Novel workflows using a QTOF MS/MS for targeted and non-targeted forensic applications

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Agenda

- Recap on TOF and high-resolution advantages
- Workflows for screening and quantitation
- Unknown identification
- Conclusions
Key Features of AB SCIEX LC/MS/MS Systems

- Quantitation
- ID with MRM ratio
- Quantitation
- ID with MRM ratio
- ID with MS/MS library searching
- Quantitation
- ID with accurate mass
- ID with MS/MS library searching
- ID true unknowns
- Retrospective data processing

increasing confidence in compound ID
In Search of Greater Selectivity…

Principle of Time-of-Flight (TOF) Mass Spectrometry

- Ion packets are pulsed and accelerated into a TOF analyzer.
- Separation of ions is based on the time to traverse the flight tube, and arrive at the detector, on a nanosecond time scale.
  - Smaller $m/z$ ions move faster than heavier $m/z$ ions
- Higher resolution is achieved with longer flight path (longer TOF tubes, reflectors, faster acceleration).
In Search of Greater Selectivity…

Principle of Time-of-Flight (TOF) Mass Spectrometry

- **TOF-MS**
  - Q1: RF only
  - Q2: RF only
  - TOF: Separate ions
In Search of Greater Selectivity…
Principle of Time-of-Flight (TOF) Mass Spectrometry

- TOF-MS/MS
  - Q1: Filter
  - Q2: Fragmentation
  - TOF: Separate ions
High Resolution Time-of-Flight (TOF) Mass Spectrometry

Triple Quadrupole MS scan

“Nominal mass” instrument, 0.7 Da FWHM

High resolution, with TripleTOF® 5600 System
Why consider TOF technology?

- Greater specificity, with high-resolution measurements

“Nominal mass” instrument measures signal from compounds in a wide mass range.

“High-Res / Accurate Mass” instrument measures signal from compounds in a narrow mass range!
Why consider TOF technology?

• Greater specificity, with high-resolution measurements

A simple example:

4 different molecular formulae with nominal mass = 28 Da

\[
\begin{align*}
\text{N}_2 & = 2 \times 14.0031 = 28.0061 \\
\text{CO} & = 12.0000 + 15.9949 = 27.9949 \\
\text{C}_2\text{H}_4 & = (2 \times 12.0000) + (4 \times 1.0078) = 28.0312 \\
\text{CH}_2\text{N} & = 12.0000 + (2 \times 1.0078) + 14.0031 = 28.0187
\end{align*}
\]

Cannot be distinguished by a “nominal mass” instrument
Accurate Mass Measurement - aiding specificity of compound identification

C\textsubscript{33}H\textsubscript{40}N\textsubscript{2}O\textsubscript{9} has a protonated ion at 609.28066

Quadrupole MS reports mass to +/- 0.1Da = 165 ppm

High Resolution MS reports to <2ppm

Possible Formulas (C,H,N,O)

- 165 ppm  209
- 10 ppm    13
-  5 ppm    7
-  3 ppm    4
-  2 ppm    2

BUT – accurate MSMS provides higher confidence in identification
John asked R&D to build a low cost QTOF with at least 20K resolution…

- affordable
- small footprint
- innovative
- high-end performance characteristics
- ease-of-use
That’s What Bob Does…

\[ t = \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_1} \left[ \sqrt{U_0 + U_1} - \sqrt{U_0} \right] + b \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1)^{\frac{3}{2}} + \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_2} \left[ \sqrt{U_0 + U_1 + U_2} - \sqrt{U_0 + U_1} \right] + d \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1 + U_2)^{\frac{3}{2}} \]

\[ \frac{\partial t}{\partial U_1} = \frac{1}{2} \cdot \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_1} \cdot (U_0 + U_1)^{\frac{3}{2}} - \frac{b}{2} \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1)^{\frac{3}{2}} + \frac{1}{2} \cdot \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_2} \left[ (U_0 + U_1 + U_2)^{\frac{3}{2}} - (U_0 + U_1)^{\frac{3}{2}} \right] \cdot \frac{d}{2} \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1 + U_2)^{\frac{3}{2}} \]

\[ \frac{\partial^2 t}{\partial U_1^2} = -\frac{1}{4} \cdot \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_1} \cdot (U_0 + U_1)^{\frac{3}{2}} + \frac{3 \cdot b}{4} \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1)^{\frac{3}{2}} - \frac{1}{4} \cdot \frac{\sqrt{2 \cdot \text{mass}}}{q \cdot E_2} \left[ (U_0 + U_1 + U_2)^{\frac{3}{2}} - (U_0 + U_1)^{\frac{3}{2}} \right] + \frac{3 \cdot d^2}{4} \cdot \sqrt{\frac{\text{mass}}{2}} \cdot (U_0 + U_1 + U_2)^{\frac{3}{2}} \]
Prototype of low-cost TOF to optimize ion mirror and focusing

Simulation to optimize focusing in N-TOF geometry
Customer feedback:

“We are very impressed with X500R’s size without sacrifice of performance. We have little room for additional mass specs.”

