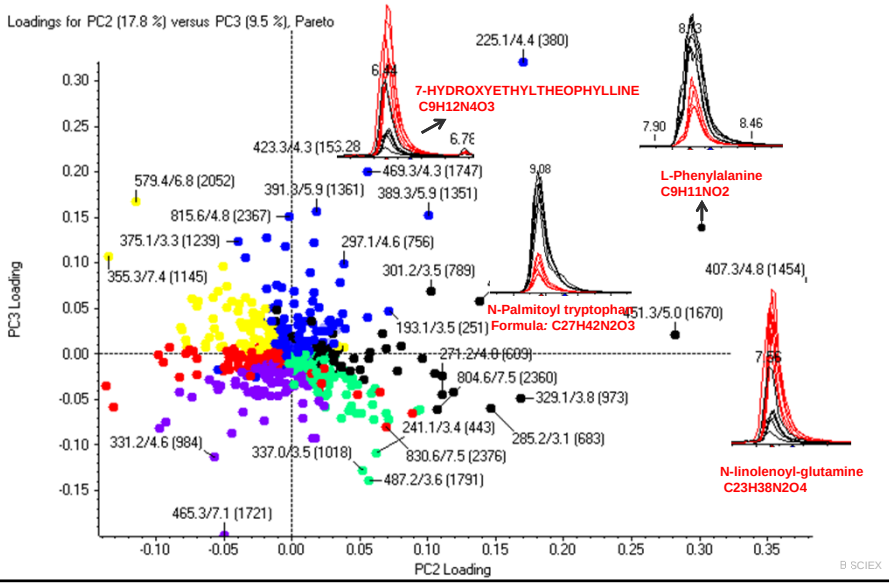
Multivariate Statistical Analysis of MS Data

- The PCA Scores (top) and Loadings (bottom) plots with Pareto scaling highlight the differences between the urine samples.
- Principle Component Variable Grouping (PCVG) is used in the loadings plot to cluster ions with similar profiles (represented here in the same colour), accounting for 70 % of the variation.
- m/z 655 is one of the peaks contributing to the difference in one of the urine samples



LC-MS Peak Alignment with Metabolite ID



Known Compound Identification

Screen of SWATH MS/MS against in-house Libraries

Library Match Strategy for Known Compound Identification

- **Identification criteria**
  1. Accurate mass TOF MS (ppm error)
  2. Retention time (min)
- **Metabolite ID Confirmation criteria**
  3. TOF MS/MS spectral matching (purity score)
     - Library MS/MS spectrum with experimental
  4. Isotope pattern match
     - theoretical vs. experimental

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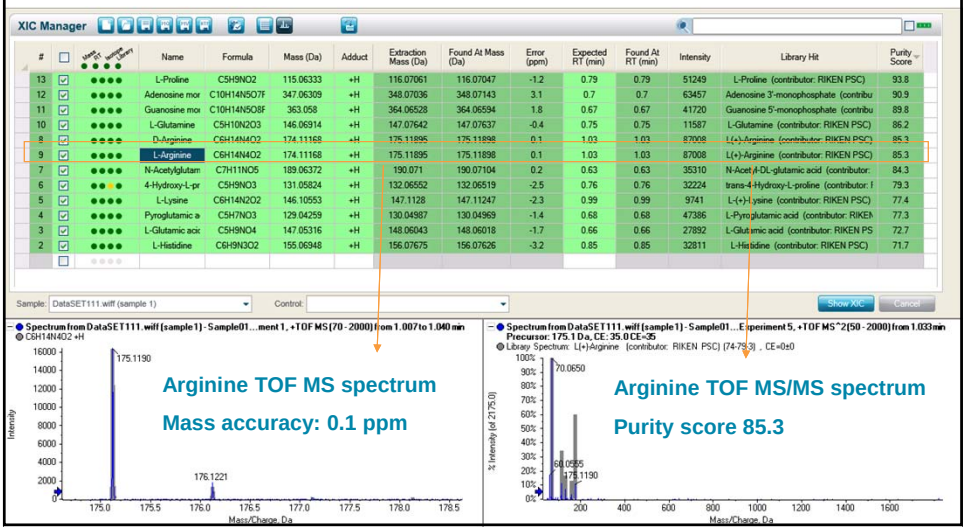
Known Compound Identification- Library Match

