




**SWATH™ Acquisition;
Accelerating Quantitative Metabolomics**

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Metabolomics Workflow Strategies

```

    graph TD
      A["Biological Sample  
(urine, blood, tissue, etc.)"] --> B["Untargeted Metabolomics  
(Metabolite Profiling)"]
      A --> C["Targeted Metabolomics  
(Monitoring Selected Metabolites)"]
      B --> D["Deconvolution and  
Marker Extraction"]
      D --> E["Identification of Relevant  
Metabolites  
(Multivariate Data Analysis)"]
      E --> C
      C --> F["Metabolite Concentration"]
      F --> G["Functional Annotation"]
    
```

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

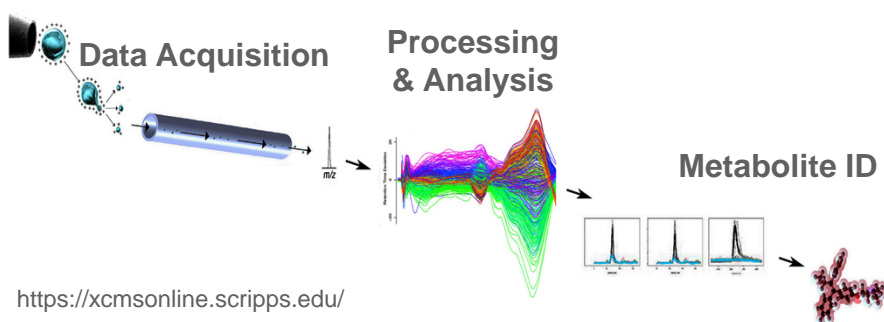
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Pave the Way Forward... Reducing Data Interpretation Bottlenecks...

PROTOCOL

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

Zheng-Jiang Zhu¹, Andrew W Schultz¹, Junhua Wang¹, Caroline H Johnson¹, Steven M Yannone², Gary J Patti³⁻⁵ & Gary Siuzdak¹

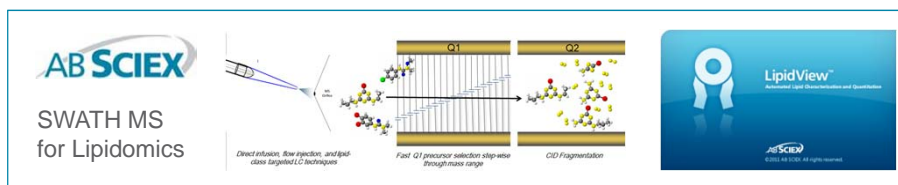


Pave the Way Forward... Reducing Data Interpretation Bottlenecks...



Lipidomics reveals a remarkable diversity of lipids in human plasma^{1[S]}

[Quehenberger et al, J Lipid Res. 2010 Nov;51\(11\):3299-305](#)



Design and Implementation of Quantitative Lipid Internal Standards

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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

G rard Hopfgartner · David Tonoli · Emmanuel Varesio

Buspirone

122 (+O)

SWATH MS for Metabolite Fishing

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

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Quantitative LC-MS Techniques

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Precursors

1 amu

CID
Transition

Fragments

1 amu

Triple quadrupole

m/z

MRM/SRM

m/z

QTOFs

1 amu

X amu

MRM^{HR}

0.01 amu

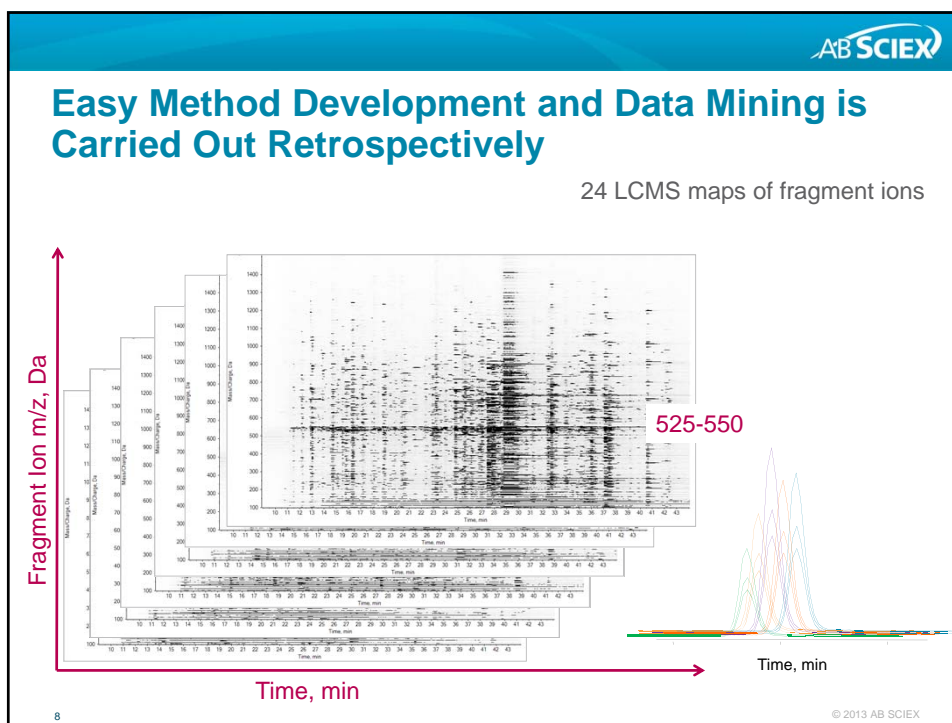
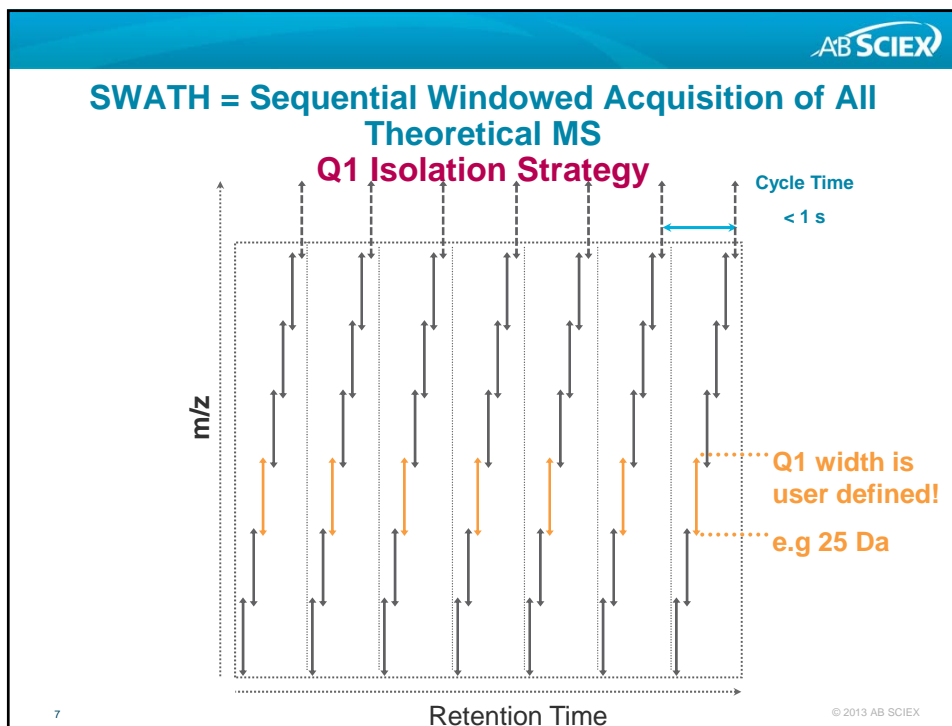
0.01 amu

