

# Emerging Technologies in Chemical Food Safety Control



[michel.nielen@wur.nl](mailto:michel.nielen@wur.nl)



Detection of Chemical Food Contaminants  
Laboratory of Organic Chemistry



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# Opportunities, challenges, *and some butts*



Introduction

UPLC/MS

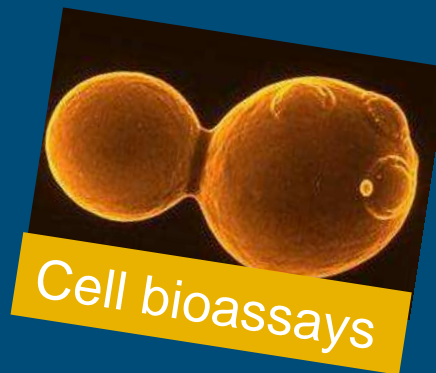
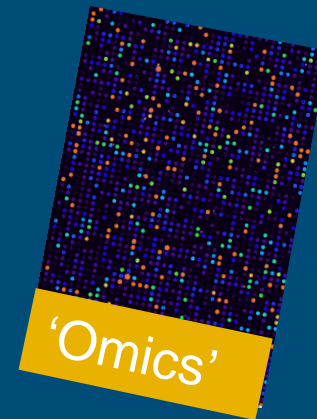
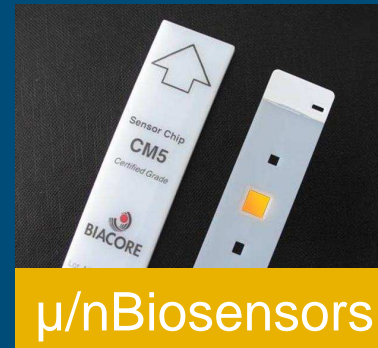
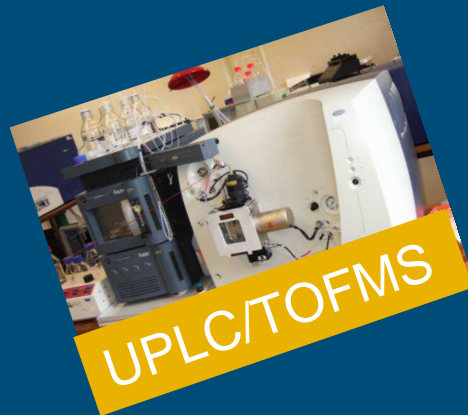
REA

Bio/MS

Omics

DESI/DART

Conclusion



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# Detection of chemical food contaminants



## Introduction

UPLC/MS

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## 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*



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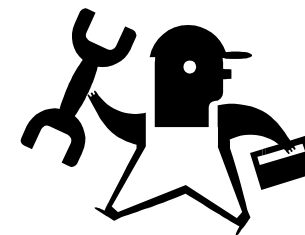


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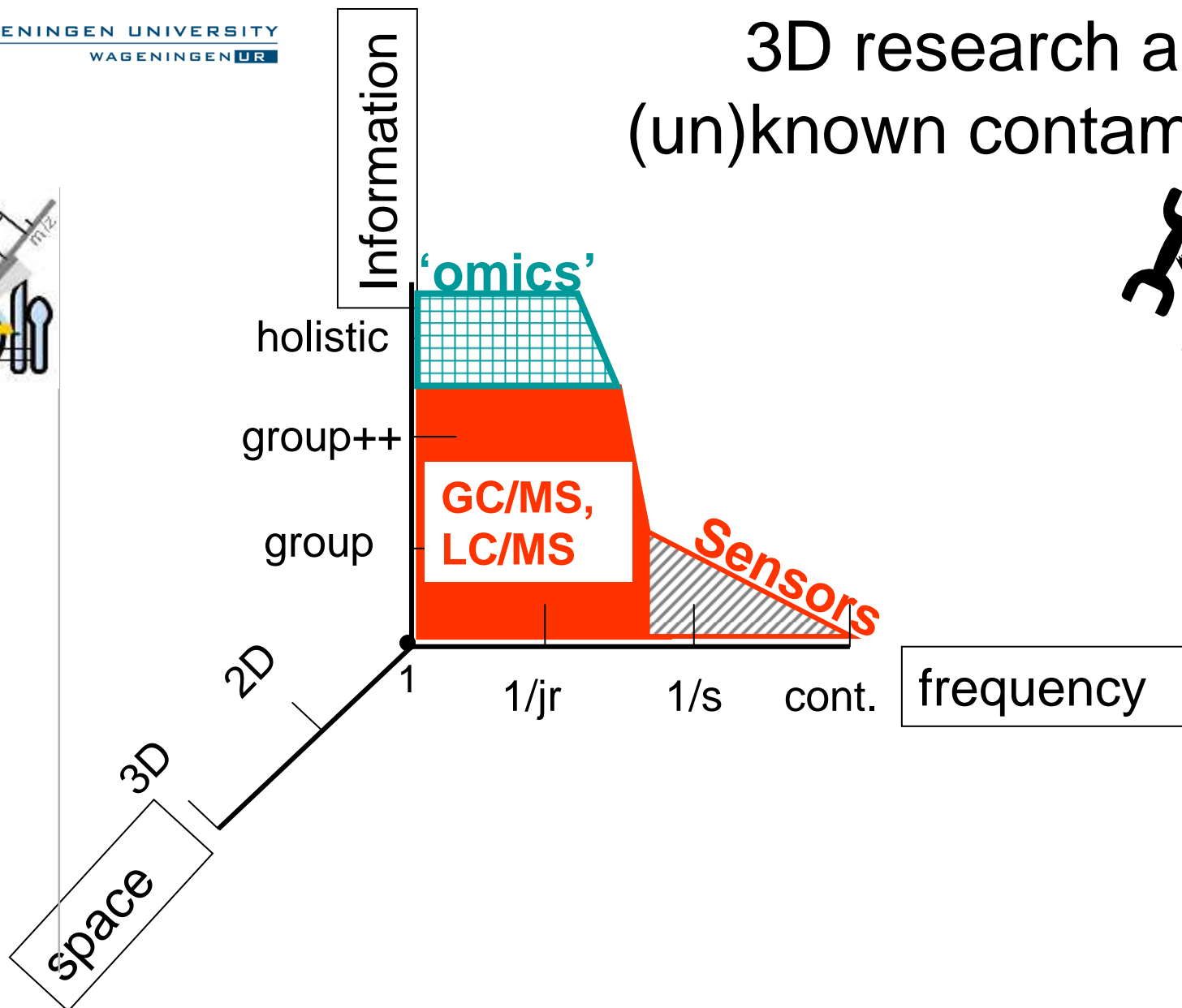
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# 3D research area for (un)known contaminants



## Introduction

- UPLC/MS
- REA
- Bio/MS
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- Conclusion



