Rapid screening of chemical (Drug and Pesticide) residues in food using (LC)-TOF-MS

Results and challenges

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Presentation overview

- LC-TOF-MS for screening of samples for veterinary medicines

- Direct TOF-MS analysis of chemical residues using an Atmospheric Pressure Solids Analysis Probe (ASAP).

- Summary of progress and future work
ToF and Veterinary Drugs

- Urine
  100 veterinary drugs

- Egg
  Macrolides.

- Honey
  8 tetracyclines.

- Milk
  >100 veterinary drugs

  150 veterinary drugs,

- Egg, fish, meat
  • 100 veterinary drugs
Project Aim

• Investigation of use of Time-of-Flight Mass Spectrometry (ToF-MS) coupled to liquid chromatography. **Screening only**

• Selection of drugs:
  • EU MRL/MRPL (tissue) compounds
  • Other Annex IV (banned) compounds
  • Related compounds
    o Historical use
    o Licensed use outside of EU
    o Potential compounds of abuse
Extraction and Clean-Up

Initial test matrix – Chicken muscle

Extraction
Oxalic acid/MeCN

Dry
Na₂SO₄

Dispersive SPE
C18
Analysis

- LC-ToF-MS
  - Agilent 1200 series LC coupled to 6224 ToF-MS
    - Column – Acquity HSS T3 50 x 2.1mm 1.8µm.
    - Mobile phase A: 0.1% formic acid in water.
    - Mobile phase B: 0.1% formic acid in methanol.
    - Gradient: 5%B to 99%B in 15 minutes.
    - Column temperature: 40 °C
    - Injection solvent: 1:1 Methanol:water
    - Mode: Electrospray
  - 2 instrument runs per sample ES+ and ES-
Validation approach (1)

- Qualitative screen, CCB only.
  - 3 spiking concentrations – 1, 10, 100 µg/kg
  - 19 out of 20 replicates detected at any concentration.

- Ruggedness - Other matrices (Bovine muscle, liver, kidney etc.)
  - 3 spiking concentrations – 1, 10, 100 µg/kg
  - 8 out of 8 replicates detected at any concentration.
Validation approach (2)– choice of spiking concentrations

**EU MRLs for muscle**

- **0 - 0.9 µg/kg**: 8 (4%)
- **1 - 9**: 16 (8%)
- **10 - 99**: 71 (37%)
- **100+**: 97 (51%)

*N=192*
Validation approach (3) – screening for MRL/MRPL compounds

Increasing concentration

Highest Concentration Not Detected | Lowest Concentration Detected

- MRL = FAIL
- MRU = UNKNOWN
- MRL = PASS
Validation Approach (4) screening for non-MRL compounds

- Possible outcomes for non-MRL compounds
  - Detected at any concentration – **PASS**
  - Not detected at 100µg/kg - **FAIL**
Ions Monitored

• ES+
  – \([\text{M+H}]\) and/or \([\text{M+Na}]\) except:
    – \([\text{M+2H}]\) (azithromycin, bacitracin A, cefquinome, ceftazidime, imidocarb, spiramycin I, tulathromycin)
    – \([\text{M+3H}]\) (bacitracin A, tulathromycin)
    – \([2\text{M+Na}]\) (ribavirin, furazolidone)
    – \([\text{M+}]\) (amprolium, dyes)
    – \([\text{M+H2O+H}]\) (succinylsulfathiazole)
    – \([\text{M+MeOH+H}]\) (3-O-acetyltlyosin, tylosin A)
    – \([\text{M-C2H2O+H}]\) (troleandomycin)
    – \([\text{M-C3N2H4+H}]\) (clotrimazole)

• ES−
  – \([\text{M-H}]\) except:
    – \([\text{M+HCO2Na-H}]\) (clavulanic acid)
### Results 1 – Poultry muscle

