Qualitative Screening and Quantitative Analysis of Pesticides Using Data Independent Acquisition High Accuracy Mass Spectrometry

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Outline

- Background
- Analysis of pesticides
  - LC-MS Technologies
  - Data-dependent Acquisition (DDA)
- Data-independent Acquisition (DIA)
- DIA Applications
  - Qualitative screening
  - Quantitative analysis
- Conclusions
Who We are – A Brief History of the CVG

- Founded in 1994 by a group of analytical scientists from the pharmaceutical industry in Toronto, Canada
- Registered as a non-profit organization in Canada in February 1999
- Become a Voting Organization Member of the United States Pharmacopeial Convention in 2009
- Expanded in 2009 to include environmental and food sectors
Common Ground

- Uses similar technologies in analysis
- Learning opportunities & cross fertilization
- Make new contacts and friends
Challenges in Routine Pesticide Analyses

- Sampling
- Standard preparation
- Analytical turnaround time
- Sample preparation
- Sample analysis
- Data processing/training
- Compound detection (target/non-target), identification and quantitation
- Quality control, quality assurance and report of uncertainty

Are you sure?
Pesticide expenditures in U.S. agriculture, 1960-2008

- Nominal
- Real (2008 dollars)

Millions of Dollars

http://www.ers.usda.gov/media/1424209/pesticideuseinusagriculture21selectedcrops.xlsx
Pesticides in the Environment: We Share

2. Renata Raina-Fulton, University of Regina
Pesticide Analyses – The Challenge

- European Union: 462 pesticides approved for use
- Japan Positive List has > 700 entries (January 2014) [http://www.m5.ws001.squarestart.ne.jp/foundation/agrall.php](http://www.m5.ws001.squarestart.ne.jp/foundation/agrall.php)
- British Crop Production Council Pesticide Manual (October 2014 update)
  - 920 main + 710 supplementary entries
  - >10,400 product names and >3,100 discontinued names
- The U.S. Environmental Protection Agency has approved approximately 10,400 of 16,000 pesticides via "conditional registration" since 2010, [http://www.rodalenews.com/herbicides-and-pesticides](http://www.rodalenews.com/herbicides-and-pesticides)

Maximum Residual Limits/Allowed Concentration (MRL or MAC): 0.01-10 ppm (mg/Kg or mg/L)
Analysis of Environmental Organic Contaminants
The General Approach

- Good screening method(s) for typical background study
  - Known-knowns (targeted) and known-unknowns (non-targeted)
  - Semi-quantitative data for trend analysis
  - Determine target pesticides for monitoring requirements

- Short and long term monitoring programs of target pesticides (quantitative analysis) to determine temporal and seasonal trends

- Develop mitigation strategies
Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS)

Targeted MS/MS Analysis
Two MRM (SRM) transitions

Collisional induced dissociation (CID)

Guideline 96/23/EG (veterinarian analysis), MS precursor = 1.0; MS² product 1.5; two MRMs get 4 identification points

Are two MRM transitions enough for identification?

False-positive liquid chromatography/tandem mass spectrometric confirmation of sebuthylazine residues using the identification points system according to EU directive 2002/657/EC due to a biogenic insecticide in tarragon

Andreas Schüermann*, Veronika Dvorak, Claudio Crúzer, Patrick Butcher and Anton Kaufmann
Data-dependent Acquisition (DDA) or Information-dependent Acquisition (IDA)

The DDA was developed for shotgun proteomic applications (circa. 2001)

- Using a survey scan, DDA selects N (N = 1 - ?) precursor ions according to user defined intensity criteria
- Collisional induced dissociation (CID) used to generate product ion mass spectra (DDMS$^2$) for each precursor for the identification of precursor ion
- Qualitative and quantitative analyses in one

Not Suitable for mega-method!