Table showing name, formula, mass accuracy, RT, intensity, library hit & purity score



Unknown Compound Identification

Steps for unknown compound ID using chemical logic

1. Elemental composition assignment using TOF MS
2. Elemental composition of fragment ion (TOF MS/MS)
3. Structure search for elemental composition in public database

   (PubChem, ChemSpider, HMDB, MassBank, Metlin etc.)
4. Structural elucidation and fragmentation correlation

*** All from a single injection**

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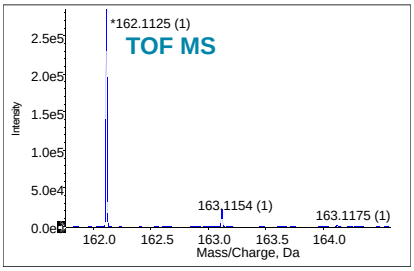
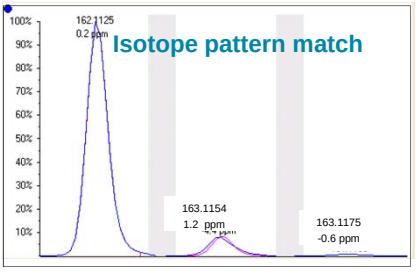
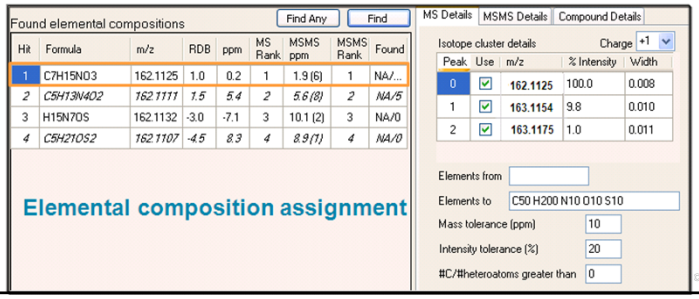
Elemental Composition Assignment using TOF MS







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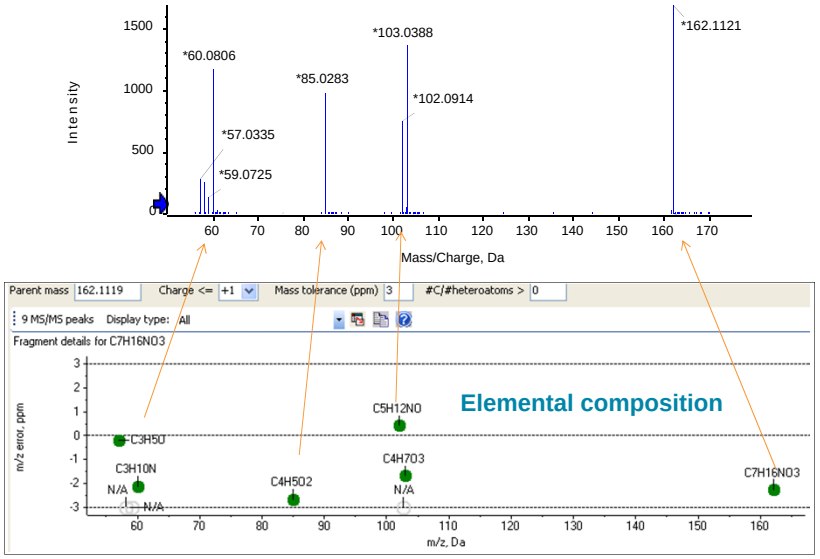
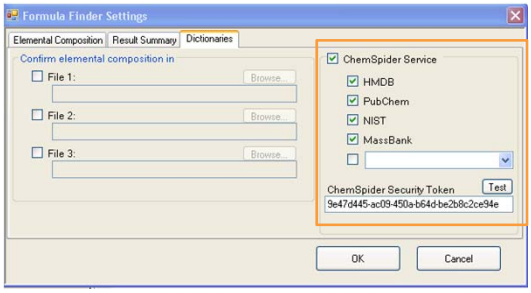
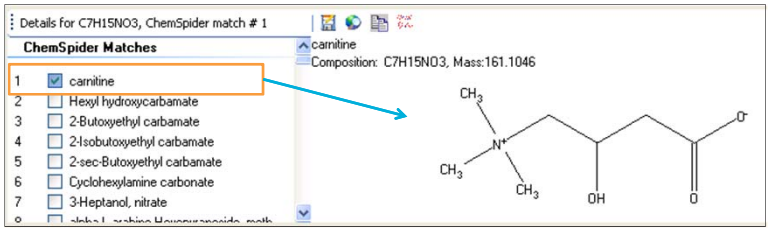
Elemental Composition Assignment of Fragment Ions

