MRG: Metabolomics Research Group

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Chris Turck: Max Planck Institute (Chair)

J Biomol Tech. 2015 Sep;26(3):83-9. doi: 10.7171/jbt.15-2603-001.

The ABRF Metabolomics Research Group 2013 Study: Investigation of Spiked Compound Differences in a Human Plasma Matrix.

Cheema AK, Asara JM, Wang Y, Neubert TA, Tolstikov V, Turck CW

Abstract

Metabolomics is an emerging field that involves qualitative and quantitative measurements of small molecule metabolites in a biological system. These measurements can be useful for developing biomarkers for diagnosis, prognosis, or predicting response to therapy. Currently, a wide variety of metabolomics approaches, including nontargeted and targeted profiling, are used across laboratories on a routine basis. A diverse set of analytical platforms, such as NMR, gas chromatography-mass spectrometry, Orbitrap mass spectrometry, and time-of-flight-mass spectrometry, which use various chromatographic and ionization techniques, are used for resolution, detection, identification, and quantitation of metabolites from various biological matrices. However, few attempts have been made to standardize experimental methodologies or comparative analyses across different laboratories. The Metabolomics Research Group of the Association of Biomolecular Resource Facilities organized a "round-robin" experiment type of interlaboratory study, wherein human plasma samples were spiked with different amounts of metabolite standards in 2 groups of biologic samples (A and B). The goal was a study that resembles a typical metabolomics analysis. Here, we report our efforts and discuss challenges that create bottlenecks for the field. Finally, we discuss benchmarks that could be used by laboratories to compare their methodologies.

Metabolomics is Concerned with the Simultaneous, Comprehensive Measurements of Small Molecules

Metabolomics is the comparative analysis of endogenous metabolites found in biological samples:

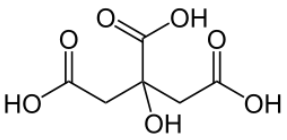
- ***Compare*** two or more biological groups
- Find and identify potential biomarkers
- Look for biomarkers of toxicology
- Understand biological pathways
- Discover new metabolites

Metabolites are the by-products of metabolism

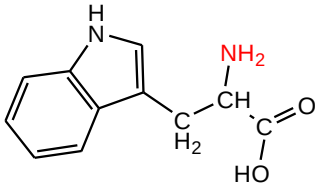
- Range of physico-chemical properties
- Classes: Amino acids, Sugars, organic acids, fatty acids, lipids…

What are the chemical differences that result in the observable difference

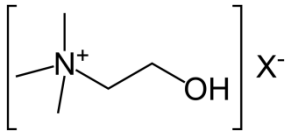
Chemical Diversity of the Metabolome



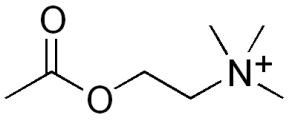
Citric acid



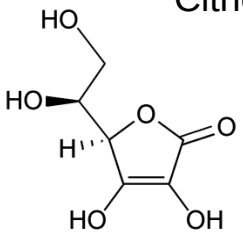
Tryptophan



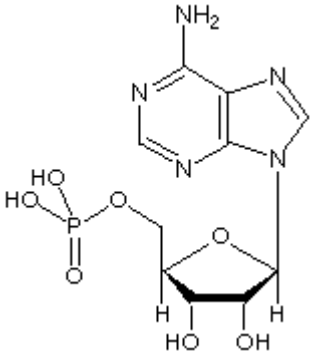
Choline



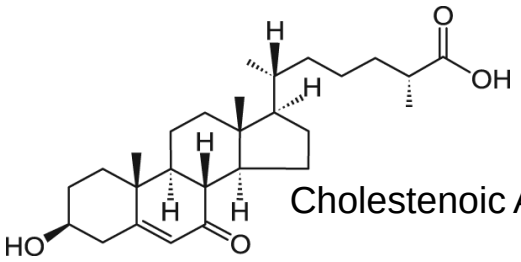
Acetylcholine



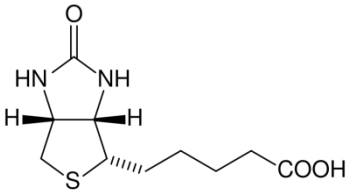
Ascorbic Acid
(Vitamin C)



