Lessons learned from the CON*ff*IDENCE project: Contaminants in food and feed – inexpensive detection for control of exposure

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SEVENTH FRAMEWORK PROGRAMME

CONFIDENCE

Contents

- Introduction to CON*ff*IDENCE
- Results
  - ✓ Highlights
  - ✓ Inclusion of new requests from DG SANCO and EFSA
  - ✓ Validation and fitness for purpose of screening methods
- Conclusions

CON*ff*IDENCE objectives

- To assure chemical safety and quality in the European food supply; support of EC policies and competitiveness of food and feed industries
  - ✓ Multi-detection: “multiplex”
  - ✓ Inexpensive screening techniques
- To speed-up analysis for factory approval of lots
- To contribute to the assessment of risks of emerging contaminants
  - ✓ e.g. shellfish toxins such as palytoxin and spirolides

CONffIDENCE passport

- FP7 Collaborative Project first call “Food, Agriculture & Fisheries, and Biotechnology”
- Duration: May 2008 – December 2012
- 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Budget: 7.5 Mio €
- Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)

RIKILT
WAGENINGEN UR

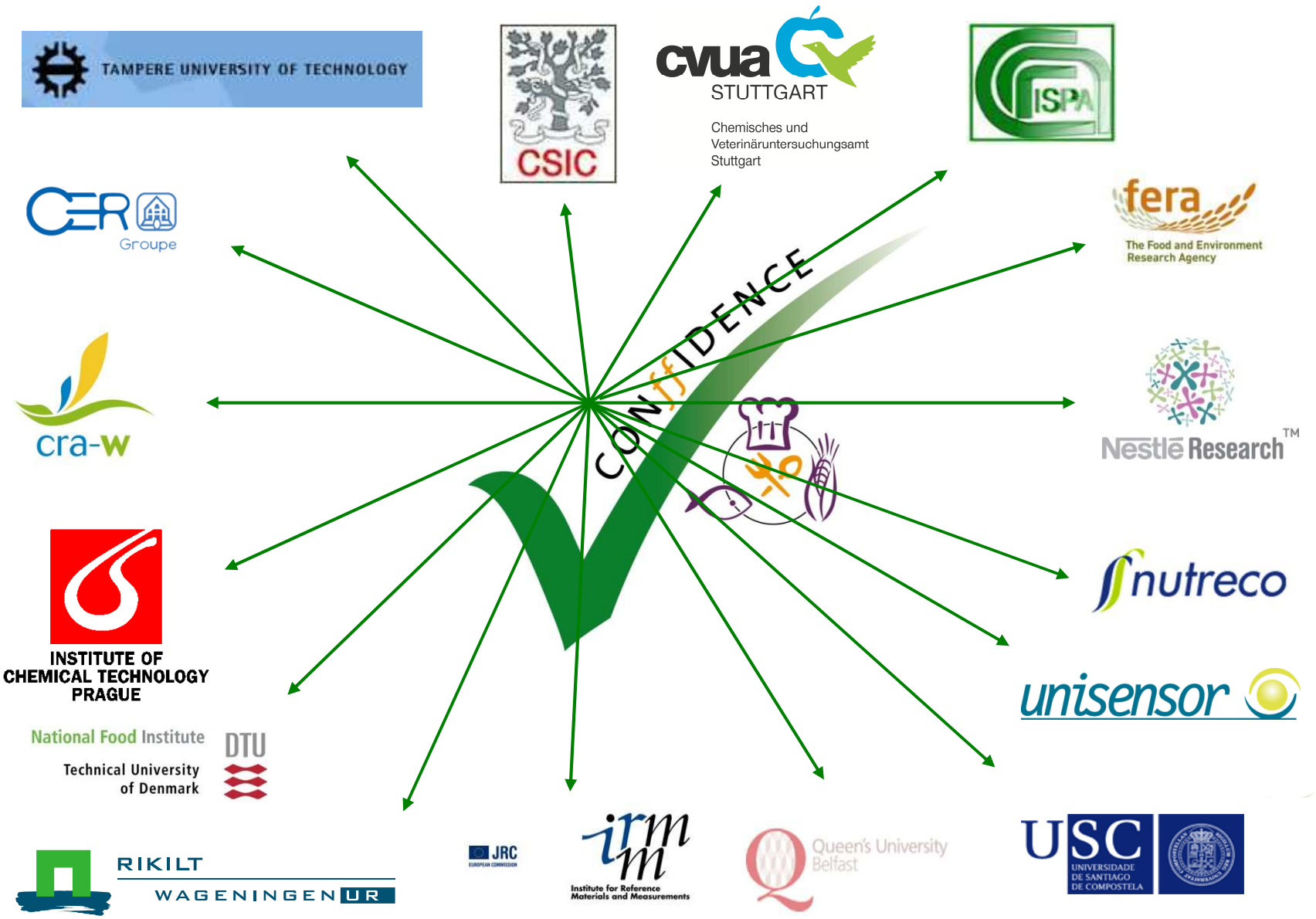
The commodities

Food	&	Feed
✓ Fish/shellfish		Fish feed
✓ Cereals		Cereal-based feed
✓ Potatoes/vegetables		
✓ Honey		
✓ Eggs		
✓ Meat		
✓ Dairy products		

The target contaminants

- Organic pollutants
  - ✓ POPs (Persistent Organic Pollutants) + PAHs
  - ✓ Perfluorinated compounds
  - ✓ Pesticides
- Veterinary drugs
  - ✓ Antibiotics
  - ✓ Coccidiostats
- Heavy metals: speciation of arsenic and mercury
- Biotoxins:
  - ✓ Alkaloids
  - ✓ Marine biotoxins
  - ✓ Mycotoxins

The consortium



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- Introduction to CON*ff*IDENCE
- **Results**
  - ✓ **Highlights**
  - ✓ **Inclusion of new requests from DG SANCO and EFSA**
  - ✓ **Validation and fitness for purpose of screening methods**
- Conclusions

CONffIDENCE Highlights (1)

- Biotoxins:
  - ✓ Multiplex dipstick for mycotoxins in cereals
  - ✓ Multiplex dipstick for ergot alkaloids in cereals and feed
  - ✓ NIR Hyperspectral Imaging for ergot sclerotia in cereals
  - ✓ Multiplex dipstick for tropane alkaloids in feed
  - ✓ Multiplex ELISA for pyrrolizidine alkaloids in honey and feed
  - ✓ Multiplex biosensor assay (SPR) for shellfish toxins
- Veterinary drugs:
  - ✓ Multiplex dipstick for antibiotics in honey
  - ✓ Multiplex flow cytometry for coccidiostats in feed and eggs

CER Groupe

Queen's University Belfast

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CONffIDENCE Highlights (2)

- Heavy metal speciation:
  - ✓ Inorganic arsenic in seafood and fish feed: SPE-AAS
  - ✓ Methylmercury in seafood and fish feed: LC-ICPMS
- Organic pollutants:
  - ✓ POPs and PAHs in seafood and fish feed: simplified integrated sample prep + GC-MS/MS or GCxGC-TOFMS
  - ✓ POPs and PAHs in fish: X-map technology
  - ✓ DESI and DART-MS of dithiocarbamates in vegetables
  - ✓ Multiplex electrochemical immunosensor for paraquat and DON in cereals
  - ✓ Perfluorinated compounds in fish and milk: Simplified sample prep and LC-MS/MS
- Cross-cutting surveys, e.g. multiple contaminants in seafood

