Pacific Northwest
NATIONAL LABORATORY
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Automated Solid Phase Extractions Coupled with Ion Mobility-Mass Spectrometry for Rapid Metabolomic Screening

Erin Shammel Baker
Xing Zhang, Xueyun Zheng, Kristin E. Burnum-Johnson, Jennifer E. Kyle, Young-Mo Kim, Erika M. Zink, Matthew E. Monroe, Yehia M. Ibrahim, Daniel J. Orton, Justin G. Teeguarden, Thomas O. Metz, and Richard D. Smith

Pacific Northwest National Laboratory

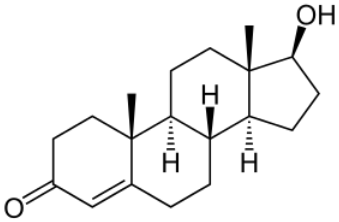
Main challenges with metabolomic measurements

1. Metabolites of interest occur from very low to high concentrations (fM-mM) so measurements with high dynamic range and sensitivity are essential

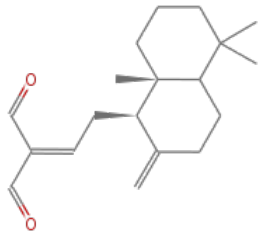
2. Biological changes are best understood when both endogenous and exogenous metabolites are analyzed

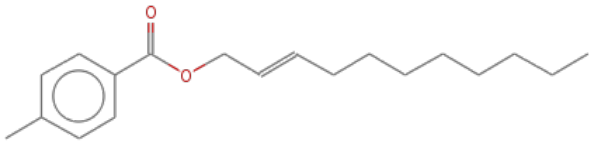
3. Untargeted measurements covering thousands of small molecules are desired to perform time course studies and analyze large cohorts

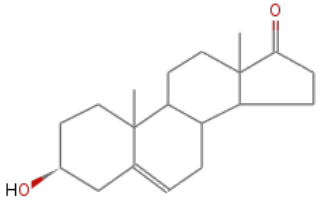
4. Many small molecules have the same masses but a different chemical makeup so distinguishing them with MS-based approaches can be difficult or impossible



Testosterone
Exact mass = 288.2089188

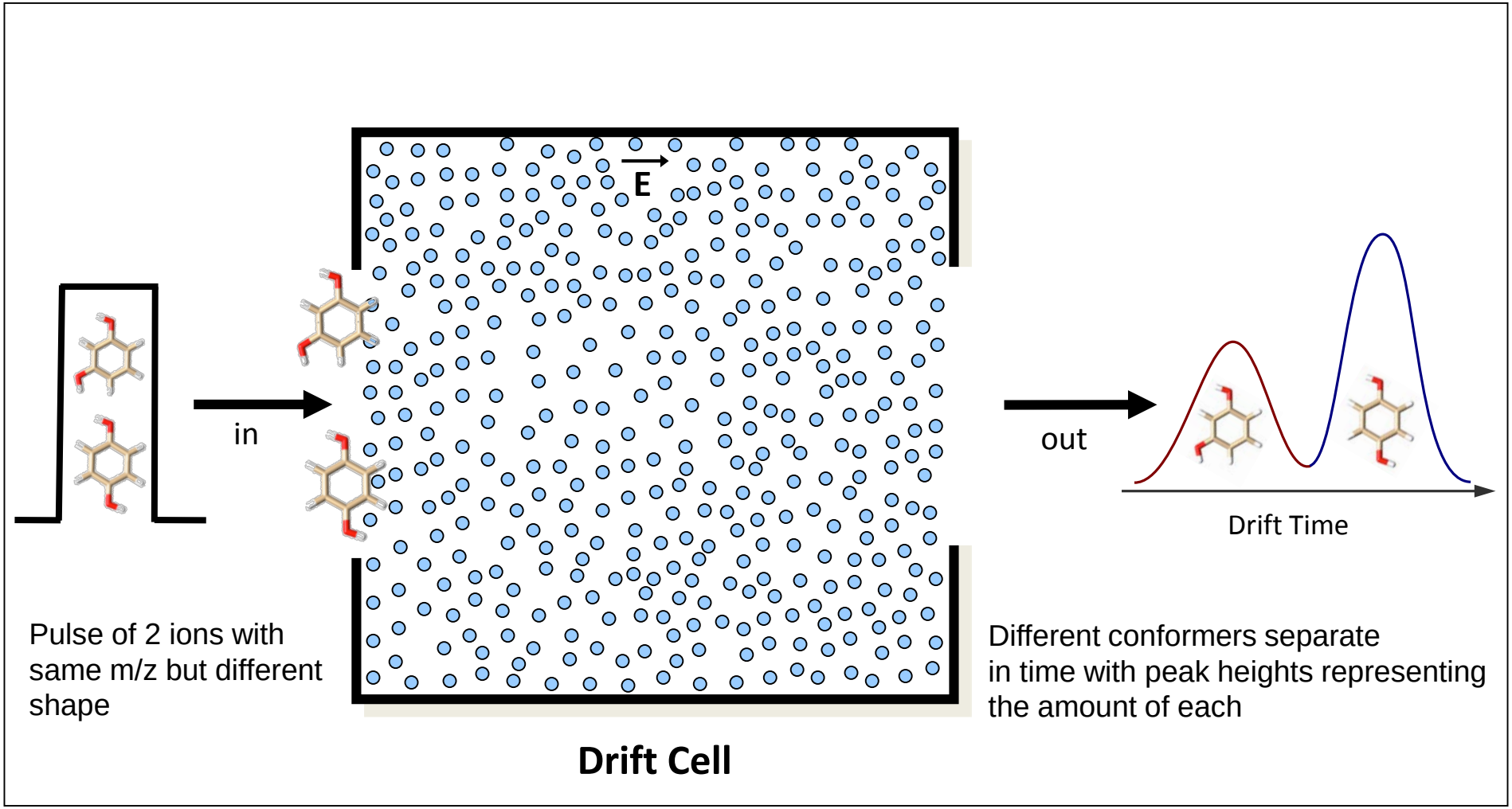






In the NIST database there are
18 different options with exact mass = 288.2089188

Ion mobility concept

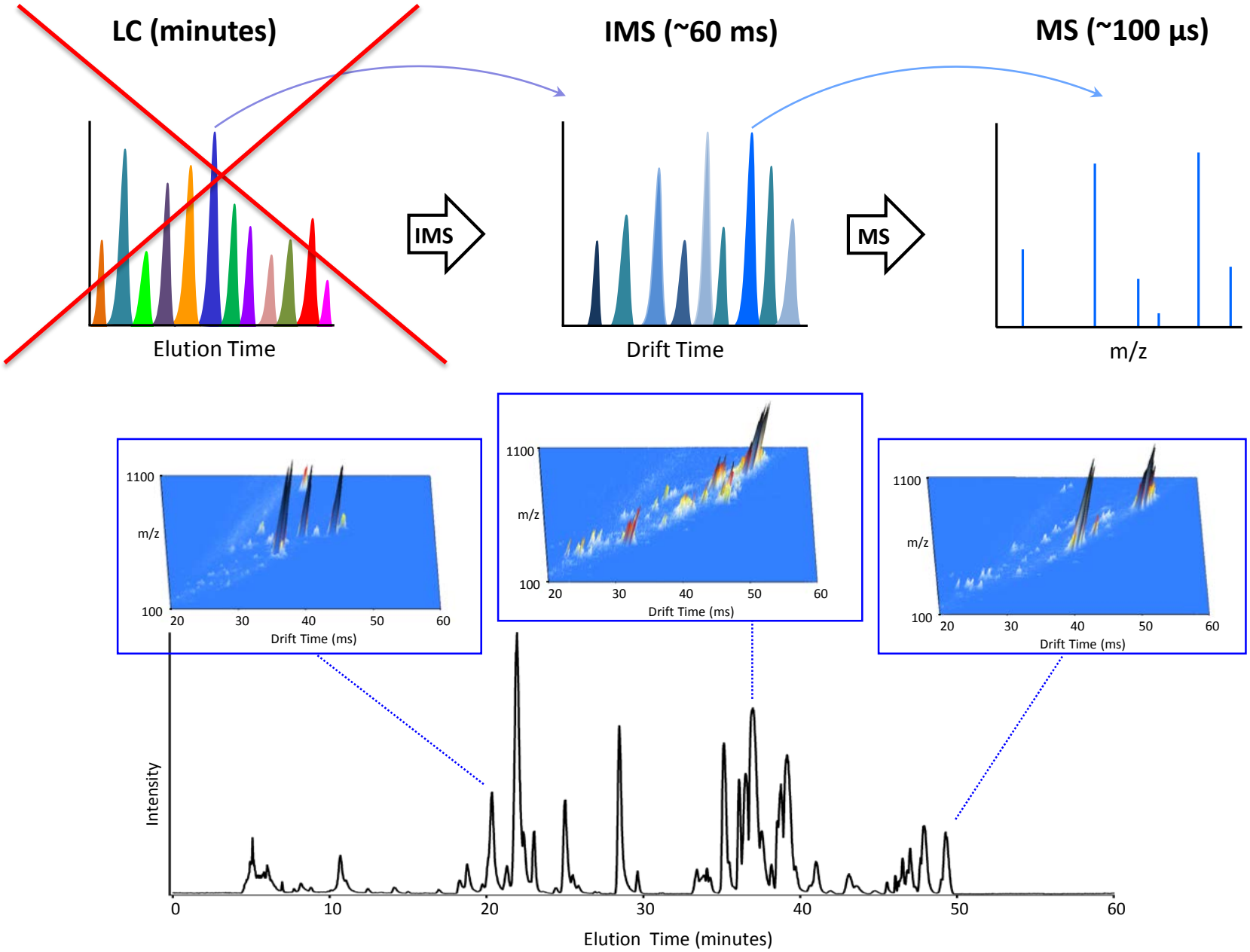


velocity is constant

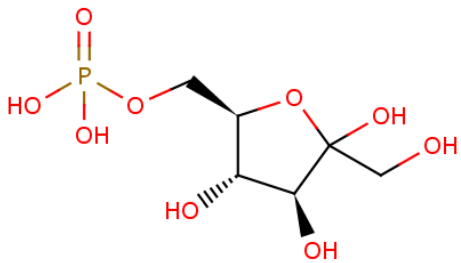
$$v = K \vec{E}$$

K = ion mobility

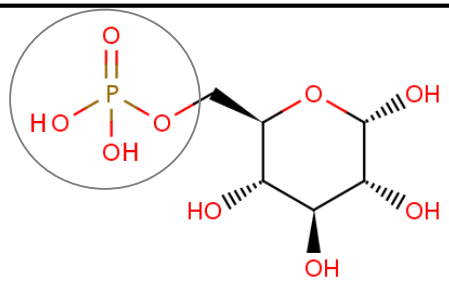
Ion mobility concept



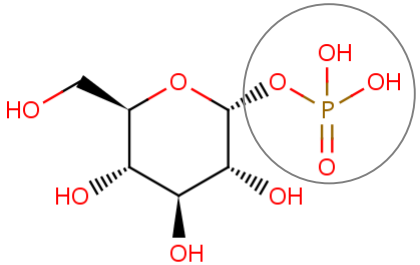
Isomer separations difficult with hydrophobic interaction liquid chromatography (HILIC)