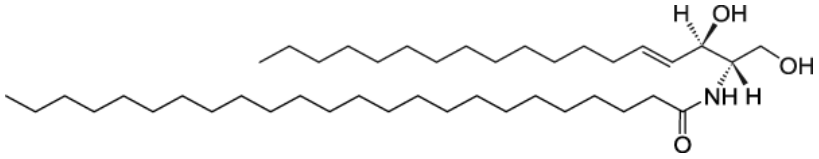
AMP



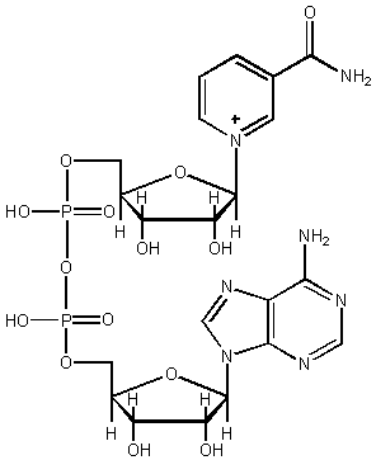
Cholestenoic Acid



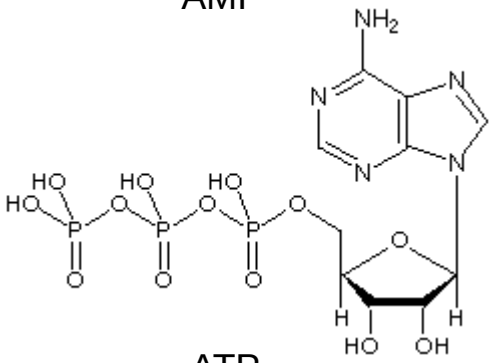
Biotin
(Vitamin B7)