## Identification of 244 pesticides using with Fit > 80%

<table>
<thead>
<tr>
<th>Top N Ions</th>
<th>18 min LC Run Time</th>
<th>25 min LC Run Time</th>
<th>30 min LC Run Time</th>
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<tr>
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<td>Conc. (ppb) # of Identified</td>
<td>Conc. (ppb) # of Identified</td>
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<td>2</td>
<td>2.5 158</td>
<td>2.5 170</td>
<td>2.5 170</td>
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<tr>
<td>8</td>
<td>100 204</td>
<td>100 212</td>
<td>100 209</td>
</tr>
</tbody>
</table>

- Only Top-N ions were analyzed
- Duty cycle limits the number of data points used to define LC peaks correctly
- Two separate analyses are required for screening and quantitative analysis
Full Scan, High Resolution Mass Analyzer
An Orthogonal Approach for Chemical Contaminant Analysis
Quantitative, targeted screening and non-targeted screening

Full scan (TIC)

Extracted Ion Chromatogram (XIC) using exact mass

XIC of precursor ion

Quantitation

Identification

dd-MS/MS
(containing product ions)

Precursor ion from inclusion list to trigger dd-MS/MS

3 step Collision energy spectrum (HCD 40 ± 50%: 20, 40, 60)

Library search using dd-MS/MS spectral and/or compound database

Data mining

Database (ChemSpider) Search Result

Non-targeted screening based on full scan MS analysis

Targeted screening using dd-MS/MS
Data-independent Acquisition (DIA)

The DIA method was proposed to overcome the limitation of the DDA in shotgun proteomic analyses (John R Yates III et. al., Nature Methods 1, 39-45 (2004))

- Developed on a ThermoElectron LTQ fast scanning Iontrap
  - DIA selects all precursor ions (above a threshold) in a m/z window (isolation window, e.g., 25 daltons)
  - do the CID and collect one product ion spectrum for all precursor ions in that window
- The same process cycles through the selected mass range (e.g., 20 windows @ 25 Daltons/window for a mass range 100-600 m/z).
- Range of windows (isolation window) may vary:
  - depending on how crowded the spectrum is in a given range
  - in the crowded part (lower m/z), a 25-dalton isolation window
  - a 100-dalton isolation window when reaches the higher m/z which tends to have fewer precursor ions and cover a larger m/z range
- Consistent sampling point allows for good qualitative and quantitative analysis from one single LC-MS analysis
- Allows for known-unknown and unknown-unknown analysis
**DDA versus DIA**

**Q 1**

- One pre-scan to select one or top-N precursor ions for MS/MS

**CID**

- Best selectivity

**DDA**

- MRM/ SRM or DDMS²

**DIA**

- One pre-scan to select precursor ions above a threshold from a window

- Multiplexity & selectivity achieved via high accuracy mass analyzer
1 Full + 20 Sequential MS\(^2\) Scans = 1.8 Sec/Duty Cycle
21 scans @ \(R_{\text{FWHH}}\) 17,500 (12 scans/sec or 0.083 sec/scan)

- Duty cycle = 1.8 second
- Isolation window 25 Da
- Isolation window 100 Da

10 scans across peak
DIA Set up

https://skyline.gs.washington.edu/labkey/project/home/support/begin.view.
Effect of DIA Parameters: LOOP and MSX
Results from four level calibration standards of 603 pesticides (N=3)

Identification criteria:
1. SANCO 12571/2013
   a) Quasi-molecular ion (< 5 ppm)
   b) 1-3 fragment ions (<5 ppm accuracy, (FDA CFSAN + EFS Library, San Jose)
2. In addition:
   • Isotopic patterns (90% fit, ± 10% intensity and <5 ppm accuracy)
   • library search (FDA CFSAN + EFS Library, San Jose)

<table>
<thead>
<tr>
<th>MSX&amp;LOOP</th>
<th>Duty Cycle (Sec)</th>
<th>Concentration (pg/mL, ppt)</th>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Average</td>
<td>RSD</td>
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<td>1.1</td>
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<tr>
<td>10</td>
<td>20</td>
<td>0.7</td>
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</table>
Chloropyrifos, $[M+H]^+ = 349.9336$

DIA MS$^2$, m/z 325-350 isolation window

TIC

XIC

350 $\rightarrow$ 294

350 $\rightarrow$ 198

350 $\rightarrow$ 115
(m/z 200-225, 25-dalton isolation window)
Seven Triazines
(m/z 200-250, 50-dalton isolation window)

- Cyanazine, 241.10
- Atrazine, 216.10
- Simazine, 202.08
- Trietazine, 230.12
- Prometryn, 242.14
- Terbuthylazine, 230.12
- Cyprazine, 227.69
Diphenamid - Selective pre-emergence herbicide

**Data Review - MSX i Loop from JW 12152014 Screening**

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Compound Name</th>
<th>Match Result Name</th>
<th>Formula</th>
<th>Adduct</th>
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<tbody>
<tr>
<td>20141215_DIA_016_1000ppm_MLSX_Scan_1</td>
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<td>Diphenamid@RT 13.82</td>
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<td>Diphenylamine@RT 15.57</td>
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<td>Dipropetryn</td>
<td>Dipropetryn@RT 15.87</td>
<td>C11H21N5</td>
<td>M+H</td>
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</table>
Cycluron (199.1805, \( \text{C}_{11}\text{H}_{22}\text{N}_2\text{O} \)) or Cymoxanil (199.0826, \( \text{C}_7\text{H}_{10}\text{N}_4\text{O}_3 \))?