0.01 amu

TripleTOFTM 5600+

SWATH-MS

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Why SWATH Analysis for Metabolomics?

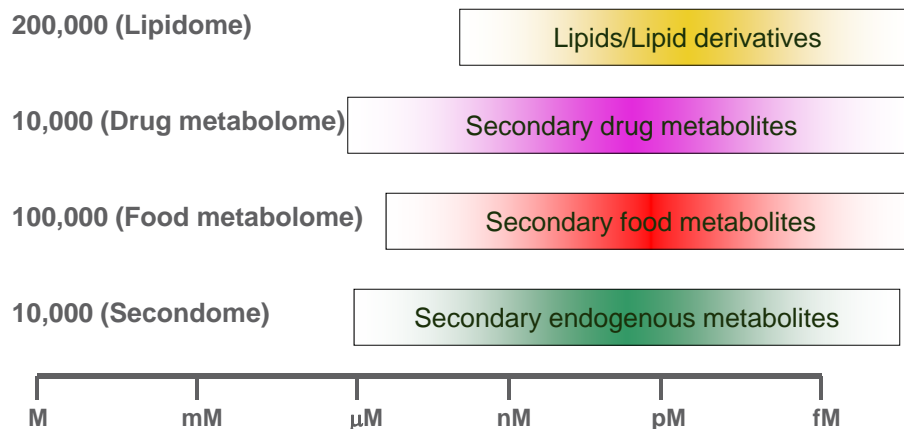
- Data Independent Acquisition – no prioritization or bias in precursor data collection given LC separations are not always ideal
- Best Quant and Best Qual metabolite ID all in one run
 - Quant is MS/MS for best selectivity and signal to noise
 - No pre-designed inclusion lists
 - No pre-set compound 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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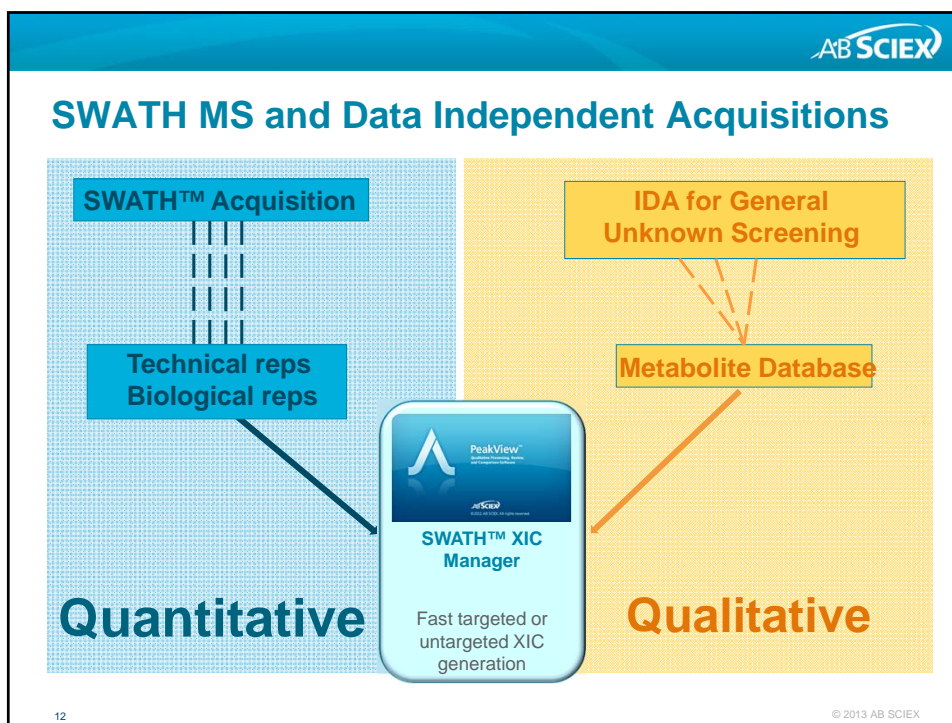
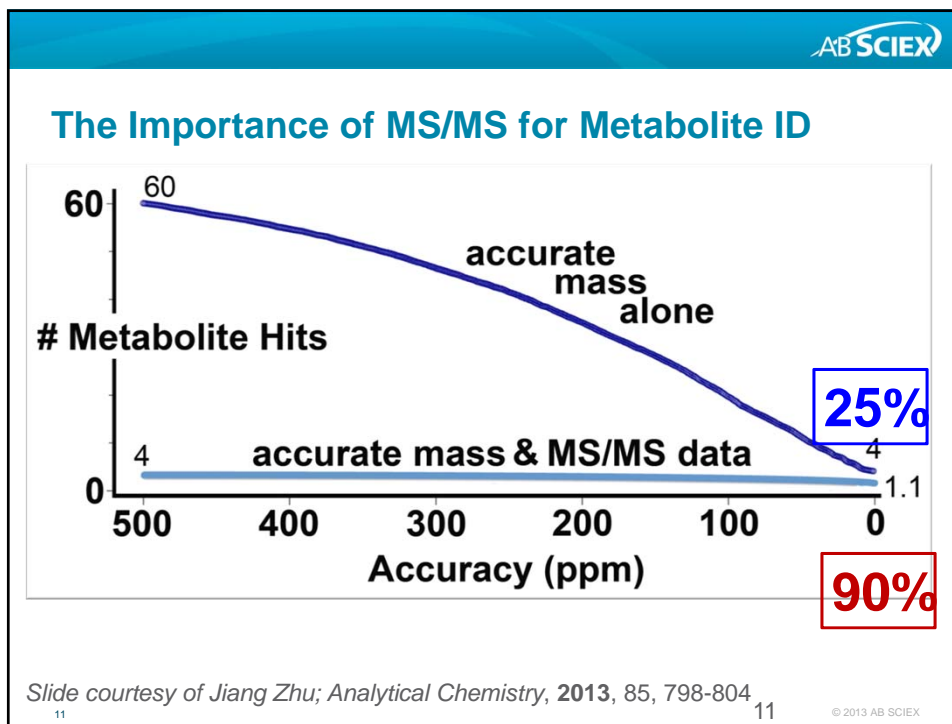
Theoretical Human Metabolomes