Mycotoxins: Commodity dedicated **multiplex dipstick tests** for the determination of major ***Fusarium* toxins**

WHEAT

OAT

WHEAT BASED BREAKFAST CEREALS

Target toxins: DON, ZEA, T-2 and HT-2 toxins

MAIZE

MAIZE FEED

MAIZE BASED BREAKFAST CEREALS

Target toxins: DON, ZEA, FB_1, FB_2, T-2 and HT-2 toxins

Target levels: EU maximum permitted levels

The assay procedure

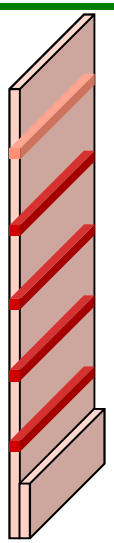
Methanol/water extraction

High speed blending

Dilution with buffer

Incubation at 40°C, 10 min
Migration, 10 min

Reading



NEG: Test Lines **darker** than CTRL line

POS: test Lines **lighter** than CTRL line

Total analysis time: 30 min for 6 mycotoxins

The commercial kit

unisensor

www.unisensor.be

MULTIPLEX: **6** mycotoxin analysed in **1** test

FAST: up to **8 samples** in **1 hour** (including sample preparation)

SENSITIVE: mycotoxin detection at levels close to EU regulatory limits

USER FRIENDLY: **5 min** for sample preparation, easily performed on site

Bee4sensor for honey

- **Multisensor**: Unique **multiplex,** antibody based dipstick **assay**, for the screening of **sulphonamides**, **fluoroquinolones**, **tylosin-A**, and **chloramphenicol** in **honey**

- Laboratory method
  - ✓ Successful Inter-lab validation with 7 European laboratories
- Field-test method
  - ✓ Proof of principle
  - ✓ Global field trial with 16 participants e.g. bee inspectors
- Rapid test for industry and enforcement authorities

fera
The Food and Environment Research Agency

unisensor

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Multisensor – bee4sensor for honey

The test kit (bee4sensor) is already marketed by unisensor and will be soon produced, based on customer demand:
http://www.unisensor.be/en/catalog/antibiotics-28/bee4sensor-45.php

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The Food and Environment Research Agency

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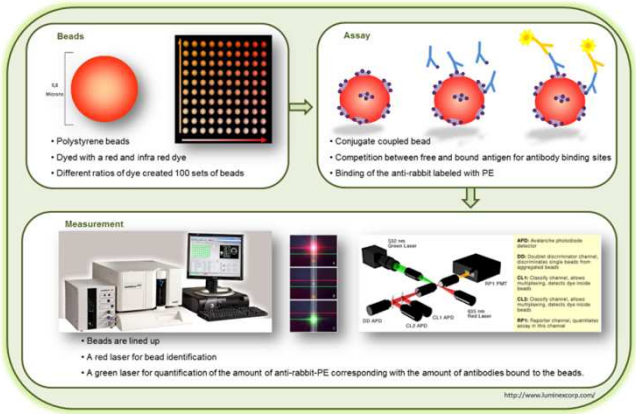
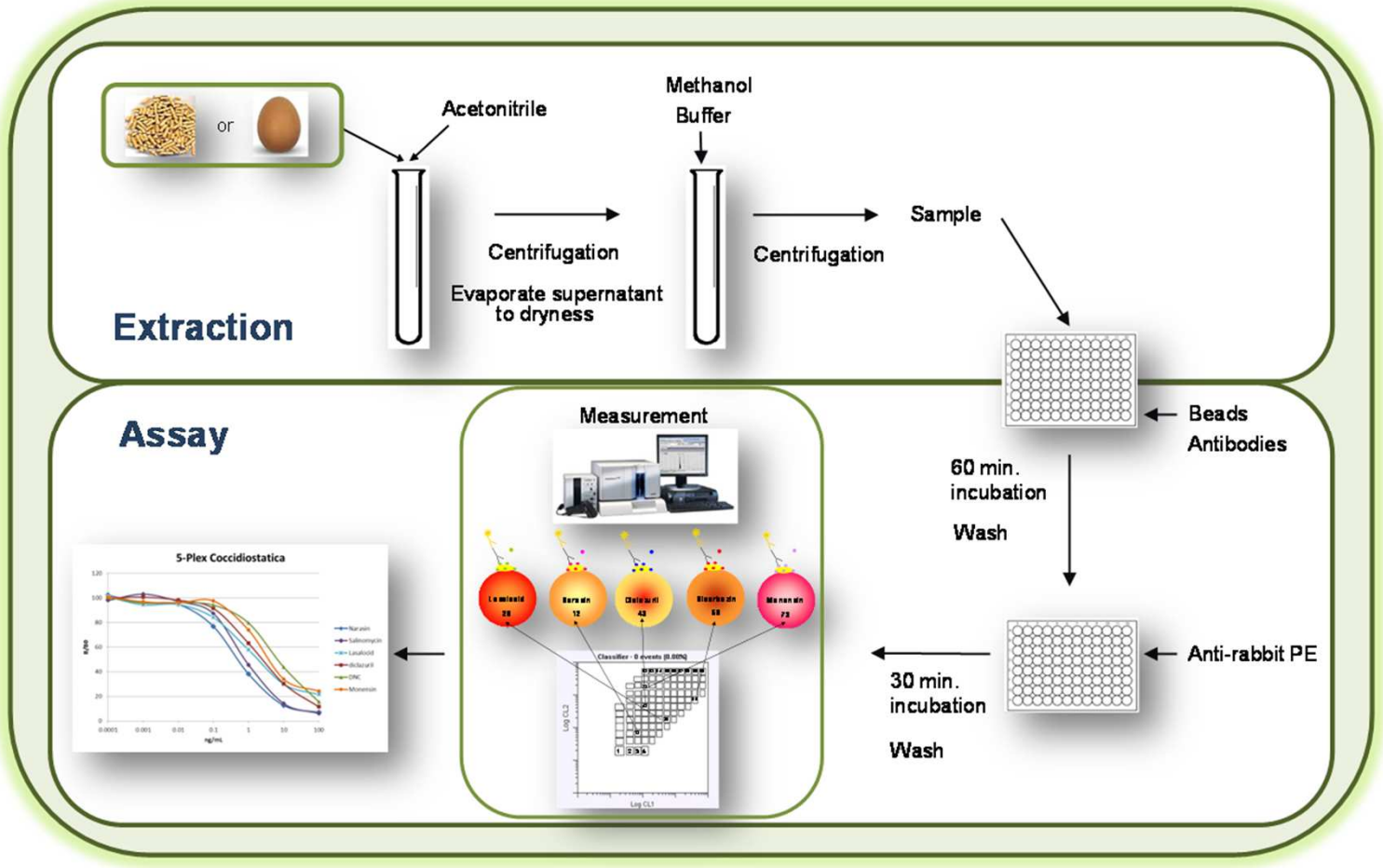
Coccidiostats in feed and eggs

- New fast and inexpensive multiplex method for the screening of:
  - ✓ Residues of coccidiostats in eggs (Regulation (EU) N° 610/2012)
  - ✓ Coccidiostats at cross-contamination levels in non-target feed (Regulation (EU) N°574/2011)

