Advances in Ion Mobility – Mass Spectrometry:

Driving New Capabilities in Discovery, Identification and Characterization Workflows

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Agilent Distinguished Scientist
Senior Director – Ion Mobility
6 June 2018
Overview of Today’s Presentation

• Introduction to Low Drift Field Ion Mobility and the Agilent 6560 IM-QTOF

• Creating Standardization and Accuracy in CCS Measurement

• Creating and Deploying Databases

• Applications in Lipidomics, Intact Protein and Targeted Proteomics
Beginnings of Ion Mobility
Explaining the Nature of Matter – *circa* 1900

- In 1897, Thomson showed that cathode rays were composed of previously unknown negatively charged particles which were 1000 times smaller than the hydrogen atom which he named corpuscles, now electrons.

- Paul Langevin went to Cambridge University and studied in the Cavendish Laboratory under Sir J. J. Thomson,[5], returning to the Sorbonne and obtained his Ph.D. from Pierre Curie in 1902.

- Thomson was awarded the 1906 Nobel Prize in Physics for his work on the conduction of electricity in gases.

- In 1912 while working with Canal Rays JJ Thompson separated Neon Isotopes, cited as the earliest mass spectrometry measurement.

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J.J. Thompson

Born 18 December 1856
Manchester, England
Died 30 August 1940
Cambridge, England

Paul Langevin

Born 23 January 1872
Paris, France
Died 19 December 1946
Not All Ion Mobility Analyzers Work the Same

Types of ion mobility spectrometers

- **Static Fields**
  1. Drift Tube IMS (DTIMS)
  2. Differential Mobility Analyzer (DMA)

- **Dynamic Fields**
  1. Field Asymmetric IMS (FAIMS)
  2. Traveling Wave IMS (TWIMS)
  3. Trapped IMS (TIMS)

Biochimica et Biophysica Acta 1811, 935–945 (2011).
What Does Ion Mobility Measure?
From Velocity to Mobility to Collision Cross Section

Mobility = \frac{\text{Velocity}}{\text{Field Strength}} = \frac{L/td}{E}

Collision Cross Section = \Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_bT)^{1/2}} \left[ \frac{1}{m_B} + \frac{1}{m_I} \right]^{1/2} \frac{1}{N} \frac{t_d}{273.2} \frac{E}{760} \frac{L}{P}

Rotationally Averaged Collision Cross Section typically expressed in nm² or Å²
Conformational Space Occupancy of Biomolecules
The Separation of Representative Classes and Analytes

Agilent 6560 Ion Mobility – QTOF Mass Spectrometer

Low Field Drift Tube Ion Mobility – for highest CCS Accuracy

78.1 cm Drift Cell
~60 Resolving Power

Quadrupole Time-of-Flight
40,000 Resolving Power
Agilent 6560 Ion Mobility – QTOF Mass Spectrometer
Low Field Drift Tube Ion Mobility – for highest CCS Accuracy
Combining the Power of Accurate Mass and Structural Characterization

*With Current UHPLC (and GC) Separations*
Separation of Structural Sugar Isomers $\text{C}_{18}\text{H}_{32}\text{O}_{16}$

Not Possible by Mass Analysis at “Any MS Resolution”

raffinose 26.68 ms

melezitose 25.76 ms

Resolution Power = 60

Separation of tri-saccharides isomers
Drift versus Retention Time Enables Separation of Complex Mixture of Oligomers by MW, Charge State, End-Group and Repeat Unit Composition
Understanding 6560 IM-QTOF Modes

QTOF Mode

Theoretical Result
• 100% duty cycle

Actual Results
• No Ion Mobility Separation

IM Mode

Theoretical Result
• Up to 100% duty cycle

Actual Results
• Trap capacity, particularly low mass ions, reached in much less than 50 ms,
  • Ions compressed in short pulse increase in intensity can saturate the TOF detector

Multiplexed IM Mode

Theoretical Result
• 50% duty cycle with demultiplexing

Actual Results
• Trap efficiently remains high capturing all thin incoming ions, including low mass
  • Shorter trapping times reduce the occurrence of TOF detector saturation
Agilent Demultiplexing Tool
Batch Support for Post Acquisition Processing

Also works with Single Field Calibration when the Tunemix is also Acquired and Demultiplexed under Identical Conditions.

