SCREENING ENVIRONMENTAL SAMPLES FOR A DIVERSE RANGE OF COMPOUND CLASSES AND STRUCTURES WITH ACCURATE MASS LC-MS AND AN INTEGRATED SCIENTIFIC INFORMATION SYSTEM

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Food & Environmental  Business Operations
Overview

The requirements for screening assays
- Introduction & background to screening analysis
- Challenges and regulatory considerations
- Performance criteria

Pesticide screening solution with UNIFI

An overview of UNIFI 1.6 software via a well water example

Summary and conclusions
The purpose of residue screening?

**Goals**
- To eliminate the **compliant** samples (≥95% samples?)
- To identify the **non-compliant** samples for subsequent confirmation and quantification (~5%?)

**Needs**
- Applicable for **high-throughput multi-residue** analysis
- **Sensitivity** in line with the relevant Regulatory limits in complex matrices
- **Validated** in accordance with legislative requirements

**Process**
- **Rapid, cost effective** and “**user friendly**” from sample preparation to results reporting...
Residue Screening Classification

- **Targeted screening (QQQ)**
  - Well defined target list of analytes
  - Selective acquisition &/or processing modes
  - Analytical standards available for every compound

- **Non-targeted (suspect) screening (Tof)**
  - Non targeted relates to the acquisition type
  - Screen against a comprehensive library of known compounds
  - Analytical standards available for most compounds

- **Unknown screening (Tof)**
  - No defined target list
  - Compound not present in the library, maybe a new chemical structure
  - Structural elucidation required
Challenges/Needs Facing Environmental Monitoring Laboratories

- Laboratories have to screen for larger numbers of compounds at very low concentrations (<ppb, ppt)
- Increase in the number of samples =
  - Shorter turn-around times required to keep pace with demand
  - Simple access
- With longer lists of priority substances, better understanding of the transformation of pesticides and POPs in the environment, many laboratories wish to extend the scope of their analytical methods beyond the compounds listed in the drinking water methods.
- Trend for **generic, multi-residue** methods
  - Trade off between selectivity and sensitivity...
SANCO/12495/2011 – performance criteria
- high resolution, accurate mass

- Sensitivity in line with the relevant Regulatory limits
  - MRLs / MRPLs / RCs / ALs / ADIs etc...

- Applicability of the screening method is defined by the false non-compliant (positive) and false compliant (negative) rates
  - A low false negative rate is critical for screening assays to avoid missing MRL violations
  - Tolerance ≤ 5% false negative rate
  - A low false positive rate is important for screening assays to reduce costly quant / confirmatory analysis
  - Desirable ≤ 5% false positive rate

- Mass accuracy tolerance = ≤5 ppm
- Mass resolution tolerance = ≥20k (FWHM)
SANCO/12495/2011 criteria for chromatography

- ...........the minimum acceptable retention time should be at least twice the void volume of the column...

- .........the retention of the analyte, should correspond to that of the calibration solution ......with a tolerance of ±0.5% for GC and ±2.5% for LC.

Chromatography is still important !!!!
A variety of MS analysers and configurations including HR-MS are recognised with differing requirements for identification depending on the inherent analytical power.
Why High Resolution Accurate Mass Screening?

To see the WHOLE picture!!
Advantages of accurate mass screening?

- Over recent years high resolution mass spectrometry has gained in popularity as a screening tool in the food and environmental sector
  
  ✓ **Ability to perform non-targeted analysis**
  - The freedom to measure compounds without prior compound specific tuning
  
  ✓ **Ability to perform historical (retrospective) data review**
  - The capability of performing structural elucidations of unknowns or suspected compounds
  
  ✓ **Ability to perform full spectral analysis**
  - Providing greater insight into the composition of a complex sample
  
  ✓ **Ability to screen for larger number of compounds and adducts**
  - Compared to QqQ based screening
  
  ✓ **Increased specificity in complex matrices**
  - Accurate mass, diagnostic fragment ions...

“HRMS strongly competes with classical tandem mass spectrometry in the field of quantitative multiresidue methods (e.g., pesticides and veterinary drugs). It is one of the most promising tools when moving towards nontargeted approaches…”

A comprehensive solution for high-throughput, multi-residue pesticides screening.