- The Technology:
  Flow cytometry based multiplex immunoassay



The assay procedure



Generic extraction; 40 samples (240 analytes) per day in routine

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Collaborative study - Overview

	Eggs	Feed
Narasin/Salinomycin	2.89	0.52
Lasalocid	0.17	2.75
Nicarbazin	0.35	10.47
Diclazuril	9.14	93.40
Monensin	2.42	1.65

Rate of false positives in the blank in %

Established at 95% confidence level (maximum rate of false negatives is 5%)

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NIR imaging method for ergot sclerotia

- NIR hyperspectral imaging method to detect and quantify ergot bodies in cereals at levels below regulatory limits
- Full conveyer belt system with belt speed of 100 mm/s allows analysis of up to 100 kg grain/hour

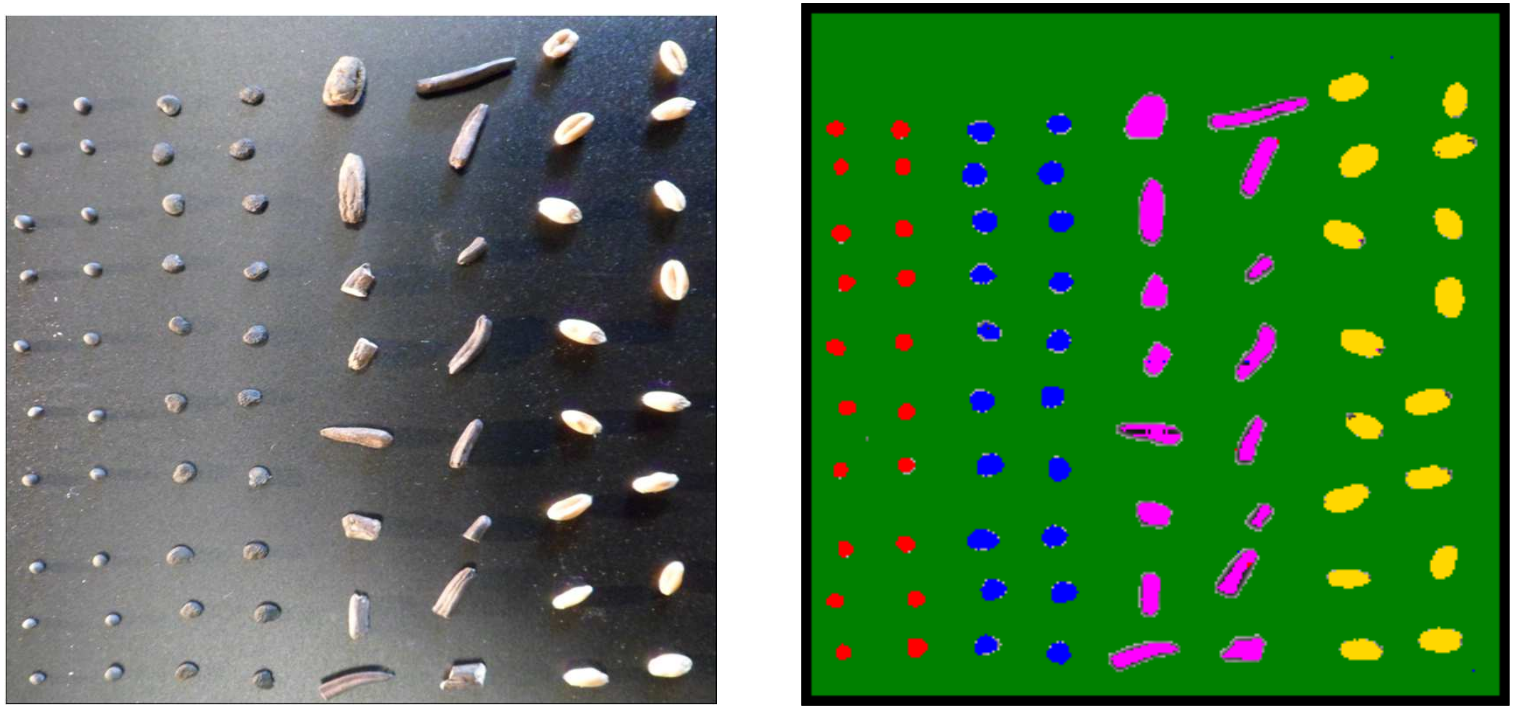
NIR line scan imaging system

Conveyor belt

Test system in operation at Nutreco

Further developments

- Multicontaminants detection: ergot, Datura, …



2 lines of rapeseed, Datura seeds, ergot sclerotia and wheat kernels, respectively

Integrated sample prep for POPs/PAHs

BFR | PCB | PAH | Non-*ortho* PCB

Extraction
Shaking (H_2O + ethyl acetate)

Isolation
10 min

Partition (transfer into organic phase)
$MgSO_4$ + NaCl

Clean up
Silica SPE minicolumn

Clean up
30 min

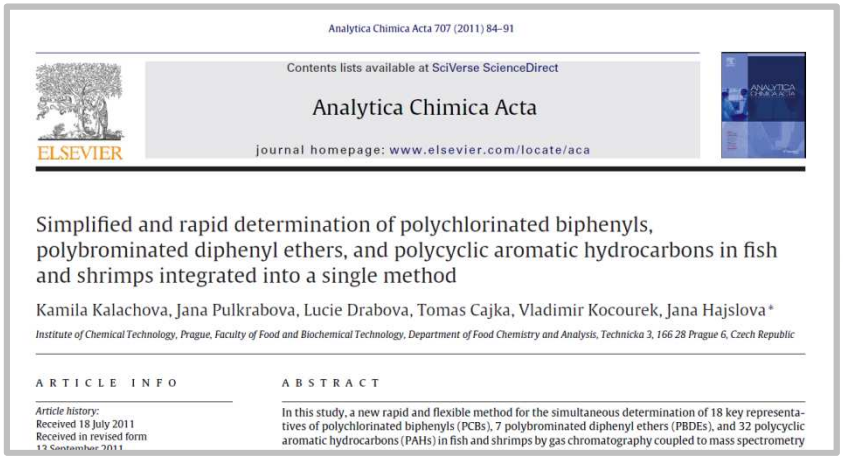
Identification & quantification
GC×GC–TOFMS (EI)
GC–MS/MS (EI)

Identification & quantification
1 h

TIME SAVING
- One sample = < 1hour
- Six samples at one time

SOLVENT VOLUME REDUCTION

COST/LABOUR SAVING



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Simplified and rapid determination of polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in fish and shrimps integrated into a single method

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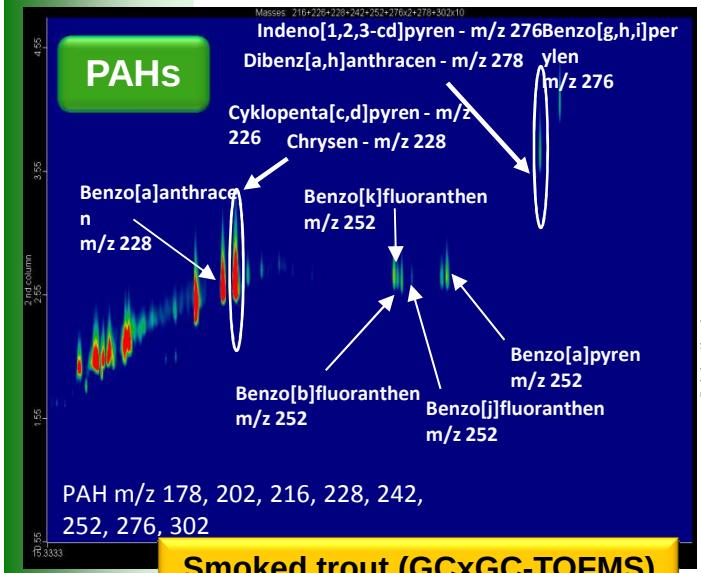
ABSTRACT

