Increasing XIC Selectivity with Narrowed Mass Selection

Fig. 4. Liver extract spiked with marbofloxacin at 50 μg L⁻¹. Monitored is always the exact mass of the [M + H]⁺ ion of marbofloxacin at 303.14028 with a resolution of 10,000 FWHM. Varied was the mass window.
• \( m/z \, 246.0109^+ \) is a background interfering ion only baseline resolved from guanfacine using a resolution \( \geq 70,000 \)
• Calculated resolution needed to resolve two ions: \( \sim 56,000 \)
Guanfacine Background Interfering Ion

Source of interferant ion may be derived from water in mobile phase. Note as water is decreased in the gradient the interfering ion is diminished.

Data acquired by Dr. Rob Sturm on Thermo Q-Exactive, Advion
Do you need HRMS for accurate mass measurement?

• No!
  – We need stable mass axis calibration and analysis
  – Beneficial to also have no interferences at the m/z of interest.

• High resolution can minimize (resolve) potential interferences from contaminant peaks at the same nominal m/z ratio.
  – The interferent can cause a shift of peak shape and thus the centroid mass which can skew the accurate mass determination
  – The higher the resolution the less this is a problem.
Peak Mass Measurement?

• Two common approaches:
  – Peak apex or top
    • Signal maximum
      – Important to have stable ion current which is not erratic or noisy
      – Often multiple scans are averaged
  – Centroid approach
    • Weighted average of peak center
    • Averaging parameters can be user controlled

If peaks are good ones with symmetry both methods work

This is not often the case!
The Analytical Horsepower of Higher Mass Resolution

Peptide mixture: [Val$^5$]-Angiotensin II
Sequence: DRVYVHPF
Formula: C$_{49}$H$_{69}$N$_{13}$O$_{12}$
Exact mass: [M+2H]$^2+ = 516.76671$
$\Delta m$ (mmu): 18.2 mmu

Lys-des-Arg$^9$-Bradykinin
Sequence: KRPPGFSPF
Formula: C$_{50}$H$_{73}$N$_{13}$O$_{11}$
Exact mass: [M+2H]$^2+ = 516.78490$
$\Delta m$ (mmu): 18.2 mmu

RP = 15,000

RP = 56,700

Lecture 6, Page 47
Techniques to Improve Elemental Composition Determination

- Rings and Double Bonds (RDB)
  - Can be used to intelligently reduce the number of possible elemental compositions of a metabolite

- Element constraints based on parent elemental composition
  - A parent compound with one Cl will not generate a metabolite with two Cl’s
  - Phase I metabolism will not increase the number of nitrogen's

- Element Ratios
  - Sulphate conjugates require minimum oxygen to sulphur ratio = 3
Rings Plus Double Bonds

Nefazadone
MH⁺ = 470.2317
RDB = 12

- RDB = Total number of rings and double bonds in the structure
- Can be calculated directly from elemental composition
- More than one structure is possible for a given elemental composition and RDB
Total Rings plus Double Bonds
\((r+db)\)

- Element valences determine this
- The elemental formula allows its calculation
- \(R + db = x - \frac{1}{2}y + \frac{1}{2}z + 1\)
- for CxHyNzOn
- Example:

  - Hexane has no rings or double bonds:
    \[ CH_3CH_2CH_2CH_2CH_2CH_3 = C_6H_{14} \]
    \[ r+db = x - \frac{1}{2}y + \frac{1}{2}z + 1 \]
    \[ r+db = 6 - \frac{1}{2}(14) + \frac{1}{2}(0) + 1 \]
    \[ r+db = 0 \]

  - Cyclohexane
    \[ r+db = 6 - \frac{1}{2}(12) + \frac{1}{2}(0) + 1 \]
    \[ r+db = 1 \]

FN: Rings plus double bonds.cdx
Calculate Rings plus Double Bonds for this Compound

R + db = x-1/2y + 1/2z + 1

C14H19N02
Rings plus double bonds Problem

MW = 233
C_{14}H_{19}NO_{2}

r + db = 14 - 1/2 (19) + 1/2 (1) +1
r + db = 14 - 8.5 + 1/2 +1
r + db = 14 - 9 + 1
r + db = 6
Number of Hits with no RBD Constraint Applied

- Nefazadone metabolite at m/z 376.2341

<table>
<thead>
<tr>
<th>Hit</th>
<th>Formula</th>
<th>m/z</th>
<th>RDB</th>
<th>ppm</th>
<th>MS Rank</th>
<th>MSMS ppm</th>
<th>MSMS Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C22H34N0.25</td>
<td>376.2305</td>
<td>7.0</td>
<td>9.5</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C15H20N1.110</td>
<td>376.2316</td>
<td>9.0</td>
<td>6.6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C18H34N0.07</td>
<td>376.2330</td>
<td>3.0</td>
<td>3.0</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C14H31N0.90Cl</td>
<td>376.2335</td>
<td>4.0</td>
<td>1.7</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C19H38N0.252</td>
<td>376.2338</td>
<td>2.0</td>
<td>0.7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C19H30N0.503</td>
<td>376.2343</td>
<td>8.0</td>
<td>0.6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C18H35N0.303Cl</td>
<td>376.2361</td>
<td>3.0</td>
<td>-5.4</td>
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<tr>
<td>8</td>
<td>C16H34N0.5038</td>
<td>376.2377</td>
<td>3.0</td>
<td>-9.5</td>
<td>7</td>
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<td></td>
</tr>
</tbody>
</table>

