A Waters igazságügyi toxikológiai feladatokra alkalmas LC-MS rendszereinek áttekintése

Bartha Richárd, Waters Kft.
Tox 2018
## WATERS MS FORENSIC SOLUTIONS

With ACQUITY® UPLC® I-Class System

<table>
<thead>
<tr>
<th>Feature</th>
<th>ACQUITY QDa*</th>
<th>Xevo® TQD</th>
<th>Xevo TQ-S micro</th>
<th>Xevo TQ-XS</th>
<th>Xevo G2-XS QToF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seized drug material, bulk chemical identification/forensic chemistry</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Targeted screening solutions for systematic toxicological analysis</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MRM screening solution available (180 drugs)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Full scan/library screening* solution (&gt;950 drugs)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Comprehensive screening with accurate mass library (&gt;1500 compounds)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Non-targeted screening</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discovery workflows for novel psychoactive substances (NPS)</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Confirmatory assays</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Routine sensitivity confirmatory assays (e.g., urine, blood)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>High sensitivity assays, alternative sample matrices (e.g., oral fluid, hair)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

**Informatics**

|---------------------------|--------------------|--------------------|--------------------|--------------------|-------------------------------------|

*Higher sensitivity may be achieved through the application of a higher amount of sample or more rigorous sample prep/sample concentration steps.

* Full scan/library searching solution on Xevo TQD or Xevo TQ-S micro requires ChromatLyntm Application Manager.
Team-Toxicology

Rob Lee

Nayan Mistry

Jeff Goshawk

Simon Cushen
(6 month placement student DIT)

Michelle Wood
• LC System: Waters® ACQUITY UPLC® I-Class
• Column: ACQUITY UPLC HSS C<sub>18</sub> Column 2.1 x 150 mm, 1.8 µm
• Column Temp: 50 °C
• Flow Rate: 400 µL/min.
• Mobile Phase A: 5 mM Ammonium Formate, pH 3.0
• Mobile Phase B: Acetonitrile with 0.1% formic acid
• Gradient: 13% Mobile phase B increasing to 95%, within analysis time
• Injection volume: 5 µL (MRM) 10 µL (Full scan)
• Run time: **15 Minutes**

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**Toxicology solution:**

ACQUITY UPLC I-Class

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**Low Dispersion Detection**

As low as 4 µL total system dispersion
UPLC optimized optical detection
PDA, UV/Vis, FLR, RI, ELS
ACQUITY QDa

**Column Management**

eCord intelligent column tracking
Active solvent preheating p to 90 °C
Up to 2, independent thermal zones

**Sample Manager – FTN-I / FL-I**

Inject from 0.1 to 1000 µL (FTN-I)
Inject from 0.1 to 250 µL (FL-I)
≤ 0.001% Carryover
Auto-addition and Auto-dilution
Optional 7,296 capacity with Sample Organizer
Optional thermally controlled fraction collection

**Binary Solvent Manager**

18,000 PSI
0.001 to 2.0 mL/min
High pressure, binary mixing
Active check valves
Gradient SmartStart
Integrated solvent select valve (+2 solvents/line)
Nominal Mass Solutions

- Xevo TQD System – Forensic Screening
  - Includes Acquity UPLC I-Class with a Xevo TQD Mass Spectrometer
- (STA) Systematic Toxicological Analysis
  - ~950 compounds
- (MRM) Targeted Multiple Reaction Monitoring
  - ~175 compounds (2 transitions/compound)
- DVD contains UPLC conditions & database libraries

Figure 1. The ACQUITY UPLC I-Class with Xevo TQD Mass Spectrometer.
**Xevo Tandem kvadrupól család**
(Relatív MRM érz. 1 : 5 : 5 × 5)

- **Xevo TQ-D**
  - Megbízható, kompakt

- **Xevo TQ-S micro**
  - Kompakt, megbízható, érzékeny

- **Xevo TQ-XS**
  - Maximum érzékenység MRM és full scan módban
Ultra trace analysis
What is in a sample?