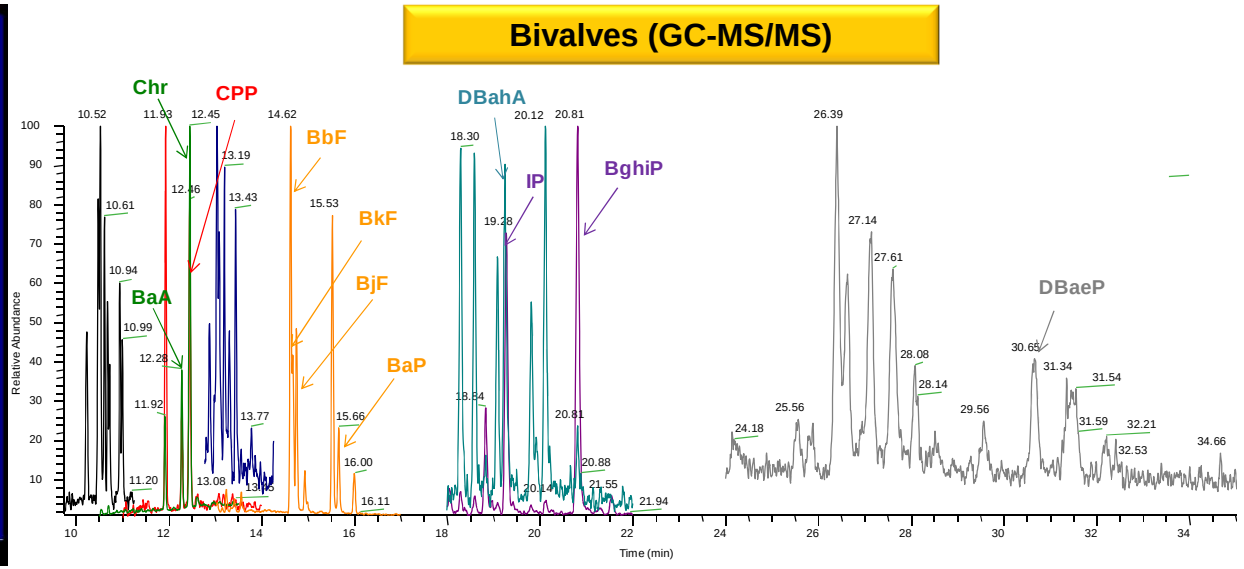
In this study, a new rapid and flexible method for the simultaneous determination of 18 key representatives of polychlorinated biphenyls (PCBs), 7 polybrominated diphenyl ethers (PBDEs), and 32 polycyclic aromatic hydrocarbons (PAHs) in fish and shrimps by gas chromatography coupled to mass spectrometry

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

GC–MS methods for POPs



Smoked trout (GCxGC-TOFMS)



Parameter / feature	GCxGC-TOFMS	GC-MS/MS
Chromatographic resolution	+++	+
Selectivity of detection	++ (deconvolution)	++ (products ions)
Detection limits	+	+++
Data handling (time demands)	-	+
Retrospective data mining	+	-
Availability in common control labs	-	++

Anal Bioanal Chem (2012) 403:2813–2824
DOI 10.1007/s00216-012-6095-3

ORIGINAL PAPER

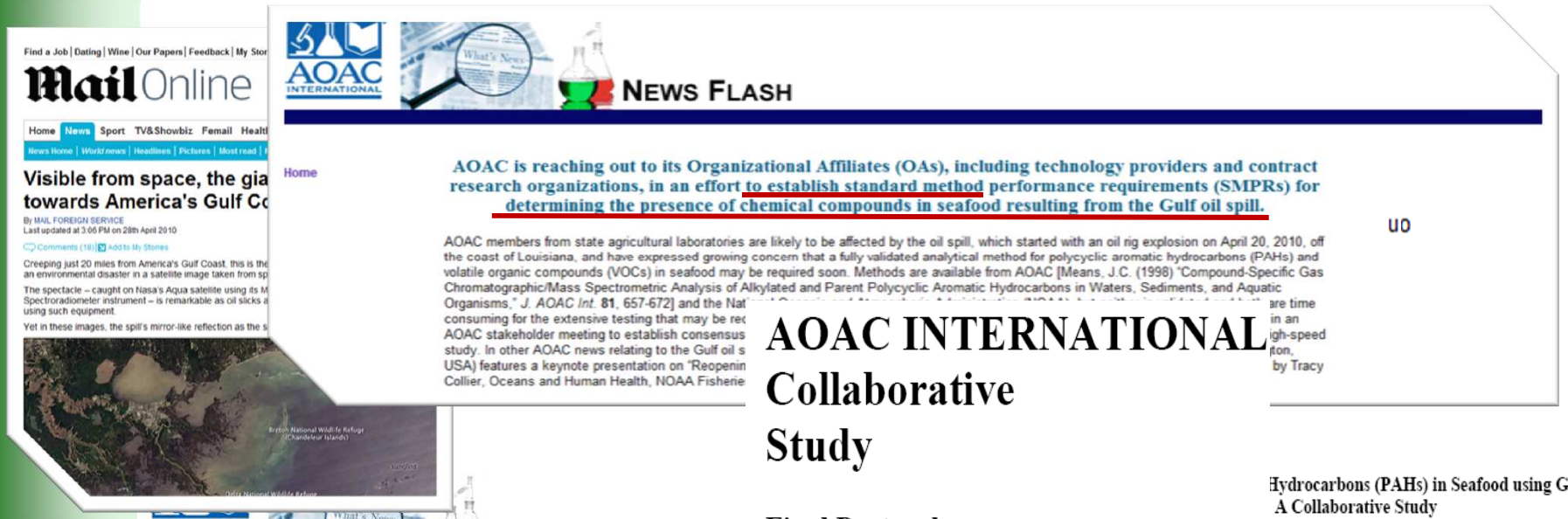
Implementation of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons in fish

Kamila Kalachova · Jana Pulkrabova · Tomas Cajka · Lucie Drabova · Jana Hajslova

Received: 7 March 2012 / Revised: 30 April 2012 / Accepted: 2 May 2012 / Published online: 29 May 2012

Abstract In the presented study, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) was shown to be a separation of all target analytes even of critical groups of PAHs (group (a): benz[*a*]anthracene, cyclopenta[*cd*]pyrene and chrysene; group (b): benzo[*b*]fluoranthene, benzo[*j*]fluo-

AOAC accepts and validates CONffIDENCE method



Cross-cutting survey of seafood (n = 150)

- Co-occurence of **POPs & PUFA, PFAS, heavy metal speciation**
- Data to be provided to **EFSA** for risk-benefit discussions