TOF MS/MS Spectrum



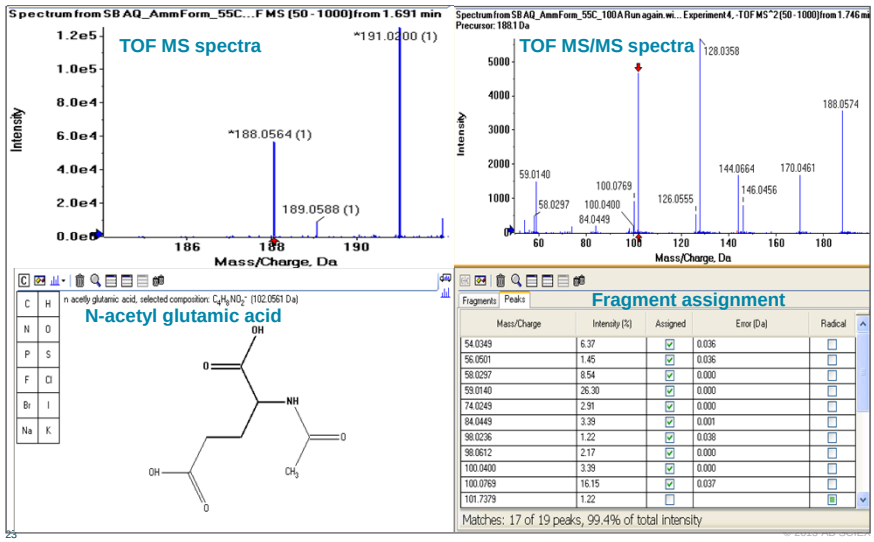
Elemental Formula & Structure Search using Metabolite Databases including METLIN





