Welcome to our E-Seminar:

The API-TOF Advantage – Routine Mass Accuracy and High Resolution
Agilent LC/MSD TOF
High Resolution - Accurate Mass by LC/ESI-TOF

Accurate Mass provides

• empirical formula
• identification and
• Confirmation

• Added Selectivity
• Resolution to separate isobaric compounds
• All spectra automatically calibrated for mass peaks within 5 ppm of exact mass
How Much Accuracy in Needed?

Reserpine \((C_{33}H_{40}N_{2}O_{9})\) has a protonated ion at 609.28066

Single quad reports mass to +/- 0.1 = 165 ppm

Number of possible formulas using only C, H, O & N:

<table>
<thead>
<tr>
<th>Accuracy</th>
<th>Formulas</th>
</tr>
</thead>
<tbody>
<tr>
<td>165 ppm</td>
<td>209</td>
</tr>
<tr>
<td>10 ppm</td>
<td>13</td>
</tr>
<tr>
<td>5 ppm</td>
<td>7</td>
</tr>
<tr>
<td>3 ppm</td>
<td>4</td>
</tr>
<tr>
<td>2 ppm</td>
<td>2</td>
</tr>
</tbody>
</table>

*Accurate mass reduces risk of investing effort on the wrong molecule*
The Potential TOF Advantage

Routine Mass Accuracy < 3ppm
  • No user interaction required
  • Delivered over broad dynamic range
  • Immune to broad swings in laboratory environment

Resolution  > 4000 at low mass, > 10,000 at higher masses

Fast scanning (> 5/second) over broad mass range
Confirmation with Accurate Mass

Accurate mass determination provides a level of selectivity independent of fragments from an MS/MS experiment.

Determination of empirical formula

An accurate mass measurement does not guarantee a unique empirical formula at 5 ppm confidence level.

Usually other information can be combined with TOF data to raise certainty.
Reserpine Confirmation

14 double bond equivalents (DBE)

Consistent isotope ratios

Dial 1-816-650-0621 for e-Seminar Audio
Agilent Design Optimizes Ion Transmission and Mass Resolving Power

- Two Stage Ion Mirror
- Low CTE Inner Flight Tube
- Optically Coupled Ion Detector
- 5 Stage Vacuum System
- Agilent Orthogonal Spray Source(s)
- Beam cooling and guidance
- Effective Flight Path Length of 2.0m

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A Time of Flight Scan

1. Pulse ions every 100 microseconds
2. Measure at detector each nanosecond
3. 100,000 data points in each transient
4. Sum 2000 – 10000 transients into one scan
5. Produces spectra with excellent ion statistics

20 µsec – m/z 118
46 µsec – m/z 622
90 µsec – m/z 2421
Some Key Hardware Design Features

Uses Agilent orthogonal spray sources and ion optics for superior sensitivity and flow rate range

Flight tube designed with minimal coefficient of thermal expansion

Temperature sensitive electronic components placed in temperature controlled environment

ADC digitization for maximum mass accuracy over broad dynamic range

Use CDS and dual sprayer for internal reference mass correction
Fundamentals of TOF Mass Assignment

Agilent Goal: Routine high mass accuracy

Hardware designed for highly stable mass measurement

Hardware and software for transparent internal reference mass correction

Mathematical error in mass assignment & calibration mass accuracy within ~1 ppm for peak finder & mass assignment algorithms

- Traditional algorithms can have errors > 10 ppm with discrete data.
- New algorithm has intrinsic mass errors < 1 ppm with discrete data and non-symmetric peaks
TDC versus ADC

Time to Digital Conversion (TDC) only measures time of arrival of first ion at a given m/z value

More sample means more ions means earlier arrival

Requires higher acquisition rate than ADC and peak intensity matching to accurately assign mass

Reduced dynamic range

Analog to Digital Conversion (ADC) records time and number of ions arriving

Sample concentration does not impact (maximum) arrival time

Provides wider dynamic range
Understanding the TDC and ADC

1. Single ion arrives at the detector. The resulting signal crosses a preset TDC threshold and the arrival time is recorded.