100 ng/L (100 ppt) pesticides in drinking water
Example Tests

- Anti-psychotics/anti-depressants
- Ethanol biomarkers
- Opiates
- Amphetamines
- Cocaine and metabolites
- Benzodiazepines
- Cannabinoids
- GHB and precursors
- Mixed-panels illicit compounds
- Hallucinogens

Example Matrices

- Urine
- Blood/plasma
- Postmortem blood
- Saliva
- Preserved oral fluid
- Sweat
- Hair
- Larvae/Pupae
- Meconium
Qualitative & Quantitative Methods:
- Amphetamines
- Cannabinoids
- Benzodiazepines
- Opiates
- Barbituates

Across several sample matrixes
- Urine
- Plasma
- Whole blood
- Hair

Commitment to providing cutting edge forensic applications
- Cannabinoids in whole blood
- “Designer drug” applications
What Do We Do in HRMS LCMS Analysis?

...4 Fundamental Questions

1. Are these compounds in my sample?
   - Screening

2. How much is in my sample?
   - Quantification

3. What else is in my sample?
   - Elucidation

4. What is the difference between my sample and another one?
   - Comparison

Set policies for a regulated environment

- Administration > Security > Global Policies

When you install UNIFI software, you decide whether you want to configure policy and audit trail settings for a regulated (GxP) or non-regulated environment. If you chose "Configure settings for a regulated environment (GxP)"*, UNIFI software configures data folders, global policies, folder policies, roles, custom fields, event properties, an administrator user account, and predefined reasons to comply with GxP (Good Practice quality guidelines and regulations). You can change these settings at any time.
LC-HRMS Analysis
ACQUITY I-Class / Xevo G2-XS QTof
The best combination of products to create the best overall solution

- ACQUITY UPLC® I-Class
- Xevo® G2-XS QTof
- UNIFI Scientific Information System
- Toxicology Exact Mass Database
- Column Chemistries
- Service and Support
MS\textsuperscript{E} Screening Analytical Workflow

1. Sample preparation
   - Dilute and shoot
   - Liquid/liquid
   - SPE

2. Collection of unrestricted dataset using UPLC-MS\textsuperscript{E}
   - 15 min gradient separation
   - Same as nominal mass chromatography
   - MS\textsuperscript{E} (Collision energy ramp 10-40eV)

3. Post-acquisition data processing
   - Matching acquired data to exact mass database
### Experimental UPLC Method (ES+)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A</th>
<th>% B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>87</td>
<td>13</td>
<td>Initial</td>
</tr>
<tr>
<td>0.50</td>
<td>87</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>10.00</td>
<td>50</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>10.75</td>
<td>5</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td>12.25</td>
<td>5</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td>12.50</td>
<td>87</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>15.00</td>
<td>87</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

**ACQUITY UPLC I Class**

<table>
<thead>
<tr>
<th>Column</th>
<th>ACQUITY™ HSS C18 1.8 µm x 150 mm @ 50 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase A</td>
<td>5 mM Ammonium Formate, pH 3.0</td>
</tr>
<tr>
<td>Mobile Phase B</td>
<td>Acetonitrile + 0.1 % formic acid</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5 µL</td>
</tr>
</tbody>
</table>

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## Experimental MS Method

### MS Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass Spectrometer</strong></td>
<td>Xevo G2-XS QToF</td>
</tr>
<tr>
<td><strong>Acquisition Mode</strong></td>
<td>MS&lt;sup&gt;E&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Ionisation mode</strong></td>
<td>ESI (+ve and –ve)</td>
</tr>
<tr>
<td><strong>Capillary voltage</strong></td>
<td>0.8 kV (ES+), 1.5 kV (ES-)</td>
</tr>
<tr>
<td><strong>Cone voltage</strong></td>
<td>25 V</td>
</tr>
<tr>
<td><strong>Desolvation temperature</strong></td>
<td>400 °C</td>
</tr>
<tr>
<td><strong>Desolvation gas flow</strong></td>
<td>800 L/h</td>
</tr>
<tr>
<td><strong>Cone gas flow</strong></td>
<td>20 L/h</td>
</tr>
<tr>
<td><strong>Acquisition range</strong></td>
<td>50-1000 m/z</td>
</tr>
</tbody>
</table>
| **Collision Energies (MS<sup>E</sup>)** | Low energy: 6 eV  
                            High energy: 10-40 eV |
| **Lockspray**                    | Leucine enkephalin 556.2766 m/z (ES+), 554.2620 m/z (ES-) |
What is it?
- Patented generation and time-alignment of fragment ions