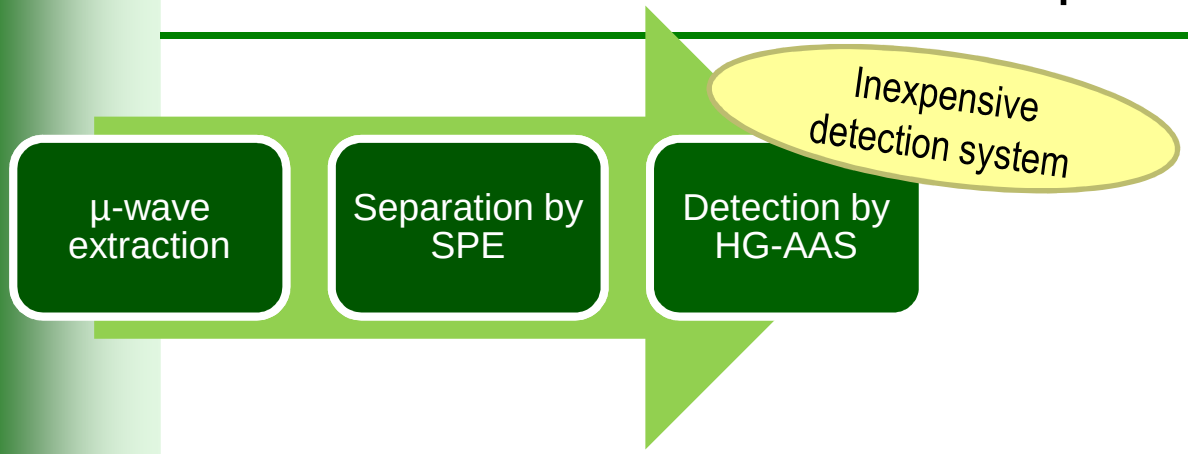
Species	Region	Lead for collection of samples
Herring	Baltic sea	DTU
	North sea	RIKILT
	Atlantic ocean	DTU
Cod/whiting/ hake	North sea	ICT
	Atlantic ocean	ICT
	Mediterranean sea	CSIC
Trout and salmon	Czech Republic	ICT
	Spain	CSIC
	Scandinavia	DTU
Bivalves	Scandinavia	DTU
	The Netherlands	RIKILT
	Mediterranean Sea	CSIC
Tuna	Canned, in water, preferably from Europe	All
Pangasius	Mostly Vietnam	All

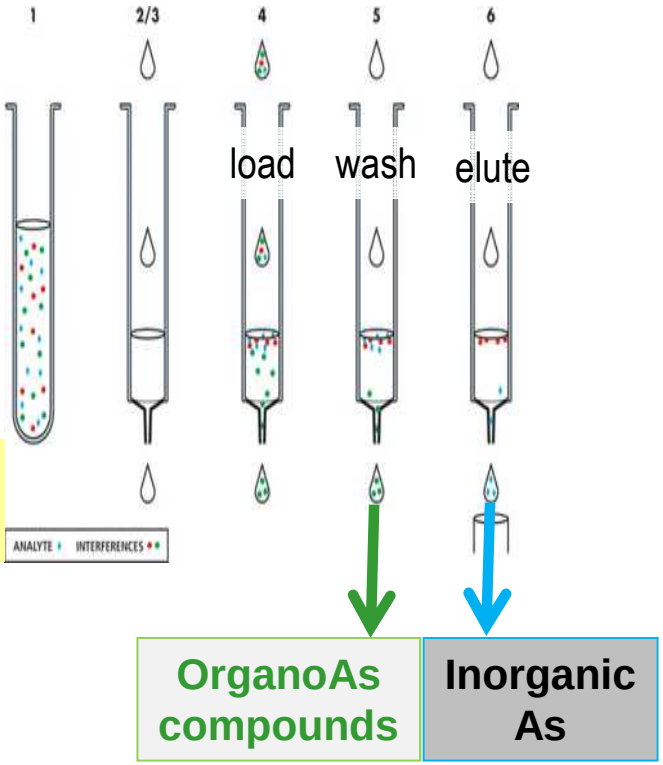
efsa
European Food Safety Authority

CONffIDENCE Highlights

- Inclusion of new requests from DG SANCO and EFSA, through the *Advisory Board*
  - ✓ Broadening the types of pyrrolizidine alkaloids in honey and feed: inclusion of *Heliotropium* alkaloids
  - ✓ Brominated flame retardants: Hexabromocyclo-dodecane (HBCDD) stereoisomers in fish and fish feed: LC-MS/MS method
  - ✓ Extending the number of ergot alkaloids in cereals
  - ✓ Inorganic arsenic in rice

SPE-HG-AAS – a novel speciation alternative…





Concept: Sequential elution for selective separation of **inorg As** from **organoAs** species by SPE

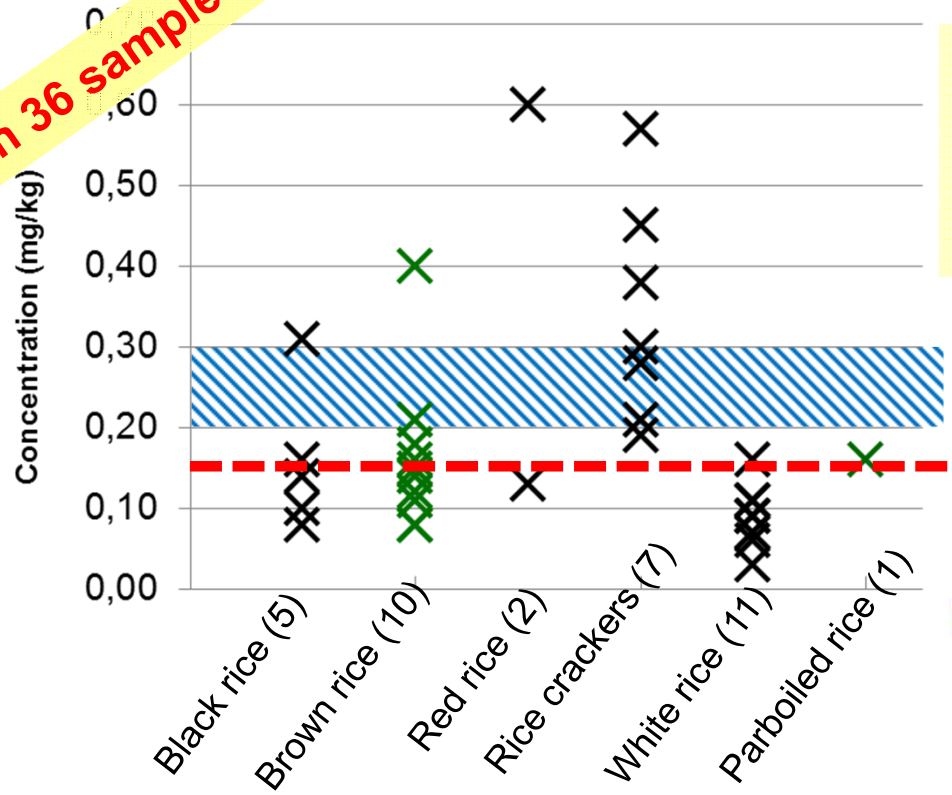
- Method for inorganic arsenic in marine samples (food and feed)
- Validated through a full-scale collaborative study (10 labs)

National Food Institute
Technical University of Denmark
DTU

Inorganic arsenic in rice – a hot food safety topic

- Tailoring of SPE HG-AAS method for rice and rice products
- Simplified extraction in waterbath for increased sample throughput (> 50 samples extracted in 1 hour)

Survey on 36 samples



Dataset provides input to:
EFSA (exposure estimation)
EU commission (legislation?)
CODEX (legislation?)