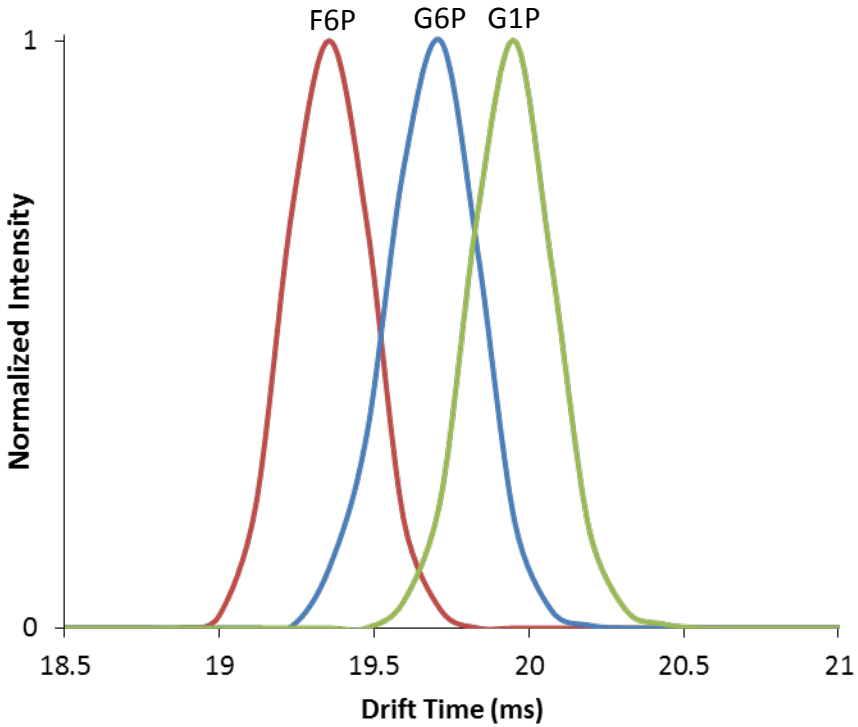
D-Fructose-6-phosphate (F6P)

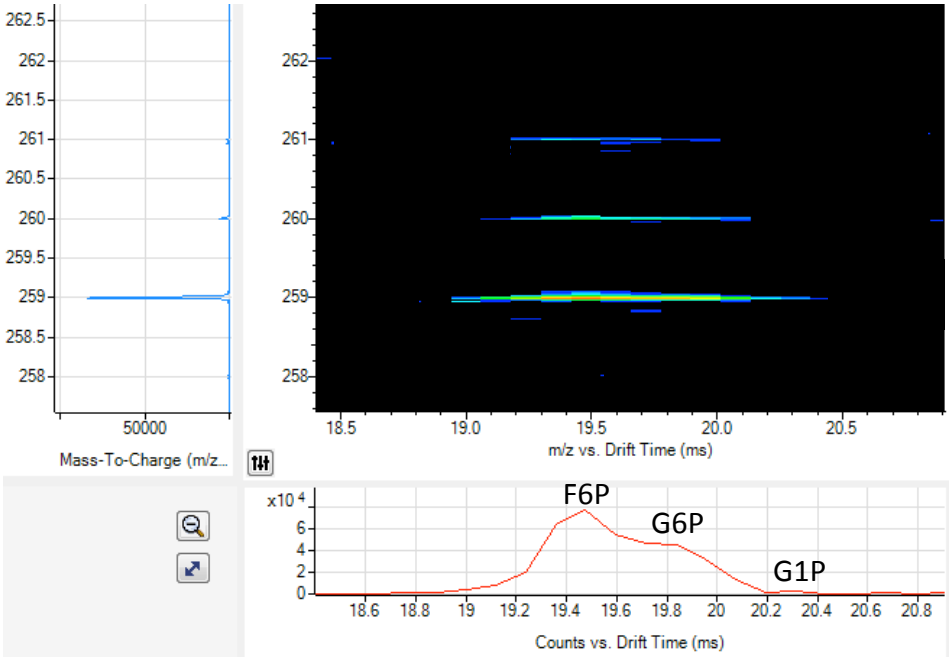


D-Glucose-6-phosphate (G6P)



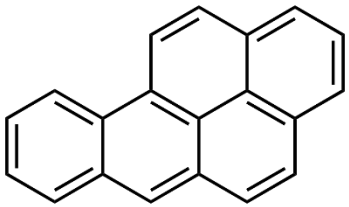
α-D-Glucose-1-phosphate (G1P)



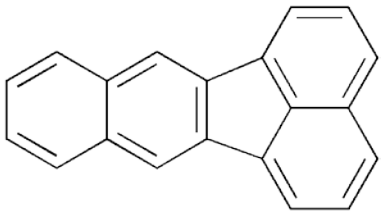


Deprotonated form $[M - H]^-$ m/z = 259.02

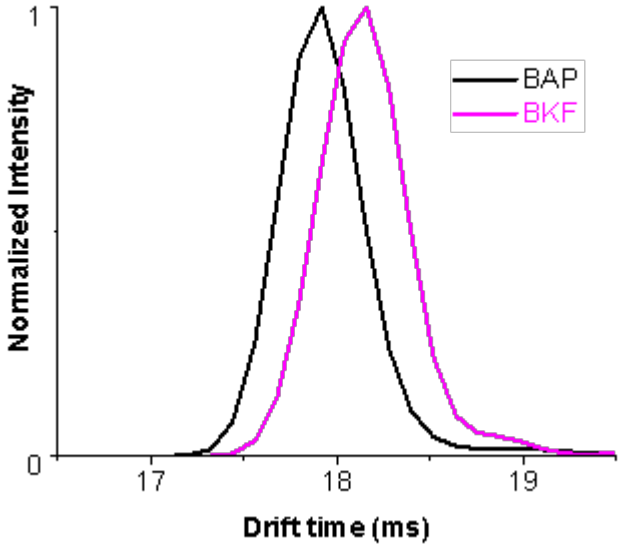
Isomeric exogenous metabolite separations with APCI



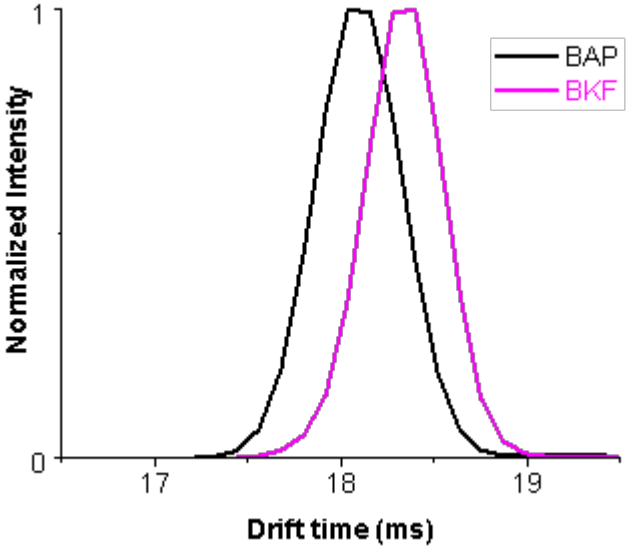
BAP:
Benzo(a)Pyrene



BKF:
Benzo(k)fluoranthene

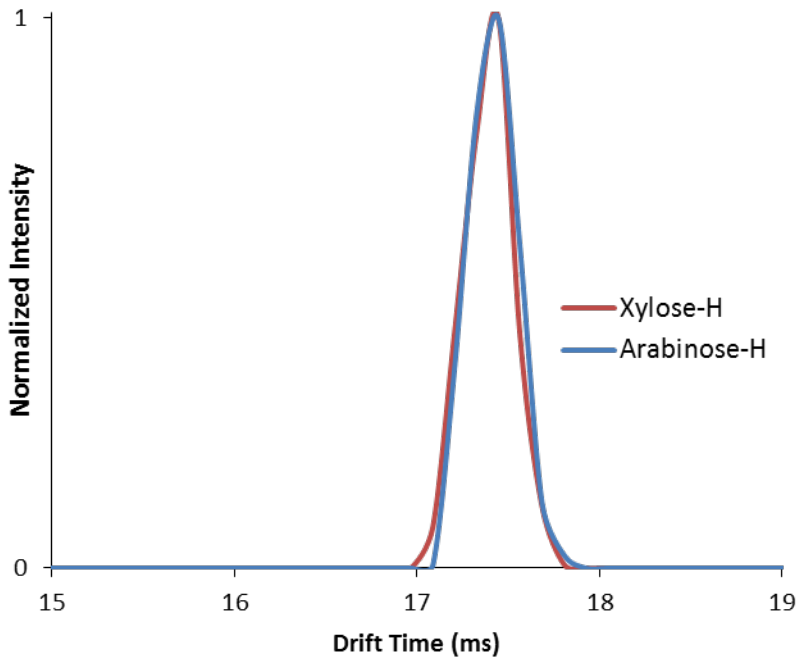


Radical form $[M]^{\cdot+}$ m/z = 252.09

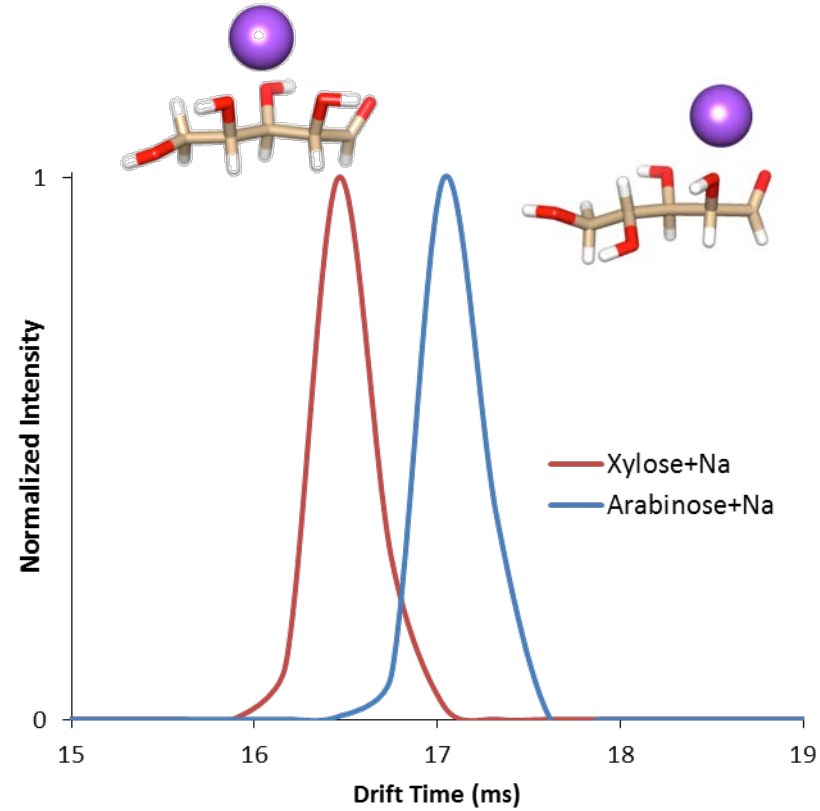


Protonated form $[M + H]^+$ m/z = 253.10

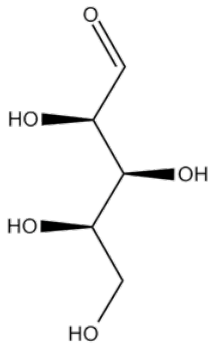
(Sugar+Na)$^+$ isomer separations with IMS