Why is it important?
- Confidence in data and improved productivity
  - reduces false positive

What are the benefits?
- Out-of the box method
  - No method development
- Provides rich qualitative data

Low collision energy mode to produce precursor ion data

High collision energy mode to produce fragment ion data
Precursor and Fragment Ion Spectra

MS\textsuperscript{E}

Collision energy ramp 10-40 V

9.4 min = clobazam
What is it?
- A database of toxicologically relevant compounds
  - Developed in collaboration with toxicology laboratories
- >1500 Compounds
  - All with RT Information
    - Robustness proved over several years
  - with verified fragments
  - with Mol Files/theoretical fragments

What are the benefits?
- Increased confidence in compound identification
- Time saving for users
Compounds in Toxicology Library

- Exact mass database contains specific compounds and fragment ion data for the following compounds:
  - AMPHETAMINES
  - ANAESTHETIC AGENTS
  - ANALGESICS ANTIARRHYTHMICS
  - ANTIASTHMATICS
  - ANTICONVULSANTS
  - ANTIEMETICS
  - ANTIEPILEPTICS
  - ANTIHISTAMINES
  - ANTIMALARIALS
  - ANTIPSYCHOTICS
  - ANTI-SEIZURE
  - ANTIDEPRESSANTS
  - ANTIHYPERTENSIVES
  - ANTITUSSIVES
  - BENZODIAZEPINES
  - BETA BLOCKERS
  - COCAINE METABOLITES
  - OPIOIDS
  - PESTICIDES
  - SSRIs
  - TCAs
  - PIPERAZINES
  - CATHINONES

- Contains “designer drug” compounds like bath salts & synthetic cannabinoids.
- Name *(chemical, common, synonyms)*
- Chemical formula, CAS
- Structure
- Retention time
- Accurate mass precursor ions
- Accurate mass fragment ions
- Isotopic patterns
- Spectra
- References
  - Identification:
    - Accurate mass error (eg. < 5 ppm) data
    - isotope match
    - existing fragments
    - RT
Analysis of a System Suitability Mixture containing 10 standards *i.e.*, a very clean sample!

**ID criteria: Elemental composition (exact mass only) ± 5 ppm**

**Database ~1000 substances: = 25 hits**

(Database ~5000 substances: = 55 hits)

**ID criteria: Elemental composition (exact mass only) ± 5 ppm and RT**

**Database ~1000 substances: = 13 hits**

**ID criteria: Elemental composition (exact mass only) ± 5 ppm and RT and fragments**

**Database ~1000 substances: = 10 hits**
Tools for finding the unknowns

- Identified components:
  - Tyrosine
  - Peracetamol
  - Ethyl theophylline, beta-hydroxy
  - Caffeine
  - MDMA
  - Cocaine
  - Nevirapine
  - Ketamine
  - Methyl/Clonazepam

- Unidentified components:

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Elucidation of unknowns

Discovery Toolkit > ChemSpider

MSMS Results > Send to ChemSpider > Review Hits > 5-Hydroxyomeprazole
Exact Mass Screening Conclusions

- Complex specimens and ‘unknown’ toxicants present significant analytical challenges to the toxicologist

- High resolution TOF provides high accuracy mass measurement

- Well defined, reproducible chromatographic method

- TOF-MS$^E$ analysis permits collection of a complete dataset

- High resolution allows differentiation between nominally isobaric compounds and other interfering substances

- Exact mass of parent molecule AND fragments provides high degree of confidence in the identification