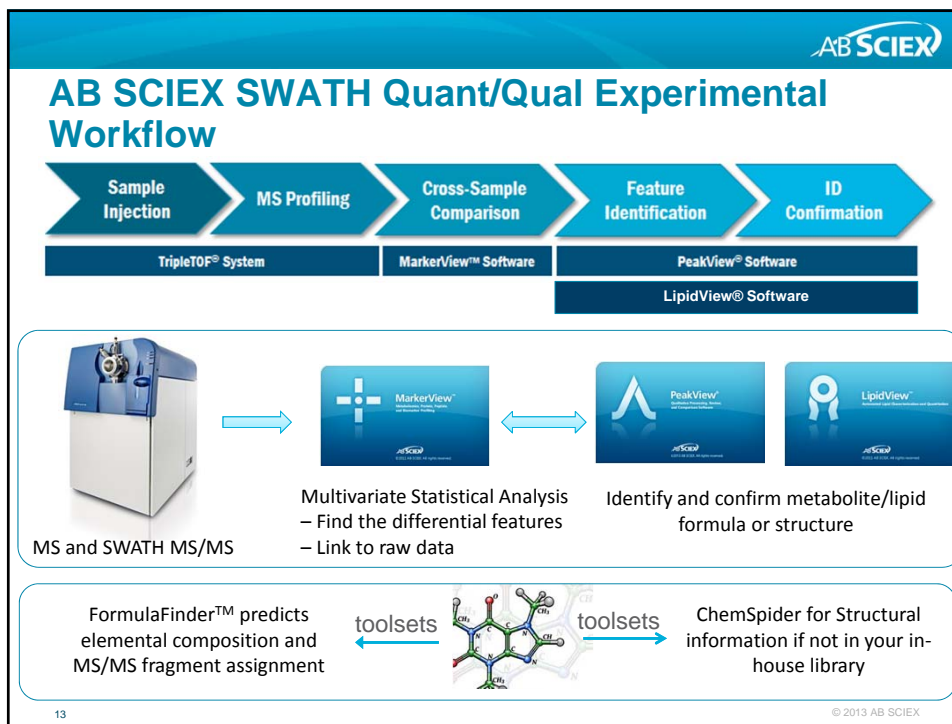
How Important is Relative Quantitation?

Wishart Bioinformatics Workshop; www.bioinformatics.ca

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Metabolomics Study Design

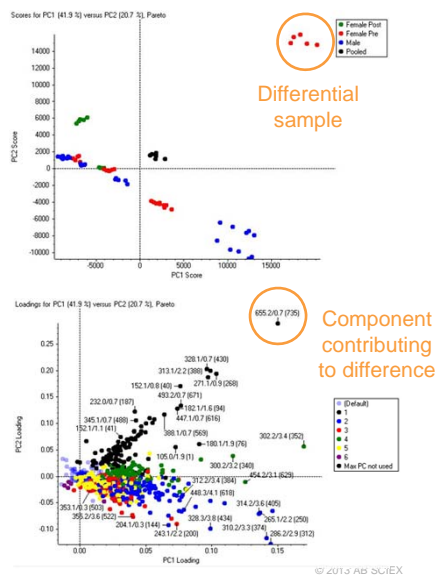
- 12 plasma samples were consensually collected.
- 6 male and 6 female samples.
- Female samples were split into 2 groups, pre-menopausal (4) and post menopausal (2).
- Urine was diluted 1:4 with distilled water and centrifuged.
- A pooled sample was prepared using an aliquot from all samples.

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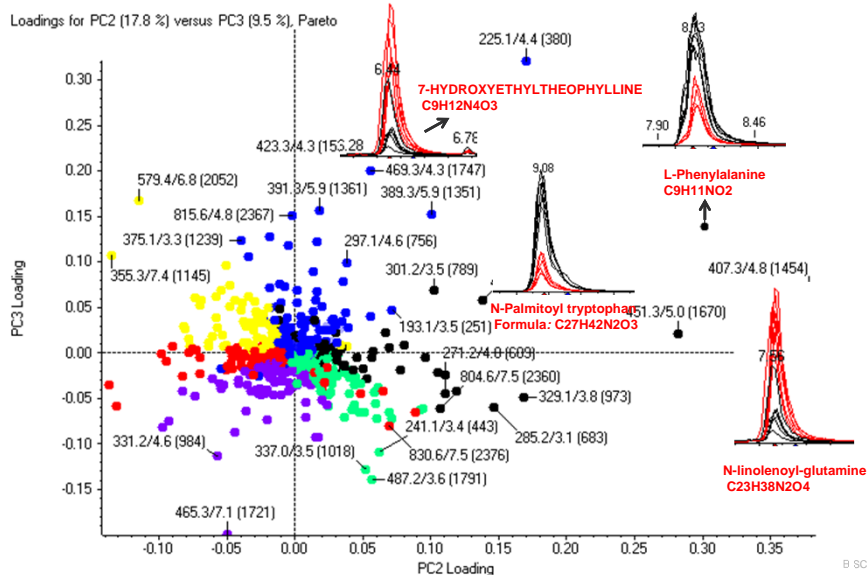
Multivariate Statistical Analysis of MS Data