# Emerging chemical food contaminants



Introduction

UPLC/MS

REA

Bio/MS

Omics

DESI/DART

Conclusion

## 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)

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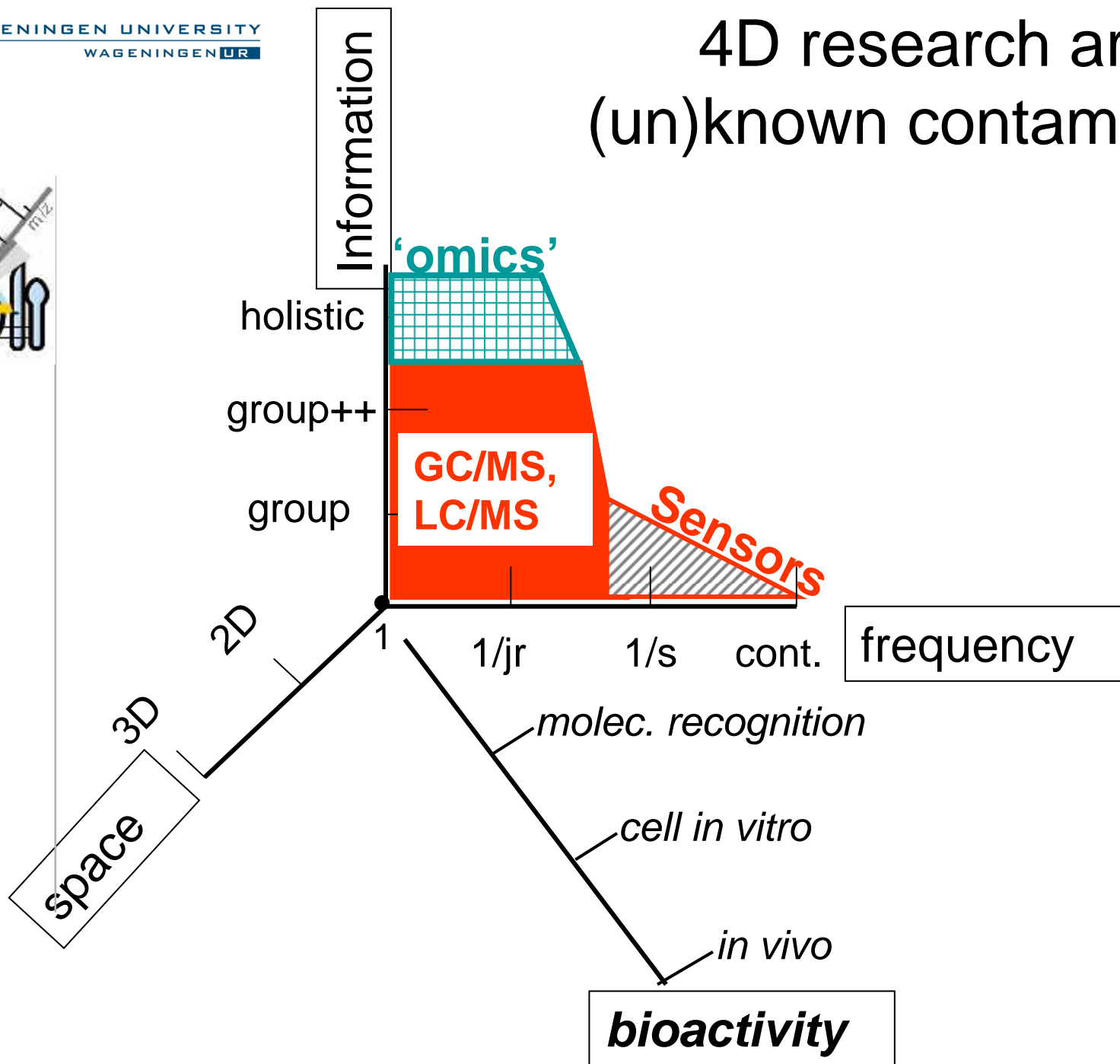


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# 4D research area for (un)known contaminants



- Introduction
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# Topic 1: instrumental multi-target screening



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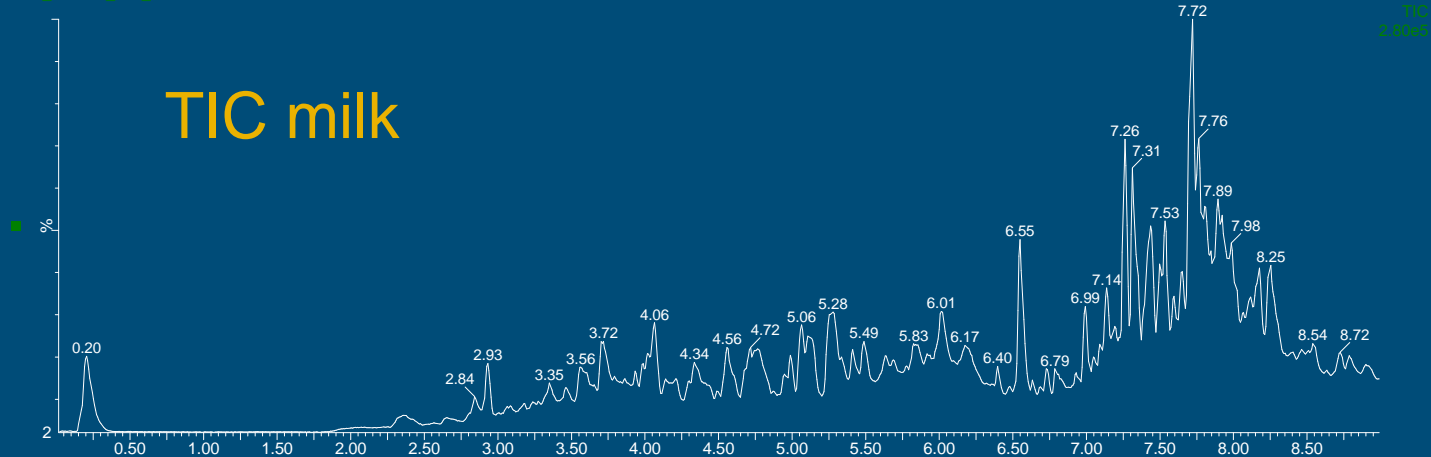
# UPLC/TOFMS of milk extracts