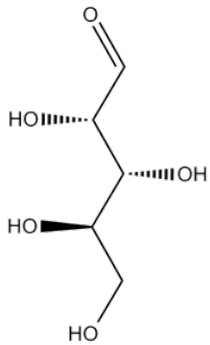
Deprotonated form $[M - H]^-$ m/z = 149.04



Sodiated form $[M + Na]^+$ m/z = 173.04



Xylose

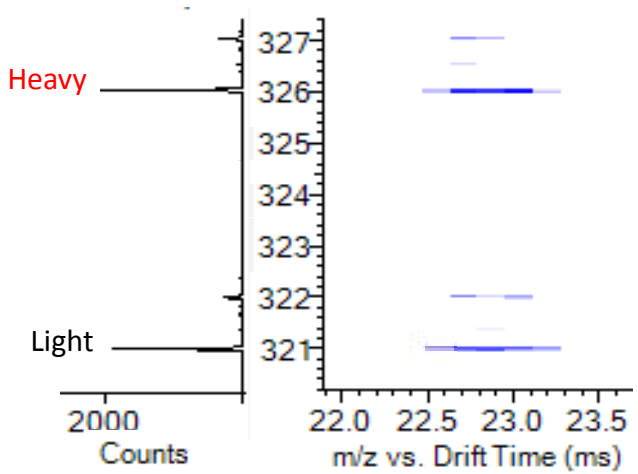


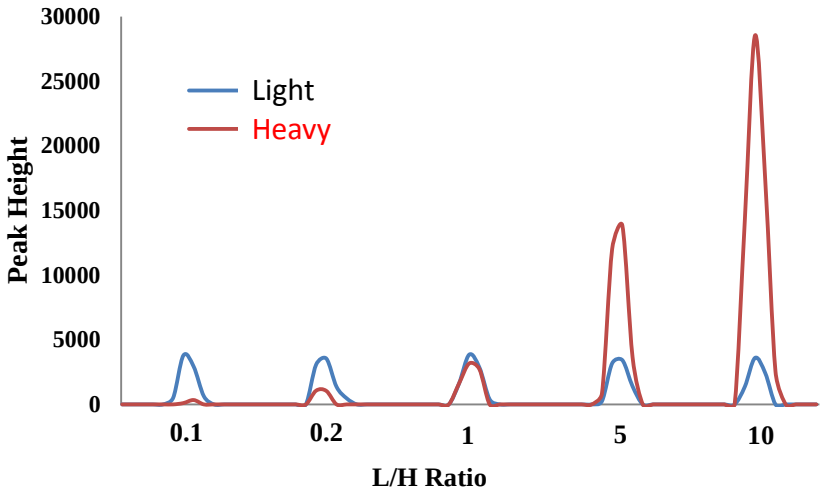
Arabinose

Richard D. Smith TOD pm 04:10
Ultrahigh IMS Resolution using Novel Traveling Wave Approaches & Long Serpentine Path Length SLIM Modules

Isotope Labeling

Chloramphenicol

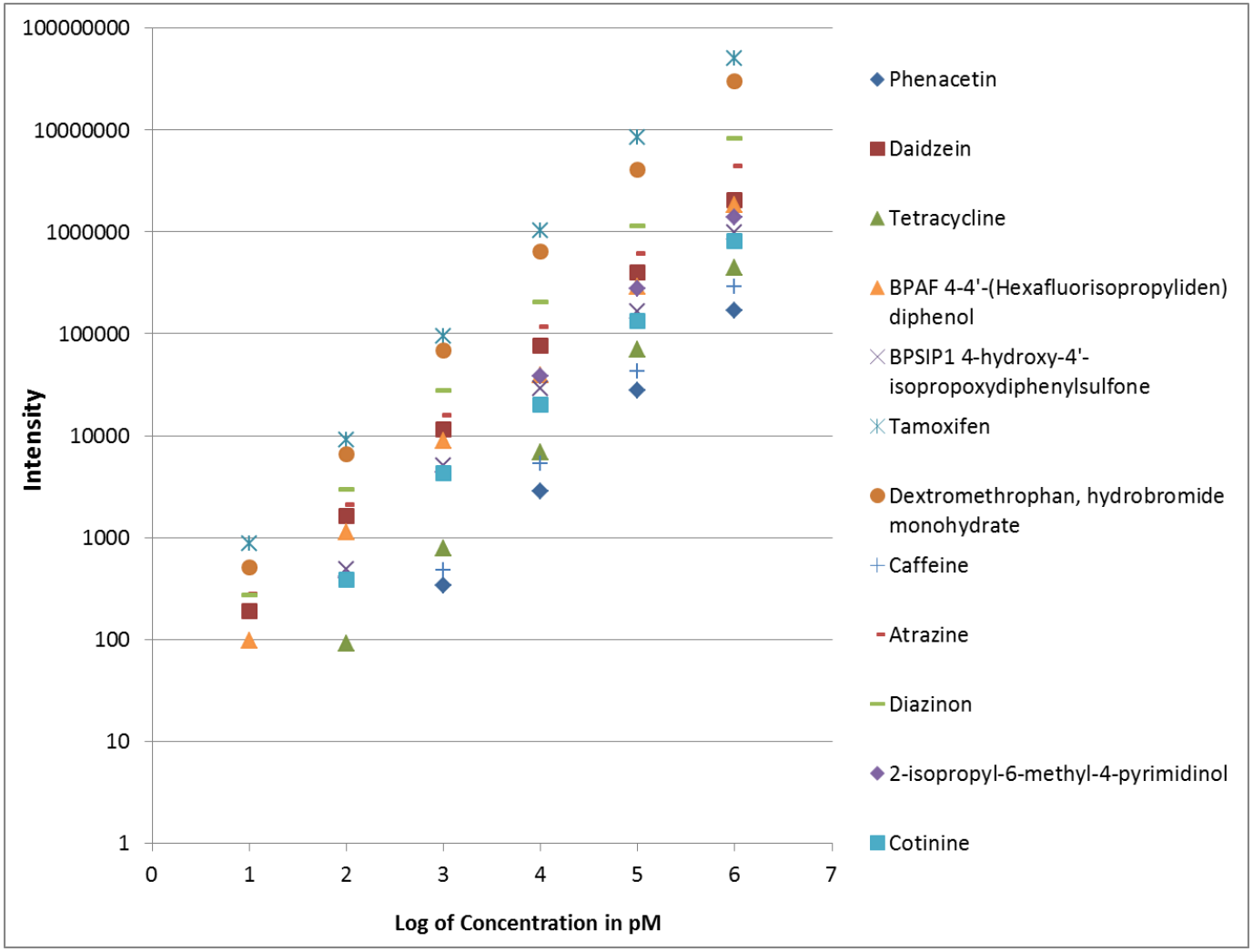




Light - unlabeled
Heavy - Labeled with 5 deuteriums

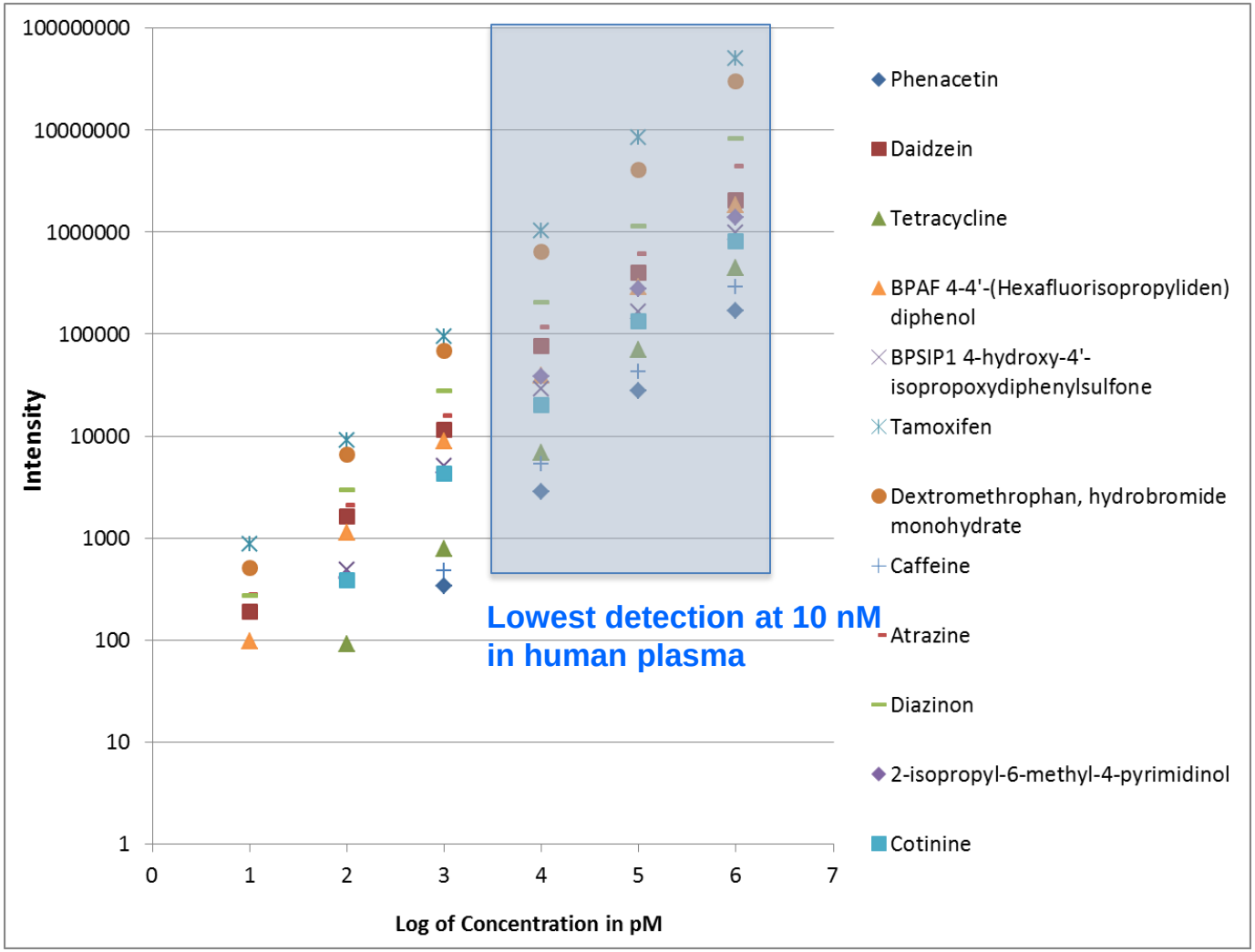
Light and heavy labelled metabolites have the same drift time