Structural Elucidation in PeakView™ Software

Match N-Acetylglutamic Acid Fragments from Structure with MS/MS Data

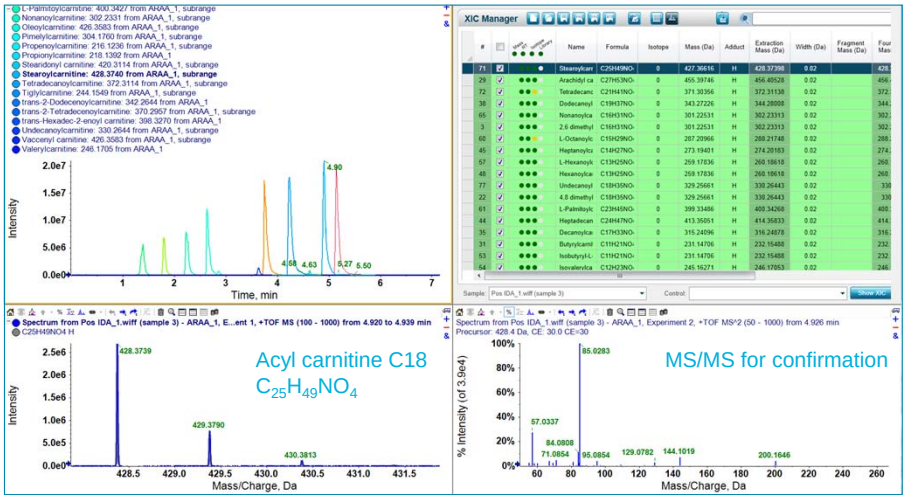


Hypothesis Driven Targeted Biochemical Pathway Workflow

1. **Targeted data processing** using a list of known metabolites in any biochemical pathway
2. **Single injection workflow** for quantitative and qualitative data
3. **Validate hypothesis** whether a specific pathway is effecting as a result of disease state or treatment or phenotype or genotype
4. **Quick answers to biologists** and fast turn around time
5. **Ready to use list of metabolites** in a given biochemical pathway like
   - Glycolysis
   - TCA cycle
   - Acyl carnitines
   - Phosphate pentose pathway
   - Oxidative stress markers
   - Eicosanoid pathway
   - Leukotriene's
   - Amino acids etc.

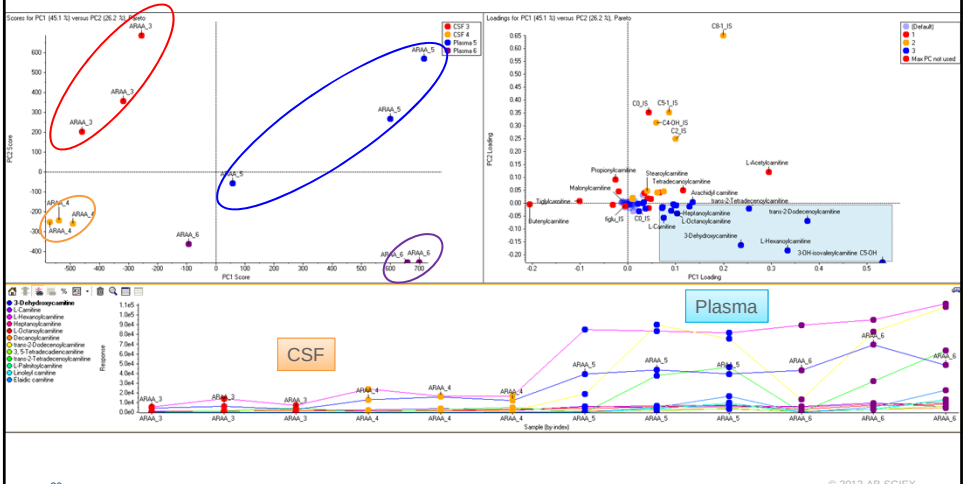
SWATH MS Quantitation of Acyl Carnitines

XIC Manager for Targeted Extraction of MS or MS/MS

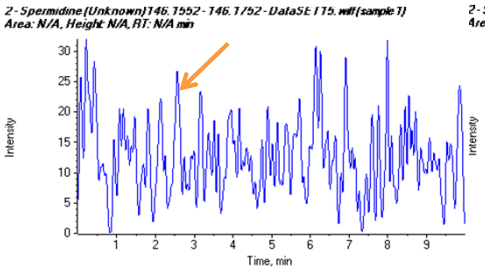


MarkerView™ Software

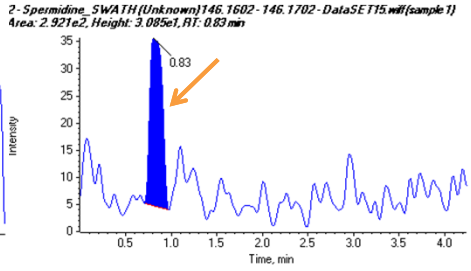
Profiling of Acyl Carnitines in CSF & Plasma Extracts