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

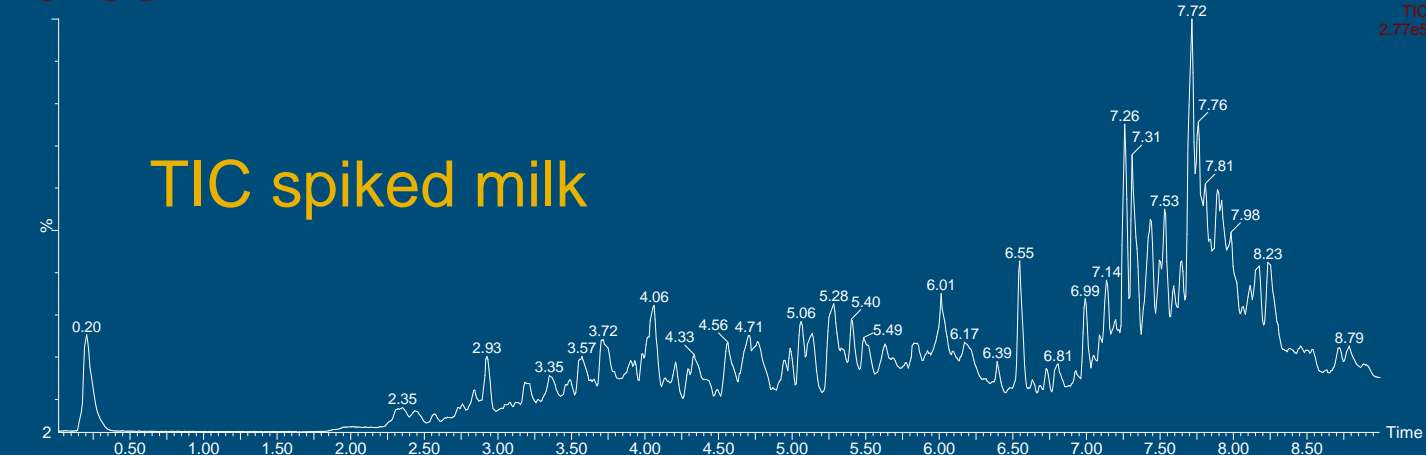
Bianco Milk  
LCT\_070612\_MM\_009

## TIC milk



LCT\_070612\_MM\_012

## TIC spiked milk



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# Quantitative validation of 101 vet drugs



Introduction

UPLC/MS

REA

Bio/MS

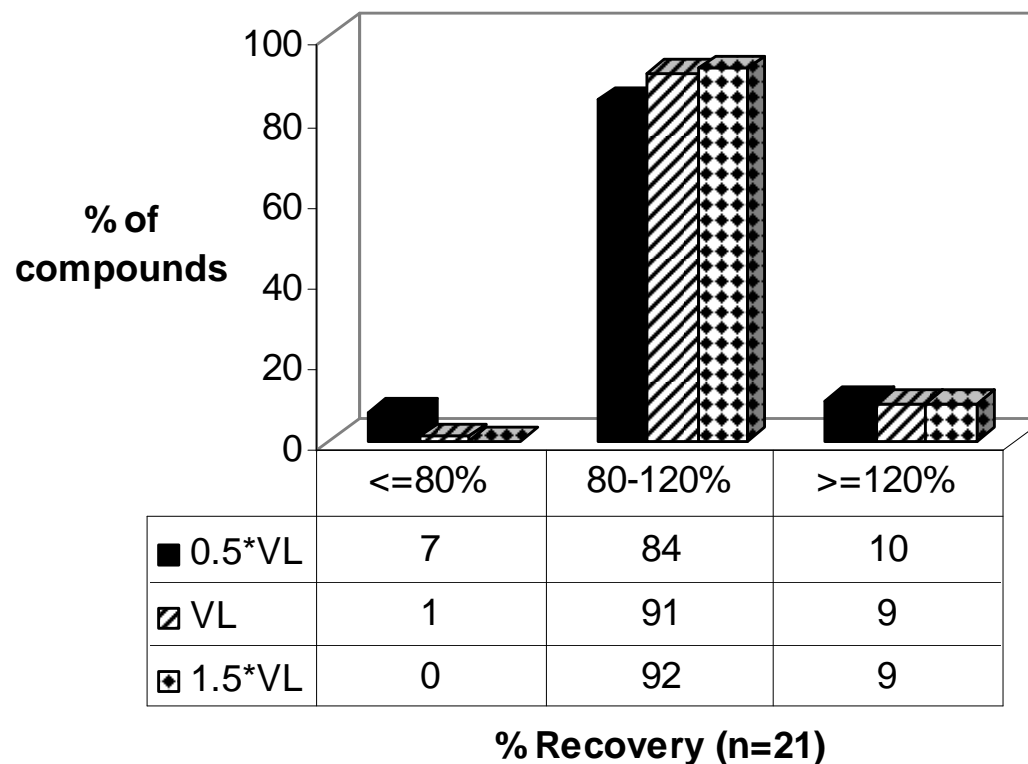
Omics

DESI/DART

Conclusion

**c**

## Accuracy



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# New views on validation concepts needed !



Introduction

UPLC/MS

REA

Bio/MS

Omics

DESI/DART

Conclusion

- LOQ: all compounds  $< 7 \mu\text{g/L}$  [#100  $< \text{MR(P)L}$ ]
- 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*

A.A.M. Stolker et al., *Anal. Bioanal. Chem.*, 391 (2008) 2309-2322

H.G.J. Mol et al., *Anal. Chem.*, 80 (2008) 9450-9459

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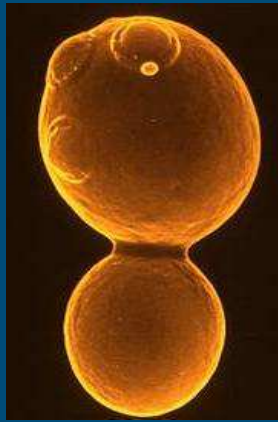
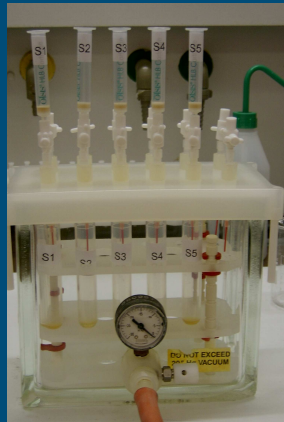
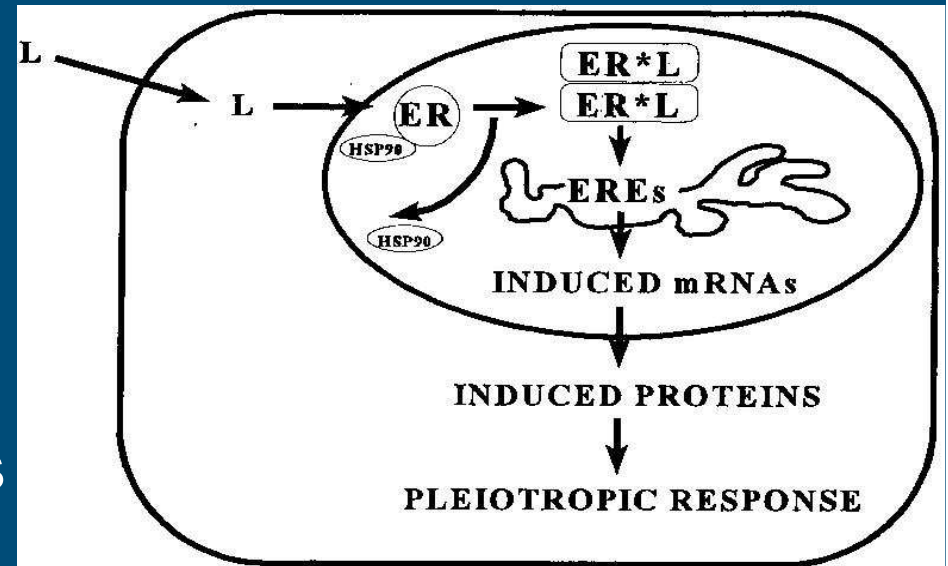