Present limit of detection in water using IMS-MS



Lowest detection at 10 pM for standards in water
Linear concentration response

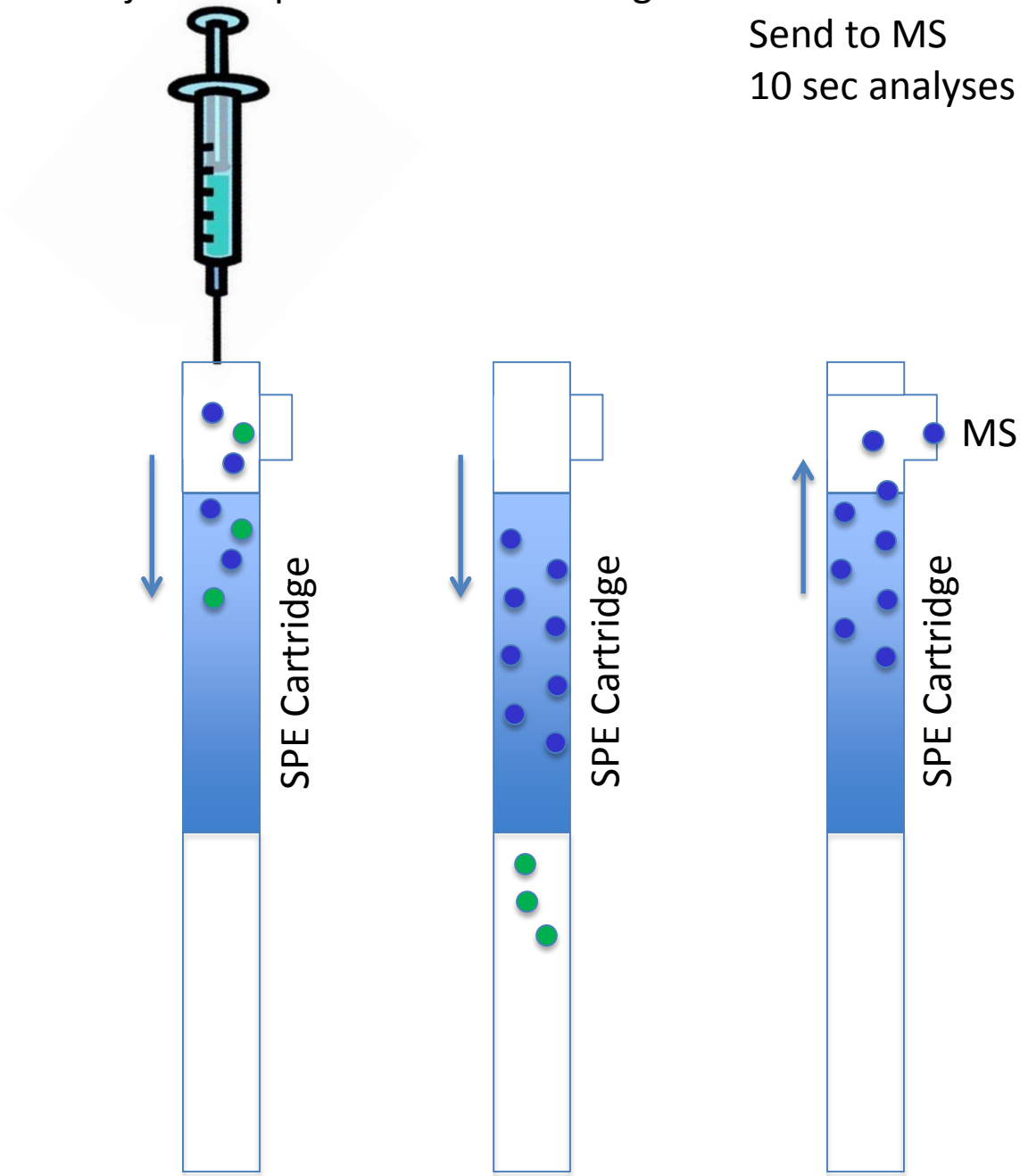
Present limit of detection in human plasma with IMS-MS