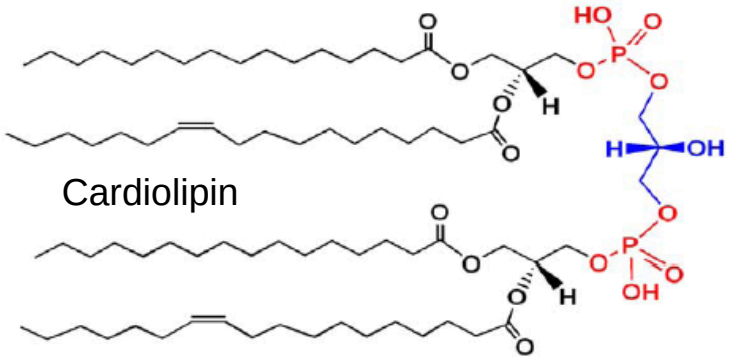
Ceramide



NAD



ATP



Cardiolipin

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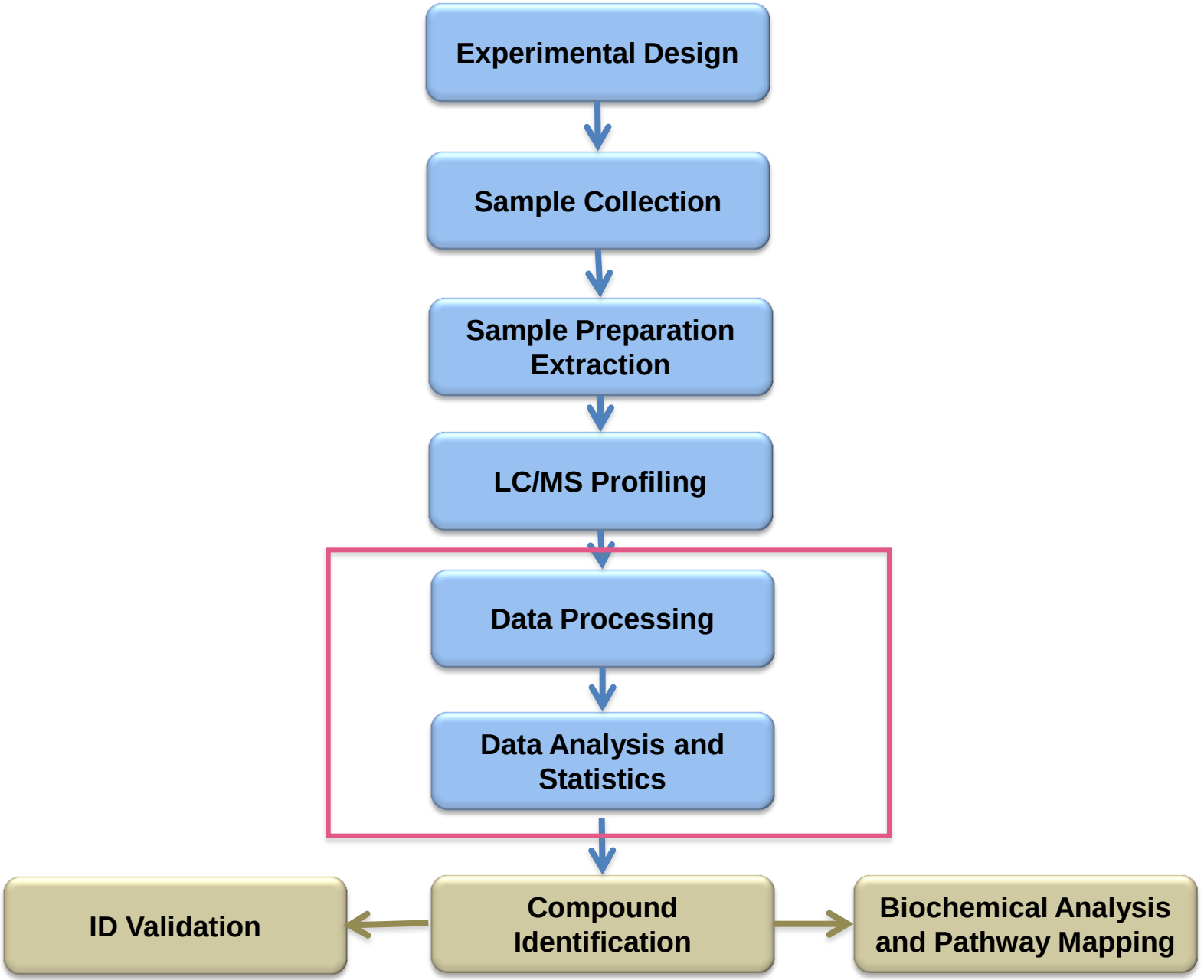
Untargeted Metabolomics Workflow



Current Challenges in Untargeted Metabolomics

- Sample Preparation
- Computational Approaches
- Compound Identification

Sample Preparation

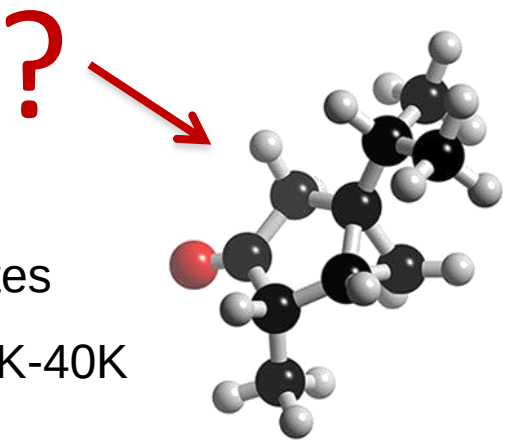
- Not a uniform approach
- Each approach needs to be validated across multiple studies
- Variable preparation approaches can enhance extraction of certain groups of metabolites
- Missed metabolites
  - Metabolite unstable
  - Sample loss

Multiple Computation Approaches for Metabolomics Data Analyses

- **Pre-processing Software**
  - Progenesis
  - XCMS
  - mzmine
  - Vendor specific software
- **Computational Approaches**
  - Online tools: Metaboanalyst; Mummichog
  - In house scripts: R-based; Matlab
  - SAS; SPSS

Untargeted Metabolomics Compound Identification Challenges

No linear "blueprint"



Playing field is ill-defined

Most metabolites uncharacterized

Limited availability of pure standards for metabolites

Custom synthesis of unavailable metabolites from $1K-40K

How many metabolites are we looking for ?