Quantitative Performance
TOF MS Quant vs. SWATH Quant

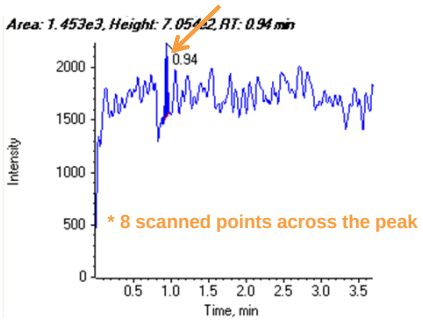


TOF MS: 1 nM- 0.14 ng/mL Spermidine



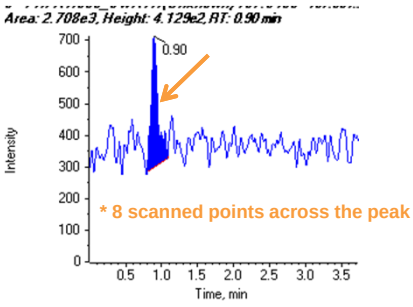
SWATH: 1 nM- 0.14 ng/mL Spermidine

Quantitative Performance
TOF MS Quant vs. SWATH Quant



TOF MS:
1 nM- Amine-1 MW: 153.05

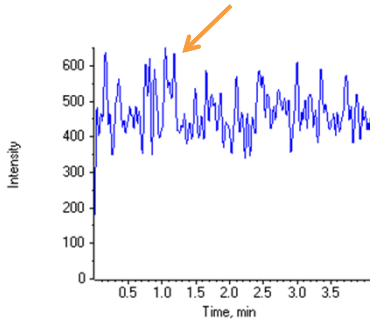
High background noise



SWATH Acquisition:
1 nM- Amine-1 MW:153.05

High Selectivity with **5X less** background noise

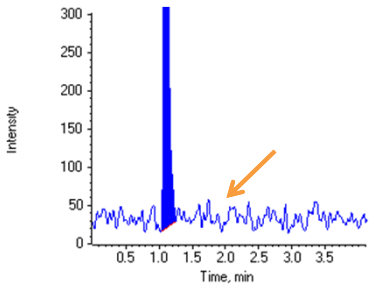
TOF MS vs. SWATH™ Acquisition Quantification



* 8 scanned points across the peak

TOF MS:
1 nM- Amine-2 MW : 177.1

High background noise



* 8 scanned points across the peak

SWATH Acquisition:
1 nM- Amine-2 MW : 177.1

High Selectivity with 10X less background noise

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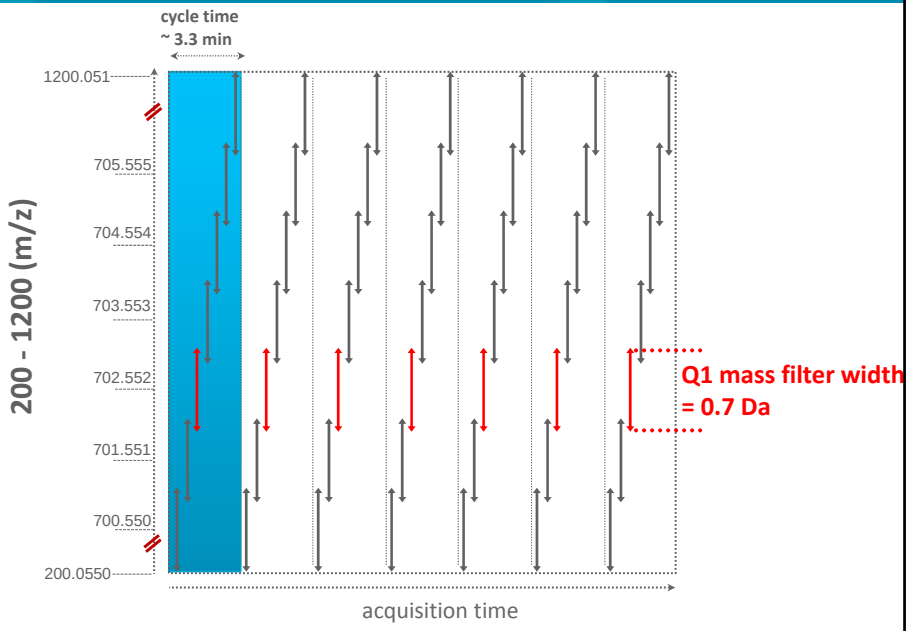
SWATH Acquisition Method Set-up for Lipidomics