Future EU max level (0.2-0.3 mg/kg)?

Chinese max level (0.15 mg/kg)

National Food Institute
Technical University of Denmark
DTU

Validation of screening methods

- Field of application
  - ✓ Analyzing a high number of samples, of which the majority is contaminated at low levels
- Main feature: Binary result (1 or 0): The sample is above or below a target level (e.g. legal limit)
- Fitness for purpose of the method:
  - ✓ Non-compliant samples should be tested positive (or better: suspect); Criterion is rate of false negative (β-error, safety aspect): should be $\leq$ 5 %
  - ✓ Compliant samples should be tested negative; Criterion is rate of false positive (α-error, economical aspect): $\leq$ 20 %
- CONffIDENCE: Quantitative methods used in a binary fashion

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Principle of quantitative methods used in a binary fashion

- The measurements include a numerical value (e.g. from dipstick reader)
- The numerical value is compared against a predefined cut off value
- Depending on whether the numerical value is below or above the cut off value, the sample is considered as suspect or negative

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Steps of validation

- Preparing blank samples, samples with the analyte below and at the target level (TL)
- From the precision experiments of the TL samples we calculate the cut-off value (with β-error = 5% by definition)
- From the precision experiments of the other samples we calculate the α-error
- Example: Mycotoxins

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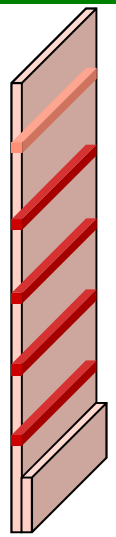
Mycotoxins: the assay procedure

Methanol/water extraction

Dilution with buffer

Incubation at 40°C, 10 min
Migration, 10 min

Reading

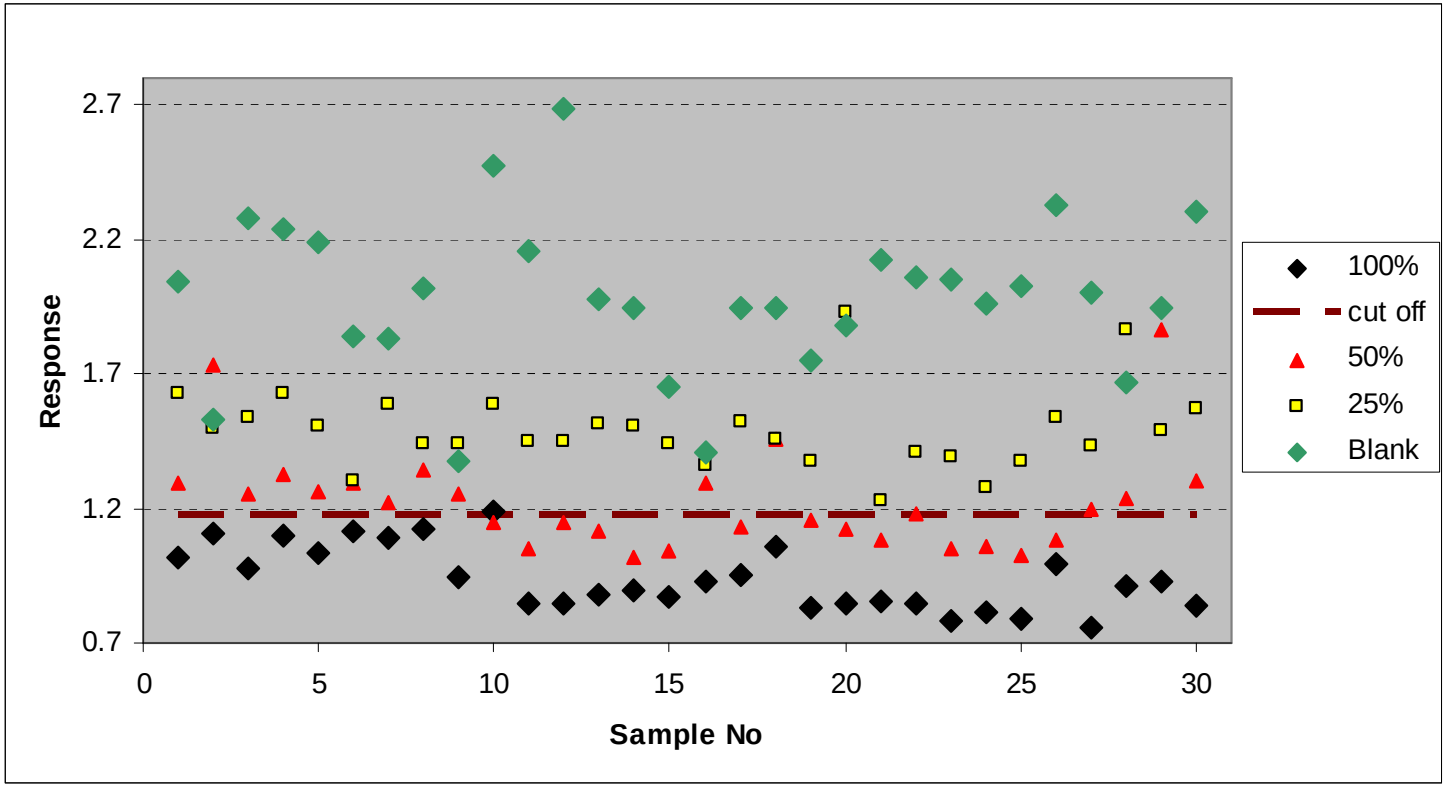


NEG: Test Lines **darker** than CTRL line

POS: test Lines **lighter** than CTRL line

Total analysis time: 30 min for 6 mycotoxins

Example: Zearelenone in maize



- Based on cut-values the rate of false positive samples is estimated (also by t-statistics)
- Rate of false positive:
  - Samples with 50 % of target level: 40 %
  - Samples with 25 % of target level: **2.2 %**
  - Blank samples: **0.6 %**

How to know the fitness for purpose?

We need to know three things:

- The performance profile including the cut-off value of the test
- The frequency distribution of the concentration of the target analyte in typical samples
- The cost per analysis of the screening test compared to the confirmatory method