Bob Haufler (R&D)
Introducing the SCIEX X500R QTOF System

Design Improvements and Details
Introducing the SCIEX OS Software

Single Software Platform for MS Control, Data Processing and Reporting
Comparison of workflows:

**IDA (Information Dependant Acquisition)** Provides MS/MS Information of about 80 - 95 % of precursors

**MS\textsuperscript{E}-Acquisition** Provides MS/MS of Everything (MS\textsuperscript{ALL})

**SWATH\textsuperscript{®} Acquisition** Provides Comprehensive MS/MS\textsuperscript{ALL}
Information Dependent Acquisition of MS/MS (IDA)

Provides MS/MS Spectra with High Selectivity (Q1 Resolution unit)

IDA can be used with and without inclusion list for target or non-target screening
## Limitations of IDA

### List of Pesticides

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<thead>
<tr>
<th>Acetamiprid</th>
<th>Dimethoate</th>
<th>Flutriafol</th>
<th>Nitenpyram</th>
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<td>Omethoate</td>
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Courtesy of Prof. Amadeo F. Alba, Universidad de Almaria
Confident Identification of Isomers using MS/MS (Prometon vs. Terbumeton)

Prometon
$$C_{10}H_{19}N_5O+H^+$$
RT = 5.44 min

MS: 226.1664 (0.63 ppm)
MS/MS: 98.5%

Terbumeton
$$C_{10}H_{19}N_5O+H^+$$
RT = 5.53 min

MS 226.1665 (0.99 ppm)
MS/MS: 95.7%

Identification based on MS/MS library searching (Q1 at unit resolution)
MS/MS\textsuperscript{ALL} using SWATH\textsuperscript{®} Acquisition

A Mode of Data Independent Acquisition Providing all MS/MS Spectra Wider than unit Q1 Isolation Window is Stepped Across the Mass Range

SWATH\textsuperscript{®} acquisition can be used with variable Q1 isolation windows across the mass range.
MS/MS\textsuperscript{ALL} with SWATH\textsuperscript{TM} Acquisition

- Simple Acquisition
- Highly Reproducible
- Selectivity similar to MRM
- Permanent record
- Takes advantage of the TripleTOF\textsuperscript{TM} and/or X500R Series
  - Speed
  - Sensitivity
  - square Q1 transmission windows
- Basis for simultaneous Quant/Qual analysis

1 cycle = 0.9 s 150 – 750 amu using fix windows of 25 amu
Sequential Window Acquisition of all Theoretical Fragment Ion Spectra (SWATH™)

Conceived at Ruedi Aebersold’s lab @ ETH Zürich
Implemented on SCIEX TripleToF™ systems
Data-Independent Acquisition (DIA)
SWATH-MS principle: Acquisition & Targeted analysis

Aebersold and coworkers, ETH Zurich
Comparing IDA and SWATH acquisition

1. Both acquisition techniques employ TOF-MS full-scan
   - Provides accurate mass measurement, and isotope pattern, for parent ions

2. IDA employs triggering product ion scans on detected compounds
   - IDA triggers off ions in the TOF-MS full-scan that meet a triggering threshold criteria. Unit resolution MS/MS (0.7 Da wide precursor isolation window) are acquired for the top candidates

3. SWATH® Acquisition Employs looped product ion scans
   - SWATH collects MS/MS for all compounds by using wide Q1 isolation windows, stepped across a mass range

### IDA

**Experimental Method**

- TOF-MS (survey scan)
  - IDA switching criteria (threshold etc.)
- Product ion scan 1
- Product ion scan 2
- ...
- Product ion scan 19
- Product ion scan 20

### SWATH

**Experimental Method**

- TOF-MS
- Product ion, 150-171
- Product ion, 170-191
- ...
- ...
- ...
- Product ion, 510-531
- Product ion, 530-550

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The SCIEX X500R QTOF System – IDA

Confident Identification of Isomers using MS/MS (Prometon vs. Terbumeton)

Prometon

C\textsubscript{10}H\textsubscript{19}N\textsubscript{5}O+H+  
RT = 5.44 min

MS: 226.1664  
(0.63 ppm)