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# Topic 2: Bioactivity-based untargeted approaches

## Bioassay screening

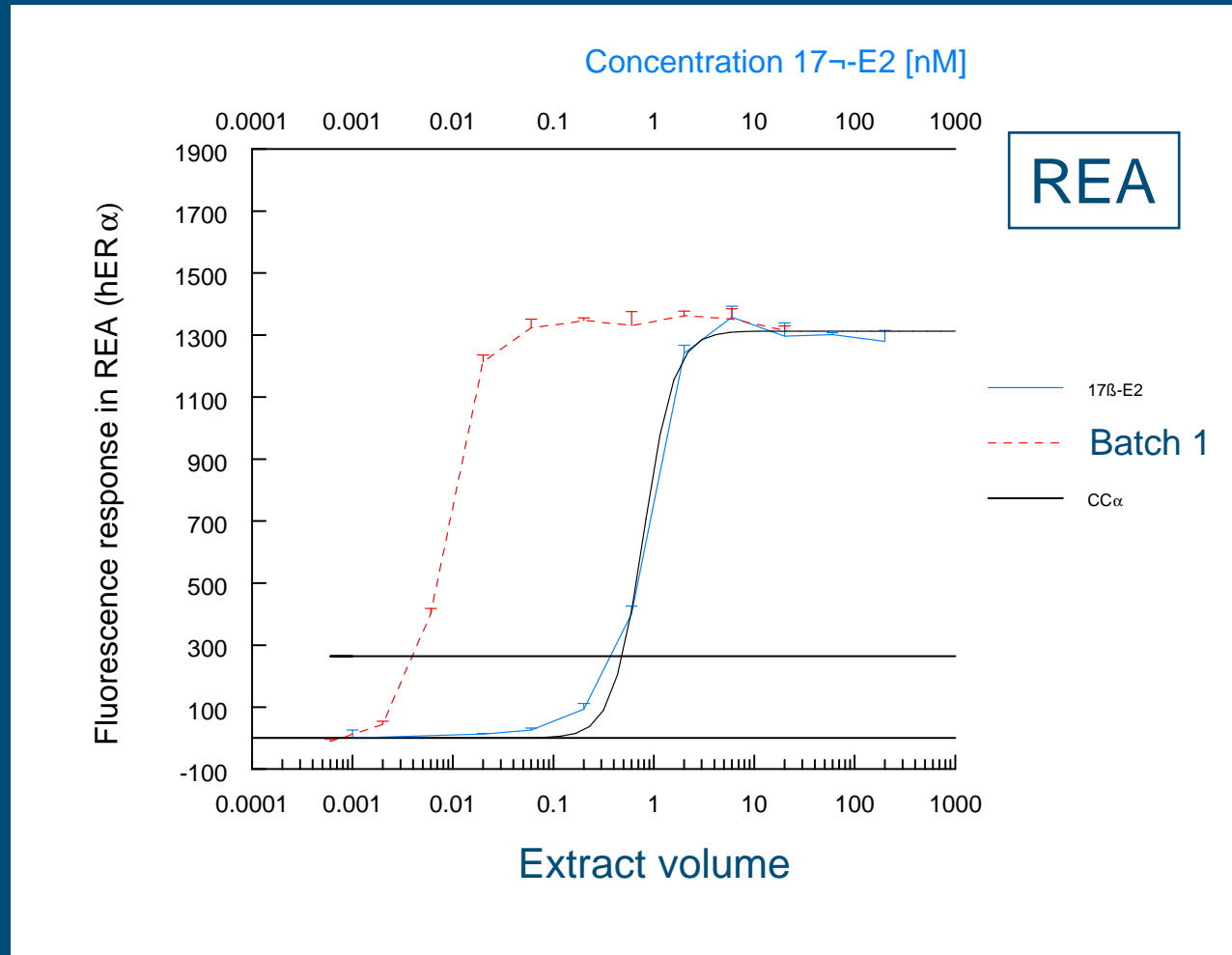
- recombinant yeast cells expressing the human steroid receptor and yGFP-reporter
- for androgens, estrogens, gestagens, glucocorticosteroids



# Food supplements: a 'pure herbal product' case



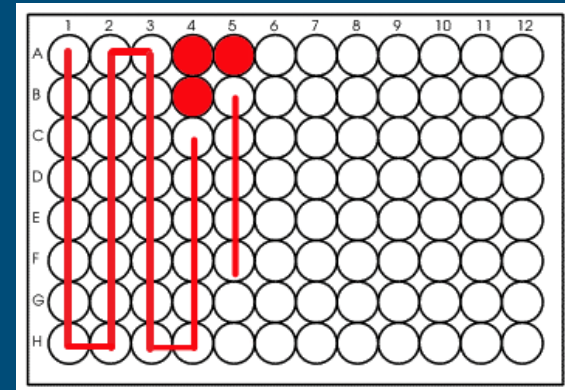
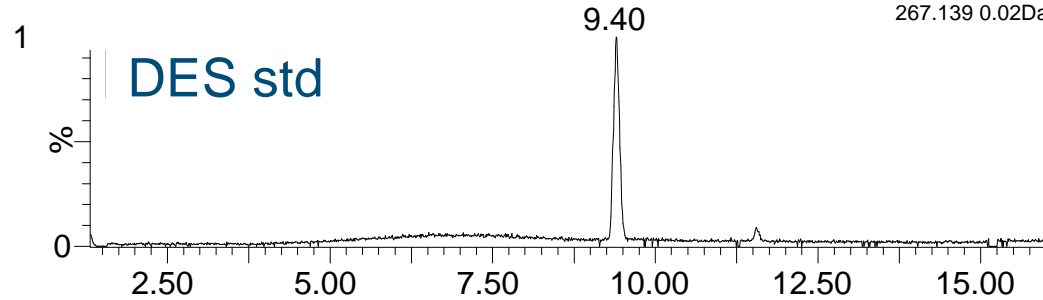
- Introduction
- UPLC/MS
- REA**
- Bio/MS
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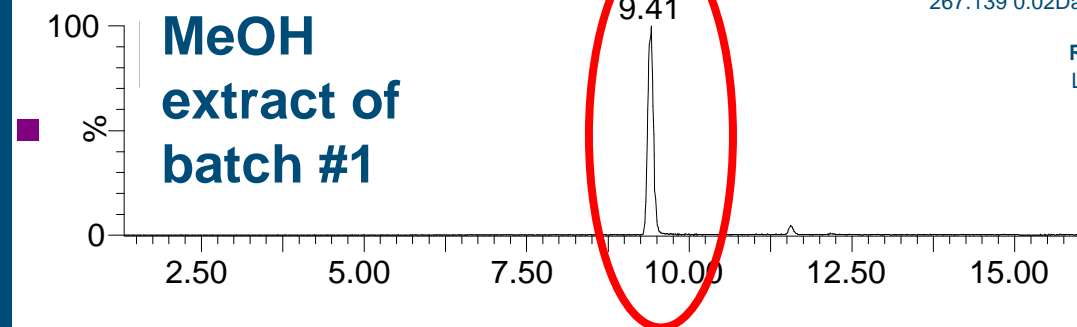
# Bioactivity-directed LC/TOFMS identification