Lowest detection at 10 pM for standards in water
Linear concentration response

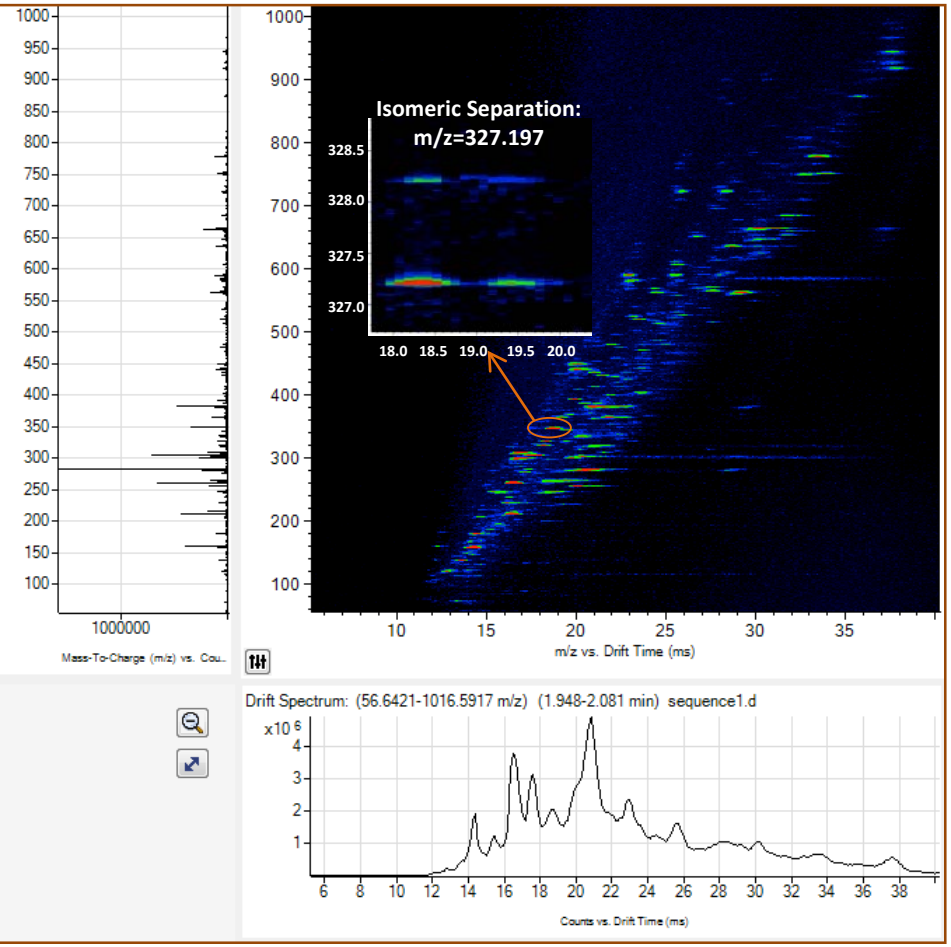
RapidFire SPE System

1. Inject Sample
2. Wash Cartridge
3. Reverse Flow
   Send to MS
   10 sec analyses

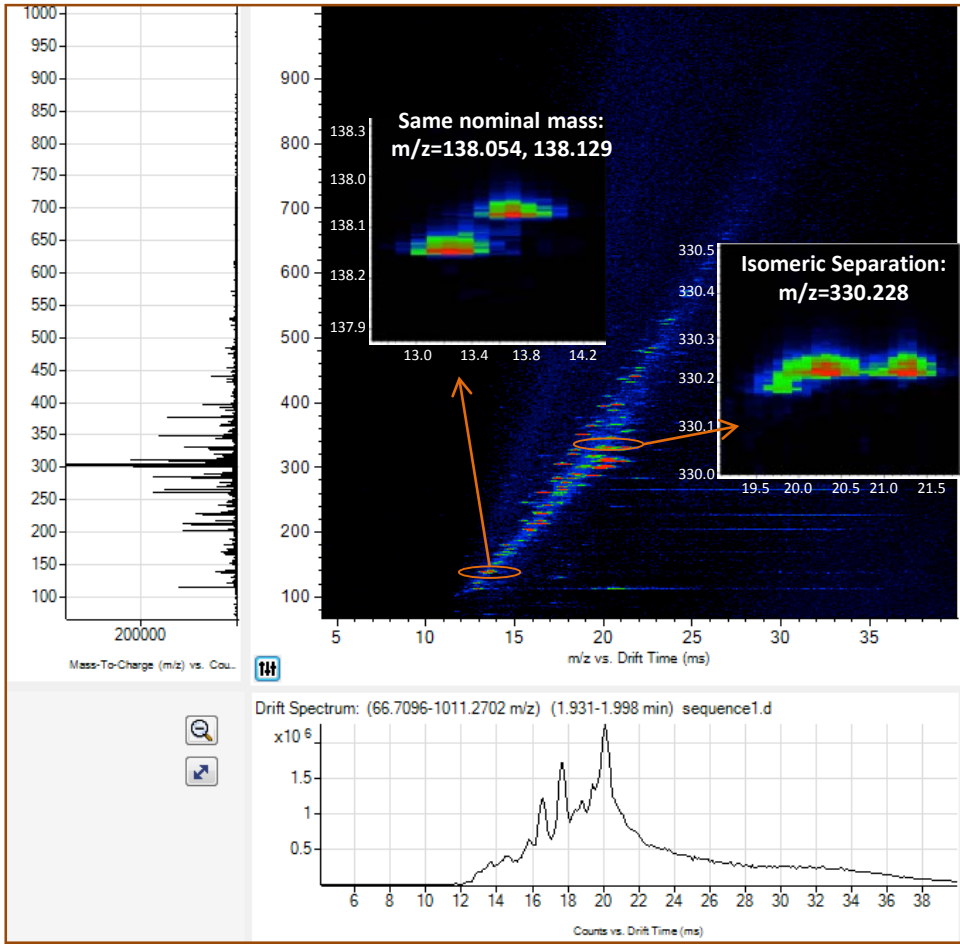


SPE-IMS-MS analyses of biological samples

Metabolites extracted from mouse plasma



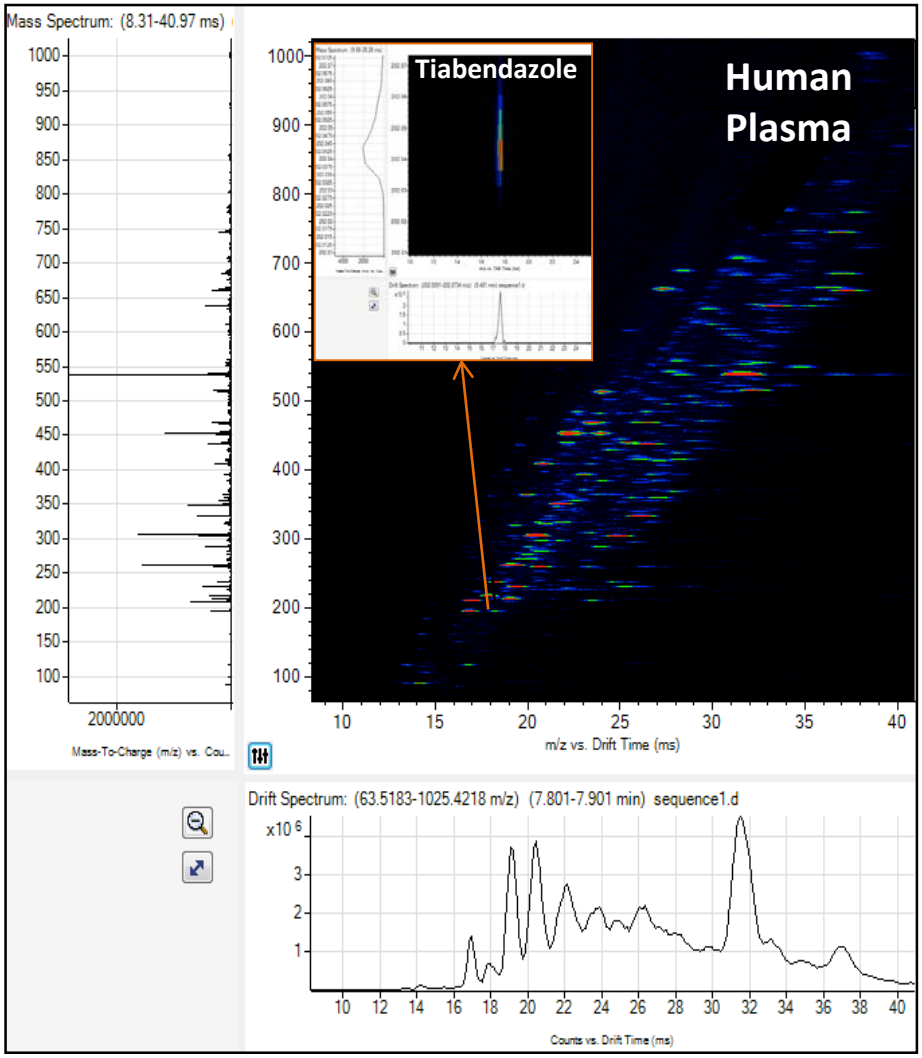
Metabolites extracted from human urine

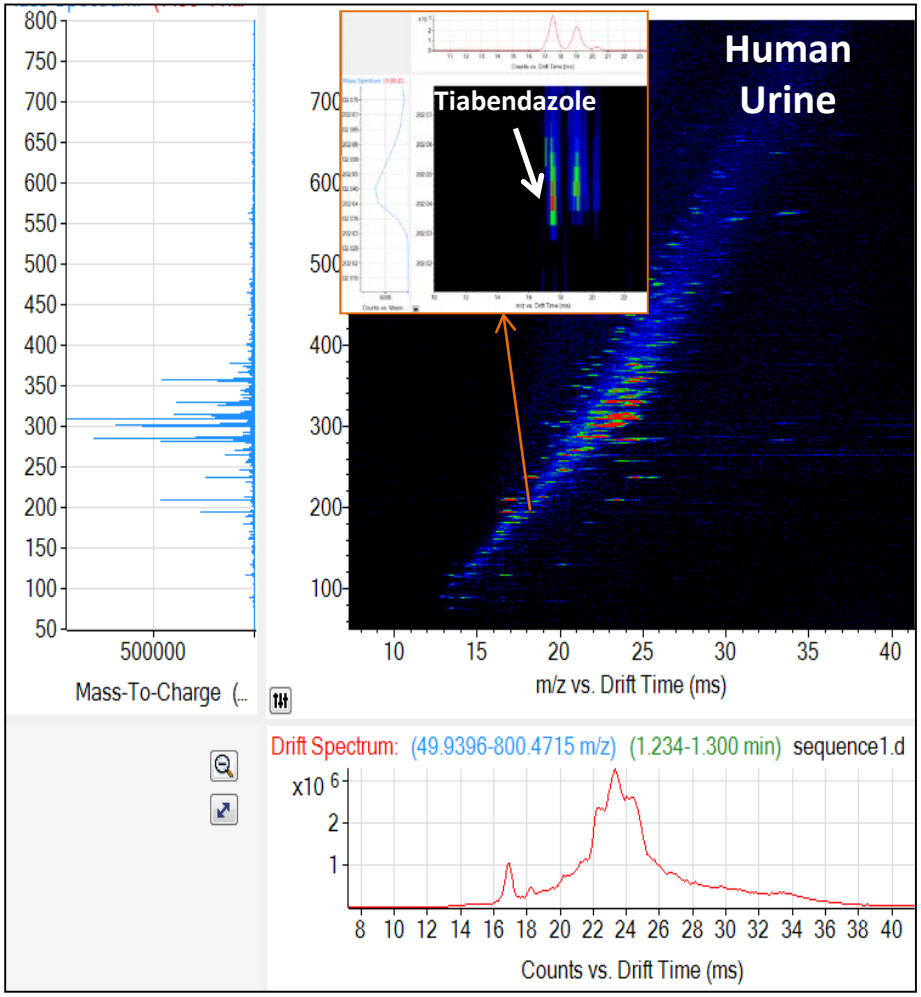


~1400 features with S/N > 5

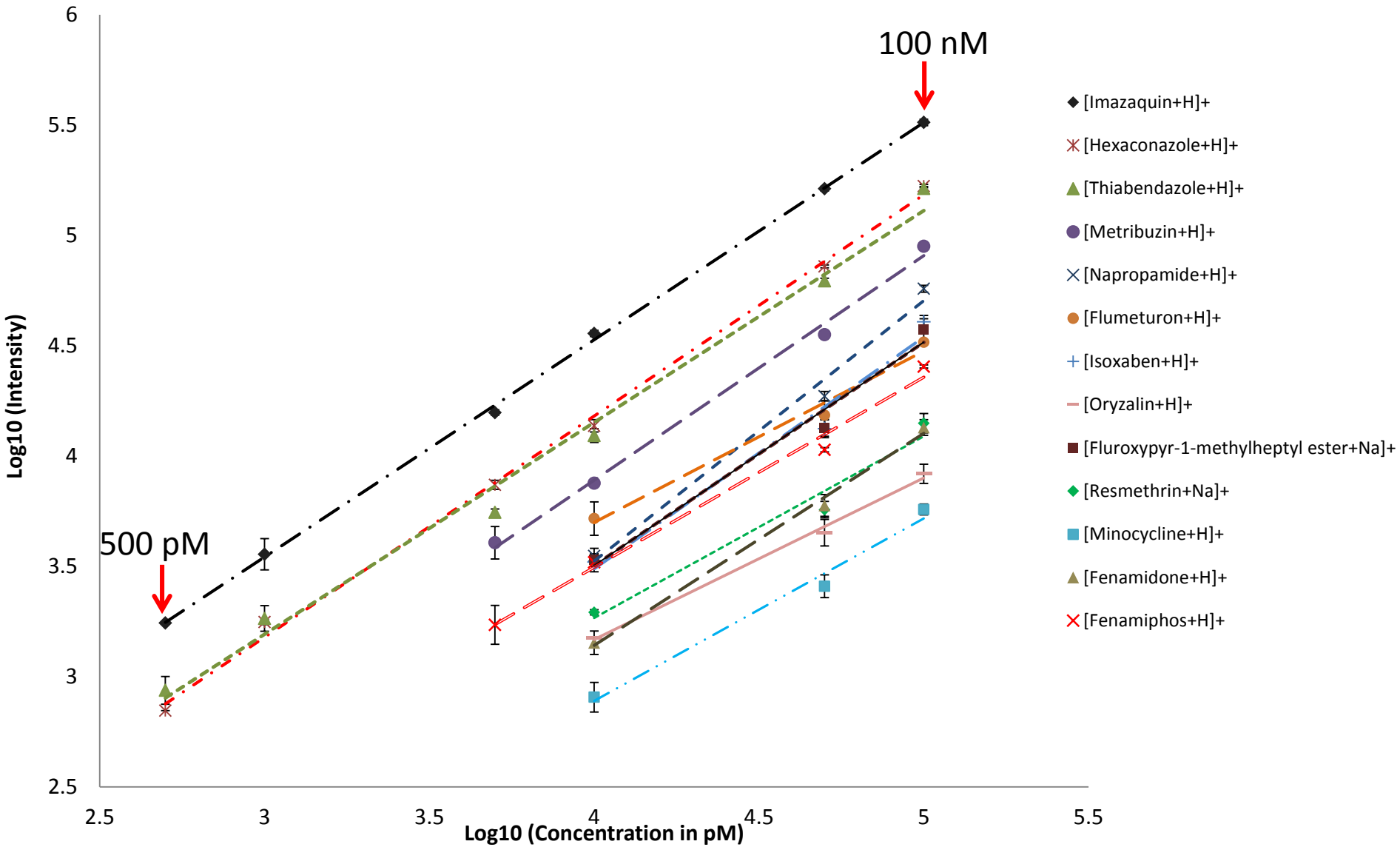
~1000 features with S/N > 5

Metabolite interferences in different matrices



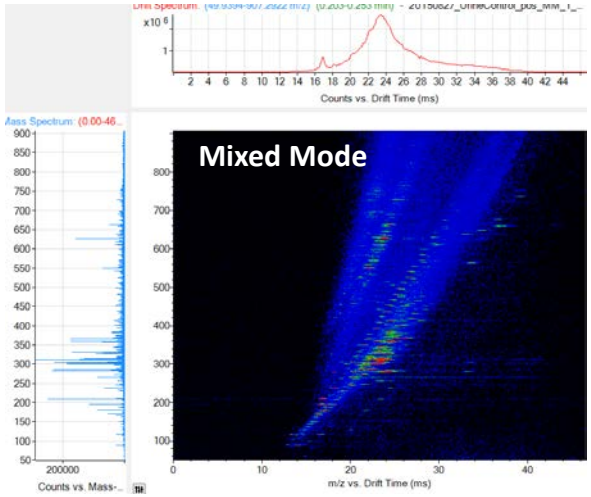


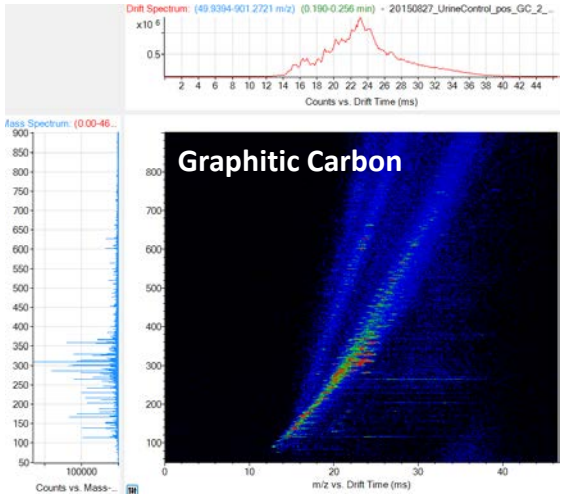
Exogenous metabolites calibration curves in plasma

