CONffIDENCE Open Day

Rapid methods for Organic pollutants

ORGANIC POLLUTANTS cluster (WP1a-c)

Noordwijkerhout, the Netherlands

January 27, 2010

WP1a - POPs

Persistent Organic Pollutants

WP leader: Jana Hajslova

VSCHT, Institute of Chemical Technology, Prague, CZ

WP deputy leader: Willem Haasnoot

RIKILT, Institute of Food Safety, Wageningen, Nl




SEVENTH FRAMEWORK PROGRAMME

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Tasks

T.1a.1 Sourcing and preparing of test matrices (fish, feeds and cereal-based food) with known content of target POPs (dioxin-like PCBs, BFRs, PAHs) (Nutreco, VSCHT)

T.1a.2 Development of a GC×GC/TOFMS comprehensive screening tool (VSCHT)

T.1a.3 Development of a simplified sample preparation strategy and delivery of extracts of test materials to the comprehensive profiling and flow cytometry based multiplex screening platforms (VSCHT, RIKILT)

T.1a.4 Development of flow cytometry TP-based inhibition assay in buffer for hydroxylated PBDEs, -dioxin-like PCBs and PAHs (RIKILT)

T.1a.5 Development of inhibition assay in buffer for parent PAHs (BaP and other selected PAHs) (RIKILT)

T.1a.6 Development of magnetic beads-based pre-concentration assay (RIKILT)

Organic pollutants cluster: WP1a-POPs

Analytes

- **Dioxin-like PCBs**
- **Brominated flame retardants (BFRs)**

Matrices

- Fish, fish feed

Justification

- Matrices representing the most important dietary source of these contaminants

Analytes

- **Polycyclic aromatic hydrocarbons (PAHs)**

Matrices

- Cereal-based food

Justification

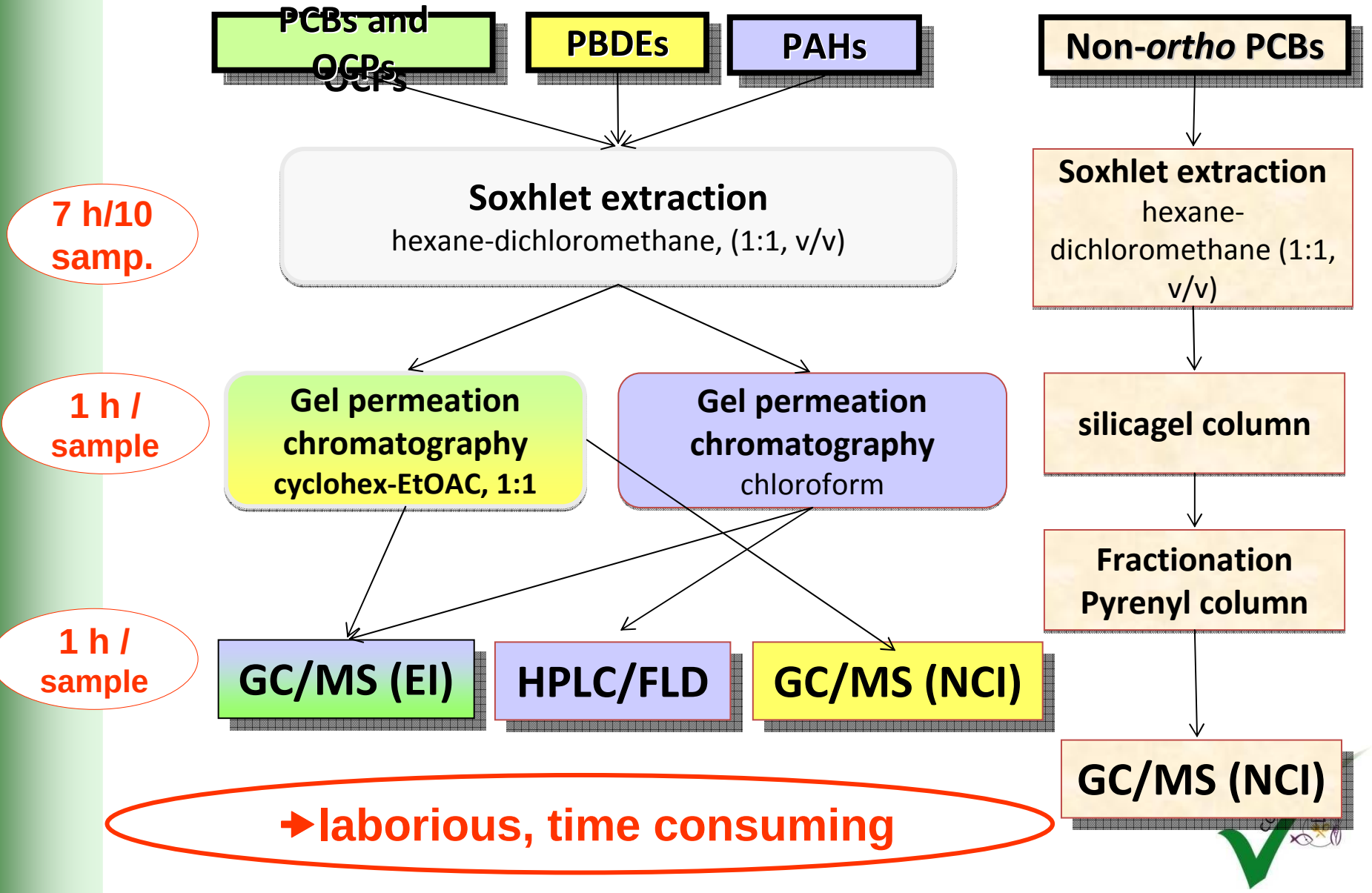
- Matrix representing a key commodity for production for vulnerable groups (infants, young children)