RIK0215677 MeOH extract (1.04 mg/ml)

LCT\_080609\_005



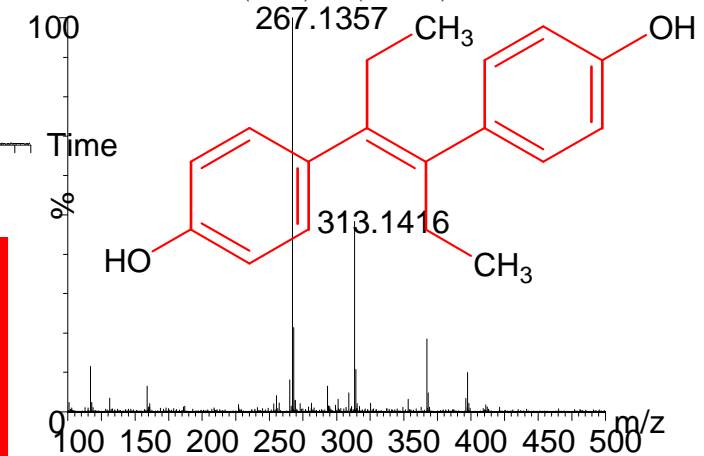
LCT\_080609\_007



RIK0215677 MeOH extract (1.04 mg/ml)

LCT\_080609\_007 785 (9.415) Cm (777:792)

1: TOF MS ES-



Diethylstilbestrol (DES)



# Scary food supplements ! (versus residue issues)



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

- 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 1<sup>st</sup> and 2<sup>nd</sup> generation**



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# Topic 3: Micro and Nano biosensor approaches



Introduction

UPLC/MS

REA

Biosensor/MS

Omics

DESI/DART

Conclusion

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*

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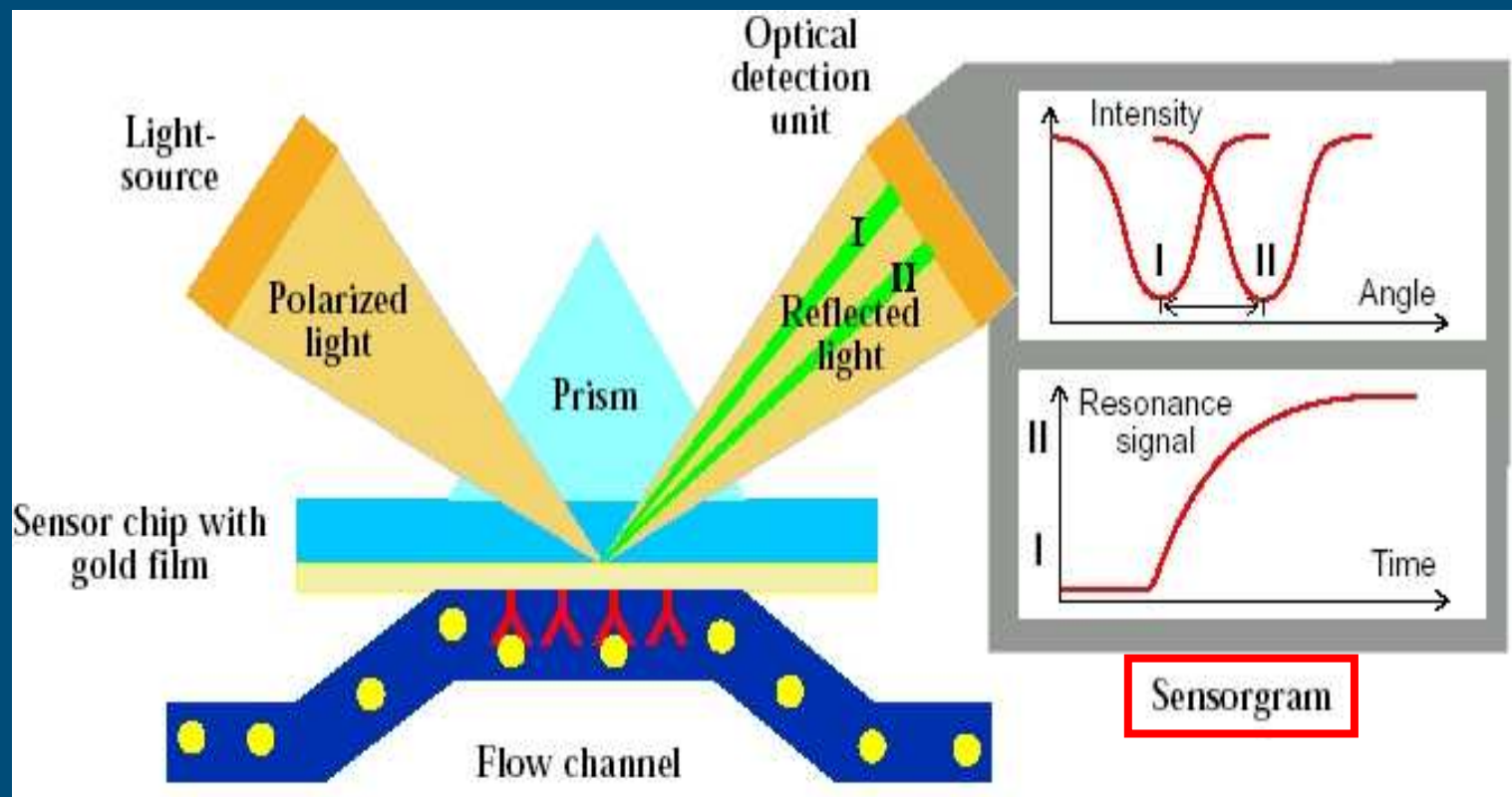
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# Example: label-free binding assay on a chip: SPR



Introduction  
UPLC/MS  
REA  
Biosensor/MS  
Omics  
DESI/DART  
Conclusion



- Big molecules: direct assay using immobilised recognition elements
- Small molecules: inhibition assay format

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# SPR biosensor screening for 13 fluoroquinolones