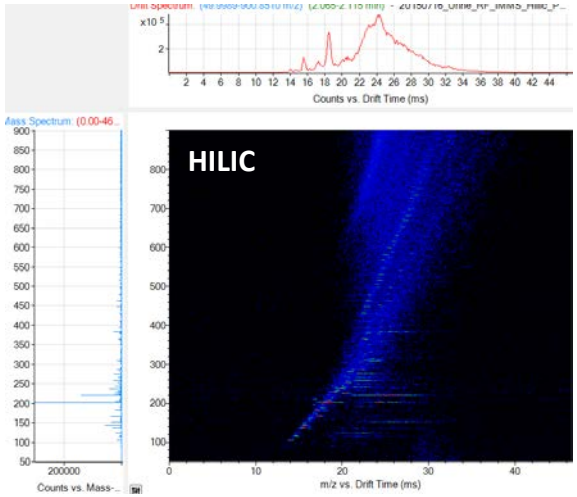


Urine metabolite analysis for type 1 diabetes

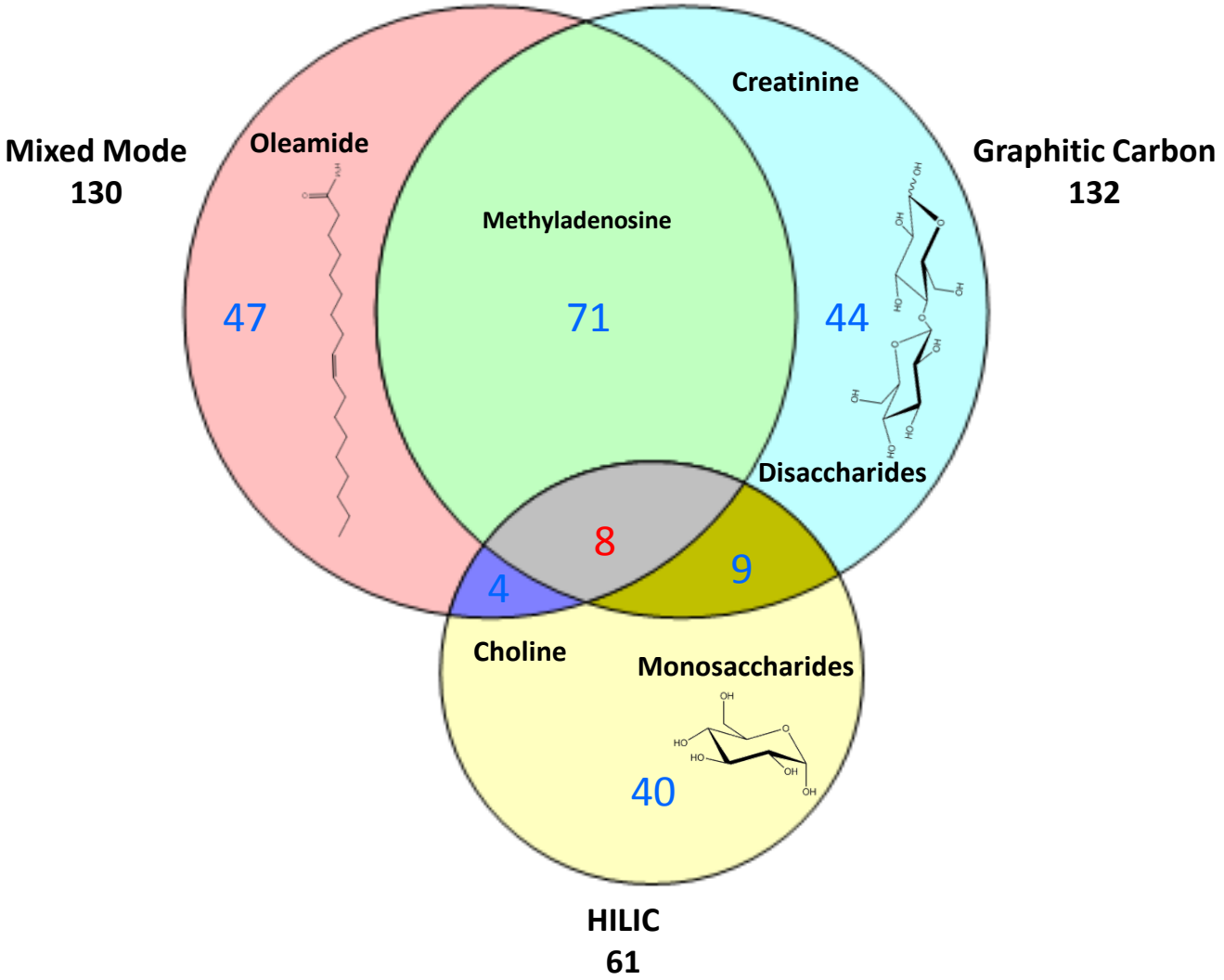
- **100 patient samples (50 pairs of T1D patients with sibling controls)**
- **Each sample were analyzed through 3 different cartridges in both positive and negative ion mode**