8 pulses are summed (transformed) to 1
All Ions IM/MS Fragmentation

Linking IM Drift time to Collision Energy for Optimal Fragmentation
Acquiring “All Ions IM/MS”
Alternating Hi/Lo Fragmentation Frames

In the Acquisition Method we can link the Collision Energy to the Drift Time.

Set the Collision Energy Table to provide the correct CE at each Drift Time.

1 volt / msec is a good default
Overview of Today’s Presentation

• Introduction to Low Drift Field Ion Mobility and the Agilent 6560 IM-QTOF

• Creating Standardization and Accuracy in CCS Measurement

• Creating and Deploying Databases

• Applications in Lipidomics, Intact Protein and Targeted Proteomics
## Separation Technologies and Identification Applicability

### Can Ion Mobility Serve as a Basis for Analyte Confirmation & Identification?

<table>
<thead>
<tr>
<th>Mass Spectrometry (TOF-MS)</th>
<th>Ion Mobility (DT-IM)</th>
<th>Gas Phase (GC)</th>
<th>Liquid Phase (LC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical Measure</strong></td>
<td>Flight Time</td>
<td>Drift Time</td>
<td>Ret. Time</td>
</tr>
<tr>
<td><strong>Property Measured</strong></td>
<td>m/z</td>
<td>CCS</td>
<td>Partition Coeff.</td>
</tr>
<tr>
<td></td>
<td>A property of the analyte</td>
<td>A property of the analyte, drift gas and temperature</td>
<td>A property of the analyte, stationary and mobile phases and temperature</td>
</tr>
<tr>
<td><strong>System Accuracy</strong></td>
<td>&lt; 1 ppm</td>
<td>until now – 2%</td>
<td>1% RRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Database Compatible</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>RRT – Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Limited</td>
</tr>
</tbody>
</table>
Refining the Analytical Precision of CCS Measurements

Historical Data (42 months) for the Agilent Tuning Mixture (VU System)

- 2014 initial prototype instrument
- 2015 drift tube/funnel field alignment
- 2015 SWARM tuning algorithm
- 2014 precision gas flow controls
- 2014 absolute pressure readings
- 2014 initial prototype instrument¹

Fundamental improvements in the instrumentation and methods have benefited the precision of the CCS measurement.

¹ May et al. “Conformational Ordering of Biomolecules in the Gas Phase…” *Analytical Chemistry* 86(4), 2107-2116 (2014)
Creating a Standard for CCS Measurement
Setting the Stage for CCS Workflows

Scientific Firsts required to support CCS as a Database Standard

- Create a reference system for highly accurate primary CCS measurements.
- Based on Agilent Tunemix publish Standard CCS Reference Values.
- Enable a Universal Calibration Equation to annotate every detected Feature
- Establish the above standards through a broadly recognized Interlab Study first presented at ASMS 2016 and now published in Analytical Chemistry.

The result: “True CCS” – traceable to the Agilent Reference Standards
Direct Collision Cross Section Determination
Measurement without the use of Calibration Standards – Stepped Field Method

\[ \Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left[ \frac{1}{m_i} + \frac{1}{m_B} \right]^{1/2} \frac{t_dE}{760} \frac{T}{P} \frac{1}{273.2} \frac{1}{N} \]

- Detector
- Optics
- Cell
- Drift Tube
- Rear Funnel
- Ion Source

\[ y = 6258x + 5.5225 \quad R^2 = 0.9999 \]
\[ y = 8019x + 7.2356 \quad R^2 = 0.9996 \]
\[ y = 5410x + 4.9269 \quad R^2 = 1.0000 \]

Drift time (ms)
P/V (Torr/V)
Colchicine
Reserpine
Ondansetron

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Stepped Field Method

Direct Collision Cross Section Determination Measurement without the use of Calibration Standards
Establishing the Agilent Reference Standard Measurement
Only Commercial System Built the can serve as a Primary Standard for CCS