<table>
<thead>
<tr>
<th>Class</th>
<th>MRL/MRPL</th>
<th>Non-MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pass</td>
<td>Fail</td>
</tr>
<tr>
<td>Ansamycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthelminitics (general)</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Antifungal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiviral</td>
<td></td>
<td></td>
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<tr>
<td>Avermectins</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Benzimidazoles</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Coccidiostats (general)</td>
<td>4</td>
<td></td>
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<tr>
<td>Diaminopyrimidines</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dyes</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Fenicols</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Ionophores</td>
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<tr>
<td>Lincosamides</td>
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<tr>
<td>Macrolides</td>
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<td>2</td>
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<tr>
<td>Nitrofurans/markers</td>
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<td></td>
</tr>
<tr>
<td>Nitroimidazoles</td>
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<td>1</td>
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<tr>
<td>Penicillins</td>
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<td>Pesticides</td>
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<td>1</td>
</tr>
<tr>
<td>Phenols/salicylanilides</td>
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<td>1</td>
</tr>
<tr>
<td>Pleuromutilins</td>
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<td></td>
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<tr>
<td>Quinolones</td>
<td>9</td>
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<tr>
<td>Quinoxalines/markers</td>
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<td>3</td>
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<tr>
<td>Sulfonamides</td>
<td>25</td>
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<td>Tetracyclines</td>
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<tr>
<td>Tranquilizers</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Unclassified</td>
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<td>3</td>
</tr>
</tbody>
</table>

Total investigated: 265
No. Pass: 196
% Pass: 74

**Reasons for Failures**
- Sensitivity (MRPLs)
- Suppression (Polarity extremes)
- Recovery
Results 2 – Other Matrices

Bovine Muscle

- Pass: 48
- Fail: 11
- Unknown: 206

Bovine Liver

- Pass: 44
- Fail: 6
- Unknown: 215

Bovine Kidney

- Pass: 44
- Fail: 9
- Unknown: 212
ES+ Quinolones

- Pipemidic acid
- Marbofloxacin
- Ofloxacin
- Enoxacin
- Norfloxacin
- Ciprofloxacin
- Enrofloxacin
- Danofloxacin
- Difloxacin
- Sarafloxacin
- Oxolinic acid
- Lomifloxacin
- Flumequine
- Ibafloxacin
- Piromidic acid
- Nequinate
- Decoquinate
- Cinoxacin
- Oxolinic acid
- Nalidixic acid
- Flumequine
ES- Various drugs

- Thiamphenicol
- Florfenicol
- Nitromide
- Clorsulon
- Tetrazole
- Succinyl sulfathiazole
- Nitrofurantoin
- Phthaly sulfathiazole
- Phthaly sulfacetamid
- Chloramphenicol
- Nitroxynil
- Niclosamide
- Cefuroxime
- Bithionol
- Fusidic acid
- Closantel
- Toltrazuril
- Zeranol
- TFM
- Thioxanthenesulfonate
- Toltrazuril Sulfoxide
- Toltrazuril Sulfone
- Toltrazuril
- Diflubenzuron
- Bithionol sulfoxide
- Tflubenzuron
- Niclosamide
- Diflubenzuron
- Chloranilone
- Oxyclozanide
- Bithionol
- Toltrazuril
- Diflubenzuron
- Bithionol sulfoxide
- Tflubenzuron
- Niclosamide
- Diflubenzuron
- Chloranilone
- Oxyclozanide
Issues

• Sensitivity v Scan speed v “points across peak” – run times (conventional v rapid)

• Instrument resolution

• Matrix suppression effects
Run times

7 min gradient
1 spectrum/sec

[M+H]
C12H17N2S
221.1107

BUT 70% response lost
MS resolution – mass 895

50ppm window

10ppm window

Peak Apex 1
Measured mass 895.5032

Peak Valley
Measured mass 895.4924

Peak Apex 2
Measured mass 895.4856

Mass Accuracies/ppm
Abamectin B1a  -24.3
Semduramicin  -0.7

-12.3  11.3  -4.7  18.9
Matrix Suppression

Closantel M-H

Solvent standard

Tissue standard
Conclusion/Future work

• >200 compounds successfully detected in a range of matrices.