**DisQuE™ Dispersive Sample Preparation Kit**
- Fast, simple pesticide extractions

**ACQUITY UPLC I-Class System**
- High resolution separations of trace analytes

**Xevo G2-S QTof**
- Accurate mass measurements for precursor and product ions

**UNIFI Scientific Library**
- The ultimate reference resource

**PREPARATION** ➔ **SEPARATION** ➔ **DETECTION** ➔ **INTERPRETATION**
Why UltraPerformance LC?

Item name: Water_005
Channel name: 1: TOF MS e (50-1200) 6eV ESI+
**Xevo G2-S QTof**  
*QuanTof technology – stability of mass accuracy*

**Repeatability** - leek spiked with pirimicarb (*n*=10) = RSD 3.2%

<table>
<thead>
<tr>
<th>Observed Mass (m/z)</th>
<th>Mass Error (mDa)</th>
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<td>0.8</td>
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<td>0.6</td>
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<td>0.6</td>
</tr>
<tr>
<td>239.1516</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**MH⁺ m/z = 239.1508**
Xevo G2-S QTof
QuanTof technology – Accurate & Quantitative

Quantitative Performance
Matrix Matched Calibration Series
(mandarin extract): Imazalil: 1 – 1000 ng/g
Quantitative accuracy and precision
n=10, run in a series of 300 injections over 60 hours

Pirimicarb in leek replicates at 50 ng/mL (n=10)

Mean calculated concentration = 51 ng/ml, RSD = 3%
Excellent quantitative accuracy and precision
Xevo G2-S QTof (MS$^E$ Technology) Spectral Alignment

Coeluting Precursor & Fragment Ions

MS$^E$ Deconvoluted Spectra

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Exact mass performance for precursor and all fragment ions generated in the UPLC/MS\textsuperscript{E} experiment.

Allows informatics to interpret data correctly and reliably = greater confidence in the validity of the results.
Screening experiments are dependent on the **quality** of data and libraries.

Critical information that is used for ID process:

- **Name** *(chemical, common, synonyms, marker residue definition)*
- **Chemical formula**
- **Structure**
- **Retention time**
- **Accurate mass precursor ions**
- **Accurate mass fragment ions**
- **Isotopic patterns**
- **Isotope intensity**
- **Expected ion ratios**
- **Theoretical spectra**
- **Drift Time**
Utilizing the component information

Accurate Mass Screening

- Precursor Formula
  - Exact Mass

- Retention Time

- MS\textsuperscript{E} Fragments
  - Exact Mass

- Isotopes

Scientific Library

- Target Library
  - Adducts
  - Biotransformations
  - Instrumental Transformations

Unidentified Components

Discover (ad-hoc)

Identified Targets

Quantification
Waters Residue Screening

- **Screening Library**
  - 2000+ compounds with structure, synonyms, formulae & accurate mass precursors
  - 600+ compounds with detection results (retention time and accurate mass fragment ions)
  - Pesticide Residues
  - Veterinary drugs, metabolites, epimers, & marker residue definitions
  - Pharmaceutical & Personal Care Products

- **Toxicology Library**
  - 1000+ compounds with structures, formulae and detection results

Both positive ion and negative ion detection results for those compounds that run in both modes

- Theoretical fragment m/z used in the Scientific Library

- User can easily build a new library or import an existing library using an Excel spreadsheet.
## Scientific library

### Importing Existing Data: Excel spreadsheet

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>Structure</th>
<th>RT</th>
<th>Frag 1</th>
<th>Frag 2</th>
<th>Frag 3</th>
<th>Frag 4</th>
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<td>137.0916</td>
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</table>
## The Component Approach

The software organises the data across all channels into components.

### Components

<table>
<thead>
<tr>
<th>ID</th>
<th>Mass</th>
<th>RT</th>
<th>Area</th>
<th>Isotopes</th>
<th>Fragments</th>
<th>Adducts</th>
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<td>Na⁺</td>
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</table>

Peak detection simplifies the data.

Components can span multiple channels of data.

The components can now be analysed.

The software organises the data across all channels into components.
Single Processing Workflow

- Workflow determines what UI is displayed

- Workflows are created for rapid data review
What are detection results and why are they important?
Using a mass accuracy threshold

- Quechers extract of a red apple spiked with 2 pesticides

**Number of Identified Pesticides (mass accuracy alone)**

- 5ppm: 272
- 3ppm: 151
- 1ppm: 48

Total Identified Pesticides: 478
The importance of retention time

- Quechers extract of a red apple spiked with 2 pesticides

**Number of Identified Pesticides**
(Using retention time plus 5 ppm window)

- ± 0.25 min: 9
- ± 0.4 min: 15
- ± 0.5 min: 20
- no Rt: 272

Even a 1 min window is better than 1 ppm mass accuracy AND with reduced risk of false negatives
How useful are fragment ions?