The Agilent 6560 IM-QTOF is the only commercially available low pressure system that conforms to the Mason-Schamp Equation – Defining CCS

\[
\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_bT)^{1/2}} \left[ \frac{1}{m_t} + \frac{1}{m_B} \right]^{1/2} \left( \frac{t_dE}{L} \right) \frac{760}{P} \frac{T}{273.2} \frac{1}{N}
\]
Precise Calibration the Agilent Reference System
Achieving sub-part per thousand Accuracies

Pressure
The Agilent Capacitance Diaphram gauge in Alt. Gas Kit has 0.2% gas pressure accuracy

Length
Precise metrology determined a drift cell length of $78.302 \pm 0.0088$ cm

Temperature
Temperature profiling established a length weighted temperature correction of 1.1 C.

Field
Precise independent voltage measurements determined a positive mode correction of 1.00284 and negative mode correction of 1.0013.
Why Bundle in the “Alt. Gas Kit”
Supports Other Gases PLUS 25x increase in Pressure Accuracy

To support Alt Gases the gauges where switched from Pirani to Capacitive Manometer.

- Measures “True Pressure” not thermal conductivity – gas composition independent
- Pressure Accuracy from 5% to 0.2%
- Added Mass Flow Controller which regulates the pressure
Looking at Pressure Read Back Accuracy

**Without Alt Gas Kit:**
- Relies on holding instrument conditions stable
- Must have constant source temperature
- Requires rough pump to be in good condition

**With Alt Gas Kit:**
- Regulation gives increased pressure stability for Single Field work
- Accurate pressure reading to support Stepped Field CCS measurements

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**Good Std (Pirani) System**

Good Std (Pirani) System

+/- 0.25%

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**Alt Gas Kit / Capacitive Manometer**

Alt Gas Kit / Capacitive Manometer

+/- <0.1%
Examining CCS Bias (error) with and without Pressure Regulation

Values are the mean shift in CCS for the Lipid standards in each Lab:
- Unregulated Cell Pressure
- Agilent
- BOKU
- Duisburg-Essen
Creating the CCS Reference Standard

**Standard CCS** values based on Agilent Tunemix

<table>
<thead>
<tr>
<th>Tune Mix Ion</th>
<th>CCS</th>
<th>%RSD</th>
<th>Tune Mix</th>
<th>CCS</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>118</td>
<td>121.30 ± 0.20</td>
<td>0.17%</td>
<td>112</td>
<td>108.23 ± 0.20</td>
<td>0.19%</td>
</tr>
<tr>
<td>322</td>
<td>153.73 ± 0.23</td>
<td>0.15%</td>
<td>301</td>
<td>140.04 ± 0.29</td>
<td>0.21%</td>
</tr>
<tr>
<td>622</td>
<td>202.96 ± 0.27</td>
<td>0.14%</td>
<td>601</td>
<td>180.77 ± 0.21</td>
<td>0.12%</td>
</tr>
<tr>
<td>922</td>
<td>243.64 ± 0.30</td>
<td>0.12%</td>
<td>1033</td>
<td>255.34 ± 0.32</td>
<td>0.13%</td>
</tr>
<tr>
<td>1221</td>
<td>282.20 ± 0.47</td>
<td>0.17%</td>
<td>1333</td>
<td>284.76 ± 0.31</td>
<td>0.11%</td>
</tr>
<tr>
<td>1521</td>
<td>316.96 ± 0.60</td>
<td>0.19%</td>
<td>1633</td>
<td>319.03 ± 0.70</td>
<td>0.22%</td>
</tr>
<tr>
<td>1821</td>
<td>351.25 ± 0.62</td>
<td>0.18%</td>
<td>1933</td>
<td>352.55 ± 0.27</td>
<td>0.08%</td>
</tr>
<tr>
<td>2121</td>
<td>383.03 ± 0.64</td>
<td>0.17%</td>
<td>2233</td>
<td>380.74 ± 0.31</td>
<td>0.08%</td>
</tr>
<tr>
<td>2421</td>
<td>412.96 ± 0.58</td>
<td>0.14%</td>
<td>2522</td>
<td>415.75 ± 0.31</td>
<td>0.07%</td>
</tr>
<tr>
<td>2721</td>
<td>441.21 ± 0.59</td>
<td>0.13%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Deriving a Calibration Equation for Single Field Operation
Based the Mason Schamp Equation – without compound class dependency