MS/MS Fragmentation patterns

Limited "library" (MS/MS) characterization

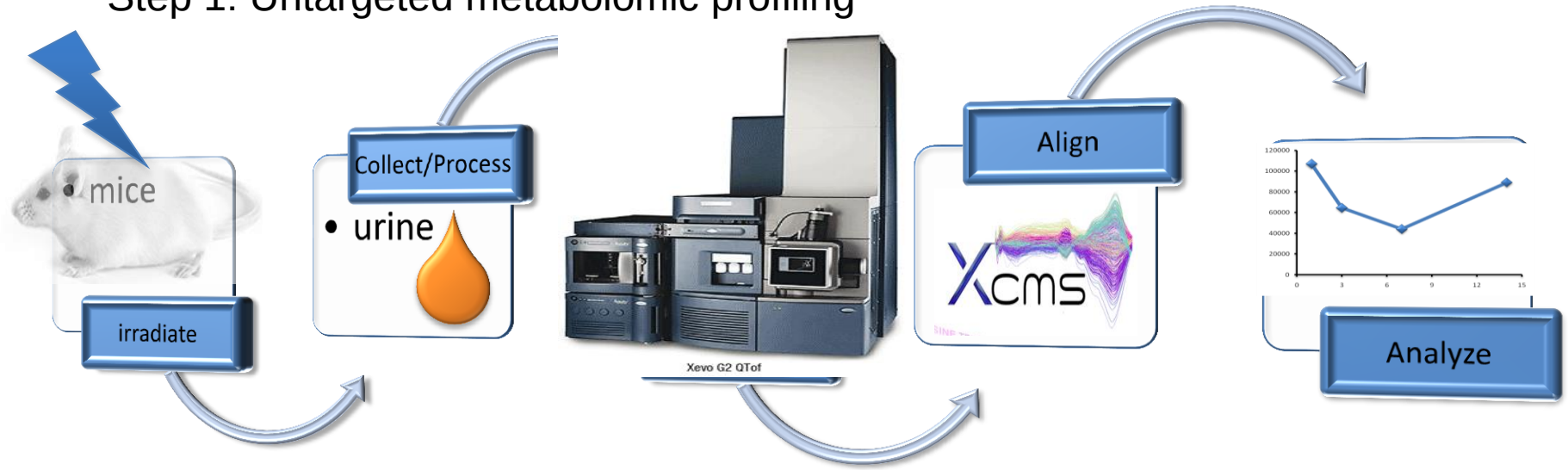
Libraries not centralized

Not predictable (as with peptides)

MSMS reference only for a single mode or adduct

MRG 2016 Study Outline

Step 1: Untargeted metabolomic profiling



Step 2: Pre-process XCMS and upload to ABRF web site

https://bioshare.bioinformatics.ucdavis.edu/bioshare/view/tthvputgo2am4yo/

Step 3: Data analysis and reporting by participant

MRG2016_Submission_Template.xlsx

https://bioshare.bioinformatics.ucdavis.edu/bioshare/download/tthvputgo2am4yo/MRG_2016_Submission_Template.xlsx

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MRG Inter-Laboratory Metabolomics Study 2016

- Design a study that resembles an untargeted metabolomics profiling experiment comparing biological changes under different conditions (exposure to Sham vs 5 Gy)

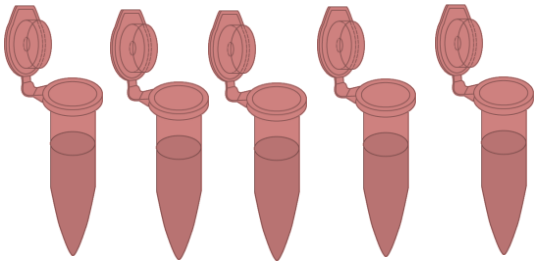
- Participants will identify statistically significant differences between groups A & B of samples in order to compare findings with varied methodologies

- Goal:
  Examine challenges, overlap and variability in results between approaches to LC-MS metabolomics data analysis

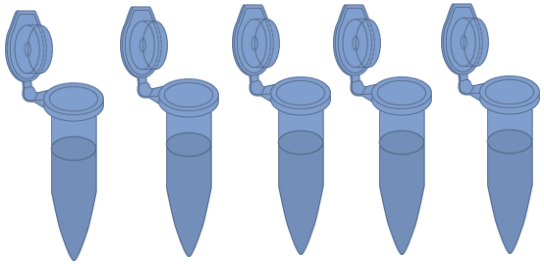
Sample Preparation

- Aliquots of urine sample were processed with extraction solution for 5 biological replicates for group A and B

Group A: Sham/0 Gy



Group B: 5 Gy



- Extraction buffer composed of (30% acetonitrile + 40% methanol + 30% water) spiked with internal standards