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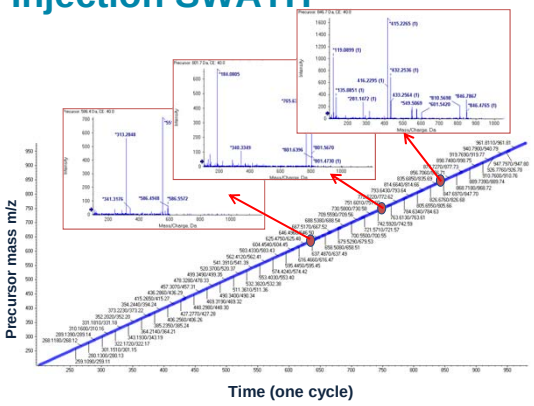
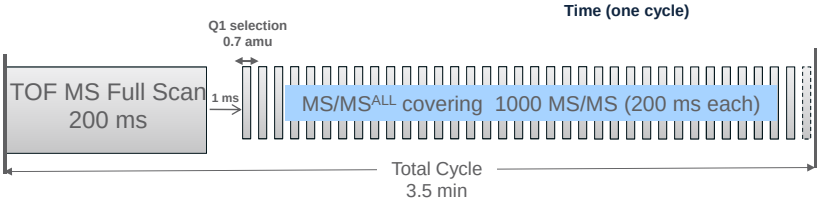
Lipidomics by Flow Injection SWATH

Method Set-up

- How to calculate cycle time?
  - Mass range
  - Step size to cover mass range
  - MS/MS accumulation time
  - Looping in a TOF MS scan (~ 200 ms)





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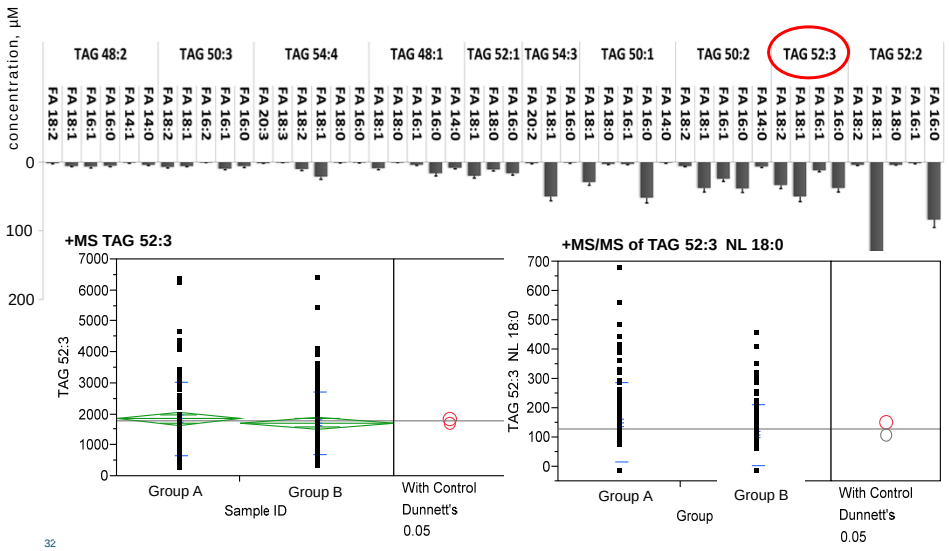
TAG Lipid Profiling in Plasma



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Conclusions

- SWATH adopting the concept of Data Independent Acquisition is the future of Quant/Qual metabolomics workflows
  - MRM quantitative data mining retrospectively
  - 100% MS/MS coverage – "The Ultimate Safety Net"
- Design a balance of sensitivity and selectivity
  - Lipidomics focuses data collection in MS/MS with narrow windows
  - Metabolomics by LC-SWATH is optimized for UPLC separations with wider windows stepped quickly across a wider mass range
  - A totally generic method – no need to change the methods, CE is ramped
- Follow up with a targeted MRM^{HR} method, + CE optimized, and targeted data processing for quantitative reporting of the important metabolites

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Acknowledgments

- University of Toronto & Toronto General Hospital
  - Johanne Allard
  - Bianca Arendt
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  - Mark Cafazzo
  - Christie Hunter
- AB SCIEX, CAN
  - Suma Ramagiri
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  - Eva Duchoslav
  - Dave Cox
  - Lyle Burton
- AB SCIEX, UK
  - Vicki Gallant, PhD
  - Baljit Ubhi, PhD

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