First Identifying all Physical and Experimental Constants

\[ \Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_bT)^{1/2}} \left[ \frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} \frac{t_d E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N} \]

When the drift gas, \( m_B \), is constant we can normalize the reduced mass term to make it dimensionless and include \( m_B \) with the other constant.

\[ \left[ \frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} = \left[ \frac{m_B+m_I}{m_I m_B} \right]^{1/2} = \left[ \frac{m_I m_B}{m_B+m_I} \right]^{-1/2} = m_B^{1/2} \left[ \frac{m_I}{m_B+m_I} \right]^{-1/2} \]

Then rearranging we have

\[ \Omega = \frac{(18\pi)^{1/2}}{16} \frac{e}{(k_bT)^{1/2}} \frac{T}{N 273.2} \frac{E 760}{P} \frac{1}{m_B} \frac{m_I}{m_I m_B}^{1/2} \times z \times \left[ \frac{m_I}{m_B+m_I} \right]^{-1/2} \times t_d \]
Examining $t_d$ and other Drift Time Contributions
Applying Mass Schamp to $t_{\text{total}}$, the Total Drift Time

*Times are estimate for m/z 622 ion. Drift Tube at 16 V/cm. Time attributed to non Ion Mobility related motion is estimated at less than 0.1 msec

Substituting $t_{\text{total}}$ as the total time through multiple zones, and $T'$, $L'$, $E'$ and $P'$ as aggregate values for all zones

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{e}{(k_b T')^{1/2}} \frac{T'}{N} \frac{1}{273.2} \frac{E'}{L'} \frac{760}{P'} m_B^{-1/2} \ast Z \ast \left[ \frac{m_I}{m_B + m_I} \right]^{-1/2} \ast t_{\text{total}}$$
Deriving the Single Field Calibration for LC/MS Compatibility

: Using Linear Regression to Calibrate $\beta$ and $t_{fix}$

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{e}{(k_b T')^{1/2}} \frac{T'}{N} \frac{1}{273.2} \frac{E'}{L'} \frac{760}{m_B} m_I^{-1/2} \times \beta \times \left[ \frac{m_I}{m_B + m_I} \right]^{-1/2} \times t_{total}$$

Combine physical and experimental constants into a single term, $\beta$ with an offset, $t_{fix}$ *

$$\Omega = \frac{1}{\beta} \times z \times \left[ \frac{m_I}{m_B + m_I} \right]^{-1/2} \times (t_{total} - t_{fix})$$

And with known CCS Stepped Field values and measured $t_{total}$ times for Tunemix ions determine $\beta$, $t_{fix}$ by calibration

* An equivalent form is

$$\frac{\beta}{z} \left[ \frac{m_I}{m_B + m_I} \right]^{1/2} \times \Omega = t_{total} - t_{fix}$$
Metabolomics and Small Molecule Application Update
Updates for B.08 Release MP, MPP, ID Browser and IM-MS Browser

**Find**
- **4D Feature Finding**: Extracts Ions and Groups as Isotopic Clusters.
  - Achieves high mass accuracy over wide dynamic range.

**Characterize**
- **IM-MS Browser**: View 4D Raw Data and Detected Features
  - Supports Stepped and Single Field Calibration
  - Individual data file feature extraction

**Process**
- **Mass Profiler**: Automation for Feature Extraction and Alignment
  - Batch extract and align across multiple runs
  - Creates Summary Table w/ CSV export.
  - Batch Extract All Ions IM/MS Fragmentation
  - Creates individual CEF files for MPP
  - Supports 2 sample compare

**Identify**
- **ID Browser & PCDLs**: Identification interface from PCDLs to MP/MPP
  - Supports ID based on RT, CCS, Accurate Mass & Fragmentation Spectra.
  - Supports databases for use as target lists for Targeted Screening in MP

**Compare**
- **Mass Profiler Professional**: Non-targeted discovery with Statistical Analysis
  - Supports CCS along with m/z and RT
  - Statistical Functions.