- 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



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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

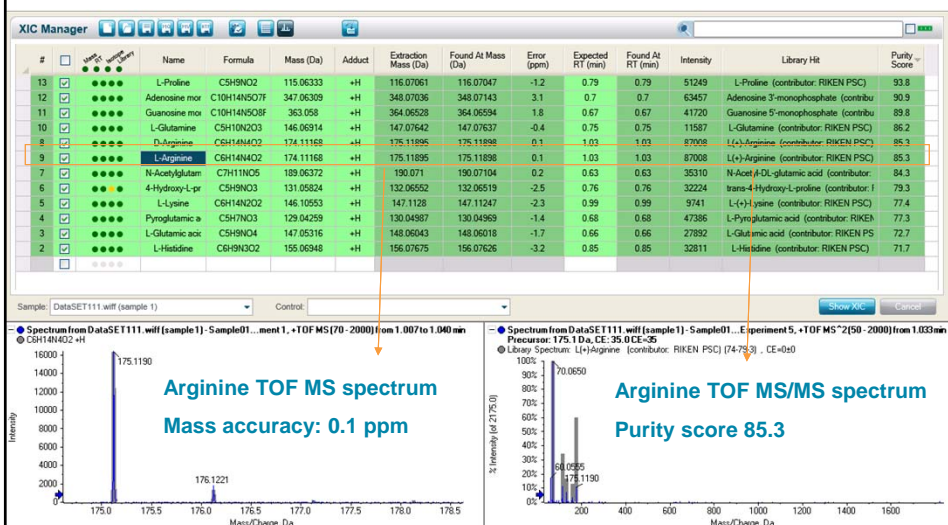
- **Identification criteria**
 1. Accurate mass TOF MS (ppm error)
 2. Retention time (min)
- **Metabolite ID Confirmation criteria**
 3. TOF MS/MS spectral matching (purity score)
 - Library MS/MS spectrum with experimental
 4. Isotope pattern match
 - theoretical vs. experimental

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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

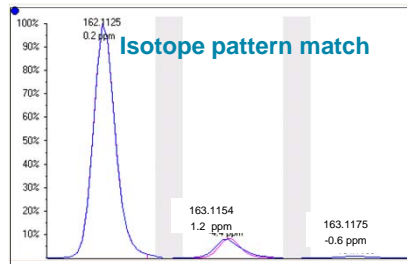
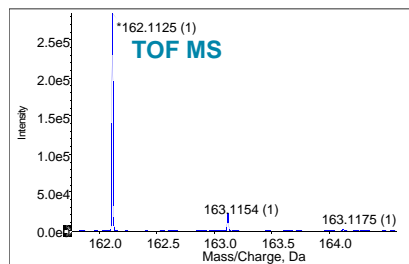
1. Elemental composition assignment using TOF MS
2. Elemental composition of fragment ion (TOF MS/MS)
3. Structure search for elemental composition in public database
(PubChem, ChemSpider, HMDB, MassBank, Metlin etc.)
4. Structural elucidation and fragmentation correlation

* All from a single injection

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Elemental Composition Assignment using TOF MS



| Found elemental compositions | | | | | | | | Find Any | Find |
|------------------------------|-----------|----------|------|------|---------|----------|-----------|----------|------|
| HR | Formula | m/z | RDB | ppm | MS Rank | MSMS ppm | MSMS Rank | Found | |
| 1 | C7H15NO3 | 162.1125 | 1.0 | 0.2 | 1 | 1.9 (6) | 1 | NA/... | |
| 2 | C5H13N4O2 | 162.1111 | 1.5 | 5.4 | 2 | 5.6 (8) | 2 | NA/... | |
| 3 | H15N7O5 | 162.1132 | -3.0 | -7.1 | 3 | 10.1 (2) | 3 | NA/0 | |
| 4 | C5H21O52 | 162.1107 | -4.5 | 8.3 | 4 | 8.9 (1) | 4 | NA/0 | |

Elemental composition assignment

| MS Details | | | | MSMS Details | | Compound Details | |
|------------|-------------------------------------|----------|-------------|--------------|--------|------------------|--|
| Peak | Use | m/z | % Intensity | Width | Charge | | |
| 0 | <input checked="" type="checkbox"/> | 162.1125 | 100.0 | 0.008 | +1 | | |
| 1 | <input checked="" type="checkbox"/> | 163.1154 | 9.8 | 0.010 | | | |
| 2 | <input checked="" type="checkbox"/> | 163.1175 | 1.0 | 0.011 | | | |

Elements from:

Elements to: C50 H200 N10 O10 S10

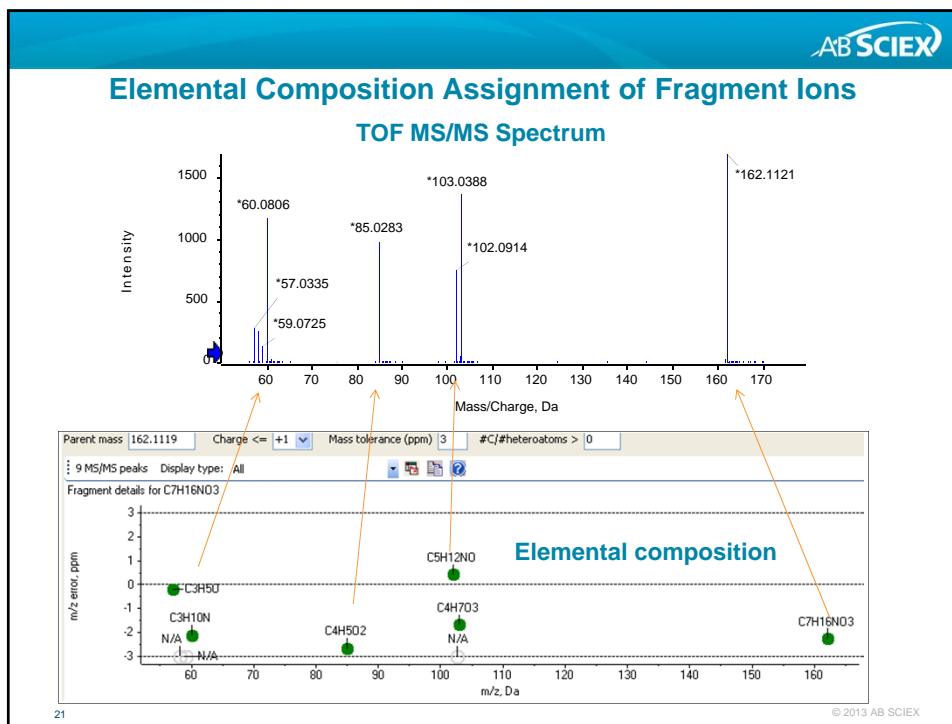
Mass tolerance (ppm):