4 hits at 5 ppm

8 hits at 10 ppm
Number of hits with Minimum RDB Constraint Applied

- Parent RDB = 12
- Conservatively set minimum RDB = 5

<table>
<thead>
<tr>
<th>Hit</th>
<th>Formula</th>
<th>m/z</th>
<th>RDB</th>
<th>ppm</th>
<th>MS Rank</th>
<th>MSMS ppm</th>
<th>MSMS Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C19H30N5O3</td>
<td>376.2343</td>
<td>8.0</td>
<td>-0.6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

One hit at 5 ppm with RDB minimum = 5

<table>
<thead>
<tr>
<th>Mass Tolerance (PPM)</th>
<th>Maximum Elements</th>
<th>RDB-min</th>
<th>RDB-max</th>
<th>No. of Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>C100 H200 N50 O50 S10 Cl50</td>
<td>1</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>C100 H200 N50 O50 S10 Cl50</td>
<td>1</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>C100 H200 N50 O50 S10 Cl50</td>
<td>5</td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>
Fully Automated Dried Blood Spot Bioanalysis by LC Coupled with High Resolution QTOF Mass Spectrometry.

Regina V. Oliveira\textsuperscript{1}, Jack Henion\textsuperscript{1}, and Enaksha Wickremsinhe\textsuperscript{2}

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\textsuperscript{2}Eli Lilly and Company, Indianapolis, IN 46285
**HRMS vs. triple quadrupole**

**QqQ**
- Quad Mass Filter (Q1)
- Octopole 1
- Lens 1 and 2
- Collision Cell
- Quad Mass Filter (Q3)
- Turbo 1
- Turbo 1
- Turbo 1
- Rough Pump
- 10KV Dynode Detector

**QTOF**
- High acquisition rate
- High mass resolution
- Increased dynamic range
- Accurate mass XIC

**QTOF:**
- High acquisition rate
- High mass resolution
- Increased dynamic range
- Accurate mass XIC
HRMS Instead of SRM MS
An alternative form of selectivity

- Collision energy optimization for each compound not needed
- Qualitative and quantitative data available
  - Full-scan HRMS mass spectra always acquired and available for later review or use of alternative XIC exact mass ion
- XIC of an exact mass is employed for the ratio with the exact mass of the IS
Automated DBS card extraction must handle a large number of cards per batch;

Track all replicates of all samples on all cards;

Provide the sensitivity required to work with such small samples;

Must provide performance at least equivalent to that of the manual system.
2D LC-HRMS Chromatogram of Fortified Blood with Loop injection of IS

LLOQ = 5.0 ng/mL for each drug and IS = 10 ng/mL

DBS card: Agilent Auto DBS

Midazolam

Midazolam-d₄

Desipramine

Desipramine-d₃

DBS card: Whatman DMPK-C

Midazolam

Midazolam-d₄

Desipramine

Desipramine-d₃

Lecture 6, Page 59
Future Directions

- Faster scanning high resolution full scan mass spectrometers that are quantitative.
  - One injection for quantitative and qualitative information
  - Eliminate the need for tuning triple quadrupoles
  - One platform for both quantitative work and qualitative work
  - Systems that are user friendly
    - Software and "Ease of Use" will be key drivers

Quantitative-Qualitative Workflows
Quan-Qual Work Flow

- Collect both quantitative and qualitative data in a single experiment.
  - Less work and more information.
- Traps, triples, TOFs have been used to do this type of experiment.
- Limitations of past mass analyzers:
  - Dynamic range (TOF and non-ICR based Traps)
  - Scan speed/Duty Cycle (QqQ and Trap)
  - Sensitivity/Selectivity (TOF and Trap)
  - Expensive (FT)
- An inexpensive, fast, full scan, accurate mass, high resolution mass spectrometer with a wide dynamic range and good sensitivity is needed.
  - (needs good software too!!)
Key Take Aways

• Evaluate the obvious information staring at you in the mass spectrum:
  – Mass defect
    • Elemental composition
    • Rings plus double bonds
  – Isotope m/z and relative abundance:
    • Consistent with proposed elemental composition?
  – Understand how to use isotope peaks for quantitative analysis purposes
Conclusions

• IDA Efficiency and data quality in TOF MS can be improved by application of advanced real time criteria
• Advanced IDA criteria are most useful in complex matrix
• Metabolite ID software tools can be used to our advantage to improve and streamline metabolite detection while helping reduce labor intensive aspects
• Addition of structure-derived data processing parameters and chemical logic assists in construction of optimum compound specific processing parameters
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• Robert Sturms
References