**Skyline** and Spectrum Mill
Extracting Analyte Separation Coordinates

“Multi-dimensional Feature Finding”

Detecting the Drift Time, m/z and z of each analyte and applying the calibration terms $\beta$ and $t_{fix}$ annotates each analyte collision cross section.
This Update – Leading in CCS Accuracy and Workflows

- Drift Tube Ion Mobility – Achieving True CCS
- Built-in Alt. Gas Kit – Accuracy and Pressure Stability
- If you want Accurate CCS – Don’t Heat Up My Ions!
- Multiplexed IM and All Ions IM/MS get new focus
- Adding Skyline – Combining Accurate CCS and Quantification
- PNNL SW Tools – Better Feature Finding and Dynamic Range
Our recent Interlaboratory Study showed 0.3% reproducibility for database creation (Stepped Field Workflow) workflows and 0.6% for analytical ID (Single Field) workflows.
Dynamic Field approaches use RF confinement and heats the analyte throughout the mobility measurement leading to shifts in CCS values.
Recent Ion Mobility Fundamentals Paper
Valerie Gabelica, 6560 IM-QTOF Customer

In IMS, the force exerted by an electric field on an analyte ion is exactly balanced by friction with the buffer gas, yielding a steady-state analyte velocity \( v_0 \). The ion mobility, \( K \) (Eq. (1)) is thus a measure of friction linked to an observable: the time \( \tau \) the ion takes to traverse the length \( L \) of the mobility cell.

\[
K = \frac{v_0}{E} = \frac{\tau L}{I_0}
\]  (1)

\( K \) depends on the collision frequency, hence on the gas number density \( N_0 \), gas temperature \( T \), and pressure \( P \). So the reduced mobility \( K_r = K N_0 P_0 = K I_0 \rho_0 (T/T_0) \) is better to compare different experiments (standard conditions, \( N_0 = 2.887 \times 10^{16} \text{ m}^{-3}, \rho_0 = 760 \text{ Torr}, T_0 = 273.16 \text{ K} \)). When \( t_0 \) is small compared to the ion thermal velocity \( v_{th} \), \( K \) can be expressed as Eq. (2) [1].

\[
K = \frac{3}{16} \sqrt{\frac{2 \pi}{\mu k_B T}} \times \frac{\pi}{N_0}
\]  (2)

\( \mu \) is the reduced mass of the ion-gas pair \( (\mu = m M/(m + M)) \), where \( m \) and \( M \) are the ion and gas-particle masses, \( k_B \) is the Boltzmann constant, and \( \sigma \) is the analyte charge.

\( \Omega \), often called the ‘collision cross section’ (CCS), is actually a momentum transfer collision integral, that is, the momentum transfer between ion and gas particles averaged over all gas-ion relative thermal velocities. While the terms tend to be used interchangeably in IMS, they are in fact not identical in a wider context.

Reference: Fundamentals of ion mobility spectrometry
Valerie Gabelica and Erik Marklund
Current Opinion in Chemical Biology 2018, 42:51–59
“… Class-dependent calibration problems in TWIMS could thus also come from ion temperatures in TWIMS differing from the calibrant DTIMS temperatures. In summary, DT and TW experiments will transpose well only if analytes and calibrant CCS values have the same temperature dependency in the buffer gases of interest. “

From: Fundamentals of ion mobility spectrometry
Valerie Gabelica and Erik Marklund
Current Opinion in Chemical Biology 2018, 42:51–59
Molecular Heating effects each analyte’s structure differently.

Different chemical class have their own temperature dependencies giving rise to class dependent CCS calibrations.

