Time of Flight  The Power of Accurate Mass
What is "Accurate Mass"?

- Mass measurements accurate to several decimal places (rather than "nominal" or "unit" mass measurements of $± 0.1 \text{ Da}$)
- Made possible by higher resolution mass analyzers
- Formerly limited to magnetic sector instruments
- Now available with Time of Flight (TOF) and Fourier Transform (FT) instruments (FT-MS and "orbitrap")
- Most Common Uses:
  - Allows confirmation of molecular formula for proposed compounds
  - Allows calculation of possible molecular formulae for unknowns
  - Allows rapid selective and specific screening for target compounds
Figures of Merit for Accurate-Mass Instrument Performance

Resolution = \( \frac{m/z}{W_{1/2}} \)

\[ e.g., \quad \frac{922.0092}{0.087} = 10,597 \]

Mass accuracy (mass error)

\[ \text{Mass accuracy} = \left( \frac{\text{measured} - \text{theory}}{\text{theory}} \right) \times 10^6 \]

\[ (1000.001-1000.000)/1000) \times 10^6 = 1 \text{ ppm} \]

Therefore: 0.001 Da (1 mDa) error

@ MW 300 Da is approximately 3 ppm
Mass Resolving Power
Why resolution contributes to accurate mass: narrow mass peak widths allow accurate centroiding

Arb. Units

<table>
<thead>
<tr>
<th>m/z</th>
<th>118</th>
<th>119</th>
<th>120</th>
</tr>
</thead>
</table>

R=4,700

(Masses from standard Agilent tune mix solution)

<table>
<thead>
<tr>
<th>m/z</th>
<th>622</th>
<th>623</th>
<th>624</th>
</tr>
</thead>
</table>

R=9,300

<table>
<thead>
<tr>
<th>m/z</th>
<th>2722</th>
<th>2724</th>
</tr>
</thead>
</table>

R=13,500
How Much Accuracy in Needed for Identification?
(new concept for LC/MS: mass accuracy in "ppm")

Reserpine (C$_{33}$H$_{40}$N$_2$O$_9$) has a protonated ion at m/z 609.28066

Single quadrupole MS reports mass to +/- 0.1 = 165 ppm

Number of possible formulas using only C, H, O & N, at various mass errors:

- 165 ppm, 209 (single quadrupole resolution)
- 10 ppm, 13 (older TOF instruments)
- 5 ppm, 7
- 3 ppm, 4
- 2 ppm, 2

Accurate mass limits the number of possible formulae for a given m/z measurement.
Agilent Time-of-Flight (TOF) LC/MS
Agilent’s orthogonal TOF design
A Time-of-Flight "Scan"

1. Pulse a packet of ions up flight tube (a transient)
2. Measure ion arrivals at detector each nanosecond
3. Large number of data points in each transient
4. Sum 2000 – 10000 transients into one scan
5. Produces spectra with excellent ion statistics (both center of mass and abundance)

Flight time is converted to m/z against calibration:
e.g., 20.010 µsec → m/z 121.0508

See an animation at:
http://www.chem.agilent.com/cag/other/lc-msd-tof.wmv
Benefits of Atmospheric Pressure Ionization (API)

Ionizing difficult compounds (API), no derivatization

Accurate mass measurement (four decimal places, < 2ppm)

High resolution for distinguishing near-isobaric masses:
- 5000 at low mass, > 10,000 at higher masses

Fast scanning
- >10 spectra/sec, many spectra across even a narrow UPLC peak

Excellent sensitivity for full-scan data (some instruments)

Wide mass range when needed

All-scan, all-the-time data allows retrospective search for compounds not originally sought.
Forensic Tox Compound Analysis
The Value of the Agilent Solution

Robust hardware
Sample Preparation Products
HPLC and UHPLC Columns
Automated software workflows
Comprehensive Accurate Mass Libraries
LC/MS Application Kits
First-class service and application support
Agilent TOF Screening Solution

Agilent 62** TOF
- 20K resolution
- 5 decades in-scan
- Low Pg Sensitivity
Qualitative Screen Report – Compound 9 (Cocaine)

![Graphs and tables showing molecular ion spectra of Cocaine.]

