Applications of novel acquisition modes and instrument geometries in Time-of-Flight Mass Spectrometer for Targeted Quantitation

Mark Wrona, Yun Alelyunas, Jayne Kirk, Martin Palmer, Nick Tomczyk, Russell Mortishire-Smith

Diego Rodriguez Cabaleiro
EBF 2015

diego_rodriguez_cabaleiro@waters.com
Overview

- TQ vs HRMS systems

- TOF Acquisition modes
  - MS
  - MSMS
  - IMS-MSMS (Ion Mobility Spectrometry)

- The additional benefits of microfluidics
Overview

- TQ vs HRMS systems

- TOF Acquisition modes
  - MS
  - MSMS
  - IMS-MSMS (Ion Mobility Spectrometry)

- The additional benefits of microfluidics
Detectors TQ vs HRMS

- Best sensitivity
- Best LDR (4-5 orders)
- Software for quick Met. Dev.
- Stablisied across departments
- Selectivity based on fragmentation

- Best sensitivity in full scan
- Good dynamic range (3-4 orders)
- Full scan or targetted quantitation

- Selectivity
  - Exact Mass, IMS, Fragments
Evolution of TOF Sensitivity

QTof Sensitivity Improvements

- Xevo G2-XS
- XevoG2-S
- Synapt G2-S
- Synapt G2
- QTof Premier
- QTof II
- Micromass QTof

Date:
- October 1995
- April 2001
- January 2004
- July 2009
- April 2012
- June 2014
Overview

- TQ vs HRMS systems

- TOF Acquisition modes
  - MS
  - MSMS
  - IMS-MSMS (Ion Mobility Spectrometry)

- The additional benefits of microfluidics
How do we select a peak using m/z?

We can choose how to sample a peak

What width we choose is related (inversely) to the resolution of the instrument and the noise around the peak of interest

Mass Tolerance:

$$m/z = 386.255 \pm 30 \text{ ppm}$$
Buspirone in Plasma (10 pg/mL)
Adjusting Selectivity of PPM tolerance

For this assay, a 30 or 50 ppm window is optimal

©2015 Waters Corporation
XIC tolerance to S/N Relationship Plot (Buspirone in Plasma)

Accuracy Mass

Nominal Mass

<table>
<thead>
<tr>
<th>ppm</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>8.9</td>
</tr>
<tr>
<td>100</td>
<td>15.1</td>
</tr>
<tr>
<td>50</td>
<td>20.0</td>
</tr>
<tr>
<td>30</td>
<td>21.3</td>
</tr>
<tr>
<td>20</td>
<td>19.6</td>
</tr>
<tr>
<td>10</td>
<td>14.1</td>
</tr>
<tr>
<td>5</td>
<td>12.0</td>
</tr>
</tbody>
</table>

30 ppm
Ion Mobility Spectrometry

- Separation based on size and shape
- Orthogonal separation different from chromatographic and mass resolution
New distinctive parameter = CCS

- Unique physicochemical property of each ion
  - chemical structure
  - 3-dimensional conformation
LC-IM-MS
Greater peak capacity

Resolution in 2 dimensions = Retention X Mass

Resolution in 3 dimensions = Retention X Mobility X Mass
How do we select a peak using CCS?

We can set a tolerance around the CCS and choose to exclude isobaric interferences (NOISE).

Buspirone
Buspirone in Plasma (100 pg/mL) Adjusting Selectivity of CCS tolerance

For this assay 10% is optimal
Combining Selectivity Factors
CCS + m/z

Both ppm window and IMS window result in S/N hotspot (20-30ppm and 10% IMS)

Buspirone in Plasma
HDMSE, 100 pg/mL
Combining Selectivity Factors
CCS + m/z + Quadrupole

Additional 2-3 fold further enhancement in S/N with Quad

Buspirone in Plasma
HDMSMS, 100 pg/mL
Applying IMS principles to other compounds in Plasma

Clopidogrel (late eluter)  Oxytocin (+2 Peptide)

20 ppm  20 ppm
No IMS  + IMS

IMS Cleanup is generically applicable  (RMS Noise Calculated)

©2015 Waters Corporation
How do we do IMS/CCS Filtering and mass filtering together?