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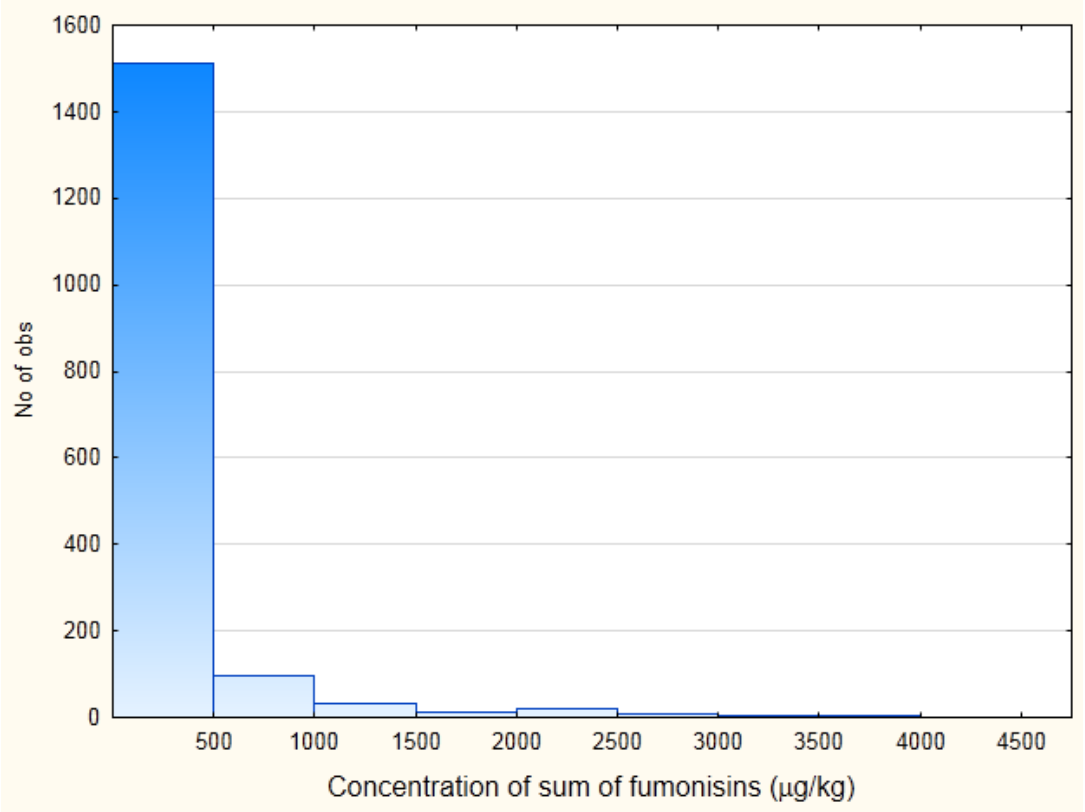
Example: Fumonisin concentrations maize – European data. **Step 1**



Most of the samples contain the analyte far below the maximum level (ML) = 4000 µg/kg (food)

Example: Fumonisin concentrations in maize – European data. **Step 2**

The "rate of false positive results" from the validation is superimposed on the frequency distribution

% of ML with validation data	Range % of ML	Range: µg/kg	No of Samples	Rate of false positive results	Number of false positive
0	0-12	0-480	1486	0.058	86
25	13-37	481-1480	153	0.12	18
50	38-62	1481-2480	36	0.64	23
100	62-100	2481-4000	19	0.95	18
		Total No of samples:	1694	Total No of false positive results	146
				Total Ratio of false positive results (%)	8.60

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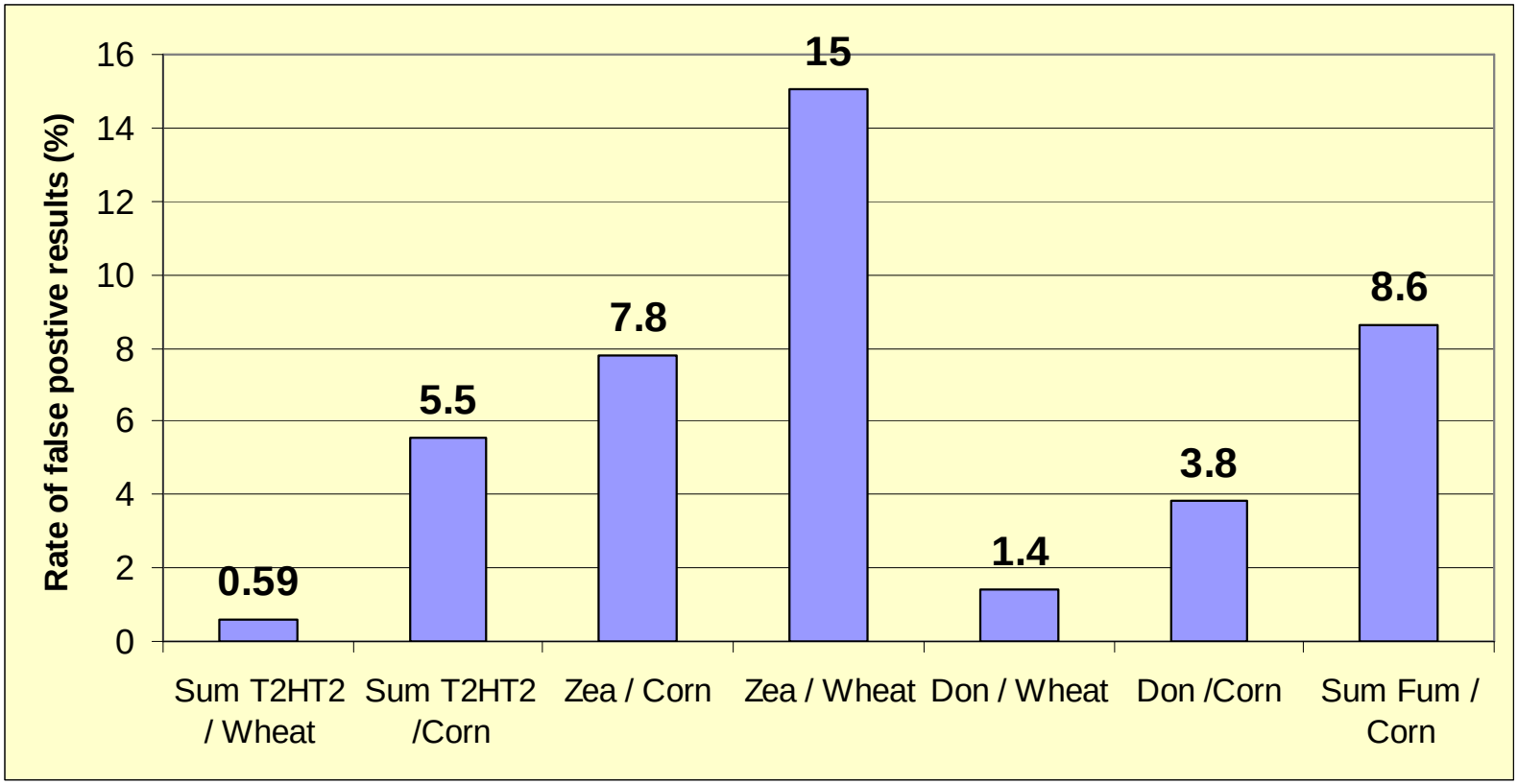
Overview for all mycotoxins