2. This time the sample is more concentrated and two ions arrive slightly spaced in time as determined by the instrument’s resolving power. The yellow line is the resulting signal.

But, the TDC threshold triggers off the first ion and the arrival of the second ion is not detected. This causes a shift to shorter arrival times for higher signal levels.

3. ADC acquisition makes a conversion each 0.001 usec of the total signal. ADC systems do not drop ions or suffer from “dead time” effects.

Ion Flight Time: Is the centroid of all detected ions
Break Number 1

For questions, at break please dial 1 on your phone, or type onto the chat screen at any time during the presentation.

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Ref. Mass Correction with $m/z$ 118 and 922

Ref. Mass
$m/z$ 922.010

“Unknown”
$m/z$ 582.319
1.6 ppm error

100,000 Arb.Units
250 Arb.Units
400x

Theoretical Mass | Abundance Arb. Units | Error (ppm)
--- | --- | ---
487.73253 | 1316 | 0.61
450.2364 | 5304 | 1.59
722.81666 | 6611 | 0.64
417.21191 | 6910 | 2.66
512.25457 | 8978 | 1.66
464.25036 | 12534 | 0.73
519.21717 | 575 | 0.06
653.3617 | 21881 | 1.38
435.91023 | 555 | -0.28
501.79513 | 10141 | -0.04
820.47251 | 842 | 0.25
526.58871 | 3100 | 0.35
674.32258 | 1881 | -1.4
480.60877 | 23885 | -2.52
582.31897 | 49027 | 1.57
710.84248 | 3863 | -1.16
627.97323 | 13881 | 0.55
474.23075 | 24508 | 1.13
507.81333 | 11016 | 0.68
740.40136 | 9671 | -0.12
784.37501 | 643 | -3.49

Unknown
784.37501 | 643 | -3.49

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Automatic Internal Referencing

Analytical Sprayer

Reference Sprayer
Mass Accuracy and Dynamic Range

XIC of +TOF MS: 609.3 amu from 10pg_res4.wiff
Max. 2395.0 cps.

10 pg Reserpine, external calibration
Mass assignment = 609.2809
Error = 0.4 ppm
Spectral Saturation and Mass Accuracy

XIC of +TOF MS: 409.3 amu

Max. 3.7e6 cps.

Actual mass 409.2598
Automatic Thresholding of Spectra

The graph shows the detection of a compound at 311.09 amu, with a highlighted peak indicating the compound of interest. The y-axis represents the intensity of the signal, and the x-axis represents time in minutes. The y-axis scale is logarithmic, ranging from 10^0 to 10^7, highlighting the dynamic range of the detected signals.
Results with 50% Threshold

Mass Accuracy Using Threshold

Ion Abundance vs. Mass Error, ppm

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Mass Resolving Power

Masses from standard Agilent tune mix solution

Arb. Units

R=4,700

118  119  120

m/z

R=9,300

622  623  624

m/z

R=13,500

2722  2724

m/z
TOF Software User Interface

32-bit client server based with modular components

Four separate panes for instrument status, real-time display, method editing and worklist management

Each pane may be sized, displayed or hidden to meet current user needs

Extensive use of right mouse click similar to other Windows applications
Easy Optimization and Calibration