- Look at the RT where our compound elutes
- View the m/z vs CCS/IMS Plot

- Isolate the peak(s) of interest
  - smallest ppm and IMS window
Peptide Quantitation
Dealing with extra complexity

Every peptide can be tracked by MASS (m/z) AND CCS
IMS Filtering of Peptide Quantitative Data

![Chromatograms](image)

- **100 ppm**
- **100 ppm + 8% IMS**

©2015 Waters Corporation
Overview

- TQ vs HRMS systems

- TOF Acquisition modes
  - MS
  - MSMS
  - IMS-MSMS (Ion Mobility Spectrometry)

- The additional benefits of microfluidics
Advantages of Integrated Microfluidics and Micro-Flow ESI

- Improved Sensitivity over 2.1
  mm scale: **10-20X**
  - Improved Sampling Efficiency
  - Improved Ionization Efficiency
  - Reduced Ion Suppression
Micro-Flow ESI vs Analytical-scale ESI
Charge State Distribution Shift

- Sharper CS Distribution
- Higher Charge States
- Increased Simplicity
- Increased Sensitivity

Standard Flow ESI
0.450 mL/min

Micro-Flow ESI
5 μL/min

Generic Affinity Capture of Humira from Rat Plasma

Humira Linear Range:
25-500 µg/mL

Deconvoluted Spectra

©2015 Waters Corporation
Trastuzumab ADC (De-Glycosylated)
High Sensitivity CSD Spectra

Trastuzumab MW:
145165.1575 g/mol

Linker and Drug:
958.5322 g/mol

1 Drug + 1 Antibody:
146123.6 g/mol

©2015 Waters Corporation
Trastuzumab ADC (De-Glycosylated) Deconvolution on ionKey/MS

**Trastuzumab MW:**
145165.1575 g/mol

**Linker and Drug:**
958.5322 g/mol

**1 Drug + 1 Antibody:**
146123.6 g/mol

!![Graph depicting mass spectrometry data with peaks at specific masses.]

- **Trastuzumab MW:** 145165.1575 g/mol
- **Linker and Drug:** 958.5322 g/mol
- **25.0 ng (on-column):** 1.88e5

Mass range: 144000 to 152000

©2015 Waters Corporation
Soon coming!

Join our ADC and Protein Therapeutic Symposium in April 2016!

April 05-07 - Wilmslow, UK

**Waters Corporation** invites you to a 3 days Symposium.
Conclusions

- **Selectivity can be exploited through**
  - Precursor selection – Quadrupole
    - Full scan modes vs MSMS or Tof MRM (not shown in this ppt)
  - Resolution – Instrument @ 32000 (for sensitivity)
  - Mass Tolerance of Peak, +/- ppm or mDa
  - CCS Tolerance of Peak, +/- drift time

- **Enabled by simple software tools to collect, process and use data effectively**

- **IonKey offers an extra step in sensitivity**
Acknowledgements

- Nick Tomczyk
- Martin Palmer
- Yun Alelyunas
- Russell Mortishire-Smith
- Henry Shion

- Craig Dorschel
- Jennifer Simeone
- Catalin Doneanu
- Stephen McDonald
- Alistair Wallace

- Keith Richardson
- Jason Wildgoose
- Kevin Giles
- Paul Rainville
- Martin Green
- Kevin Cook
- Alan Millar
- Diane Diehl
- Simon Cubbon
- Steve Cubbedge
- Gordon Kearney

- Emma Marsden-Edwards
- Jim Henriksen
- Jeff Robataille
- Rong Xie
- Logan Umberger
- Kelly Doering
- Beth Hazell
- Weibin Chen
- Liuxi Chen

©2015 Waters Corporation