## 1. Screening chip



Introduction

UPLC/MS

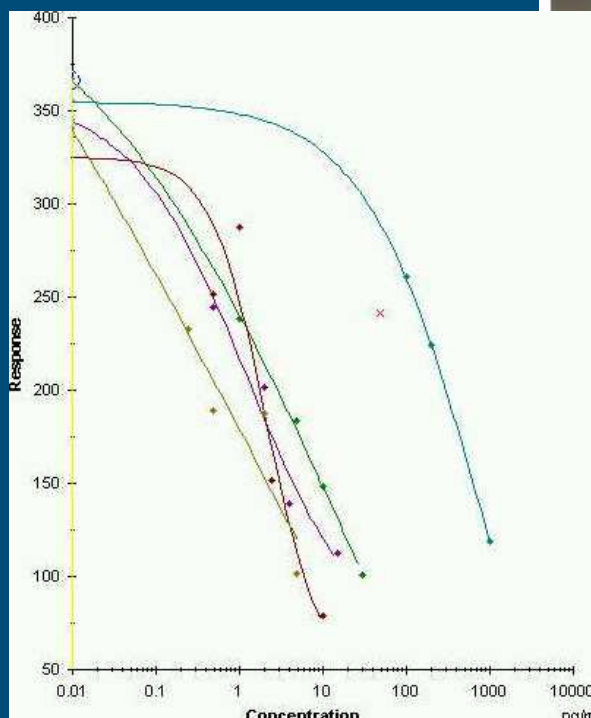
REA

Biosensor/MS

Omics

DESI/DART

Conclusion



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

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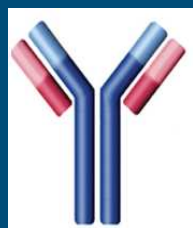
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# Purification of suspect non-compliance plus confirmation

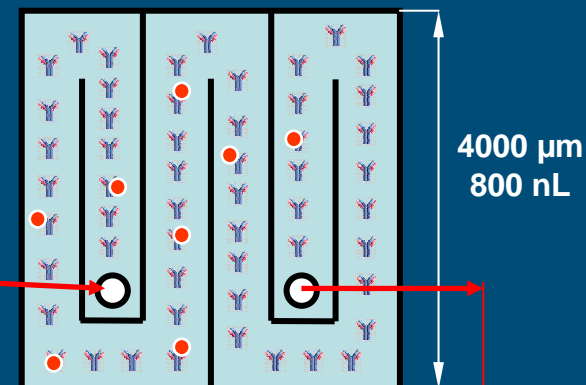


- Introduction
- UPLC/MS
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- DESI/DART
- Conclusion

## 2. Suspect onto recovery chip

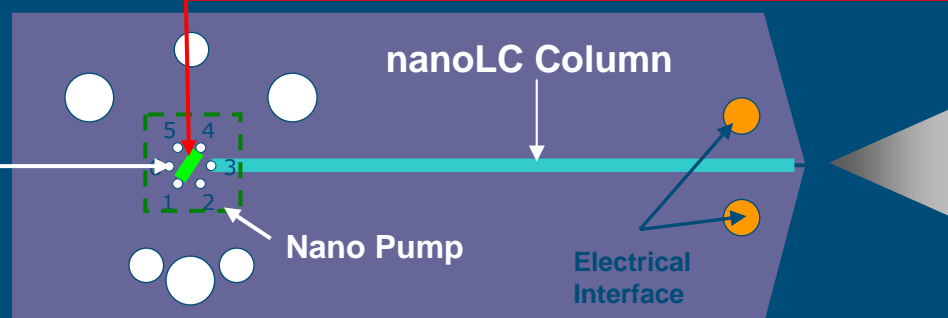


Affinity purified specific IgG fraction immobilized



## 3. to nanoLC/ESI chip

Nano trapping column



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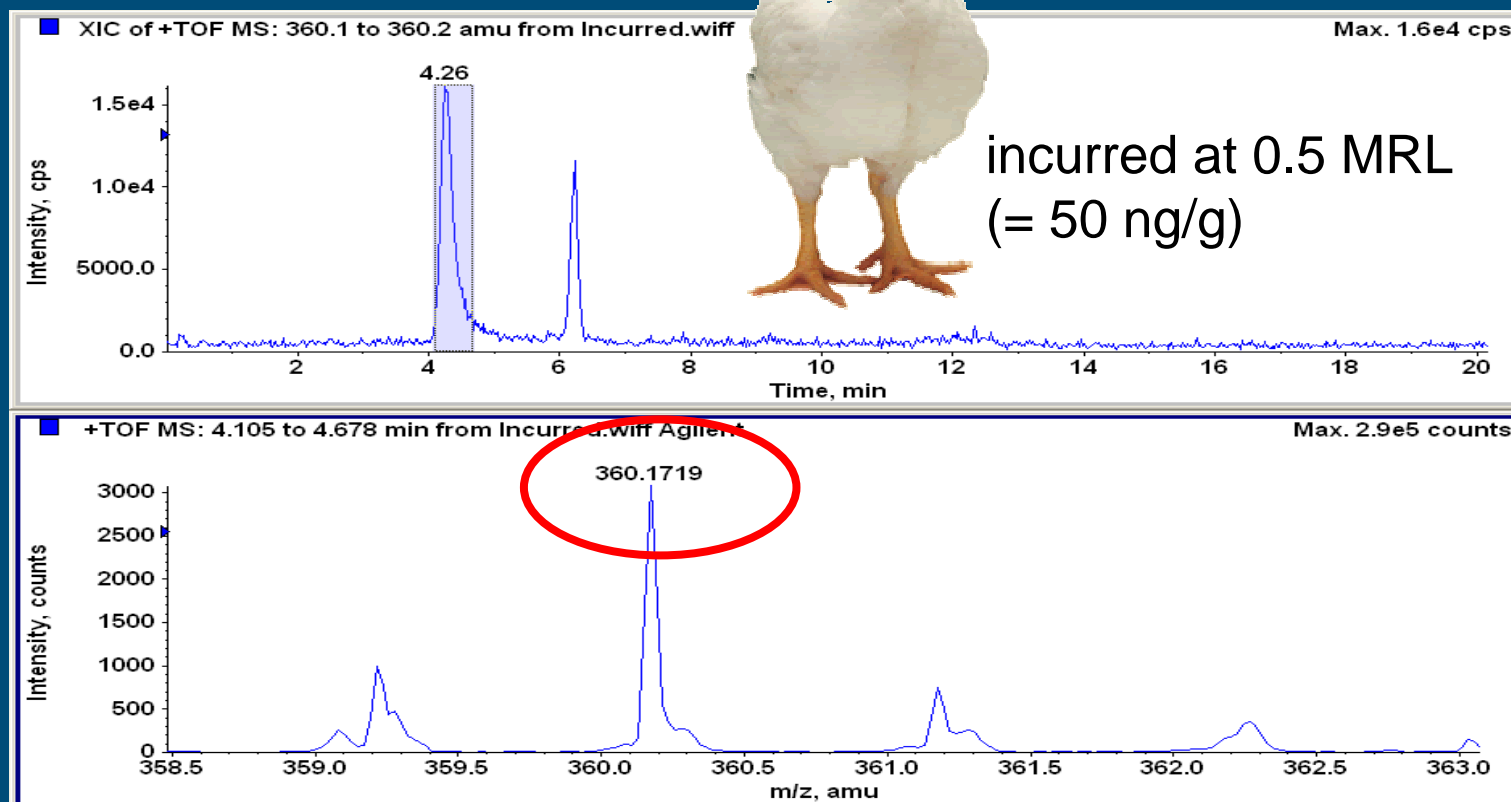
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# Proof of concept: antibiotics in chicken muscle



