Rapid Metabolomic and Lipidomic Analyses Utilizing Ion Mobility Spectrometry

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Understanding health risks

Health Risks

Specific External Environment

Internal Environment

General External Environment
Understanding health risks

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Specific External Environment

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Internal Environment
**Main challenges with small molecule measurements**

1. Small molecules 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 metabolites and xenobiotics 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

Pulse of 2 ions with same m/z but different shape

Drift Cell

Different conformers separate in time with peak heights representing the amount of each

velocity is constant

\[ v = K \vec{E} \]

K = ion mobility
Ion mobility concept

LC (minutes) → IMS (~60 ms) → MS (~100 µs)

Elution Time → Drift Time → m/z

Intensity

0 10 20 30 40 50 60

Elution Time (minutes)

20 30 40 50 60

Drift Time (ms)

100 1100

m/z
Isomers difficult to separate with hydrophobic interaction liquid chromatography (HILIC)

D-Fructose-6-phosphate (F6P)

D-Glucose-6-phosphate (G6P)

α-D-Glucose-1-phosphate (G1P)

Deprotonated form \([M - H]^-\)  \(m/z = 259.02\)
Different ionization methods

- ESI
- APPI
- APCI

Organic Acids
- Sugars
- Lipids
- Nucleosides
- Nucleotides

Nonpolar Very Polar

Molecular Weight

10,000
1,000
100
10

PAHs
- Herbicides
- Pesticides
- OH-PAHs
- Antibiotics

ESI: Electrospray ionization
Different ionization methods

- **ESI**: Electrospray ionization
- **APCI**: Atmospheric pressure chemical ionization
- **APPI**: Atmospheric pressure photoionization

Molecular Weight

- 10
- 100
- 1,000
- 10,000

Polarity

- Nonpolar
- Very Polar

- PAHs
- OH-PAHs
- Antibiotics
- Herbicides
- Pesticides
- Fatty Acids
- Sterols, Steroids
- Drugs
- Nucleosides
- Nucleotides
- Sugars
- Lipids
- Proteins
- Peptides
- Amino Acids
- Sugars
- Proteins

- ESI: Electro spray ionization
- APCI: Atmospheric pressure chemical ionization
- APPI: Atmospheric pressure photoionization

- Antibiotics

- Molecular Weight:
  - 10
  - 100
  - 1,000
  - 10,000

- Polarity:
  - Nonpolar
  - Very Polar
Isomeric xenobiotic separations

BAP: Benzo(a)Pyrene

BKF: Benzo(k)fluoranthene

Radical form [M]+ m/z = 252.09
IMS collision cross section (CCS) precision

- Compare CCS accuracy across 4 international labs
- Analyze 80 molecules (metabolites, lipids, peptides and proteins) to determine CCS agreement

Interlab comparison

- Ran triplicate injections at all 4 labs
- Analyzed in positive and negative ion mode
- Mean %RSD of 0.24%
Limit of detection in water using IMS-MS

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

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

Approximately 1400 features with S/N > 5
Approximately 1000 features with S/N > 5

Calibration curve for xenobiotics in plasma

Small molecule pipeline

Extraction

Methanol/Chloroform Extraction
(100 samples/day)

Instrumental Analysis

SPE-IMS-MS
(8200 samples/day)

Data Processing & Analysis

Database Matching & False Discovery Assessment
(? days)

<table>
<thead>
<tr>
<th>ID</th>
<th>Mass</th>
<th>Intensity</th>
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Collision cross section database

![Diagram showing m/z vs. $^{DT}_{CCS N_2} (\text{Å}^2)$ for different types of molecules including Oligosaccharides, Nucleotides, Peptides, and Lipids.]

Lipids

Oligosaccharides

Nucleotides

Peptides
Collision cross section database

DTCCS$_{Ne}$ (Å$^2$)

m/z

>600 small molecules

Lipids

Oligosaccharides

Nucleotides

Peptides

Metabolic pathways

**GLYCOLYSIS**
- Glucose
- Glucose-6-Phosphate
- Fructose-6-Phosphate
- Fructose-1,6-Biphosphate
  - Glyceraldehyde-3-Phosphate
  - Dihydroxyacetone Phosphate
  - 1,3-Bisphosphoglycerate
  - 3-Phosphoglycerate
  - Phosphoenolpyruvate
  - Pyruvate

**PENTOSE PHOSPHATE**
- Glucose-6-Phosphate
  - 6-Phosphogluconolactone
  - 6-Phosphogluconate
  - Ribulose-5-Phosphate
- Ribose-5-Phosphate
  - Xylulose-5-Phosphate
  - Sedoheptulose-7-Phosphate
- Fructose-6-Phosphate
  - Erythrose-4-Phosphate
  - Xylulose-5-Phosphate
  - Fructose-6-Phosphate
  - Glyceraldehyde-3-Phosphate