Intensity tolerance (%):

#C/#heteroatoms greater than:

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Elemental Formula & Structure Search using Metabolite Databases including METLIN

Formula Finder Settings

Elemental Composition Result Summary Dictionaries

Confirm elemental composition in

File 1:

File 2:

File 3:

ChemSpider Service

HMDB

PubChem

NIST

MassBank

ChemSpider Security Token:

Details for C7H15NO3, ChemSpider match # 1

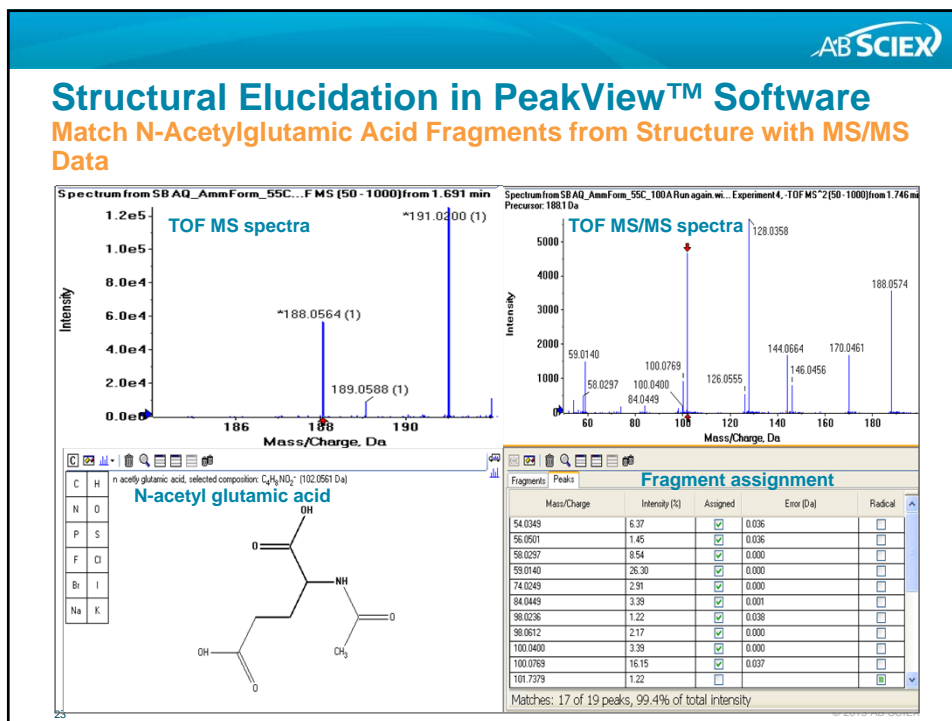
ChemSpider Matches

- 1 carnitine
- 2 Hexyl hydroxycarbamate
- 3 2-Butoxyethyl carbamate
- 4 2-Isobutoxyethyl carbamate
- 5 2-sec-Butoxyethyl carbamate
- 6 Cyclohexylamine carbonate
- 7 3-Heptanol, nitrate
- 8 2-ethyl-2-hydroxypropanoic acid, methyl ester

carnitine
Composition: C7H15NO3, Mass:161.1046

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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.

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SWATH MS Quantitation of Acyl Carnitines

XIC Manager for Targeted Extraction of MS or MS/MS

- L-Palmitoylcarnitine: 400.3427 from ARAA_1, subrange
- Nonanoylcarnitine: 302.2331 from ARAA_1, subrange
- Oleoylcarnitine: 426.3383 from ARAA_1, subrange
- Pimelylcarnitine: 304.1700 from ARAA_1, subrange
- Propionylcarnitine: 216.1236 from ARAA_1, subrange
- Propionylcarnitine: 218.1392 from ARAA_1
- Stearoylcarnitine: 428.2749 from ARAA_1, subrange
- Tetradecanoylcarnitine: 372.3114 from ARAA_1, subrange
- Tylloylcarnitine: 344.1549 from ARAA_1, subrange
- Trans-2-Dodecanoylcarnitine: 342.2644 from ARAA_1
- Trans-2-Tetradecanoylcarnitine: 370.2667 from ARAA_1, subrange
- Trans-Hexadec-2-enoylcarnitine: 368.3270 from ARAA_1
- Undecanoylcarnitine: 300.2644 from ARAA_1, subrange
- Vaccenyl carnitine: 426.3383 from ARAA_1, subrange
- Valeryl carnitine: 246.1705 from ARAA_1