<table>
<thead>
<tr>
<th>Time Window</th>
<th>Number of Identified Pesticides without fragment ions considered</th>
<th>Number of Pesticides Identified with Fragment Ion Confirmation (at least 1 fragment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>± 0.25 min</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>± 0.4 min</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>± 0.5 min</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>no Rt</td>
<td>272</td>
<td>5</td>
</tr>
</tbody>
</table>
Automated screening of pesticides based on retention time and the exact mass of one diagnostic ion resulted in too many false positives to enable efficient screening. Relative response thresholds or the requirement of the detection of one second diagnostic ion effectively reduced this to acceptable numbers. The two-ion approach was considered most useful in daily practice. As secondary ion, the use of another

- High Resolution with one diagnostic ion not enough
- Fragment ions are essential for minimizing false positive rates
- Fragment spectra are essential when searching for unknowns
Well Water Example
UPLC Acquisition Parameters

LC System: Waters ACQUITY I-Class
Column: ACQUITY UPLC HSS C₁₈, 1.8 mm, 2.1x150 mm
Column Temp: 50 °C
Flow Rate: 0.40 mL/min.

Mobile Phase A: H₂O with 5 mM NH₄HCO₂ (pH 3.0 with formic acid)
Mobile Phase B: Acetonitrile with 0.1 % (v/v) formic acid.
Total run time: 17 min
Injection volume: 100 µL

Gradient Conditions

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<tr>
<th>Time</th>
<th>Flow rate</th>
<th>Composition A</th>
<th>Composition B</th>
<th>Curve</th>
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<td>87.0</td>
<td>13.0</td>
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</tr>
<tr>
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<td>0.400</td>
<td>50.0</td>
<td>50.0</td>
<td>6</td>
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<td>5.0</td>
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<td>0.400</td>
<td>87.0</td>
<td>13.0</td>
<td>6</td>
</tr>
</tbody>
</table>
### MS conditions

- **MS system:** Xevo G2-S QTof MS
- **Resolution:** 30,000 FWHM
- **Ionisation mode:** ESI+
- **Capillary voltage:** 1 kV
- **Sample cone:** 20 V
- **Source temp:** 120 °C
- **Desolvation temp:** 550 °C
- **Desolvation gas:** 1000 L/H
- **Reference Mass:** Leucine Enkephalin \([M+H]^+ = 556.2766\)
- **Acquisition range:** \(m/z\) 50-1200
- **Acquisition rate:** 10 spectra/second
- **MS\(^E\) Low collision energy:** 4.0 V
- **Collision energy ramp:** 10-45 V
Example Classes Describing PPCPs

Steroids

Fluoroquinones

Antibiotics

Macrolides

Sulphonamides

Beta Blockers

Anti-inflammatories

Anthelmintics

Anti-convulsants

Anti-histamines

Illicit drugs

Vasoactives

Decongestants

Anti-bacterials

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Sample Preparation

- **PPCP Standards**: High Level Standards (10 µg/L) in UPLC water.

- **Extracted Blank** - Treated as a reference. A UHPLC-grade water sample (Fisher Optima™), enriched using SPE protocol (next slide).

- **Extracted well water sample** - Treated as the unknown. A well water sample, enriched as above.

- **Extracted well water sample, post spike** - Enriched as above and post spiked with the 35 PPCPs at a level of 1 µg/L.

- **Extracted well water sample, pre spike** - Well water sample pre-spiked with the 35 PPCPs (at 1 ng/L) then enriched as above. Pre-spike samples were prepared in duplicate.

- **Non-extracted well water** - Well water sample neither enriched nor spiked.

- **Extracted calibration standards** - Eight levels (1.0, 2.0, 2.5, 5.0, 10.0, 25.0 and 50.0 ng/L) of the 35 PPCPs were spiked into an extracted UPLC-grade water sample.