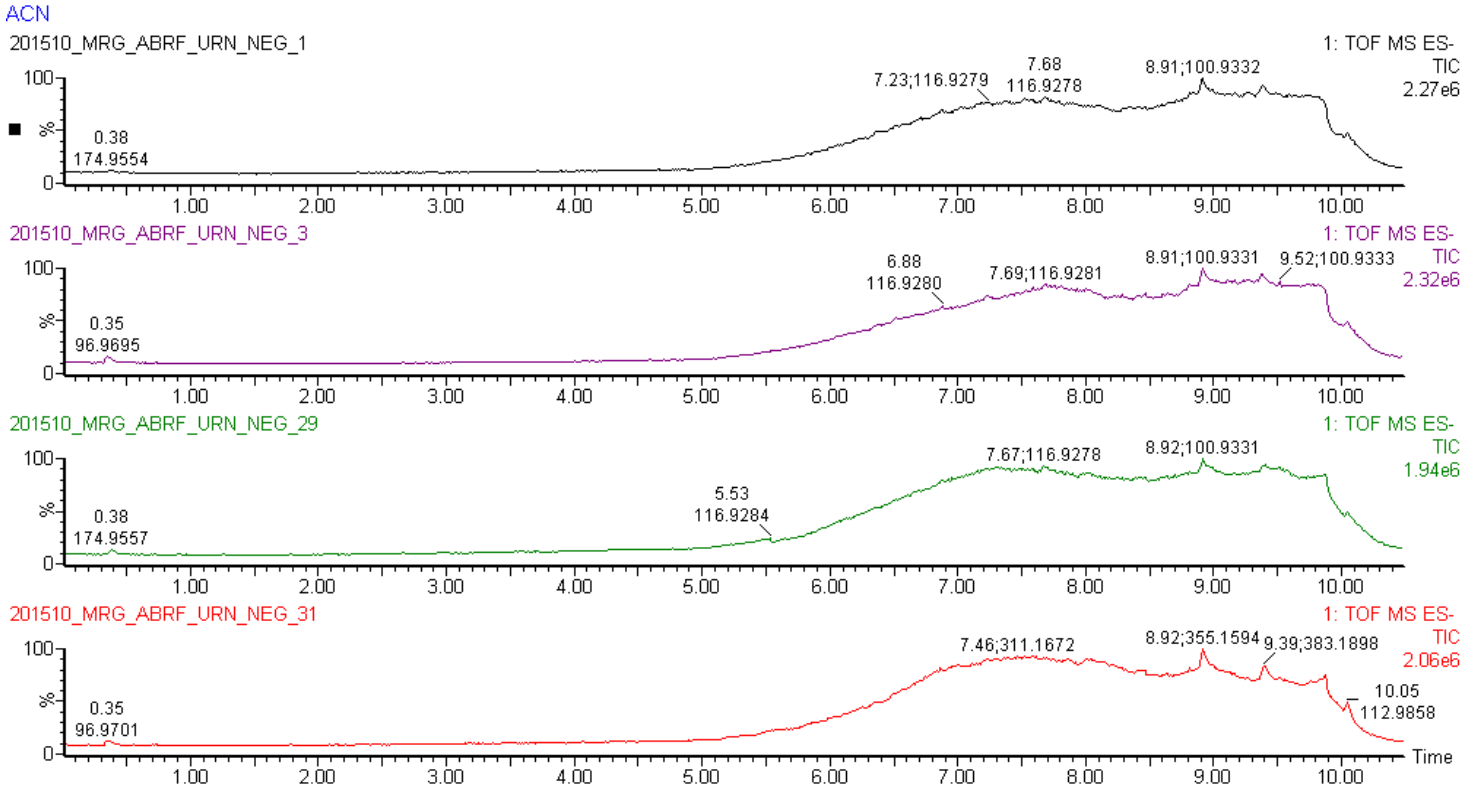
- Internal standards:
  - 4-nitrobenzoic acid ($O_2NC_6H_4CO_2H$; MW: 167.12), m/z 166.0141 $(M-H)^-$
  - debrisoquine ($C_{10}H_{13}N_3$; MW: 175.23), m/z 176.1187 $(M+H)^+$

