Development of a New Uniform-Field Ion Mobility Quadrupole Time-of-Flight Mass Spectrometer

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What Does Ion Mobility Bring to Mass Spectrometry?

**Separation**
- 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

**Confirmation**
- Improves confidence in compound identification and ion structure correlation through accurate collision cross section measurements
IMS Q-TOF 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 source maintained at ground potential (no voltage offset)

Uniform low static electric field drift ion mobility

- 80 cm long, approximately 20 V/cm with 4 Torr Nitrogen
- Mobility resolution approaches theoretical limit
- Minimizes ion excitation or heating (helps to maintain ion structures and conformations)

Uniform low field ion mobility allows direct determination of accurate CCS (Ω)

Ion fragmentation using CID enables an all ion experiment with the precursor ions separated by drift times and product ions analyzed by high resolution QTOF
Ion funnel technology brings sensitivity
It’s All About Separation

Chromatography → Ion Mobility → Mass

minutes   60 milli-seconds   160 µ-seconds
Resolving Structural Sugar Isomers $C_{18}H_{32}O_{16}$

Resolving two isobaric trisaccharides

$m/z$ drift time (ms)

melezitose

raffinose

Resolving two isobaric trisaccharides

$m/z$ 527 $M+Na^+$ 528 529
All Ion MS using 20 Volt Fragmentation Energy

Collective drift spectrum includes all ions generated from 3 compounds
Drift Time Separated Fragmentation

Simultaneous separation and fragmentation for colchicine
Collision Cross Section Correlates with Structure

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drift time (ms)</th>
<th>Ω (lit. Å²)</th>
<th>Ω (obs. Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ondansetron</td>
<td>20.09</td>
<td>172.7</td>
<td>171.0</td>
</tr>
<tr>
<td>Colchicine</td>
<td>23.03</td>
<td>196.2</td>
<td>196.2</td>
</tr>
<tr>
<td>Reserpine</td>
<td>29.89</td>
<td>254.3</td>
<td>249.1</td>
</tr>
</tbody>
</table>

Drift tube IM provides a direct accurate method to calculate collision cross sections $\Omega$

Mason-Schamp equation

$$
\Omega = \frac{(18\pi)^{1/2}}{16} \frac{z e}{(kT)^{1/2}} \left[ \frac{1}{m_i} + \frac{1}{m_B} \right]^{1/2} t_d E \frac{760}{L} \frac{T}{P \cdot 273.2} \frac{1}{N}
$$

Variables: Temperature, Pressure, Drift voltage, Drift tube length
Ion mobility separation of MMA and SA

<table>
<thead>
<tr>
<th></th>
<th>MCCS Value:</th>
</tr>
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<tbody>
<tr>
<td>MMA</td>
<td>152.3 Å</td>
</tr>
<tr>
<td>SA</td>
<td>167.8 Å</td>
</tr>
</tbody>
</table>

Counts

Drift time in ms

$m/z$ 10^5

Counts

m/z
BSA Digest with LC Separation

t_D = 20.26 ms
m/z = 435.9099

Precursor ion spectrum

\( t_R = 14.36 \) min
BSA Digest All Ion Fragmentation with LC Separation

$m/z = 435.9099 \ [HLVDEPQNLIK + 3H]^{3+}$

Total fragment ion spectrum
30 V

Drift separated fragment ion spectrum
t_D = 20.26 ms
HSA Digest Targeted MS/MS with LC Separation

$m/z = 547.3174$ (HSA) 
$[KVPQVSTPTLVEVSR + 3H]^{3+}$

Conformers
$t_D = 21.14\ ms\ 23.08\ ms$
IM Ubiquitin Charge States

Structural studies of proteins possible with CCS calculation
IM Ubiquitin Charge States
Cross Section Calculation of Ubiquitin Charge States

Automated collision cross section calculation without the use of calibration curves

### Cross Section Calculation of Ubiquitin Charge States

<table>
<thead>
<tr>
<th>Ion</th>
<th>Charge State</th>
<th>CCS experimental (Å²)</th>
<th>CCS literature (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+5H]^5+</td>
<td>5</td>
<td>1196</td>
<td></td>
</tr>
<tr>
<td>[M+6H]^6+</td>
<td>6</td>
<td>1431, 1658</td>
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</tr>
<tr>
<td>[M+7H]^7+</td>
<td>7</td>
<td>1755, 1886</td>
<td>1910</td>
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<tr>
<td>[M+8H]^8+</td>
<td>8</td>
<td>1966</td>
<td>1990</td>
</tr>
<tr>
<td>[M+9H]^9+</td>
<td>9</td>
<td>2008</td>
<td>2090</td>
</tr>
<tr>
<td>[M+10H]^10+</td>
<td>10</td>
<td>2114, 2197</td>
<td>2200</td>
</tr>
<tr>
<td>[M+11H]^11+</td>
<td>11</td>
<td>2239, 2348</td>
<td>2340</td>
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<tr>
<td>[M+12H]^12+</td>
<td>12</td>
<td>2412, 2511</td>
<td>2480</td>
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<tr>
<td>[M+13H]^13+</td>
<td>13</td>
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<td>2600</td>
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<tr>
<td>[M+14H]^14+</td>
<td>14</td>
<td>2680, 2726</td>
<td></td>
</tr>
</tbody>
</table>

Automated collision cross section calculation without the use of calibration curves

Summary

Added dimension of separation based on size, charge and ion structure

Resolve and characterize complex samples using LC/IM/MS analysis while maintaining high sensitivity

Direct method to calculate accurate collision cross sections for added analytical confirmation