Emerging Technologies in Chemical Food Safety Control

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Opportunities, challenges, and some buts
Stakeholders ask for:

- fast and simplified, one or a few parameters
- comprehensive, many parameters including emerging contaminants and unknowns
- fit-for-purpose, confirmed, validated, accredited; withstand in court cases
- scientists: risk-benefit, combi-tox, etc

Innovation of the toolbox for detection of chemical contaminants is urgent…… and fit-for-purpose analysis desired
3D research area for (un)known contaminants

- Introduction
- UPLC/MS
- REA
- Bio/MS
- Omics
- DESI/DART
- Conclusion

Information

- ‘omics’
- holistic
- group++
- group

GC/MS, LC/MS

Sensors

3D research area

space

2D

3D

1

1/jr

1/s

cont.

frequency
Emerging chemical food contaminants

EC/178/2002: proactive search for emerging risks

1. Recognised, for example by EFSA
   - Perfluorinated organic substances (FP7: PERFOOD)
   - Brominated flame retardants (FP7: CONffIDENCE)
   - Nanoparticles FP7: NanoImpactNet; NanoLyse, NanoValued

2. Not recognised yet: unknown contaminants
   - Originating from illegal production (melamine, hormones, …)
   - Changing natural toxins
   - Masked contaminants (esters, ethers, glycosides)
4D research area for (un)known contaminants

- Information
- ‘omics’
- GC/MS, LC/MS
- Sensors

- holistic
- group++
- group

- 3D
- 2D
- 1D

- bioactivity
- molec. recognition
- cell in vitro
- in vivo
- frequency

Introduction
UPLC/MS
REA
Bio/MS
Omics
DESI/DART
Conclusion
2 ml milk
Protein precipitation
2 ml acetonitrile
centrifugation
dilute 2 ml supernatant 10x with water
Generic SPE
StrataX
Elute with 3 ml of methanol, evaporate, redissolve
UPLC/TOFMS

101 vet.drugs in milk in 10 minutes
UPLC/TOFMS of milk extracts

**Introduction**

**UPLC/MS**

**REA**

**Bio/MS**

**Omics**

**DESI/DART**

**Conclusion**

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Detection of Chemical Food Contaminants
Laboratory of Organic Chemistry
Wageningen University
Quantitative validation of 101 vet drugs

Introduction

UPLC/MS

REA

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Omics

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Detection of Chemical Food Contaminants
Laboratory of Organic Chemistry

WAGENINGEN UNIVERSITY

RIKILT
INSTITUTE OF FOOD SAFETY
New views on validation concepts needed!

- LOQ: all compounds < 7 µg/L [#100 < MR(P)L]
- Linearity: 100 compounds >0.9 [#80 >0.99]
- Robustness, specificity: passed
- Application: 100 independent milk samples: no false positives, nor false negatives.
- Challenges: sample prep and data handling/reporting

*Now extended to >250 pesticides, mycotoxins, vet.drugs in feed and honey*

Bioassay screening

- recombinant yeast cells expressing the human steroid receptor and yGFP-reporter
- for androgens, estrogens, gestagens, glucocorticosteroids

2002/657/EC validated, ISO 17025 accredited, transferable, interlab tested in EU
Food supplements: a ‘pure herbal product’ case

Introduction

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Concentration 17-α-E2 [nM]

Fluorescence response in REA (hERα)

Extract volume

17β-E2

Batch 1

CCα
Bioactivity-directed LC/TOFMS identification

RIK0215677 MeOH extract (1.04 mg/ml)

LCT_080609_005

1: TOF MS ES-
267.139 0.02Da

DES std

100

0

%  

0 2.50 5.00 7.50 10.00 12.50 15.00

%  

0 2.50 5.00 7.50 10.00 12.50 15.00

LCT_080609_007

1: TOF MS ES-
267.139 0.02Da

MeOH extract of batch #1

100

0

%  

0 2.50 5.00 7.50 10.00 12.50 15.00

%  

0 2.50 5.00 7.50 10.00 12.50 15.00

LCT_080609_007 785 (9.415) Cm (777:792) 1: TOF MS ES -
267.1357 313.1416

Diethylstilbestrol (DES)
Scary food supplements! (versus residue issues)

- Batch #1 of the capsules contained about 0.9 mg/g
- Batch #2 of the capsules contained about 4.1 mg/g
- Batch #3, recently released tablets, contained no estrogenic compounds (< 5 ng/g)

DES: cancer incidences in 1st and 2nd generation
Future screening and confirmation according to the current Commission Decision 2002/657/EC ?!

- Biorecognition screening assay on a chip, *plus*
- isolation and purification of suspect on a chip, *plus*
- confirmation of identity using nanoLC/MS on a chip.