### Data Review - POCI5 Pest DIA 10122014 Screening

#### Compounds

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<thead>
<tr>
<th>#</th>
<th>Selected</th>
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<th>LS</th>
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<td>✔️</td>
<td>✔️</td>
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<td>●</td>
<td>▲</td>
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<td>▲</td>
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<td>Cycluron@RT 9.6</td>
<td>C11H22N2O</td>
<td>M+H</td>
</tr>
</tbody>
</table>

### Chromatogram

**DIA_POCI5_Pos_Pest_LO_DPI_1_#7 Cycluron**

NL: 2.60E7 m/z: 199.1795 - 199.1815

**FTMS + p Full ms [150.00-1000.00]**

#### Spectrum

**#1 Cymoxanil**

M+H @ POCI5_Pest_LO_DPI_1_#7 @ 1821 RT: 3.46

F: FTMS + p E01 Full ms m/z 137.84 @ h/e 0.00 107.84 @ h/e 30.00 [50.00-225.00]

**#2 Cycluron**

M+H @ POCI5_Pest_LO_DPI_1_#7 @ 1821 RT: 3.46

F: FTMS + p E01 Full ms m/z 137.84 @ h/e 0.00 107.84 @ h/e 30.00 [50.00-225.00]
Case #1: Neonicotinoid Pesticides

- Nicotine-based systemic insecticides, persistent in the environment and can be taken up by plant roots/leaves and transported throughout the plant.
- Widely used in agriculture including vegetables, fruit trees, berries, cereals and seed treatment; and are believed to be responsible for pollinator decline.
  - The European Commission suspended three neonicotinoids in 2013.
  - The US EPA is expediting its review because of suspected harmful effect on bees.
  - Ontario will reduce the use by 80% with neonicotinoid-treated seed by 2017.

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Products</th>
<th>Million US $ (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>Bayer</td>
<td>Confidor, Admire, Gaucho</td>
<td>1,091</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>Syngenta</td>
<td>Actara, Platinum, Cruiser</td>
<td>627</td>
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<tr>
<td>Clothianidin</td>
<td>Sumitomo/Bayer</td>
<td>Poncho, Dantosu, Dantop</td>
<td>439</td>
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<tr>
<td>Acetamiprid</td>
<td>Nippon Soda</td>
<td>Mospilan, Assail, Tristar</td>
<td>276</td>
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<tr>
<td>Thiacecloprid</td>
<td>Bayer</td>
<td>Calypso</td>
<td>112</td>
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<tr>
<td>Dinotefuran</td>
<td>Mitsui</td>
<td>Starkle, Safari, Venom</td>
<td>79</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>Sumitomo</td>
<td>Capstar, Guardian</td>
<td>8</td>
</tr>
</tbody>
</table>
Neonicotinoid Pesticides

“Declines in insectivorous birds are associated with high neonicotinoid concentrations”

“... we show that, in the Netherlands, local population trends were significantly more negative in areas with higher surface-water concentrations of imidacloprid. At imidacloprid concentrations of more than 20 nanograms per litre, bird populations tended to decline by 3.5 per cent on average annually.”
MOE Passive Sampling

- Sample a broad range of chemicals:
  - Hydrophobic/ Bioaccumulative (SPMD/PE)
    - PCBs, PAH
    - BFRs, fragrances, surfactants
  - Soluble in Water (POCIS)
    - Pharmaceuticals
    - Hormones
    - Current-use pesticides
    - Fungicides
POCIS Sampling

Lake Ontario

Lake Erie

- Lake Ontario POCIS - 2012
- Lake Erie POCIS - 2013
- Tributary POCIS - 2013
Preparation of POCIS Samples?

1. Field samples (whole) were transferred into glass centrifuge tubes.

2. Surrogates and 20mL actonitrile:H2O (0.1% acetic acid, 70:30 v/v, 1 mM EDTA) shake vigorously for 5 min., and ultrasonically extracted for 20 min.