• A number of issues identified

• Introduction of new technology – Agilent ‘Jetstream’ source, other accurate mass instruments.

• Validation requirements for multi-residue screening methods need to be better defined.
Presentation overview

- LC-TOF-MS for screening of samples for veterinary medicines

- Direct TOF-MS analysis of chemical residue using an Atmospheric Pressure Solids Analysis Probe (ASAP).

- Summary of progress and future work
ASAP-ToFMS

- Low cost accessory
- Fast change-over
- Easy to use
- Rapid analysis *(no chromatography)*
- Manual
- Enclosed source/safe
Atmospheric Pressure Solids Analysis Probe: overview

- Analyte response dependent on gas temperature and positioning of probe

Schematics courtesy of Waters
ASAP in Practice

- Bake probe (500°C) to remove contamination

- Load sample onto probe (wipe or 1µ soln)

- Insert probe (start acquisition 20 secs before analysis)

- Total analysis time = < 5 minutes

- Possible to re-use probe
Applications – ‘proof of principle’

• Strobilurin fungicides in grain
  - whole grains
  - milled grains
  - solvent extracts of grain material

• Pesticides in solvent extract of orange

• Dye contamination of spice

• Field application - diffusion of pesticides
ASAP- Selected strobilurins in solvent standard

[M+H]$^+$ mass spectra

ASAP – LCT Premier XE : Waters

LCT :  W mode (10-11000 FWHM)
Polarity :  positive
Gas Temp:  250 - 300°C
ASAP- Direct analysis of incurred whole wheat grain

TIC (sample containing azoxystrobin)

Elemental Composition Report

<table>
<thead>
<tr>
<th>Measured Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>Formula</th>
</tr>
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<tbody>
<tr>
<td>404.1230</td>
<td>404.1246</td>
<td>-1.6</td>
<td>-4.0</td>
<td>C_{22}H_{18}N_{3}O_{5}O_{3}</td>
</tr>
</tbody>
</table>

Azoxystrobin 4.5 mg/kg
ASAP- Analysis of QuECHERS crude solvent extracts of whole wheat grain

Elemental Composition Report

<table>
<thead>
<tr>
<th>Measured Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>Formula</th>
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</thead>
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<tr>
<td>404.1240</td>
<td>404.1246</td>
<td>-0.6</td>
<td>-1.5</td>
<td>C_{22}H_{18}N_{3}O_{5}O_{3}</td>
</tr>
</tbody>
</table>
ASAP: direct analysis of milled wheat grain

Elemental Composition Report

<table>
<thead>
<tr>
<th>Measured Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>Formula</th>
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<tbody>
<tr>
<td>404.1406</td>
<td>404.1246</td>
<td>16.0</td>
<td>39.6</td>
<td>C_{22}H_{18}N_{3}O_{5}</td>
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ASAP Analysis of QuEChERS acetonitrile extracts of milled wheat grain

Elemental Composition Report

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<th>PPM</th>
<th>Formula</th>
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<td>C_{22}H_{18}N_{3}O_{5}</td>
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</table>
## ASAP – Initial results summary

### Linearity - azoxystrobin

![Graph showing linearity](image)

<table>
<thead>
<tr>
<th>ASAP Method</th>
<th>LC-MS/MS mean mg/kg</th>
<th>ASAP mean mg/kg</th>
<th>Repeatability (RSD, n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain – Probe</td>
<td>4.5</td>
<td>Detected (response equiv to 12 ng)</td>
<td>12</td>
</tr>
<tr>
<td>Whole grain – Acetonitrile</td>
<td>4.5</td>
<td>3.5</td>
<td>40</td>
</tr>
<tr>
<td>Whole grain – Methanol</td>
<td>4.5</td>
<td>3.4</td>
<td>7</td>
</tr>
<tr>
<td>Milled grain – QuEChERS</td>
<td>0.45</td>
<td>0.4</td>
<td>14</td>
</tr>
</tbody>
</table>
ASAP - solvent extracts of orange