LC-MS Experiment

- Platform: Waters Xevo G2 QTOF-MS with Acquity H UPLC
- All 10 samples were run with duplicate technical replicate
- Three pooled QC injections (one every 10 injections)
- Both ESI Positive and Negative
- Binary gradient: Water +0.1% Formic Acid/ Acetonitrile +0.1% Formic Acid
- Column: Acquity UPLC BEH C18 1.7um 2.1 x 50mm at 40C

Quality Control

- Blanks examine carryover throughout run



MS Quality Control

Small Molecule Standard Cocktail:
Pre- and Post- MS Analysis Ensure Mass Accuracy <5ppm

Component	Empirical Formula	Exact mass [M+H]+	Exact mass [M-H]-
Acetaminophen	C8H9NO2	152.0712	150.0555
Sulfaguanidine	C7H10N4O2S	215.0603	213.0446
Sulfadimethoxine	C14H14N4O4S	311.0814	309.0658
Val-Tyr-Val	C19H29N3O5	380.2185	378.2029
Terfenadine	C32H41NO2	472.3216	470.3059
Leucine enkephalin	C28H37N5O7	556.2771	554.2615

Positive Mode

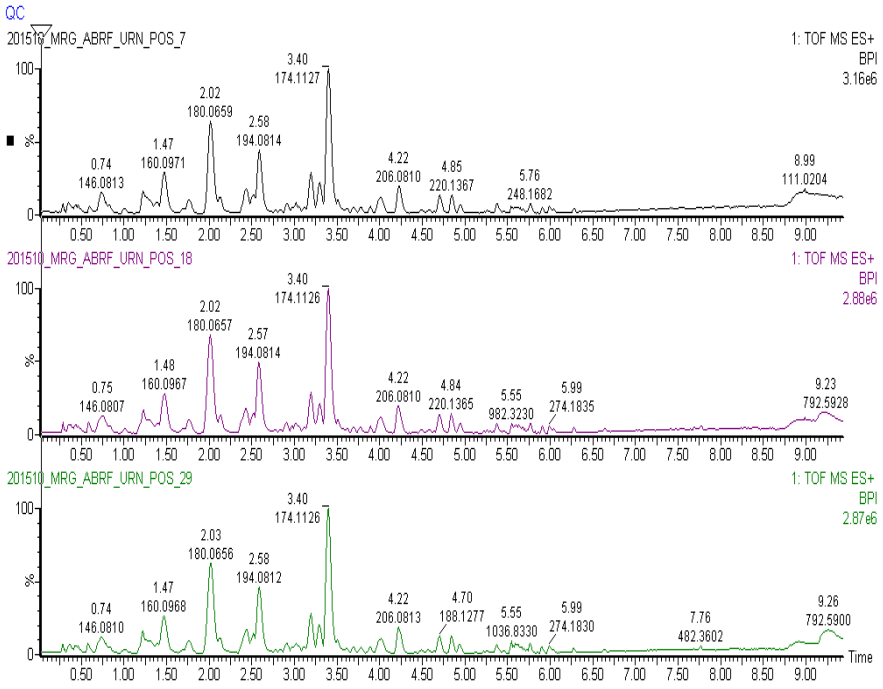
Initial				Final		
Expected m/z	Actual m/z	Mass Error (ppm)		Expected m/z	Actual m/z	Mass Error (ppm)
152.0712	152.0709	2.0		152.0712	152.0708	2.6
215.0603	215.0603	0.0		215.0603	215.0599	1.9
311.0812	311.0814	-0.6		311.0812	311.0803	2.9
380.2185	380.218	1.3		380.2185	380.2172	3.4
472.3216	472.3216	0.0		472.3216	472.3207	1.9
556.2771	556.2767	0.7		556.2771	556.2776	-0.9