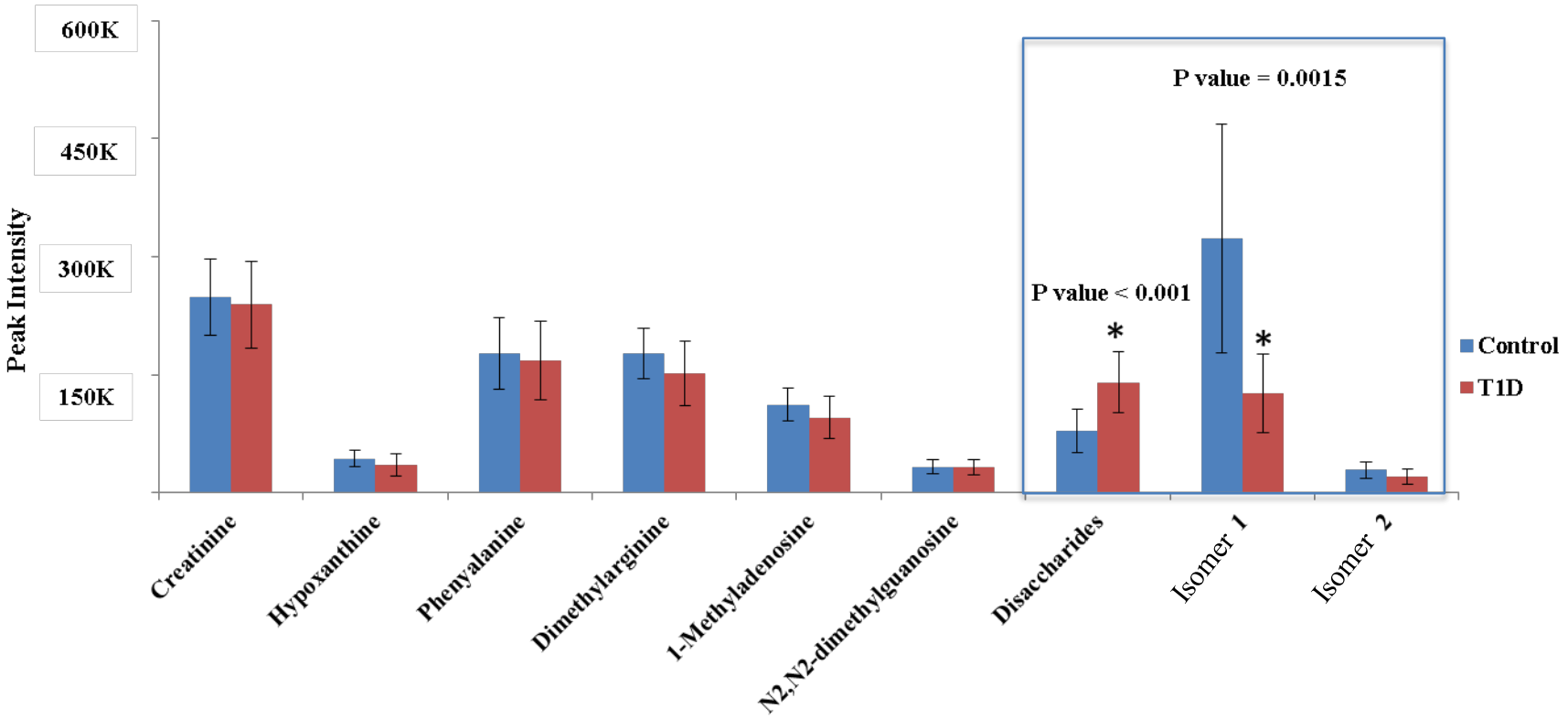


Urine metabolite analysis for type 1 diabetes

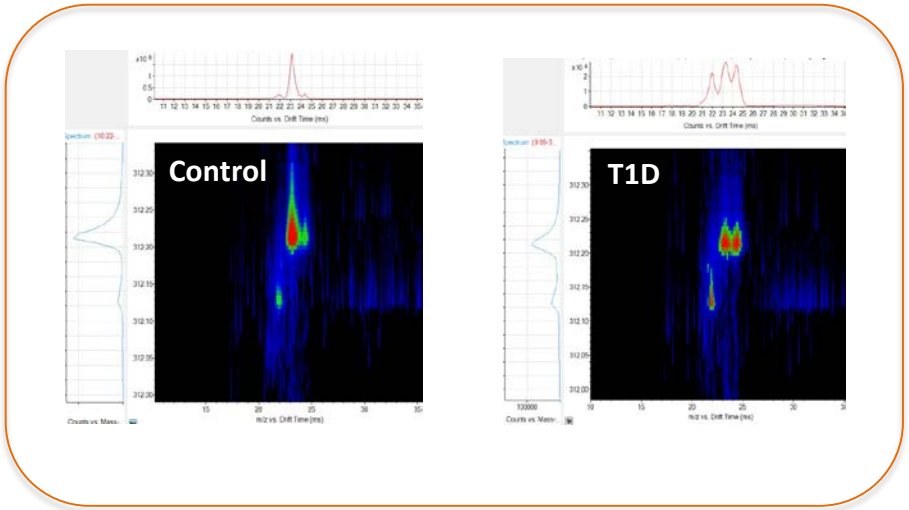


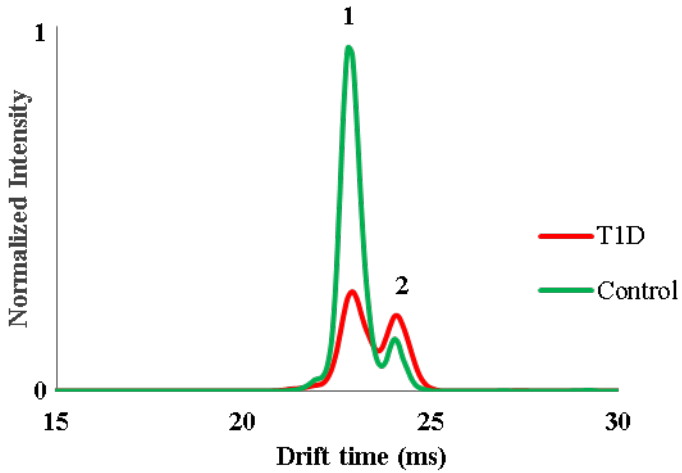
Extracted metabolites with S/N > 200

Statistically significant urine metabolites

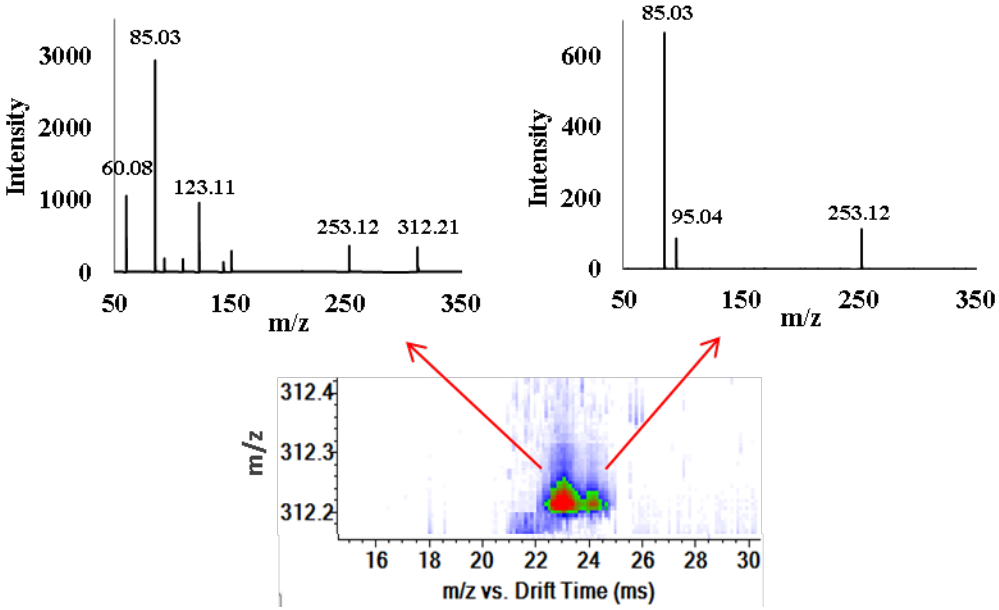


Statistically significant urine metabolite isomers





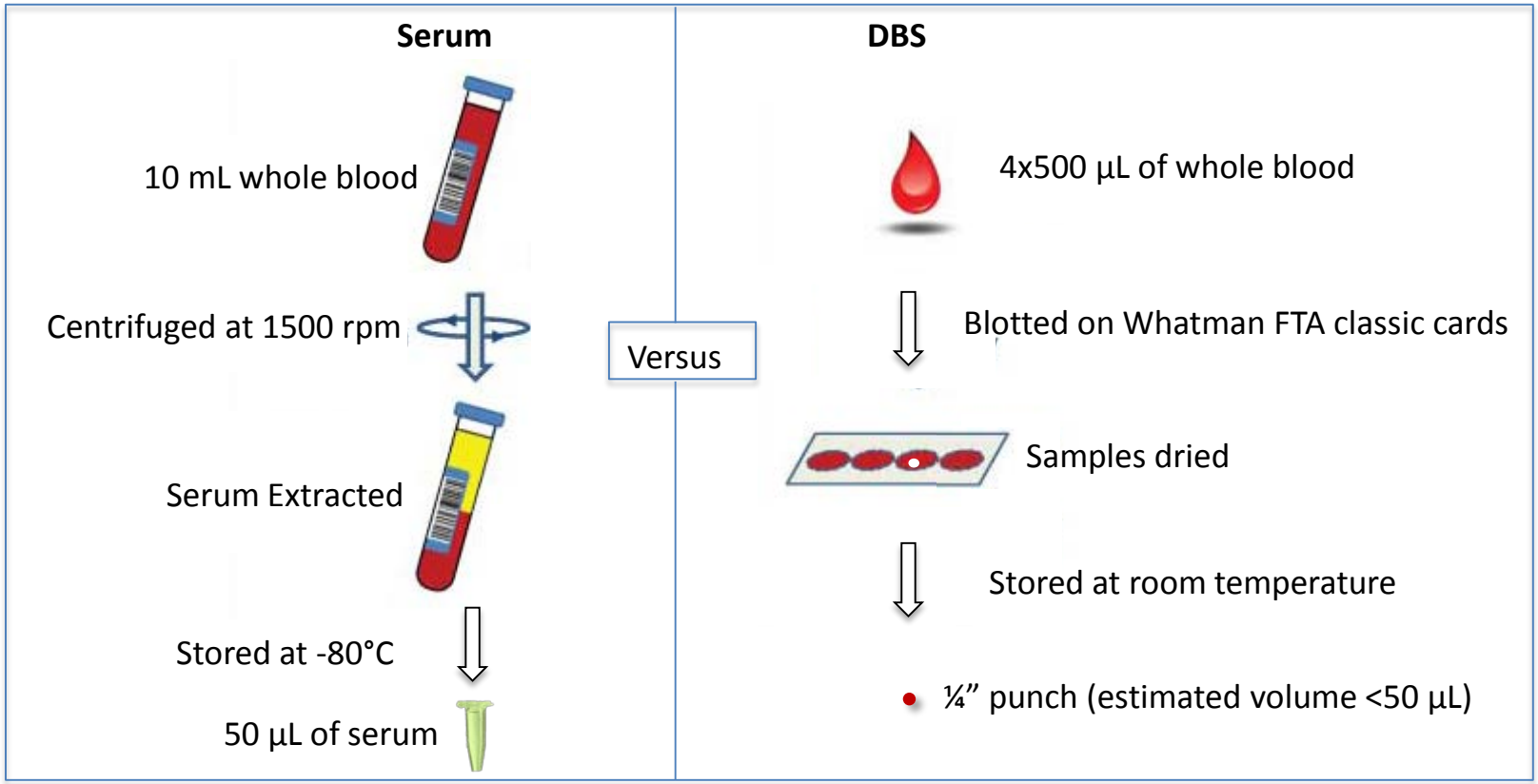
MS/MS



Serum and dried blood spot (DBS) analyses

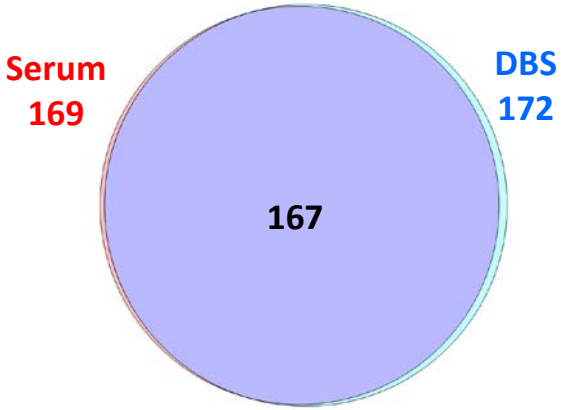
Preliminary study to compare 10 year old serum and DBS samples from OHSU

- 10 matched serum and DBS from fasting men between 55-65 years old
    - 5 patients – normal weight, lipid levels and glucose concentrations
    - 5 patients – BMI>30, triglycerides>150, hyperglycemia, diabetes