3. Sample tubes were centrifuged for 8 min at 3500 rpm. The supernatant was collected.

4. The cycle was repeated using 20 mL of Acetonitrile: Acetone, 50:50 v/v.

5. Combined extracts were brought up to 50 mL, filtered through 0.2 micron syringe filter.

6. Evaporate 10 mL of the extract to dryness; reconstitute in 100 µL internal standard solution without any cleanup and HPLC-HRMS analysis.
Typical System Performance

- 10 μL injection volume
- calibrated from 0.5 to 40 ppb (5 – 400 pg on-column)
- Estimated LOD was 25 pg Total
- XICs were obtained from the 0.5 ppb standard

Typical POCIS has a sampling volume of 15-20 L. Assuming the use of 10-L sampling volume, the LOC can be calculated at 2.5 pg/L (5 ppq)
## Analytical Results
(57 Field Samples + 7 Field Blanks)

<table>
<thead>
<tr>
<th>Compound</th>
<th># Occurrence</th>
<th>% Occurrence (N=57)</th>
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<tbody>
<tr>
<td>Acetamiprid</td>
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<tr>
<td>Clothianidin</td>
<td>19</td>
<td>33.3%</td>
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<tr>
<td>Dinotefuran</td>
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<tr>
<td>Flonicamid</td>
<td>5</td>
<td>8.8%</td>
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<tr>
<td>Imidacloprid</td>
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</tr>
<tr>
<td>Nitenpyram</td>
<td>9</td>
<td>15.8%</td>
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<tr>
<td>Thiacloprid</td>
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<tr>
<td>Thiamethoxam</td>
<td>0</td>
<td>0.0%</td>
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**Case #2: Analysis of Triazine Pesticides**  
Ontario Drinking Water Quality Standards (ODWQS) under Ontario Regulation 169/03, Triazine

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>CAS Number</th>
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<tbody>
<tr>
<td>De-ethyl Simazine</td>
<td>1007-28-9</td>
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<td>Prometone</td>
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<td>Atrazine + N-dealkylated metabolites</td>
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</table>
The SDWA’s main features include:

- Drinking-water quality standards
- Licensing for water-testing laboratories
- Approvals process for private water supply systems
- Duties on owners, operating authorities and laboratories to immediately report adverse water test
- Enforcement mechanisms
- An annual drinking-water report published by the Minister.

Section 16-3, par. 3 of Schedule 16 also requires laboratories to report “a result indicating the presence of a pesticide not listed in Schedule 2 to the Ontario Drinking Water Quality Standards in a sample of drinking water at any concentration”.
Comparison of Triazine Analytical Methods
Two-laboratory study

**Laboratory #1**
- C18 LC Column, 2.1 x 50mm, 1.7µm particle size, 0.5 mL/min flow rate, 5 mL on-line SPE
- Column temperature @ 40°C
- Tandem MS analysis using 2 MRMs for 15 target compounds
- D14-propazine as surrogate
- Quantitated by seven level calibration standards (1 to 100 ng/L) and the primary MRM.
- MDL in the 100 – 800 ppt (ng/L) range

**Laboratory #2**
- C18 LC Column, 1.0 x 100mm, 1.9µm particle size, 0.32 mL/min flow rate, 65 µL direct aqueous injection
- Column temperature @ 35°C
- HRMS DIA analysis for 15 target compounds
- Target compounds identified by accurate mass (5 ppm), one fragment ion, library search and LC RT
- D5-atrazine and D5-cyanazine as internal standard/surrogate
- Quantitated by eight level calibration standards (2.5 to 500 pg/mL) and the molecular ion
# Method Performance

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<th>Compound</th>
<th>Avg pg/mL</th>
<th>Expected pg/mL</th>
<th>RSD %</th>
<th>Rec</th>
<th>Avg pg/mL</th>
<th>Expected pg/mL</th>
<th>RSD %</th>
<th>Rec</th>
<th>Avg pg/mL</th>
<th>Expected pg/mL</th>
<th>RSD %</th>
<th>Rec</th>
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</tbody>
</table>
Atrazine @ 14 ng/L
Deethyl atrazine @ 8 ng/L
Simazine @ 17 ng/L
Thank you for Your Attention

Alex Krynitsky & Kai Zhang
Jon Wong
Zhengwei Jia
Jim Chang
Paul Helm
Jian Wang
Charles Yang & Dipanka Ghosh