Negative Mode

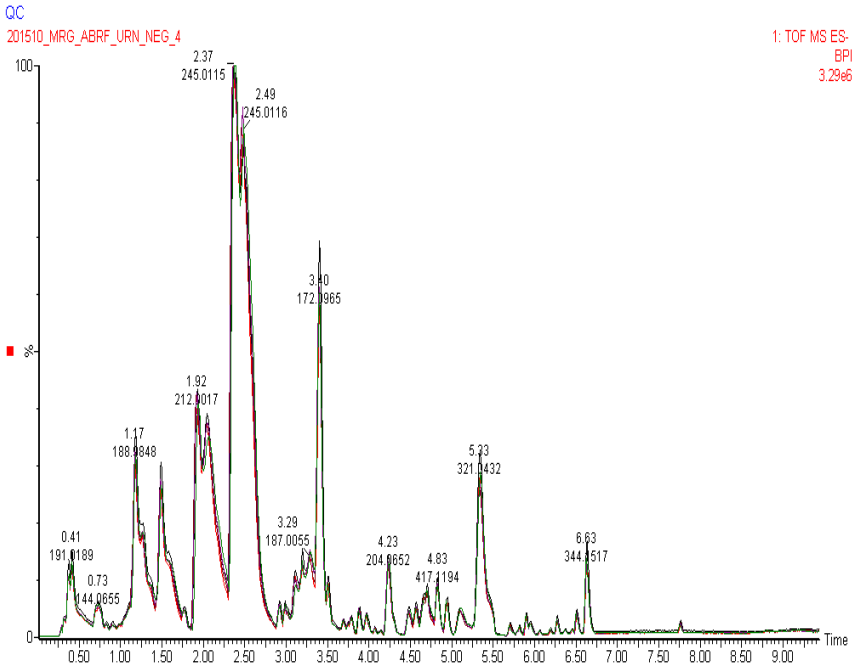
Initial				Final		
Expected m/z	Actual m/z	Mass Error (ppm)		Expected m/z	Actual m/z	Mass Error (ppm)
213.0446	213.0440	2.8		213.0446	213.0443	1.4
309.0658	309.0659	-0.3		309.0658	309.0668	-3.2
378.2029	378.2028	0.3		378.2029	378.2031	-0.5
554.2615	554.2614	0.2		554.2615	554.261	0.9

Quality Control

Base Peak Intensity for pooled QC injections compared to examine response throughout the entire experiment



Overlays utilized to examine reproducibility and consistency



Data Processing

- RAW files converted to netcdf
- Peak picking and alignment performed using XCMS
- Data uploaded to and accessible through Bioshare FTP *bioshare.bioinformatics.ucdavis.edu/bioshare/view/tthvputgo2am4yo/:*
  - ❖ Masslynx RAW data files
  - ❖ netcdf files
  - ❖ XCMS-based preprocessed csv

Validation and Characterization

- Additional rounds of compound vetting and validation upon completion of reporting
- Compound validation with commercially available standards
- MS/MS libraries, and predicted fragmentation (e.g. Mass Fragment)

Result Reporting

- Finding should be reported using the ***MRG2016_Submission_Template.xlsx*** (accessible from the FTP site https://bioshare.bioinformatics.ucdavis.edu/bioshare/download/tthvputgo2am4yo/MRG_2016_Submission_Template.xlsx)
- Additional procedures outlined in report ***ABRF-MRG2016 Metabolomics Research Group Data Analysis Study***
- MS Analysis should include:
  - m/z, RT, ion mode of each compound (mass spectrometry)
  - Molecular formula (or multiple formulas if ambiguous)
  - Fold-change
  - Statistical metrics for difference detection
  - Putative identity of the compound (based on accurate mass)

ABRF-MRG Survey

•Participant need to complete the brief online survey accessible through:

https://www.surveymonkey.com/r/abrfmet2016

•Please provide a detailed description of your methodology in the appropriate textboxes.

•MRG requests that each participant prepare a short write-up that summarizes the approach that was taken, the methods that were used, and the key findings that were obtained.

•These anonymous write-ups will be posted online and linked to each participant's results.

•Please e-mail your anonymous write-up as a pdf file along with to completed ***MRG2016_Submission_Template.xlsx*** to:

anonymousmrg2016@gmail.com

•The file name should include the **5-digit code** that you entered at the beginning of the online survey.

Expected Outcomes

The primary objective of this study is to examine reproducibility and optimal data analysis strategies for metabolomics studies:

- Compare the relative quantitative metabolite differences across two sample types reported by participants
- Examine effects of different computational techniques on the determination of significantly altered metabolites in the two groups.
- Assess the level of confidence and consistency in the results obtained from unique computational and chemometric approaches.
- Compare ability of software to determine differences across samples or help analyze data from metabolomics experiments
- Compare databases used for assigning metabolite ID

Acknowledgements

- **MRG members:**
  Amrita, Allis, Maryam, Tytus, Andrew

- **ABRF**
  Susan DeCourcey, Jonathan Korman, Brett Phinney

- **Cheema Lab: Tyrone Dowdy**

QUESTIONS???

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