SWATH™ Acquisition; Accelerating Quantitative Metabolomics

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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
Pave the Way Forward…
Reducing Data Interpretation Bottlenecks…

Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database
Zheng-Jiang Zhu¹, Andrew W Schultz¹, Jianhua Wang¹, Caroline H Johnson⁵, Steven M Yannoni⁵, Gary J Patti¹,⁵ & Gary Stuezdler¹

Data Acquisition

Processing & Analysis

Metabolite ID

https://xcmsonline.scripps.edu/

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

Lipidomics reveals a remarkable diversity of lipids in human plasma¹

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…

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

Gérard Hopfgartner - David Tondi - Emmanuel Vercesi

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.

Quantitative LC-MS Techniques

Precursors → CID → Fragments

- Triple quadrupole
- QTOFs
- TripleTOF™ 5600+

MRM/SRM → MRM^HR → SWATH-MS
SWATH = Sequential Windowed Acquisition of All Theoretical MS

Q1 Isolation Strategy

Cycle Time
< 1 s

Q1 width is user defined!
e.g. 25 Da

Retention Time

Easy Method Development and Data Mining is Carried Out Retrospectively

24 LCMS maps of fragment ions
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.

Theoretical Human Metabolomes
How Important is Relative Quantitation?

- 200,000 (Lipidome) - Lipids/Lipid derivatives
- 10,000 (Drug metabolome) - Secondary drug metabolites
- 100,000 (Food metabolome) - Secondary food metabolites
- 10,000 (Secondome) - Secondary endogenous metabolites
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

Quantitative

Technical reps
Biological reps

SWATH™ Acquisition

Qualitative

IDA for General Unknown Screening

Metabolite Database

Fast targeted or untargeted XIC generation

SWATH™ XIC Manager
AB SCIEX SWATH Quant/Qual Experimental Workflow

Sample Injection → MS Profiling → Cross-Sample Comparison → Feature Identification → ID Confirmation

- TripleTOF® System
- MarkerView™ Software
- PeakView® Software
- LipidView® Software

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

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

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.
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

- The PCA Scores (top) and Loadings (bottom) plots with Pareto scaling highlight the differences between the urine samples.

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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

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

Arginine TOF MS spectrum
Mass accuracy: 0.1 ppm

Arginine TOF MS/MS spectrum
Purity score 85.3
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
Elemental Composition Assignment of Fragment Ions

TOF MS/MS Spectrum

Mass/Charge, Da

Intensity

Elemental Formula & Structure Search using Metabolite Databases including METLIN
Structural Elucidation in PeakView™ Software
Match N-Acetylgutamic 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**

**SWATH Acquisition:**
- 1 nM Amine-2 MW : 177.1
- High Selectivity with 10X less background noise

**TOF MS:**
- 1 nM Amine-2 MW : 177.1
- High background noise

* 8 scanned points across the peak

**SWATH Acqusition Method Set-up for Lipidomics**

- Cycle time: ~3.3 min
- Q1 mass filter width: = 0.7 Da
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)

TAG Lipid Profiling in Plasma
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\textsuperscript{HR} method, + CE optimized, and targeted data processing for quantitative reporting of the important metabolites

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