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Accurate mass</th>
<th>PPM</th>
<th>Detected</th>
<th>GC-MS</th>
<th>LC-MS/MS</th>
<th>LC-TOF</th>
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<tbody>
<tr>
<td>ASAP</td>
<td>ASAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>buprofezin</td>
<td>306.1618</td>
<td>7.2</td>
<td>Y</td>
<td>nd</td>
<td>Y</td>
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</tr>
<tr>
<td>chlorpyrifos</td>
<td>349.9378</td>
<td>-10.6</td>
<td>Y</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>chlorpyrifos-methyl</td>
<td>321.9030</td>
<td>-0.6</td>
<td>Y</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>cyproconazole</td>
<td>292.1245</td>
<td>-9.6</td>
<td>nd</td>
<td>Y</td>
<td>Y</td>
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<td>cyprodinil</td>
<td>226.1334</td>
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<td>Y</td>
<td>nd</td>
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<tr>
<td>dimethoate</td>
<td>230.0070</td>
<td>1.7</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>β-endosulfan</td>
<td>nd</td>
<td>-</td>
<td>Y</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

ASAP detected 27 out of approximately 50 compounds

LC-MS/MS detected a total of 27 compounds
ASAP—
detection of contaminant in saffron

sample 400 C
260609_005 20 (0.797) Cm (19:23-(32:35+10:14))

1: TOF MS AP+
2.43e4
### Elemental Composition Report

**Single Mass Analysis**

- **Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0**
- **Selected filters: None**

#### Maxima

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>50.0 PPM</th>
<th>50.0 DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>268.1805</td>
<td>268.1814</td>
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<td>-3.4</td>
<td>8.5</td>
<td>1185.6</td>
<td>C17 H22 N3</td>
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<tr>
<td>268.1792</td>
<td>268.1774</td>
<td>1.3</td>
<td>4.8</td>
<td>-0.5</td>
<td>7005.2</td>
<td>C11 H27 N3 O2 35Cl</td>
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<tr>
<td>268.1832</td>
<td>268.1774</td>
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<td>268.1774</td>
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<td>268.1760</td>
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<td>16.8</td>
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<td>1995.9</td>
<td>C11 H26 N O6</td>
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</tbody>
</table>

**Tentative identification of dye sample 400 C**

1: TOF MS AP+  
268.181 0.05Da  
7.57e3  

![Diagram of auramine molecule]
ASAP - auramine standard

**Elemental Composition Report**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
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<th>50.0 PPM</th>
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<td>286.6</td>
<td>C18 H22 N O</td>
<td></td>
</tr>
</tbody>
</table>

**Graphical Representation**

- **Time** range: 0.20 to 2.00
- **m/z** range: 0 to 1000
- **Intensity** range: 0 to 100

**Graph Details**
- **auramine std**
- **auramine std**
- **260809_001**
- **1: TOF MS AP+**
- **268.181 0.05Da**
- **7.20e3**

**Graph Elements**
Optimisation of temperature

300°C

350°C

400°C

auramine in sample of saffron: m/z 268.1814
Field applications

- Prevent contamination of probe before use
- Avoid contact with surface of probe after sampling

Transportation of probe samples to the laboratory?
A simple practical solution
Detection of pesticides on surface of leaves in field

Tebuconazole
M+H 308.1530 calc
308.1523 measured
ASAP – Summary to date

• Qualitative screening capability – surface residues
• Matrix effects in complex extracts
• Possibilities for ‘multiple sampling in the field’
  - *Increased probability of detecting ‘hotspots’*
• Destructive ‘No repeat analysis’ (requires duplicates for positive and negative modes)
• Many possible niche applications
Thank you for listening and acknowledgements to;

BioCop Colleagues (Michel W. F. Nielen and Jana Hajslova, Matthew Sharman and teams) (FP6 project: FOOD-CT-2005-006988)

Fera Colleagues (Simon Hird)

Waters (Ramesh Rao, Hiliary Major, Sandra Rontree)

UK Veterinary Medicines Directorate

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