- faster, less reagent consumption, more sensitive when sample availability is limited
Example: label-free binding assay on a chip: SPR

- Big molecules: direct assay using immobilised recognition elements
- Small molecules: inhibition assay format
# SPR biosensor screening for 13 fluoroquinolones

## 1. Screening chip

### Specifications of TA met

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity</th>
<th>cc beta µg/kg</th>
<th>MRL µg/kg</th>
<th>Specifications of TA met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>82 %</td>
<td>0.6</td>
<td>100</td>
<td>✓</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>50 %</td>
<td>1.0</td>
<td>200</td>
<td>✓</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>45 %</td>
<td>1.1</td>
<td>300</td>
<td>✓</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>118 %</td>
<td>0.4</td>
<td>100</td>
<td>✓</td>
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<tr>
<td>Flumequine</td>
<td>0.7 %</td>
<td>68</td>
<td>400</td>
<td>✓</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>80 %</td>
<td>0.6</td>
<td>150 (bov./por.)</td>
<td>✓</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>100 %</td>
<td>0.5</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td>30 %</td>
<td>1.7</td>
<td>10 (fat)</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 (liver)</td>
<td>✓</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>26 %</td>
<td>1.9</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>43 %</td>
<td>1.2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>65 %</td>
<td>0.8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>5 %</td>
<td>11</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>116 %</td>
<td>0.4</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Introduction

UPLC/MS

REA

Biosensor/MS

Omics

DESI/DART

Conclusion
Purification of suspect non-compliants plus confirmation

2. Suspect onto recovery chip

Affinity purified specific IgG fraction immobilized

3. to nanoLC/ESI chip
Proof of concept: antibiotics in chicken muscle

3. nanoLC/ESI chip + TOFMS

incurred at 0.5 MRL (= 50 ng/g)
Proof of concept: antibiotics in chicken muscle

<table>
<thead>
<tr>
<th>Formula</th>
<th>Calculated Mass</th>
<th>mDa Error</th>
<th>ppm Error</th>
<th>RDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>C19 H23 N3 O3 F</td>
<td>360.1723</td>
<td>-0.44511</td>
<td>-1.23584</td>
<td>9.5</td>
</tr>
<tr>
<td>C24 H24 O3</td>
<td>360.1725</td>
<td>-0.64488</td>
<td>-1.79048</td>
<td>13</td>
</tr>
<tr>
<td>C19 H24 O2 F4</td>
<td>360.1712</td>
<td>0.65676</td>
<td>1.823463</td>
<td>6</td>
</tr>
<tr>
<td>C22 H22 N3 O2</td>
<td>360.1712</td>
<td>0.697816</td>
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<tr>
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<tr>
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<td>-4.96377</td>
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<tr>
<td>C22 H23 O F3</td>
<td>360.1701</td>
<td>1.79969</td>
<td>4.996753</td>
<td>10</td>
</tr>
</tbody>
</table>

Enrofloxacin

SciFinder element composition search: 1 structure option

### Topic 4: ‘Omics’-based untargeted approaches

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA oral</td>
<td>DHEA intramuscular</td>
<td>DHEA oral</td>
</tr>
<tr>
<td>N=3</td>
<td></td>
<td>DHEA intramuscular</td>
</tr>
<tr>
<td>Controls (6 months)</td>
<td></td>
<td>Controls (13-14 months)</td>
</tr>
<tr>
<td>- Male Holstein Frisians, 9-14 months, 210-430 kg</td>
<td></td>
<td>- Exposed for 7 days, 1 gram DHEA per day</td>
</tr>
</tbody>
</table>

- Male Holstein Frisians, 9-14 months, 210-430 kg
- Exposed for 7 days, 1 gram DHEA per day
Metabolomics of urine samples: UPLC/TOFMS

**Challenges:**
- robustness
- alignment
- MVA statistics
- validation of ‘biomarkers’
- identification
- biovariability!

ANOVA $p < 0.01$: 1565 mass peak loadings; additional criteria: 180 robust candidates.

Validation for false negative/false positive: 7 comply with CCβ and are highly relevant!

Crude ethyl acetate extract of 50 ug/kg spiked wheat

DESI MS and DART of fungicides in wheat

- C18 tip methanol percolate of Azoxystrobin incurred wheat

**DESI/LITMS**

m/z 404->372->

![Chemical Structure](image)

Conclusion

1. Instrumental methods catch-up in terms of speed
   - simplified generic sample extraction protocols are crucial
   - intelligent data evaluation software needed
   - hundreds of contaminants in 10 minutes
   - initially expensive but very low cost per analyte

2. More bioactivity-related multiplex assays needed
   - essential for recognizing the unexpected and unknowns
   - potential for simplified on-site rapid analysis as well
   - (bio)nanotechnology formats expected
3. Omics technologies in food control

- essential for recognizing biological effects and the presence of the unexpected and unknowns
- inherently slow
- many data handling, statistics and validation challenges
- real-life includes biovariability!

4. Forensic technologies (DESI, DART) in food control

- very fast pre-screening
- (semi) quantitative under certain conditions
- validation of false-negative rate!
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