Autotune
Quicktune
Checktune
Calibrate
CheckTune Report – Expanded View

Mass Calibration Table

<table>
<thead>
<tr>
<th>Expected Mass</th>
<th>Actual Mass</th>
<th>Height</th>
<th>Resolution</th>
<th>Resolution Limit</th>
<th>FWHM</th>
<th>Mass Error (m/z)</th>
<th>Mass Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>118.0863</td>
<td>118.0865</td>
<td>92589.0</td>
<td>4333.0</td>
<td>&gt;= 4000</td>
<td>.0273</td>
<td>.0002</td>
<td>1.7</td>
</tr>
<tr>
<td>322.0481</td>
<td>322.0483</td>
<td>184246.0</td>
<td>6726.0</td>
<td>&gt;= 5000</td>
<td>.0479</td>
<td>.0002</td>
<td>.6</td>
</tr>
<tr>
<td>622.0290</td>
<td>622.0294</td>
<td>76694.0</td>
<td>8879.0</td>
<td>&gt;= 6000</td>
<td>.0701</td>
<td>.0004</td>
<td>.7</td>
</tr>
<tr>
<td>922.0998</td>
<td>922.0998</td>
<td>58372.0</td>
<td>10350.0</td>
<td>&gt;= 7000</td>
<td>.0891</td>
<td>.0000</td>
<td>0</td>
</tr>
<tr>
<td>1221.9906</td>
<td>1221.9904</td>
<td>93518.0</td>
<td>11389.0</td>
<td>&gt;= 8000</td>
<td>.1073</td>
<td>-.0002</td>
<td>-2</td>
</tr>
<tr>
<td>1521.9715</td>
<td>1521.9718</td>
<td>101175.0</td>
<td>11734.0</td>
<td>&gt;= 9000</td>
<td>.1297</td>
<td>.0003</td>
<td>.2</td>
</tr>
<tr>
<td>1821.9523</td>
<td>1821.9531</td>
<td>163007.0</td>
<td>12063.0</td>
<td>&gt;= 9500</td>
<td>.1510</td>
<td>.0008</td>
<td>.4</td>
</tr>
<tr>
<td>2121.9332</td>
<td>2121.9335</td>
<td>135606.0</td>
<td>12312.0</td>
<td>&gt;= 10000</td>
<td>.1724</td>
<td>.0003</td>
<td>.1</td>
</tr>
<tr>
<td>2421.9140</td>
<td>2421.9141</td>
<td>141159.0</td>
<td>12590.0</td>
<td>&gt;= 10000</td>
<td>.1924</td>
<td>.0001</td>
<td>.1</td>
</tr>
<tr>
<td>2721.8948</td>
<td>2721.8963</td>
<td>118754.0</td>
<td>13203.0</td>
<td>&gt;= 10000</td>
<td>.2082</td>
<td>.0004</td>
<td>.2</td>
</tr>
</tbody>
</table>

Underlined values (in red) do not conform to the limits imposed
Break Number 2

For questions, at break please dial 1 on your phone, or type onto the chat screen at any time during the presentation.
Identification of Unknowns

- Empirical formula determination can greatly assist the identification of unknowns

- The list of possibilities is smaller with mass >250
  - At 5 ppm accuracy the possibilities are few at low mass
  - The use of isotope patterns can reduce the list of possibilities even further by specifying or eliminating atoms, determining approximate carbon content etc.

- At > 250 mass units, 3 ppm accuracy reduces the possibilities
Identifying Trace Contaminants in Pharmaceuticals

100 – fold magnification
Major Contaminant

Use knowledge of major product and synthesis process to reduce possible formula
Using In-Source CID Information

Sulfachloropyridazine - $\text{C}_{10}\text{H}_9\text{N}_4\text{O}_2\text{SCl}$

Fragmentor 220 volts

$[\text{C}_6\text{H}_6\text{NO}_2\text{S}]^+$

$\text{MH}^+$

$\text{M}+\text{Na}^+$

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Using Adduct Information

\[ C_{73}H_{108}O_{12} \] - MW 1176.7841

\[ M+Na^+ \]

\[ M+NH_4^+ \]

- 0.5 ppm error

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Confirmation with Accurate Mass

Accurate mass determination provides a level of selectivity independent of fragments from an MS/MS experiment

Determination of empirical formula

• An accurate mass measurement is NOT exclusive of all empirical formulas at 5 ppm confidence level

• A proposed compound ID or structure IS exclusive

• That is there is only one empirical formula for a proposed compound- CONFIRMATION
Empirical Formula Conformation Report

Input one or more formulas for each sample

Edit method to choose adducts, thresholding, report destination

System calculates target mass, extracts ions and calculates mass error

Reports can be printed or viewed from Internet Explorer

Complete Worklist results output in CSV format
Expanded View of Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Target Mass (amu)</th>
<th>Measured Mass (amu)</th>
<th>Mass Error (m/z)</th>
<th>Mass Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]^+</td>
<td>59329.42</td>
<td>285.02075</td>
<td>285.02019</td>
<td>-.00056</td>
<td>-1.97</td>
</tr>
<tr>
<td>[M+Na]^+</td>
<td>2799.10</td>
<td>307.00270</td>
<td>307.00248</td>
<td>-.00021</td>
<td>-.70</td>
</tr>
</tbody>
</table>
Quantification with ESI-TOF

The high resolution achieved (>5000 at $m/z=250$ and 10,000 at $m/z=1000$) increases selectivity tremendously!