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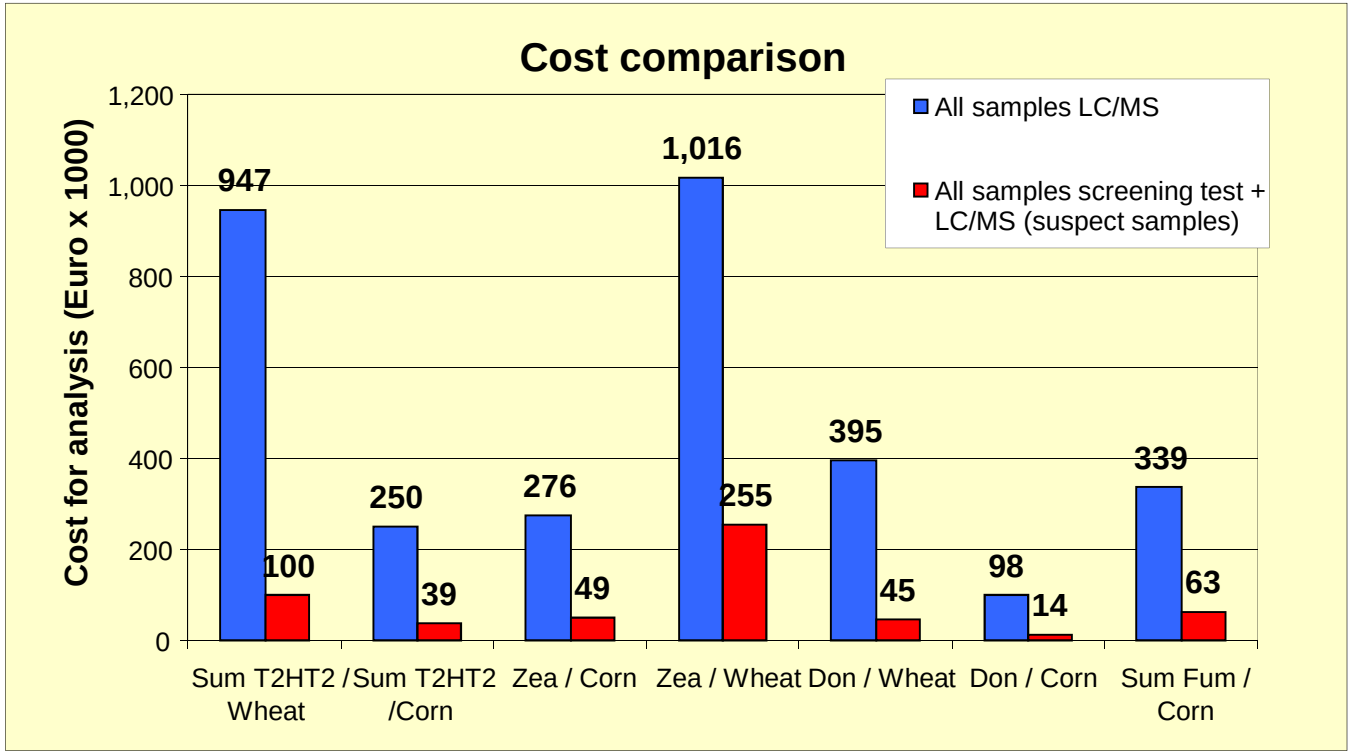
Cost estimation

Estimating total analytical costs for two options:

- Analysing all samples with LC/MS
- Analysing all samples with the screening test and all suspect samples additionally with LC/MS

LC/MS:
200 Euro/sample
Screening test:
20 Euro/sample



The test presented here is considered fit for purpose

Summary for validation and fitness for purpose of screening methods

- Safety first: The specific experimental design applied ensured that the rate of false negative results is not above 5 %
- The validation exercise also delivered information about the rate of false positive results
- Final criteria for fitness for purpose of the screening method:
  1. The actual prevalence of contaminated samples in the ground population
  2. Economical consideration of screening versus confirmatory methods

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Conclusions (1)

- Some multi-dipsticks are already commercially available

unisensor

- Multi-dipstick tests can be used in field applications
- Many tests have been validated through small-scale collaborative studies: transferability to other laboratories has been demonstrated

Conclusions (2)

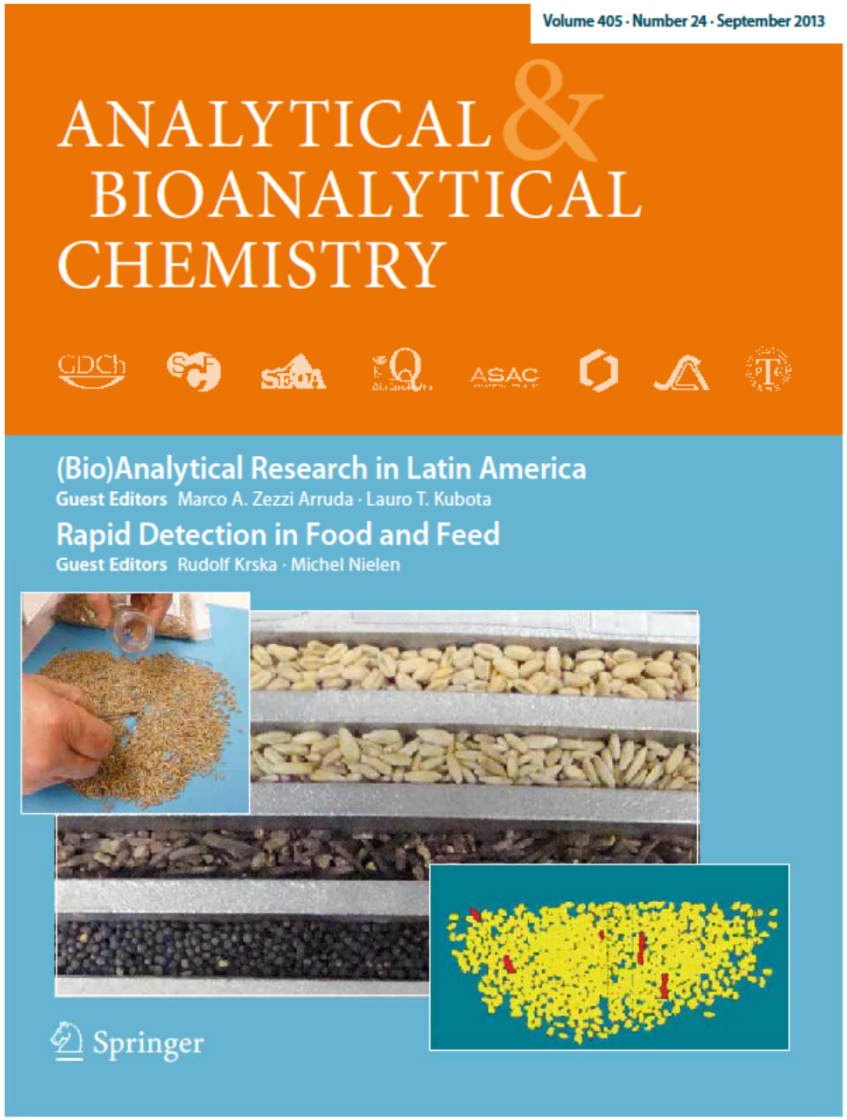
- Some tests have been validated through full collaborative studies, viz. mycotoxins dipstick, inorganic arsenic, perfluorinated compounds
  - ✓ Methods can be adopted by CEN for European standardization
- Major advances in simplified sample prep methods, reducing overall costs and speeding up analysis
- New insights in validation and fitness for purpose of screening tests
- Cross-cutting surveys, viz. fish & seafood: POPs, PFCs, heavy metals and PUFAs
- New topics have been included in the work programme in order to improve the relevance to EU policies

Dissemination

- More than 115 oral presentations and 115 posters at international conferences
- 36 Peer reviewed publications

- Website: **www.conffidence.eu**

Special volume of ABC



Acknowledgements

- ✓ The CON*ff*IDENCE partners
- ✓ The Advisory Board (DG SANCO, EFSA, FAO/IAEA, NOAA, AOAC)
- ✓ The CON*ff*IDENCE project has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°KBBE-211326

Thank you for your attention !

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