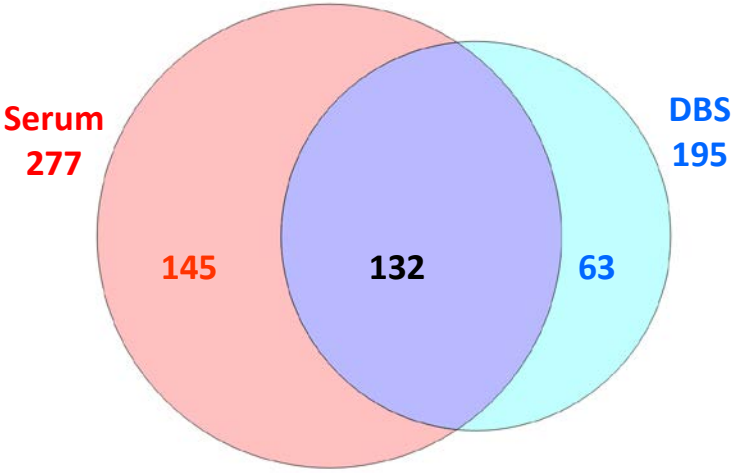


Metabolites & lipid identifications

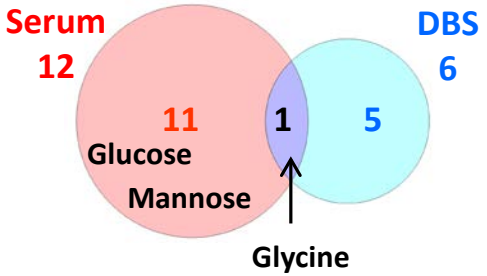
Total Metabolite Ids = 174



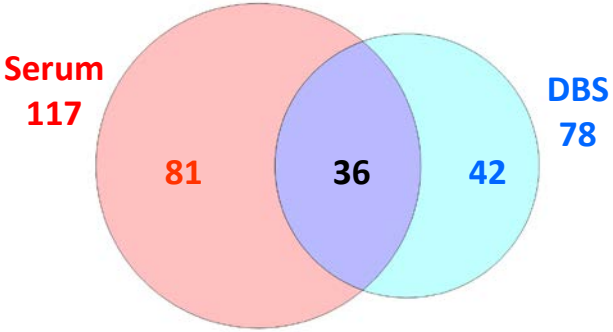
Total Lipid Ids = 340



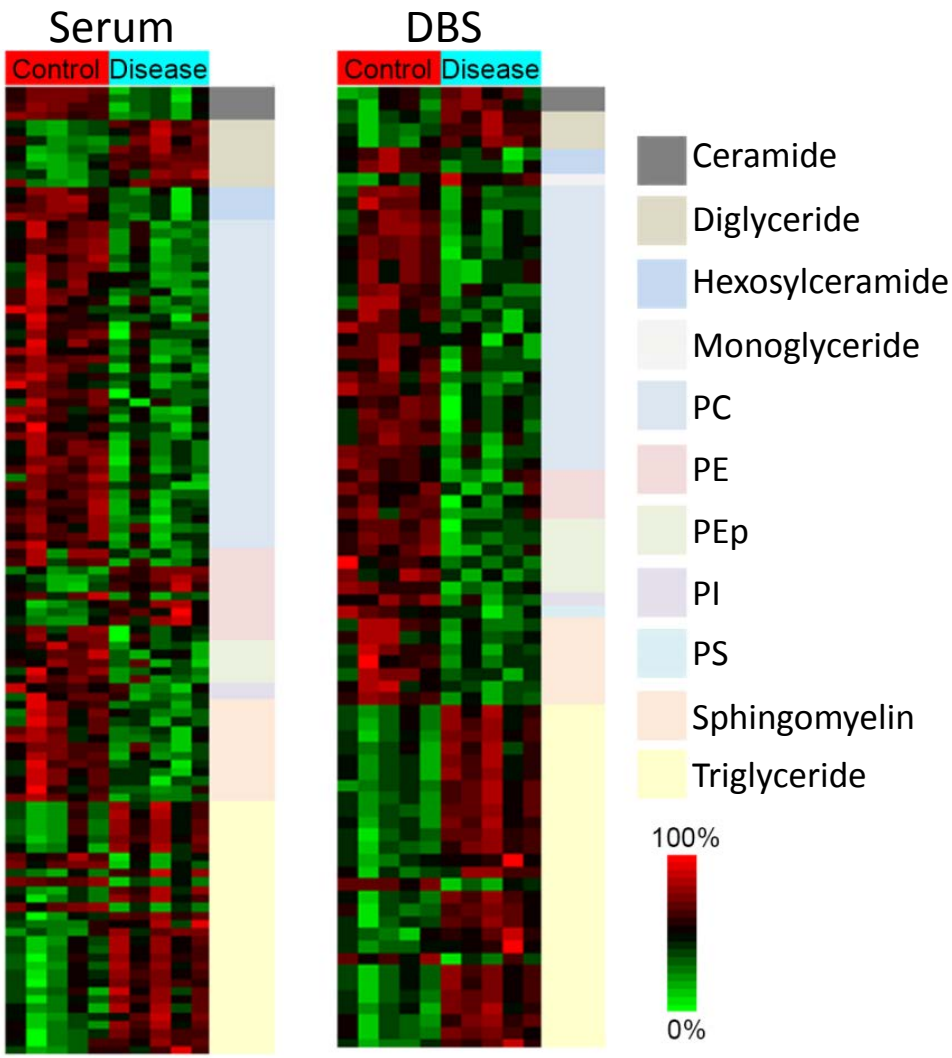
Statistically Significant Metabolites



Statistically Significant Lipids

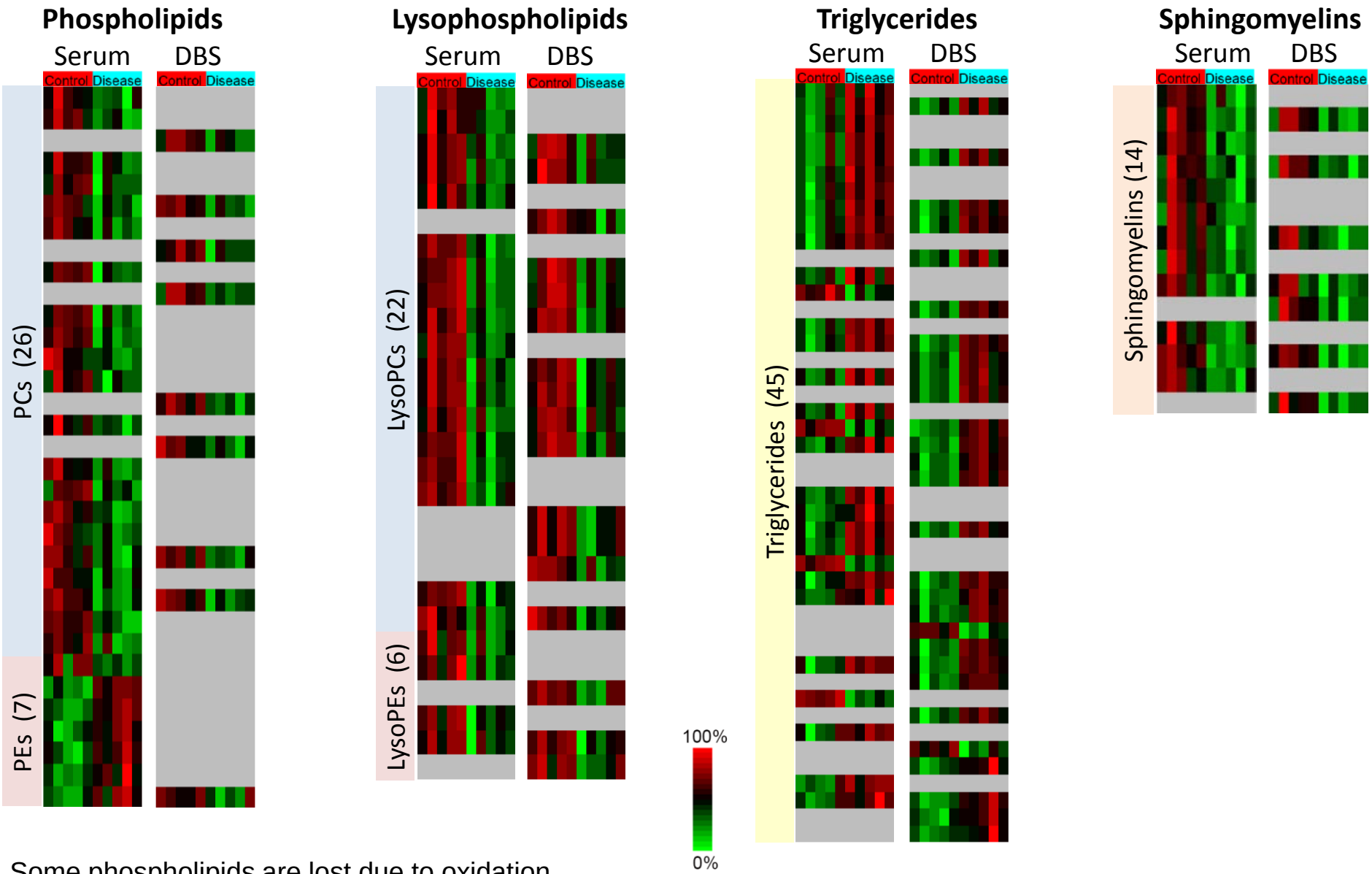


Statistically significant lipids



Lipid classes have same trends for serum and DBS

Statistically significant lipids classes



Some phospholipids are lost due to oxidation

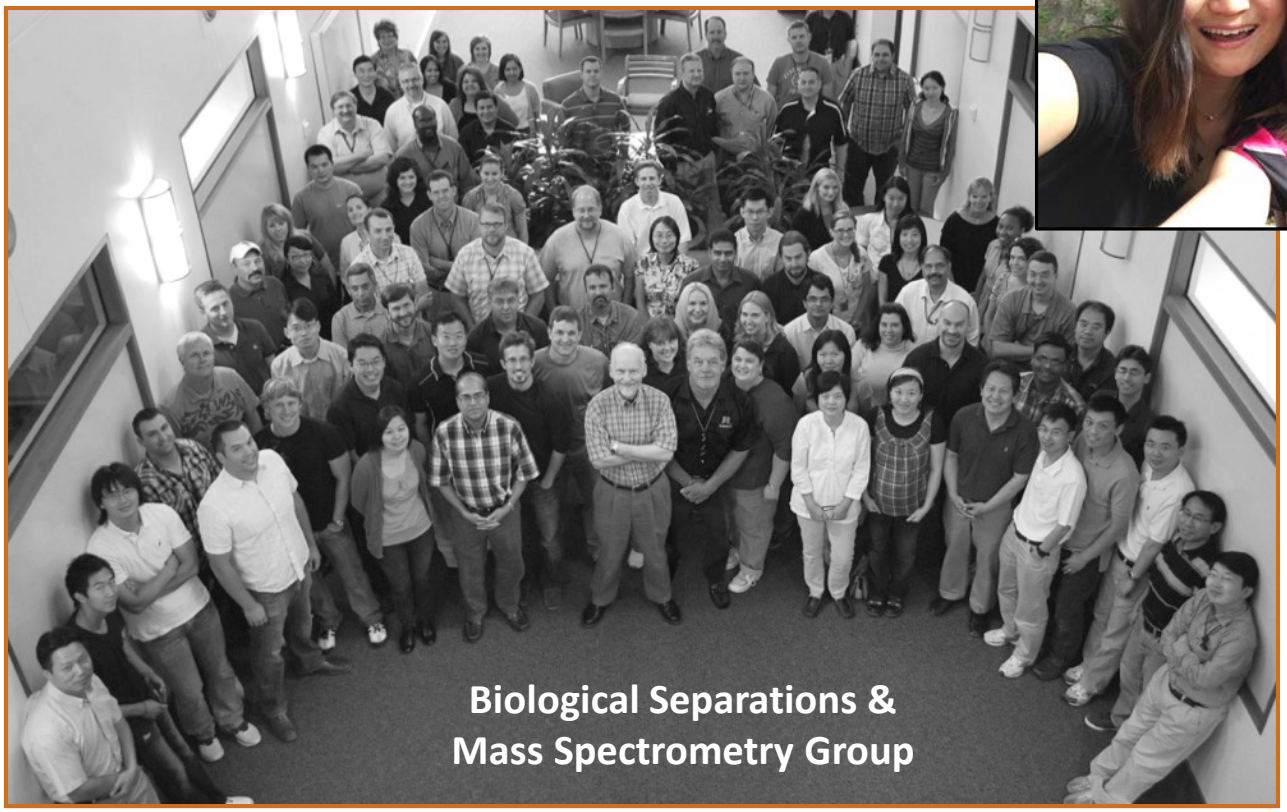
Sphingomyelins, triglycerides and lysoPCs had the most overlap between serum and DBS

Summary

- SPE-IMS-MS measurements allow sensitive, high throughput studies without LC (360 analyses/hr)
  - LODs of ~500 pM in plasma and urine
  - Intensity CV values ≤3%

- Distinct SPE cartridges can be used to target specific metabolites or several cartridge can be run for more comprehensive coverage

- Metabolites from 10 year old DBS samples degraded, however, many statistically significant lipids from serum remained

Acknowledgements

- Agilent Technologies
- National Institute of Environmental Health Sciences of the NIH (R01ES022190)
- NIH: General Medical Sciences Proteomics Center at PNNL (2 P41 GM103493-11) and National Cancer Institute
- PNNL Laboratory Directed Research and Development Program
- Environmental Molecular Sciences Laboratory