The dynamic range is greatly enhanced with the use of analog to digital conversion (ADC)!

All FULL SCAN SPECTRA are autocalibrated in the saved data file with the seamless reference mass addition.
Drugs of Abuse-LC/ESI-TOF

TIC of +TOF MS: from drug screen

Max. 8.2e6 cps.

Intensity, cps

Time, min

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<table>
<thead>
<tr>
<th>Rt (min)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.37</td>
<td>hydrocodone</td>
</tr>
<tr>
<td>3.40</td>
<td>unknown with M+H =166.1221</td>
</tr>
<tr>
<td>3.64</td>
<td>Oxycodone</td>
</tr>
<tr>
<td>3.70</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>3.81</td>
<td>Codeine</td>
</tr>
<tr>
<td>3.94</td>
<td>Methamphetamine</td>
</tr>
<tr>
<td>5.41</td>
<td>Cocaine</td>
</tr>
<tr>
<td>6.12</td>
<td>CE</td>
</tr>
<tr>
<td>6.36</td>
<td>PCP</td>
</tr>
<tr>
<td>8.04</td>
<td>Propoxyphene</td>
</tr>
<tr>
<td>8.18</td>
<td>Methadone</td>
</tr>
<tr>
<td>8.81</td>
<td>Alprazolam</td>
</tr>
<tr>
<td>9.00</td>
<td>Nordiazepam</td>
</tr>
<tr>
<td>9.46</td>
<td>Methaqualone</td>
</tr>
<tr>
<td>10.86</td>
<td>Diazepam</td>
</tr>
</tbody>
</table>

* Special thanks to the Lucas Zarwell of the Washington D.C. Medical Examiner’s Office for kindly providing the reference materials.
Extracted Ion Chromatogram of m/z 300 and Spectra of Two Isomers

- XIC of +TOF MS: 300.0 to 300.2 amu
  - Max. 6.9e5 cps.

- Hydrocodone
- Codeine

+TOF MS: 3.786 to 3.844 min
  - 299.0 299.1 300.0 301.0
  - 4.0e4 8.0e4 1.2e5 1.6e5

+TOF MS: 3.363 to 3.401 min
  - 299.0 300.0 301.0 302.0
  - 4.0e4 8.0e4 1.2e5 1.6e5
Drugs of Abuse Mass Accuracy at 3 orders of Magnitude Concentration