**TCA**
- Acetyl-CoA
  - Citrate
  - Oxaloacetate
  - Malate
  - α-Ketoglutarate
  - Fumarate
  - Succinyl-CoA
  - Succinate

**UREA**
- Ornithine
- L-Argino-succinate
  - Urea
  - Arginine
  - Fumarate
Primary metabolite trend lines

Protonated form

- Amino Acids
- Nucleotides
- Steroids
Primary metabolite trend lines

Protonated form

Deprotonated form

DT CCS N2 (Å²)

m/z

Amino Acids
Nucleotides
Steroids

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Deprotonated form

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Amino Acids
Fatty Acids
Lipid Mediators
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Steroids
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Website - http://panomics.pnnl.gov/metabolites/

Lipidomics

Glycerolipid (MG, DG, TG)

Glycerophospholipids (PC, PE, PG, PS, LP)

Saccharolipids (DGDG, MGDG)

Sterols (Cholesterol)

Polyketides (Secondary Metabolites)

Prenols (Vitamins, Quinones)

Fatty Acyls (Eicosanoids)

Sphingolipids (Ceramides, SM)
Lipid isomers

1. Class isomers (PC and PE)

\[
\text{PE 36:2} < \text{PC 33:2} \quad \text{but has the same elemental composition, } C_{41}H_{78}NO_7P
\]

![Ethanolamine](image1) < ![Choline](image2)

2. cis/trans double bond orientations

![PE(18:1/18:1) (cis-\(\Delta^9\))](image3) < ![PE(18:1/18:1) (trans-\(\Delta^9\))](image4)

3. sn-1/sn-2/sn-3 fatty acyl positions

\[
\text{PC(14:0/16:0)} < \text{PC(16:0/14:0)}
\]

4. double bond locations

\[
\text{HO} \quad \text{cis-\(\Delta^9\)} < \quad \text{HO} \quad \text{cis-\(\Delta^6\)}
\]
Ozonolysis or OzID

Parent Lipid

Diagnostic Ions $\Delta mass = 16$ Da

Steve Blanksby

Criegee
Ozonolysis on IMS instrument
ESI-OzID-IMS-MS of \([\text{PE(18:1/18:1)-H}]^-\)

**No Ozone**

**Ozone**

Both double bonds trans-\(\Delta 9\)
ESI-OzID-IMS-MS of PE(18:1/18:1)

cis, cis

trans, trans
LC-OzID-IMS-CID-MS in human plasma

PC 34:1

Intensity

Retention Time (minute)

760.58
LC-OzID-IMS-CID-MS in human plasma

PC 34:1

PC (16:0/18:1)
LC-OzID-IMS-CID-MS in human plasma

**PC 34:1**
- OzID @ RT 19.2 min
- 760.58

**PC 16:0/18:1 (n-7)**
- Aldehyde 678.47
- Criegee 694.47

**PC 16:0/18:1 (n-9)**
- Aldehyde 650.44
- Criegee 666.44

**Retention Time (minute)**
- 19.2
- 19.36

**m/z**
- [M-183+H]+ 577.52
- [M-256+H]+ 504.34
- [M-282+H]+ 478.33
- [M+H]+ 760.58
- [M-59+Na]+ 723.50
- [M-256+H]+ 577.52
- [M-282+H]+ 504.34
- [M-183+H]+ 496.34
- [M+Na]+ 782.57

**OzID @ RT 19.2 min**

**OzID @ RT 19.36 min**
LC-OzID-IMS-CID-MS in human plasma

**PC 34:1**

- OzID @ RT 19.2 min
- OzID @ RT 19.36 min

**PC 16:0/18:1 (n-7)**

- [M-183+H]^+ 577.52
- [M-256+H]^+ 504.34
- [M+H]^+ 760.58
- [M-59+Na]^+ 723.50

**PC 16:0/18:1 (n-9)**

- Aldehyde 678.47
- Criegee 694.47
- [M-282+H]^+ 478.33
- [M-183+H]^+ 577.52
- [M+H]^+ 760.58
- [M+Na]^+ 782.57

**PC (16:0/18:1)**

- NL 282
- NL 256
- NL 183
- NL 59

**PC (16:0/18:1)**

- [M-282+H]^+ 478.33
- [M-183+H]^+ 577.52
- [M+H]^+ 760.58
- [M+Na]^+ 782.57

**Retention Time (minute)**

- 19.2
- 19.6

**Intensity**

- 0.0
- 0.1
- 0.1
Summary

• Combining multiple separations and methods enables faster and better small molecule identifications as demonstrated with SPE-IMS-MS and LC-OzID-IMS-CID-MS analyses.
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Noor Aly

Ion Mobility R&D Group