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

## 3. nanoLC/ESI chip + TOFMS



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# Proof of concept: antibiotics in chicken muscle



Introduction

UPLC/MS

REA

Biosensor/MS

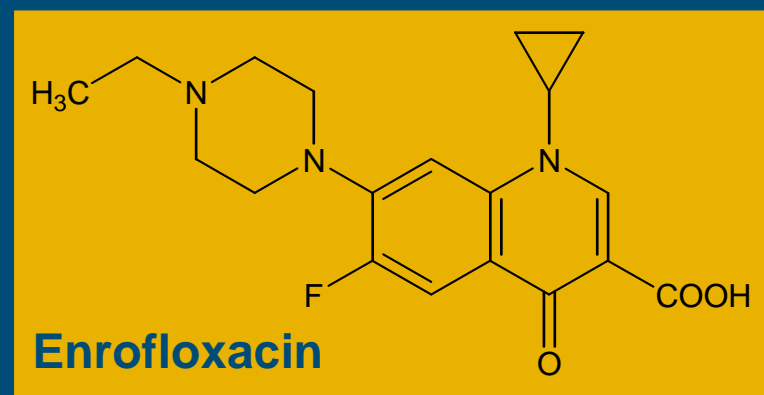
Omics

DESI/DART

Conclusion

Formula	CalculatedMass	mDa Error	ppm Error	RDB
<b>C19 H23 N3 O3 F</b>	<b>360.1723</b>	<b>-0.44511</b>	<b>-1.23584</b>	<b>9.5</b>
C24 H24 O3	360.1725	-0.64488	-1.79048	13
C19 H24 O2 F4	360.1712	0.65676	1.823463	6
C22 H22 N3 O2	360.1712	0.697816	1.937453	13.5
C16 H24 N3 O4 F2	360.1735	-1.58804	-4.40913	5.5
C21 H25 O4 F	360.1737	-1.78781	-4.96377	9
C22 H23 O F3	360.1701	1.79969	4.996753	10

SciFinder element  
composition search:  
1 structure option



G.R. Marchesini et al., *Anal. Chem.*, 80 (2008) 1159-1168

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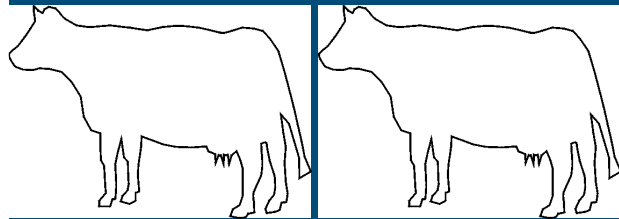
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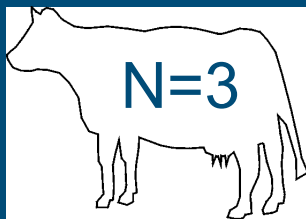
# Topic 4: 'Omics'-based untargeted approaches

## Experiment 1



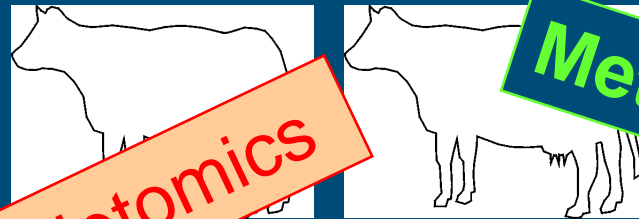
DHEA oral

DHEA  
intramuscular



Controls (6 months)

## Experiment 2



DHEA oral

DHEA  
intramuscular

Transcriptomics

Metabolomics

- Male Holstein Frisians, 9-14 months, 210-430 kg

- Exposed for 7 days, 1 gram DHEA per day

## Experiment 3



DHEA oral

DHEA  
intramuscular



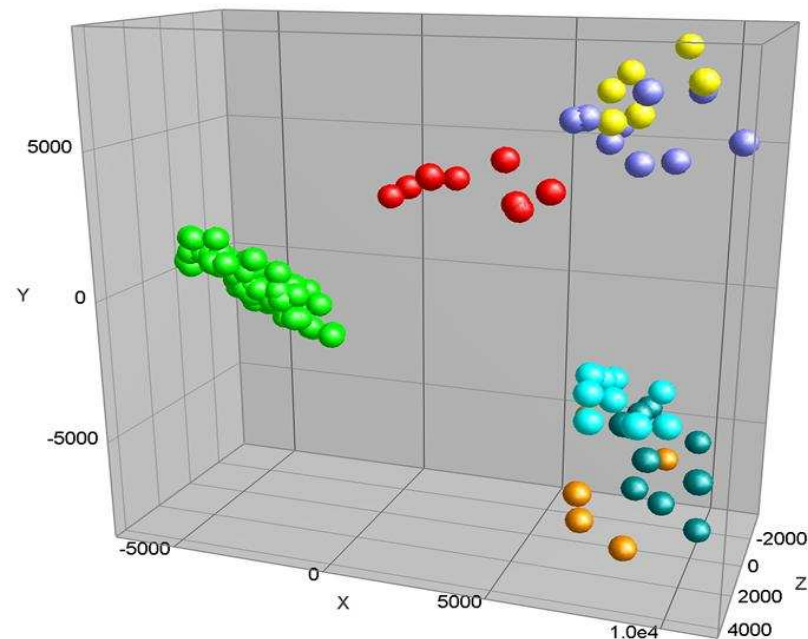
Controls (13-14 months)