From: Fundamentals of ion mobility spectrometry
Valerie Gabelica and Erik Marklund
Current Opinion in Chemical Biology 2018, 42:51–59
CCS Accuracy Summary
Low Field Drift Cell Ion Mobility – Unique Differentiation

Agilent’s Low Field Drift Cell 6560 Offers:

• Stepped Field CCS – Independent CCS Measurement, NO additional calibration
• Published Inter-Lab Study with 0.3% CCS agreement
• Highly accurate (0.2%) pressure gauges and drift pressure regulation
• Single Field Method – Universal for analyte type with 0.6% agreement
• Low Townsend (thermal heating) CCS measurement that preserves CCS accuracy

Recognized as the most accurate measure of CCS
A structural examination and collision cross section database for over 500 metabolites and xenobiotics using drift tube ion mobility spectrometry

The Significance of High CCS Accuracy

Reducing the number of candidate ID’s


Fig. 7 Increasing identification confidence for human urine features by adding CCS information. Features having candidate identifications in both our PNNL database and HMDB were compared. In most cases, the number of candidate molecules within an m/z tolerance of 15 ppm (orange and green bars) was significantly reduced when CCS matching was incorporated (turquoise and purple bars). The importance of highly accurate CCS values (0.6% error) was shown to greatly decrease the number of candidate molecules versus those for 5% error, which were similar to m/z only matching.
Once trained, supports rapid CCS determination for extended populations.

Recent publication focuses on Lipids. Newer training sets predict for CCS for 200,000 lipids.
Applying Ion Mobility to the Analysis of Lipids
Erin Baker, 6560 Collaborator, PNNL

Various Lipid Classes and Isomers can be resolved and identified through the use of Ion Mobility
Fatty acids are key components of lipids, giving them their hydrophobic properties.

Fatty acid analysis with IMS

Lowest energy structures from AMBER molecular dynamics simulations

Normalized Intensity

Drift Time (ms)
sn-1/sn-2 positional analysis

Size Comparison (Relative cross sections)

PC(14:0/16:0) < PC(16:0/14:0)
PC(16:0/18:0) < PC(18:0/16:0)
PC(18:1/16:0) < PC(16:0/18:1)
Software Updates – All Ions IM-MS Workflow

LC

Low Energy MS

High Energy MS

Drift time aligned fragments

PC’s
PC 14:1 & PC 16:0

Characteristic
184 Fragment
Software Updates – All Ions IM/MS included in CEF files…

**CEF files support Interoperability of Mass Profiler feature finding with**
- MPP
- ID Browser
- SimLipid
- ZhuLab Suite (under way)
- More…

**Feature Summary export allows for exchange to**
- Skyline
Software Updates – ID Browser
Adding Fragment Ion Measurement for All Analytes
“Alt. Frame High/Low Collision Energy - “All Ions MS/MS”

Lipid Samples and Results from Julian Griffin Lab, Cambridge Univ.

Precursors shown in Green
Fragments shown in Red
Lipid Identification – Structural Resolution

Our goal is to identify lipids only to the level of structural resolution where we are confident.

<table>
<thead>
<tr>
<th>Structural Resolution</th>
<th>Example</th>
<th>MS</th>
<th>MS/MS</th>
<th>IM-MS</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbons and Double Bonds</td>
<td>PC(22:2)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty Acid Constituents</td>
<td>PC(10:2_12:0)</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positional Isomers</td>
<td>PC(10:2/12:0)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Double Bond Position</td>
<td>PC(10:2(4,6)/12:0)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ozone</td>
</tr>
<tr>
<td>Double Bond Cis vs. Trans</td>
<td>PC(10:2(4E, 6E/12:0)</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Comp. Predict.</td>
</tr>
</tbody>
</table>

Jeremy P Koelmel from the Yost Lab (University of Florida) Aiding Future Lipid Solutions Based on his LipidMatch Software
Characterizing Antibody Therapeutics
Comparison of Rituximab-1 (Innovator) and Rituximab-2 (biosimilar)
Characterizing Antibody Therapeutics
Comparison of Rituximab-1 (Innovator) and Rituximab-2 (biosimilar)

