Advantages of Ion Mobility QTOF for Characterization of BioPharma Molecules

Add a New Dimension to your Research Capability with Agilent’s New Drift Ion Mobility QTOF System

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IM-QTOF Instrument Overview

System sensitivity optimized using electrodynamic ion funnels to focus and transmit ions

Ion Mobility resolution optimized while maintaining QTOF performance (mass resolution and accuracy)

Ion Fragmentation can be selected using standard QTOF collision cell (CID)

Bandwidth of QTOF data acquisition and processing channel was increased by 10 fold to match the ion mobility data rates
**Ion Mobility System Design**

**Ionization source**: Ion generation (ESI, AJS, Nano ESI, ChipCube, APCI etc.)

**Front ion funnel**: Efficient ion collection, desolvation and excess gas removal

**Trap funnel**: Ion accumulation and introducing ion packets into drift cell

**Drift cell**: Uniform low field ion mobility allows direct determination of accurate CCS (Ω)

**Rear funnel**: Efficient ion refocusing and introduction into mass analyzer
IM Q-TOF/MS operational modes

- Mobility Separated Precursor Ion Mode
- Mobility Separated All Ions Fragmentation Mode
- Mobility Separated Targeted Precursor Ion Mode
- Mobility Separated Targeted MS/MS Mode

m/z selection ON/OFF

Fragmentation ON/OFF

Mobility separation
Basic Operational Principle of Ion Mobility
For Conventional DC Uniform Field IMS

Electric Field

Stacked ring ion guide gives linear field

$$v = KE \propto \frac{eE}{P \sqrt{T} \Omega}$$
Benefits of Adding Ion Mobility to LC/Q-TOF/MS

Adds Additional Separation Power

- A new dimension of separation for increased mass spectral purity especially for complex mixture analysis

Improves Detection Limits

- Helps to eliminate interference from other analytes and background in the sample mixture
- Efficient ion focusing and transfer through the ion optics maximizes sensitivity for the overall system

Enhances Compound Identification

- Improves confidence in compound identification and ion structure correlation through accurate collision cross section measurements

Provides Native Molecule Structural Information

- Differentiates various protein conformers (native vs. S-S mis-matched)
It’s All About Separation

Chromatography ➔ Ion Mobility ➔ Mass

~ minutes ➔ ~ 60 milli-seconds ➔ ~ 100 µ seconds
Ion Mobility Provides Greater Specificity

Integrated Mass Spectrum:

Mobility-Filtered Mass Spectrum:

S/N increased significantly!

Crude bacterial extract
(Prof. John McLean, Vanderbilt Univ.)
Resolving Structural Sugar Isomers C$_{18}$H$_{32}$O$_{16}$

Raffinose
(DT: 26.68 ms)

Melezitose
(DT: 25.76 ms)

Resolving two isomeric tri-saccharides
Carbohydrates Analysis by IM-MS

Oligosaccharide mixture

(Profs. John McLean and Jody May, Vanderbilt Univ.)
Carbohydrates -- Great complexity by linkage

Source: Blixt et al., PNAS, 2004

4D (MS, DT, RT & TIC) Feature Finding or Library searches
Detecting Miss-formed Disulfide Bonds: Siamycin II

(Pros. John McLean and Jody May, Vanderbilt Univ.)
IM Analysis of Cytochrom C (+8):
(Uniform Drift Tube)

Drift Spectrum: (1533.5668-1560.4894 m/z) (0.214-2.956 min) - CytoC_RF90V_250C_IM_Pos_001.d

S1: Native
S2-S5: Denatured

- RF 90V
- RF 150V

Preserve protein native structures!
- Due to the much lower ions heating effects.

2015 ASMS
IM-QTOF workshop 06/03/15
All charge ions of IgG-2 under denatured condition (+45 to +70) posed the much smaller drift times than the charge ions (+20 to +35) of native IgG-2.
IM Q-TOF Comparison of IgG-1 and IgG-2 under native condition

mAb Structure: (Paul Schnier, Anal. Chem. 2010)

IgG-2 (22+ charge state) has more B isoform
IM Q-TOF/MS analysis of IgG-1 and Herceptin under the native condition
IgG-1 posted slightly lower % of isoform B at its 22+ charge state. Overall, Herceptin has slightly larger CCS values than IgG-1 with the same charge states.
IM Q-TOF Comparison of Rituximab-1 (Innovator) and Rituximab-2 (Biosimilar):