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amphetamine</th>
<th>Methamphetamine</th>
<th>Hydrocodone</th>
<th>Codeine</th>
<th>Oxycodone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal m/z</td>
<td>136.1</td>
<td>150.1</td>
<td>300.15</td>
<td>300.15</td>
<td>316.15</td>
</tr>
<tr>
<td>Conc (ng-injected)</td>
<td>measured</td>
<td>measured</td>
<td>measured</td>
<td>measured</td>
<td>measured</td>
</tr>
<tr>
<td></td>
<td>error (ppm)</td>
<td>error (ppm)</td>
<td>error (ppm)</td>
<td>error (ppm)</td>
<td>error (ppm)</td>
</tr>
<tr>
<td>50</td>
<td>-4.71</td>
<td>-3.01</td>
<td>0.37</td>
<td>0.37</td>
<td>0.04</td>
</tr>
<tr>
<td>50</td>
<td>-4.53</td>
<td>-3.03</td>
<td>0.47</td>
<td>0.37</td>
<td>-0.16</td>
</tr>
<tr>
<td>50</td>
<td>4.53</td>
<td>-3.05</td>
<td>0.3</td>
<td>0.3</td>
<td>-0.75</td>
</tr>
<tr>
<td>25</td>
<td>3.53</td>
<td>-2.49</td>
<td>1.17</td>
<td>1.60</td>
<td>0.27</td>
</tr>
<tr>
<td>*2.5</td>
<td>-5.57</td>
<td>-2.88</td>
<td>0.60</td>
<td>0.27</td>
<td>-0.43</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
<td>-2.78</td>
<td>1.6</td>
<td>1.6</td>
<td>-0.42</td>
</tr>
<tr>
<td>0.5</td>
<td>-4.23</td>
<td>-2.2</td>
<td>1.34</td>
<td>1.90</td>
<td>0.04</td>
</tr>
<tr>
<td>0.5</td>
<td>-5.01</td>
<td>-2.48</td>
<td>1.11</td>
<td>1.27</td>
<td>-0.33</td>
</tr>
<tr>
<td>0.25</td>
<td>-5.7</td>
<td>-2.69</td>
<td>0.95</td>
<td>1.27</td>
<td>-1.16</td>
</tr>
<tr>
<td>0.05</td>
<td>5.00</td>
<td>-5.42</td>
<td>3.6</td>
<td>2.26</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Codeine Response from 50 pg to 25 ng
On-column

Codeine Linearity

Area

Conc ng/ul
Food Safety Examples

Chloramphenicol (CAP) Antibiotic banned in Foods at 0.1 ppb
**Chloramphenicol (negative ion mode)**
**mass accuracy in ppm**

<table>
<thead>
<tr>
<th>pg injected</th>
<th>rep 1</th>
<th>rep 2</th>
<th>rep 3</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>19000</td>
<td>-2.48</td>
<td>-2.54</td>
<td>-3.03</td>
<td>2.7</td>
</tr>
<tr>
<td>3900</td>
<td>-2.35</td>
<td>-2.94</td>
<td>-2.63</td>
<td>2.6</td>
</tr>
<tr>
<td>770</td>
<td>-0.84</td>
<td>-2.13</td>
<td>-1.58</td>
<td>1.5</td>
</tr>
<tr>
<td>154.4</td>
<td>-1.49</td>
<td>-1.66</td>
<td>-3.36</td>
<td>2.2</td>
</tr>
<tr>
<td>31</td>
<td>0.58</td>
<td>-0.03</td>
<td>-3.18</td>
<td>0.9</td>
</tr>
<tr>
<td>6.2</td>
<td>ND</td>
<td>ND</td>
<td>-3.08</td>
<td>3.1</td>
</tr>
</tbody>
</table>
Chloramphenicol Response from 6 pg to 4 ng On-column
Nitrofuran Metabolites in Poultry

THE SELECTIVITY OF HIGH RESOLUTION ACCURATE MASS MEASUREMENT!!
NPAMOZ at Unit Mass Resolution
335.1352 -0.7+0.3

Slide 46
NPAMOZ  335.1352 ± 0.1

S/N = 70:1
NPAMOZ 335.1352
+- 20ppm(335.1262-335.1396)

S/N = 214:1
Chromatographic and Spectral Data

ESI TOF Positive ion

20 MICROLITER INJECTION OF WATER SAMPLE 119
TIC and BPC OF WATER SAMPLE 119

Slide 50
EICs OF WATER SAMPLE 119

Slide 51
EICs OF WATER SAMPLE 119

**SULFAMETHOXAZOLE**

C10H11N3O3S.....[M+H]+ = 254.0699

254.0603, 255.1123, +1.4239 PPM
Conclusions

• Agilent LC/MSD ESI-TOF offers routinized accurate mass measurement
  ✓ Seamless addition of lock-mass
  ✓ Auto-calibration of every stored scan
  ✓ Better than 5 ppm at low mass and 3 ppm at higher mass

• Confirmation and identification of unknowns

• Quantitative response
  ✓ up to 3 orders of magnitude linearity (within range of ESI)
  ✓ Greater selectivity
    ➢ From higher mass resolution (>4000 for low mass and 10000 for high mass)
    ➢ And accurate mass automatically stored for every scan