The average size of glycans on the Rituximab-1 were slightly smaller than those on the Rituximab-2. The CCS of the 27+ molecule was larger for the Rituximab-2. Ion mobility can provide not only the size but also the molecule structural information in the biosimilar study.
CIU: Collision Induced Unfolding

CIU is ion mobility analysis across a range of activation energies

CIU on the Agilent 6560 IM-Q-ToF

- Drift tube IM *before* collision cell
- Source modifications for enhanced CIU

Dr. Ruwan Kurulugama, Agilent
CIUSuite 2: Enabling the Next Generation of CIU

**Goals:**

- Analyze noisy and low signal (challenging) CIU data
- Enable high-throughput acquisition of CIU data
- Support a broad range of CIU analyses with user-friendly, extensible platform
Stability Analysis: Features and CIU50

- Fit sigmoid to features to determine precise midpoint (CIU50)

“CIU50”: 50% intensity transition point between features
Targeted Data Analysis and Quantification
Moving from Feature Finding to Extracted Ion Chromatograms

Skyline

Advanced Targeted Mass Spectrometry with Agilent TOF and Skyline

Prof. Mike MacCoss
Brendan MacLean
Brian Pratt
Mouse Liver Tissue Proteome Samples
Tracing Deuterium Enrichment Through the Proteome

Non-Enriched Samples
QTOF mode AutoMS/MS
- 32,640 MS/MS Spectra Triggered
- Extracted, ID’d and Validated in Spectrum Mill

2\textsuperscript{H}-Enriched Samples

Followed by…

IM-QTOF “Profile” and
“All Ions MS/MS” analysis
Results of Protein Database Search.

Starting Standard Protein/Peptide ID Workflow – Data Dependent Acq.

Lowest level peptides from Spectrum Mill Search

Selecting one of the very lowest ID’d peptides

Peptide Sequence: QLLQEEVGPVGVTMR m/z: 892.961
Examining A Low Level Peptide – S/N and Interference
Precursor m/z from AutoMS/MS Acquisition +/- 10ppm Extraction Window

Peptide and isotopes: QLLQEEVGPVGVETMR

QTOF AutoMS/MS Run
+/- 10ppm mass window
Moving the Experiment from Discovery to Targeted
Annotating Peptide Database with CCS Values

QTOF / Orbitrap Protein ID (DDA) Pooled Sample

(Spectrum Mill) Database Search

Import Peptide Database in to Skyline

Annotate Database Entries with CCS

Quantify Individual IM-QTOF Samples Using AM / RT / CCS

IM-QTOF “High Res Profile” Pooled Sample

or

IM-QTOF “All Ions MS/MS” Pooled Sample
Examining Mass Selectivity and Response in Ion Mobility Mode

Sample Low Level Peptide +/- 10ppm \textit{plus} +/- 0.5ms Drift Window

Peptide and isotopes (IM Mode): QLLQEEVGPVGVTMR

IM QTOF Run
 +/- 10ppm mass window
 +/- 0.5ms drift window

Dramatically Fewer Interferences as compared to mass only extraction
Direct Comparison of QTOF and IM-QTOF Sensitivity

Ion Mobility achieves a large increase in sensitivity and quantitative reproducibility

QTOF AutoMS/MS Run
+/- 10ppm mass window

IM QTOF Run
+/- 10ppm mass window
+/- 0.5ms drift window

Comparing precursor EIC’s for the peptide QLLQEEVGPVGVETMR
Comparing Non-Enriched and $^2$H-Enriched Samples
Note Shifts in Isotope Abundances

From IM QTOF Runs
+/- 10ppm mass window
+/- 0.5ms drift window
Conclusions

• Low Drift Field Ion Mobility-Mass Spectrometry has now advanced with standardization and improved accuracy so that can be a powerful tool and assist in the identification of trace analytes in LC/MS applications.

• Ion Mobility offers the possibly to selectively quantity analytes where previously MS/MS techniques were required.

• Important informatics tools are now in place to support both discovery and targeted analytical workflows.