MS/MS: 98.5%

Terbumeton

C\textsubscript{10}H\textsubscript{19}N\textsubscript{5}O+H+  
RT = 5.53 min

MS 226.1665  
(0.99 ppm)

MS/MS: 95.7%

Identification based on MS/MS library searching (Q1 at unit resolution)
The SCIEX X500R QTOF System – SWATH™

Confident Identification of Isomers using SWATH (Prometon vs. Terbumeton)

Prometon
226/184.1193
RT = 5.44 min

MS: 226.16641 (0.77 ppm)

Identification based on unique fragment ions and their ratios

Terbumeton
226/170.1036
RT = 5.54 min

MS 226.16641 (0.77 ppm)
SWATH Identifies More Compounds than IDA

IDA
With IDA data collection, some positive compounds have no MS/MS data acquired. Here it is difficult to confidently say that the compound is present without knowing other information like retention time.

SWATH
With SWATH data collection, every compound has MS/MS data acquired. Here we can see the positive library match (with fit scoring) which allows more confidence in determining the compound.
SWATH Identifies More Compounds than IDA

IDA
With IDA data collection, some positive compounds have no MS/MS data acquired. Here it is difficult to confidently say that the compound is present without knowing other information like retention time.

SWATH
MSMS data is generated, however there is no library match, so here we have the benefit of SWATH avoiding a false positive. Norephedrine has 2 strong fragment ions at 91 and 115, which are completely missing from this MS/MS data → NO MATCH.
Scheduled MRM<sup>HR</sup> (vs TOF scan)

Buphedrone (5 ng/mL in urine, 10-fold dilution, 10 μL injection)

**MRM<sup>HR</sup>: 178.1 → 131.070 ± 0.01 m/z**

**TOF-MS: 178.1226 ± 0.005 m/z**
TOF vs. SWATH™ vs. MRM³

OTC in low spiked manure (undiluted)

TOF MS

\[ S/N = 5 \]

SWATH™

\[ S/N = 12 \]

Equal to 2x increase in S/N

MRM³

\[ S/N = 92 \]

5 to 10x increase in S/N

SWATH Quant benefits from Selectivity of MS/MS fragments (MRM³ like)

MRM³ Quant benefits from Selectivity of optimized MS/MS fragments
Applying ‘Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra’ (SWATH) for systematic toxicological analysis with liquid chromatography-high-resolution tandem mass spectrometry

Kathrin Arnhard · Anna Gottschall · Florian Pitterl · Herbert Oberacher

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Abstract Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become an indispensable analytical technique for the analysis of complex matrices in forensic toxicology. Data-dependent acquisition strategies combine high sensitivity with high specificity, yet they do not cover the full range of the spectrum. A recent innovation in high-resolution mass spectrometry, the SWATH technique, is capable of identifying more compounds at lower concentration levels.
Identification and Quantitation in SCIEX OS Software

Review Quantitative and Quantitative Results

Flagging when above MRL and traffic lights for confidence

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<thead>
<tr>
<th>Index</th>
<th>Sample Name</th>
<th>Sample Type</th>
<th>Component Name</th>
<th>Actual Concentr.</th>
<th>Calculated Concentration</th>
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Review of peak integration, spectra and calibration line
Filtering the results table to only show the positive findings

- Ion Ratio and RT
- Mass error and Isotope Pattern
- MS/MS Library Matching
- Quantification

$r^2 = 0.98845$
Unknown Identification in SCIEX OS Software

Automatic Sample-Control Comparison and Compound Identification

Automatic formula finding and MS/MS library searching
Unknown Identification in SCIEX OS Software

ChemSpider Searching and Structure Elucidation

ChemSpider searching of found formulae
Automatic structure elucidation using HR-MS/MS spectra
Unknown Identification in SCIEX OS Software

ChemSpider Searching and Structure Elucidation

ChemSpider searching of found formulae
Automatic structure elucidation using HR-MS/MS spectra
Unknown Identification in SCIEX OS Software

1. Automatic Sample-Control Comparison and Compound Identification

[Image of a software interface showing automatic formula finding in a data analysis context]

- Blank Control
- Sample

Automatic formula finding
Unknown Identification in SCIEX OS Software

2. Automatic Sample-Control Comparison and Compound Identification

ChemSpider searching of found formulae
Automatic structure elucidation using HR-MS/MS spectra

Loss of OH group

For Research Use Only. Not for use in diagnostic procedures.
Thank you for listening!
I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions. I will use Google before asking dumb questions.