<table>
<thead>
<tr>
<th>Atenolol</th>
<th>Corticosterone</th>
<th>Ofloxacin</th>
<th>Ticlopidine</th>
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<tbody>
<tr>
<td>Azithromycin</td>
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<td>Oxprenolol</td>
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<tr>
<td>Benzocaine</td>
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<td>Cocaine</td>
<td>Miconazole</td>
<td>Salbutamol (albuterol)</td>
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</tbody>
</table>
**SPE Sample Preparation Protocol**

- **1:1000 enrichment**

---

**Loading**
- Condition 1: 5 mL MeOH
- Condition 1: 5 mL Water
- Load: 1 Litre @ 10mL/min

**Washing**
- Disconnect Stack
- Wash MAX: 5 mL 100 % H₂O + 2 % NH₄OH
- Wash MCX: 5 mL 100% H₂O + 2 % Formic acid

**Eluting**
- Elute 1 MAX: 5 mL 100 % MeOH
- Elute 2 MAX: 5 mL 100 % MeOH + Formic acid
- Elute 3 MCX: 5 mL 100 % MeOH + NH₄OH

**Post Elution**
- Pool all 3 elutions
- Evaporate to dryness (N₂)
- Reconstitute 1000 uL 100 % H₂O + 10 mM NH₄Formate
- Inject 100 uL

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Ditiazem Spiked into Well Water

<table>
<thead>
<tr>
<th>Expected Fragments Count</th>
<th>Identified High Energy Fragments</th>
<th>Isotope Match Mz RMS PPM</th>
<th>Isotope Match Intensity RMS Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>1.05</td>
<td>6.48</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.63</td>
<td>8.32</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.82</td>
<td>6.64</td>
</tr>
</tbody>
</table>
Cocaine Spiked into Well Water - Quantitation
Component Plot
- Carbamazepine & Potential Metabolites

Item name: Extracted Well Water Blank

Retention time [min]

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention Time</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine + O</td>
<td>4.33</td>
<td>4.24 ppm</td>
</tr>
<tr>
<td>Carbamazepine-CHNO(d...</td>
<td>5.03</td>
<td>5.03 ppm</td>
</tr>
<tr>
<td>Carbamazepine + O</td>
<td>5.81</td>
<td>5.81 ppm</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>7.49</td>
<td>7.49 ppm</td>
</tr>
</tbody>
</table>

Chromatograms

Item name: Extracted Well Water Blank
Channel name: Identified Components

Spectra

Item name: Extracted Well Water Blank, Channel name: Low energy
Time 7.4875 +/- 0.029...

Description:

Integral [Counts]

Retention time [min]

<table>
<thead>
<tr>
<th>Mass</th>
<th>Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>194.09756</td>
<td>2.5e5</td>
</tr>
<tr>
<td>237.10157</td>
<td>4.26e5</td>
</tr>
<tr>
<td>275.05669</td>
<td></td>
</tr>
<tr>
<td>314.27830</td>
<td></td>
</tr>
<tr>
<td>368.20512</td>
<td></td>
</tr>
</tbody>
</table>
Common Fragment Search

Spectra

Item name: Extracted Well Water... Channel name: Low energy: Time...
Description:

<table>
<thead>
<tr>
<th>Mass [m/z]</th>
</tr>
</thead>
<tbody>
<tr>
<td>180.08120</td>
</tr>
<tr>
<td>253.09752</td>
</tr>
<tr>
<td>309.06376</td>
</tr>
<tr>
<td>359.14784</td>
</tr>
</tbody>
</table>

Parameters

Fragment Mass: 210.09131 Da
Mass Tolerance: 5 mDa

Observed mass [m/z]

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Metabolism Localization Tool
Adding a Metabolite to the Scientific Library
Summary

- Information rich MS\textsuperscript{E} acquisition and an integrated scientific information system make it possible to screen for the presence of compounds of interest, their adducts and potential metabolites in a routine laboratory environment.

- Several compounds of interest were detected at sub ppt levels during a HRMS screening of a locally sourced well water sample enriched using the SPE protocol outlined.

- Mixed-mode SPE allowed for the analysis of acidic, basic and neutral compounds of interest in a single sample.
Summary

- The presence of retention times and accurate mass fragment ions in Scientific Libraries within UNIFI allowed identifications to be made on more information than accurate mass of the precursor ions alone. This proves critical for reducing false detection rates and enabling rapid data review for screening experiments.

- Using the metabolite identification functionality of UNIFI, several metabolites of carbamazepine were identified in an environmental water, in addition to the parent.

- Subsequent scientific library additions of identified metabolites is simplified, enabling the user to expand identification information for multi residue screening experiments.
Thanks

Questions???

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ken_rosnack@waters.com

Food & Environmental Business Operations