Intensity vs Time, min

XIC Manager

| # | Name | Formula | Isotope | Mass (Da) | Abduct | Extraction Mass (Da) | Width (Da) | Fragment Mass (Da) | Four Mas |
|----|------------------------|-----------|---------|-----------|--------|----------------------|------------|--------------------|----------|
| 71 | Stearoylcarnitine | C25H49NO4 | 0 | 427.3616 | H | 428.3739 | 0.02 | 428 | |
| 29 | Arachidyl ca | C27H52NO4 | 0 | 455.39146 | H | 456.40256 | 0.02 | 456 | |
| 72 | Tetradecanoylcarnitine | C21H42NO4 | 0 | 371.33564 | H | 372.34674 | 0.02 | 372 | |
| 38 | Dodecanoylcarnitine | C19H37NO4 | 0 | 343.27226 | H | 344.28336 | 0.02 | 344 | |
| 66 | Nonanoylcarnitine | C15H31NO4 | 0 | 301.22131 | H | 302.23241 | 0.02 | 302 | |
| 3 | 2,6-dimethyl-C18H35NO4 | C18H35NO4 | 0 | 302.25531 | H | 302.23313 | 0.02 | 302 | |
| 60 | L-Oleoylcarnitine | C19H37NO4 | 0 | 367.29966 | H | 368.31076 | 0.02 | 368 | |
| 45 | Heptanoylcarnitine | C14H27NO4 | 0 | 273.19401 | H | 274.20511 | 0.02 | 274 | |
| 67 | L-Palmitoylcarnitine | C17H33NO4 | 0 | 299.17736 | H | 300.18846 | 0.02 | 300 | |
| 48 | Hexanoylcarnitine | C12H25NO4 | 0 | 239.19336 | H | 240.20446 | 0.02 | 240 | |
| 77 | Undecanoylcarnitine | C15H31NO4 | 0 | 329.26661 | H | 330.27771 | 0.02 | 330 | |
| 22 | 4,8-dimethyl-C18H35NO4 | C18H35NO4 | 0 | 329.26661 | H | 330.26443 | 0.02 | 330 | |
| 61 | L-Palmitoylcarnitine | C17H33NO4 | 0 | 309.24406 | H | 310.25516 | 0.02 | 310 | |
| 44 | Heptanoylcarnitine | C14H27NO4 | 0 | 413.26551 | H | 414.26761 | 0.02 | 414 | |
| 35 | Decanoylcarnitine | C13H27NO4 | 0 | 315.24996 | H | 316.24878 | 0.02 | 316 | |
| 31 | Butyrylcarnitine | C11H23NO4 | 0 | 231.14706 | H | 232.15488 | 0.02 | 232 | |
| 63 | Isobutyryl-C12H25NO4 | C12H25NO4 | 0 | 231.14706 | H | 232.15488 | 0.02 | 232 | |
| 54 | Isobutyryl-C12H25NO4 | C12H25NO4 | 0 | 246.16271 | H | 246.17953 | 0.02 | 246 | |

Spectrum from Pos IDA_1_well (sample 3) - ARAA_1, Experiment 2, +TOF MS² (50 - 1000) from 4.920 to 4.939 min

Spectrum from Pos IDA_1_well (sample 3) - ARAA_1, Experiment 2, +TOF MS² (50 - 1000) from 4.926 min

MS/MS for confirmation

% Intensity (of 3.9e4) vs Mass/Charge, Da

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MarkerView™ Software

Profiling of Acyl Carnitines in CSF & Plasma Extracts

Scores for PC1 (65.1%) versus PC2 (26.2%)

Loadings for PC1 (65.1%) versus PC2 (26.2%)

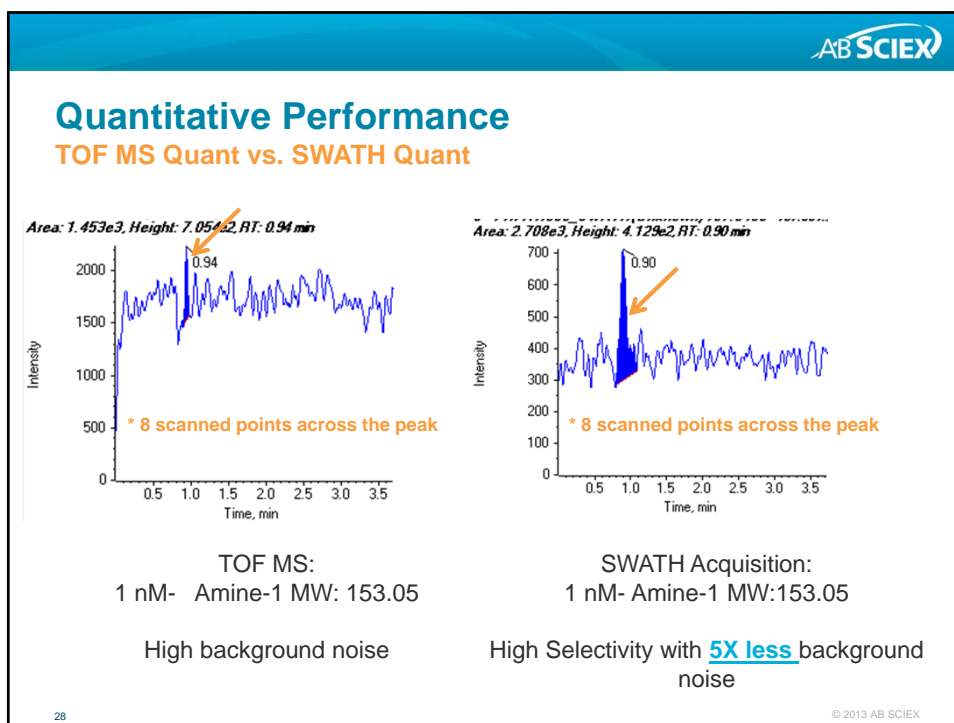
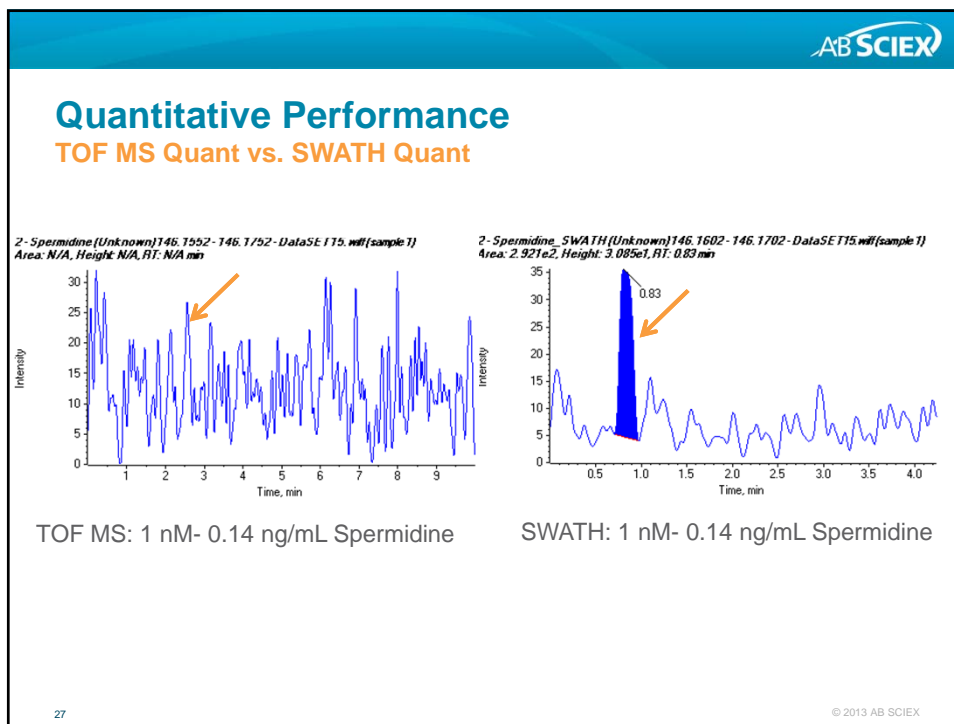
MarkerView