Rituximab-1

Rituximab-2

Mass Spectrum: (32.42-54.12 ms) (1.27-3.04 min) - Rituximab-1_10XD_04.d

Mass Spectrum: (0.00-59.76 ms) (1.308-2.888 min) - Rituximab-2_10XD_04.d

27+
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.
IM Q-TOF Comparison of Herceptin and ADC

Herceptin

ADC
The deconvoluted spectrum showed 8 major drug attachments and the calculated drug antibody ratio (DAR) was ~3.4
Mass Spectrometric Analysis of Bovine Glutamate Dehydrogenase (GDH) Complex (Hexamer)

Native condition

GDH is a hexamer of 500 residues with a molecular weight of ~56 kDa/each

337.65 kDa
IM Q-TOF/MS analysis of Bovine Glutamate Dehydrogenase (GDH) Complex (Hexamer)
## Ion Mobility Q-TOF Comparison

<table>
<thead>
<tr>
<th>Feature</th>
<th>Drift Tube Ion Mobility (Agilent)</th>
<th>Travelling Wave Ion Mobility</th>
<th>Drift Mobility advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility Resolution</td>
<td>Highest (can be &gt; 80) 80cm drift tube (L)</td>
<td>Generally around 30 10cm drift in TriWave, Multi-section device RF fields</td>
<td>Over 2X the IM resolution of T-wave</td>
</tr>
<tr>
<td></td>
<td>Higher voltage (E) No RF fields, Uniform low DC field</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>High efficiency ion funnels - trapping and rear</td>
<td>Step wave lens Pressure barrier between Q and TriWave</td>
<td>10X to 50X better than T-wave</td>
</tr>
<tr>
<td>Collision Cross Section (CCS)</td>
<td>Direct determination of $\Omega$ Low electric field and constant drift</td>
<td>$\Omega$ cannot be directly determined from drift time. Need calibration tables.</td>
<td>1-2% precision</td>
</tr>
<tr>
<td>measurement ($\Omega$)</td>
<td>tube pressure</td>
<td></td>
<td>Much better than Synapt (5-10%)</td>
</tr>
<tr>
<td>Molecular structures</td>
<td>Lower RF fields, less ion heating.</td>
<td>Higher RF fields, tendency for higher fragmentation and ion heating</td>
<td>Lower RF allows preservation of molecular structures</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>IM cycle time 10 to 100 ms is fully compatible with LC and MS duty cycles</td>
<td>Duty cycle 1 to 10 ms. No analytical benefit.</td>
<td>Drift IM is 10 to 50 more sensitive</td>
</tr>
</tbody>
</table>
Summary

- Next generation of IM Q-TOF Technology
- Added dimension of separation based on size, charge and molecular conformation
- Resolve and characterize the complex samples
  -- Increased peak capacity
- Direct determination collision cross sections
- Preservation of molecular structures
## Dual AJS ESI Source Settings: 6560 IM Q-TOF MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td>Dual Agilent Jet Stream</td>
</tr>
<tr>
<td><strong>Acquisition Mode</strong></td>
<td>Positive, Extended (10000 m/z) Mass Range (2 GHz)</td>
</tr>
<tr>
<td><strong>Gas Temp</strong></td>
<td>250 °C</td>
</tr>
<tr>
<td><strong>Gas Flow</strong></td>
<td>5 L/min</td>
</tr>
<tr>
<td><strong>Nebulizer</strong></td>
<td>20 psig</td>
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<tr>
<td><strong>Sheath Gas Temp</strong></td>
<td>275 °C</td>
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<td><strong>Sheath Gas Flow</strong></td>
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<tr>
<td><strong>VCap</strong></td>
<td>4000 V</td>
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<td><strong>Nozzle Voltage</strong></td>
<td>2000V</td>
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<tr>
<td><strong>Fragmentor</strong></td>
<td>400 V</td>
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<tr>
<td><strong>Mass Range</strong></td>
<td>300-10000 m/z</td>
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<tr>
<td><strong>Scan Rate</strong></td>
<td>0.9 frames/s</td>
</tr>
<tr>
<td><strong>IM Trap Fill Time</strong></td>
<td>50,000 us</td>
</tr>
<tr>
<td><strong>IM Trap Release Time</strong></td>
<td>300 us</td>
</tr>
</tbody>
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