**Peak List**

<table>
<thead>
<tr>
<th>m/z</th>
<th>z</th>
<th>Abund</th>
<th>Name</th>
<th>Formula</th>
<th>Ion</th>
<th>Score (DB)</th>
<th>Hits (DB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304.154</td>
<td>1</td>
<td>101926</td>
<td>Cocaine</td>
<td>C17H21N04</td>
<td>(M+H)^+</td>
<td>99.68</td>
<td>3</td>
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<tr>
<td>305.158</td>
<td>1</td>
<td>19552</td>
<td></td>
<td>C17H22N04</td>
<td>(M+H)^+</td>
<td></td>
<td></td>
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<tr>
<td>306.160</td>
<td>1</td>
<td>2441</td>
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<td>(M+H)^+</td>
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<tr>
<td>307.1638</td>
<td>1</td>
<td>407</td>
<td></td>
<td>C17H22N04</td>
<td>(M+H)^+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results For Compound 9
– PCD Search Confirmed as Cocaine

EIC of Cocaine
Mass 303.1471 (50 ng/ml)

0.99931 linear correlation
Using TOF for Quantitation
Agilent Personal Compound Database - Forensics/Toxicology

Database containing >800 Forensic/Doping Analytes

- Automated Reporting
- Controlled Substances
- Explosives
- WADA Exclusion List
- Accurate Mass Confirmation
- Retention time Confirmation
- Spectral Match Scoring (based on Isotopic Pattern & Accurate Mass spacing)
Isobars – The Need for Adequate Chromatographic Separation.

Isobaric Mass Ions – “Defined as different ions that have identical mass”

Morphine (C\textsubscript{17}H\textsubscript{20}NO\textsubscript{3})
\[ [M+H]^+ = 286 \text{ m/z} \]

Hydromorphone (C\textsubscript{17}H\textsubscript{20}NO\textsubscript{3})
\[ [M+H]^+ = 286 \text{ m/z} \]

Codeine (C\textsubscript{18}H\textsubscript{22}NO\textsubscript{3})
\[ [M+H]^+ = 300 \text{ m/z} \]

Hydrocodone (C\textsubscript{18}H\textsubscript{22}NO\textsubscript{3})
\[ [M+H]^+ = 300 \text{ m/z} \]

Methamphetamine (C\textsubscript{10}H\textsubscript{16}N)
\[ [M+H]^+ = 150 \text{ m/z} \]

Phentermine (C\textsubscript{10}H\textsubscript{16}N)
\[ [M+H]^+ = 150 \text{ m/z} \]
Isobaric Pairs

- Morphine
- Codeine
- Hydromorphone
- Hydrocodone
Target Compound Screening Results: RT and mass matches

Narrow-mass EIC for compound m/z

Spectrum with overlaid structure, theoretical isotope abundance pattern
TOF screen results: opiates (isobars are separated)

- morphine
- codeine
- hydrocodone
- oxymorphone
- hydromorphone
- oxycodone
- hydrocodone
- 6-AM
ALL IONS
ADDING FRAGMENT CONFIRMATION
All Ions MS/MS - What is it?

Step 1: Fragmentation without Isolation on TOF or Q-TOF

a) First Scan: Low fragmentation energy to analyze precursors
b) Second Scan: High fragmentation energy to analyze fragment ions
All Ions MS/MS - What is it?

Step 2: Software Extracts, Correlates, and Confirms

a) Find by Formula uses library to extract precursors from low energy MS channel

b) All Ions MS/MS uses library to extract fragments from high energy channel, gives coelution score

Software automatically matches precursors with fragment ions:
Coelution Plot with Coelution Score
Evaluation of All Ions MS/MS Automated Workflow
Untargeted Drug Screening in Postmortem Blood

Step 1: Find by Formula Extracts Precursors using PCDL database
Step 2: Extract Corresponding Fragment Ions

Cpd 11: zopiclone: +ESI HighE Scan (4.862-5.023 min, 20 Scans) O4406_alpra+meth.d...

Fragments from PCDL
Step 3: Precursor and Fragment Correlation/Coelution

Overlaid Precursor and Fragment Ion Chromatograms

Coelution Plot
Differentiation and Identification of Isomers
Untargeted Drug Screening in Postmortem Blood

Coeluting with ~50x Concentration Difference
Differentiation and Identification of Isomers
Untargeted Drug Screening in Postmortem Blood

Similar precursor \( m/z \) values and overlapping isotopic clusters
Compound Confirmation-Methadone
Untargeted Drug Screening in Postmortem Blood

Red outline overlays theoretical isotopic pattern and abundance with acquired precursor data for methadone

Methadone confirmed with seven qualified fragments
Red outline overlays theoretical isotopic pattern and abundance with acquired precursor data for alprazolam.

Alprazolam confirmed with two qualified fragments, despite low concentration.
High Productivity: Compound Details View

- Co-elution Plot
- Overlaid Chromatograms
- Compound List
- Compound ID Results
- Low Energy (MS) Spectrum
- High Energy Fragment Spectrum
All Ions on Random Dollar Bill for Cocaine
What do you think?