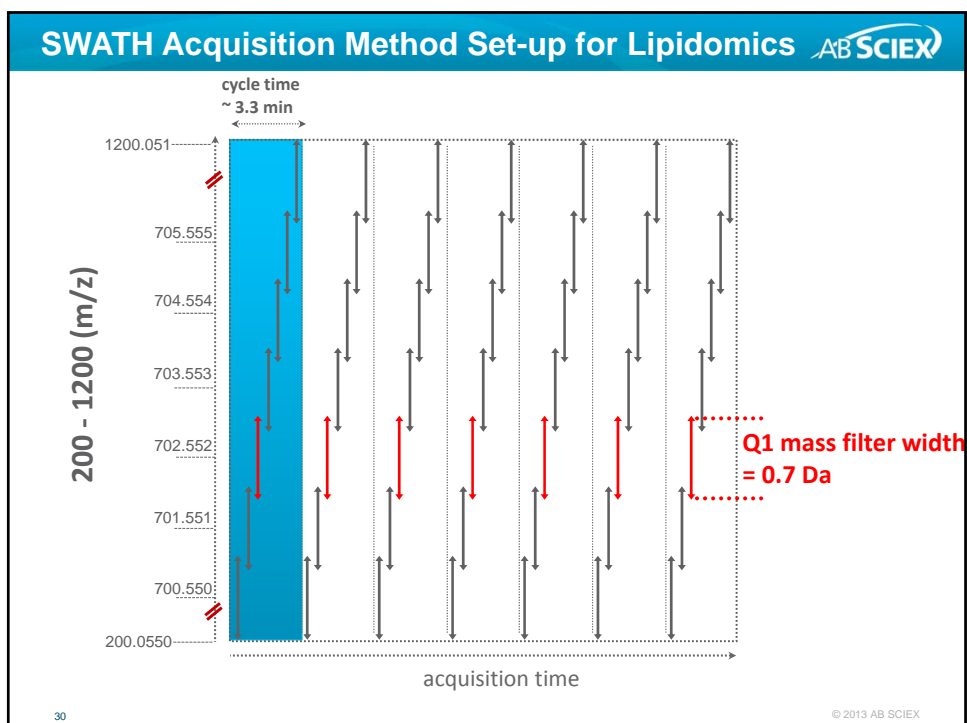
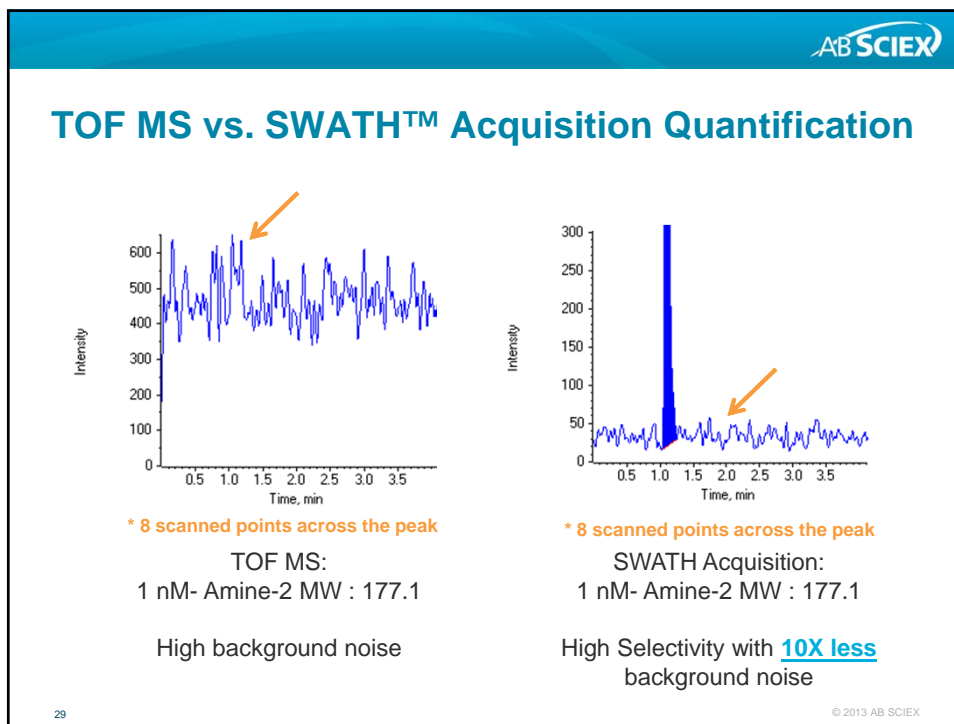
Acyl Carnitines