# 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  $CC\beta$  and are highly relevant!

J.C.W. Rijk et al., *Anal. Chem.*, 81 (2009) submitted

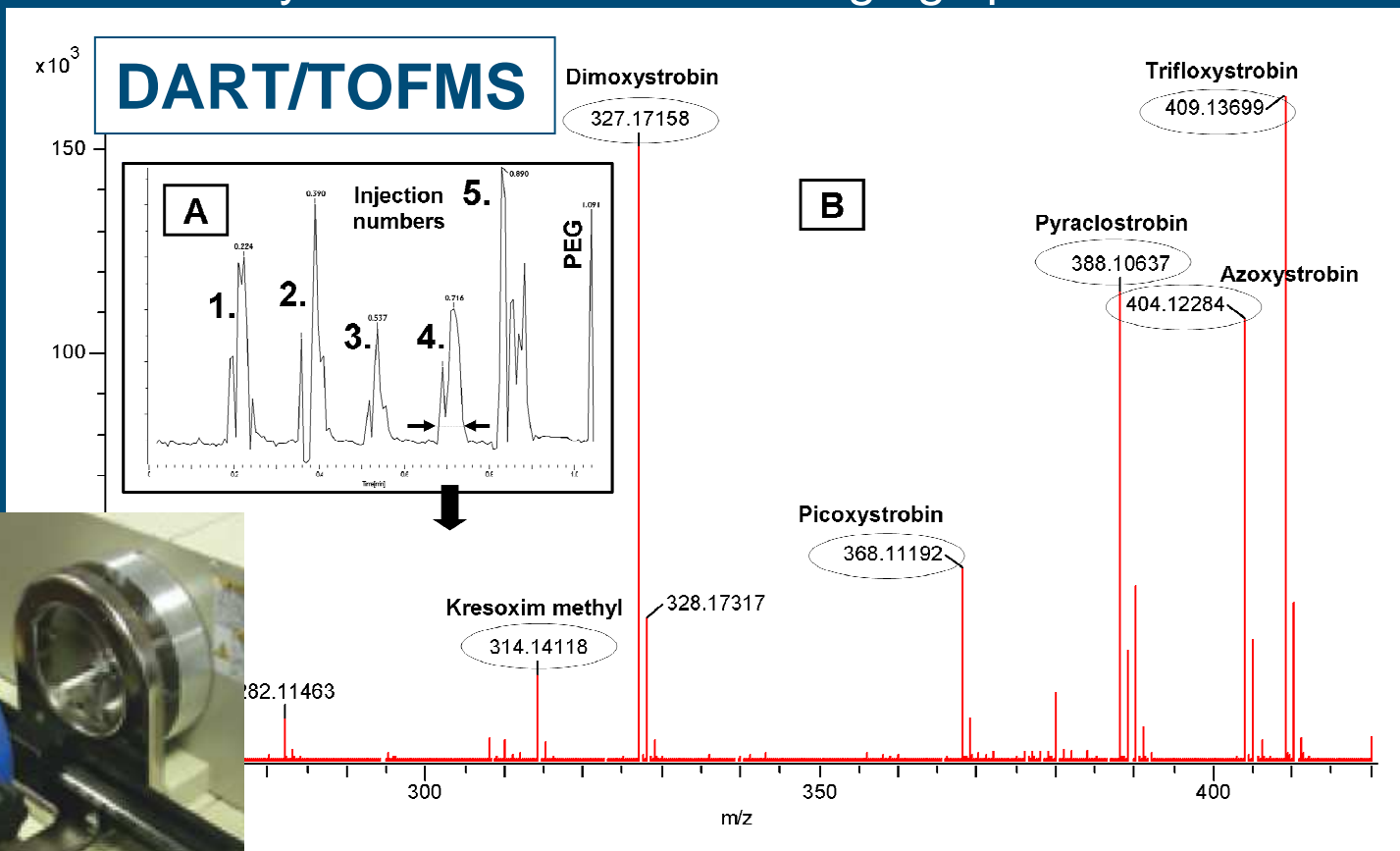


# Topic 5: Emerging technologies from forensics

- Crude ethyl acetate extract of 50 ug/kg spiked wheat



Introduction  
UPLC/MS  
REA  
Biosensor/MS  
Omics  
DESI/DART



J. Schurek et al., *Anal. Chem.*, 80 (2008) 9567-9575

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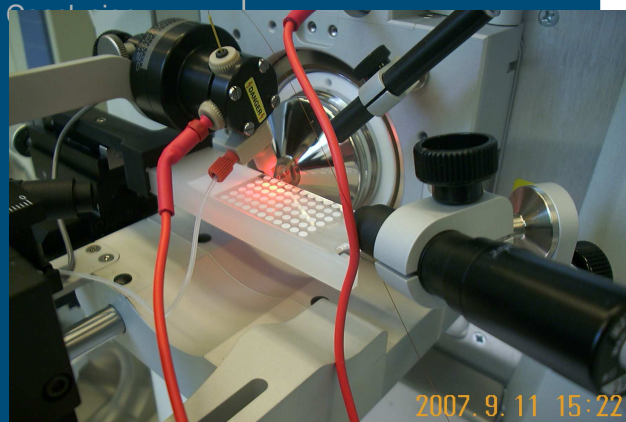
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# DESI MS and DART of fungicides in wheat

- C18 tip methanol percolate of Azoxystrobin incurred wheat

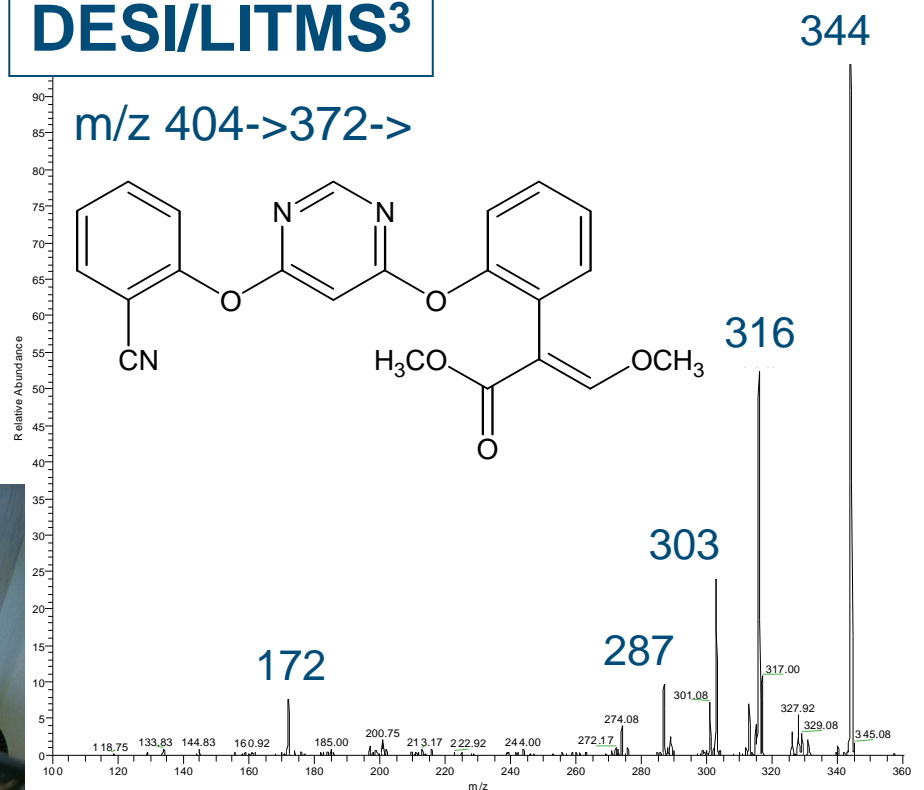


Introduction  
UPLC/MS  
REA  
Biosensor/MS  
Omics  
DESI/DART



## DESI/LITMS<sup>3</sup>

m/z 404->372->



10  
identification  
points !

J. Schurek et al., *Anal. Chem.*, 80 (2008) 9567-9575

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# Conclusion



Introduction

UPLC/MS

REA

Biosensor/MS

Omics

DESI/DART

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

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# Conclusion



Introduction

UPLC/MS

REA

Biosensor/MS

Omics

DESI/DART

Conclusion

## 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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# Acknowledgements

- |  |         |                   |
|--|---------|-------------------|
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| ▪ Jeroen Rijk  | -RIKILT | metabolomics      |
| ▪ Toine Bovee  | -RIKILT | bioassays         |
| ▪ Dick Hooijerink                                      | -RIKILT | DESI              |
| ▪ Jakub Schurek  | -ICT    | DART              |
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