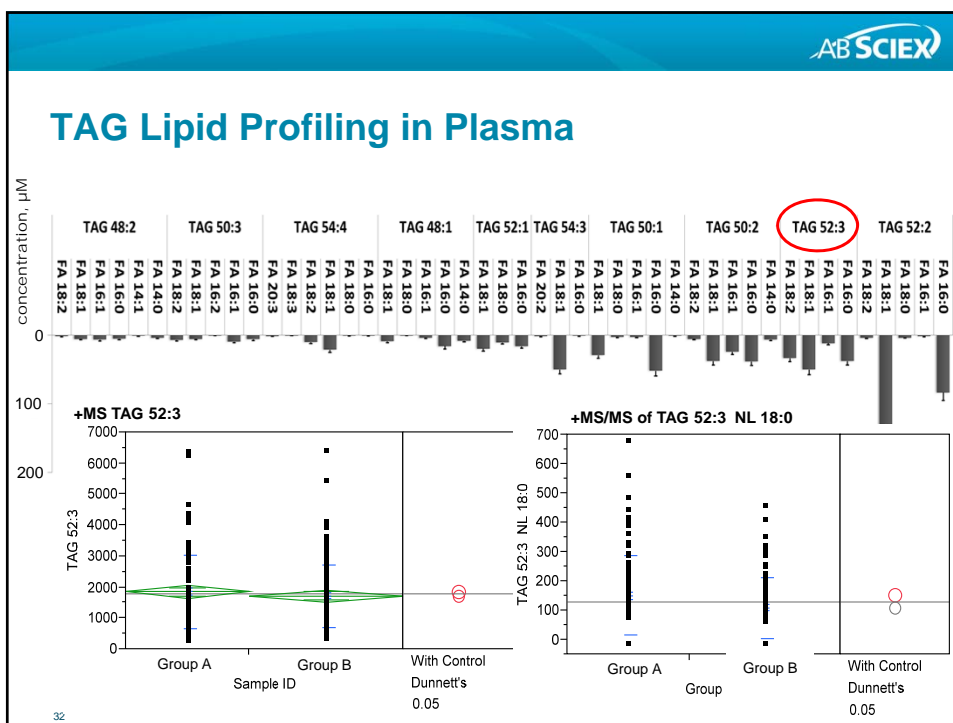
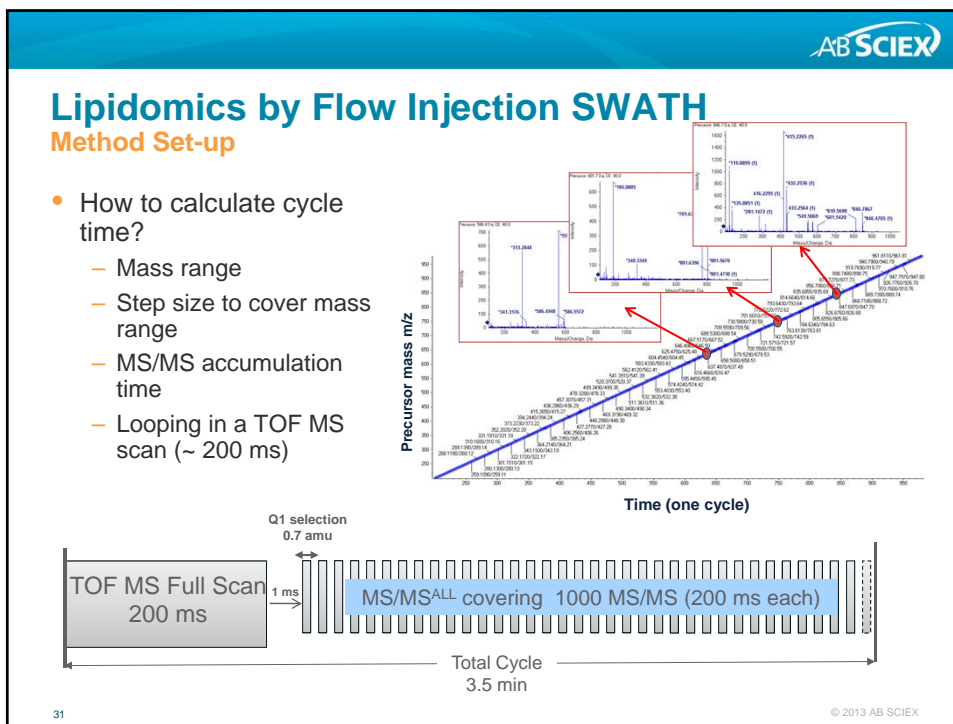
CSF

Plasma

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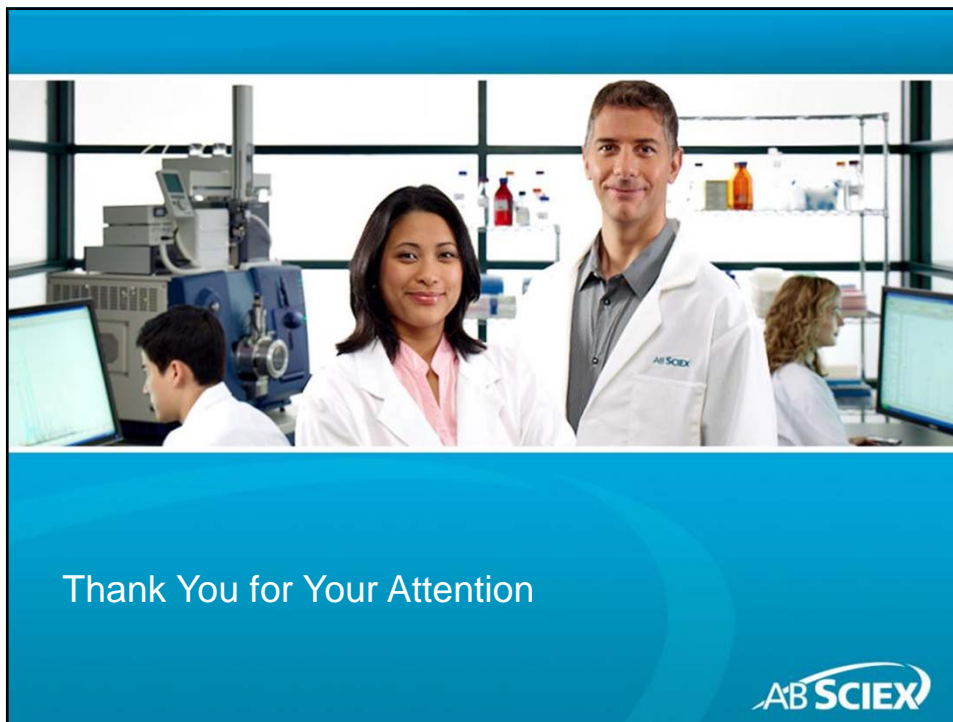


Conclusions

- 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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