Target performance characteristics

- **LODs**
  - **BFRs:** ≤0.1 µg/kg (wet fish tissue)
  - **Dioxin-like PCBs:** 1 ng WHO-PCB-TEQ/kg (fish tissue)
    1 ng WHO-PCB-TEQ/kg (feed)
  - **B[*a*]P:** ≤0.5 µg/kg (cereal based food)
    1 µg/kg (fish)
- **Maximum false positive rate**
  50%
- **Maximum false negative rate**
  5%
- **Desired repeatability**
  ±15%

Several POP groups in a single run?

FISH: „conventional“ sample preparation methods for POPs groups



FISH: New, rapid sample prep method for halogenated POPs using PLE

PCBs & OCPs | **PBDEs** | **Non-*ortho* PCBs**

25 min

Pressurized liquid extraction

hexan-dichloromethan (Na_2SO_4, silicagel - fat retainer)

35 min

HRGC-TOFMS (EI) or GC×GC-TOFMS (EI)

PLE extraction

Dioxin-like PCBs

- non-*ortho* congeners #77, 81, 126, 169
- mono-*ortho* congeners #105, 114, 118, 123, 156, 157, 167, 189
- **86 – 110%, RSD max. 12%**

Brominated flame retardants

- Polybrominated diphenylethers: congeners #28, 47, 99, 100, 153, 154, 183
- Hexabromocyclododecane
- Polybrominated biphenyl: congener # 153
- **80 – 90%, RSD max 12%**

Polycyclic aromatic hydrocarbons

- benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, cyclopenta(c,d)pyrene, dibenz(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,h)-pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, indeno(1,2,3-cd)pyrene and 5-methylchrysene, Benzo(c)fluorene

- **55 – 75 %, RSD up to 28 % -** ☹

FISH: new, rapid sample prep for all analytes

Extraction
10 g fish muscle tissue + 5 mL H_2O
10 mL **EtOAC**

← *surrogate BDE 37 and ^{13}C-PCB 77*

Isolation of analytes 10 min

↓ *Shaking (1 min)*

Addition 4 g $MgSO_4$ + 2 g NaCl

6 samples prepared in 1 h

↓ *Shaking (1 min)*
Centrifugation (5 min, 11 000 rpm)
5 mL aliquot of EtOAC layer
Evaporation and reconstitution of sample in 1 mL hexane

Clean up 30 min

Clean-up on Silicagel mini-column (1 g)
1)Conditionation with 6 mL Hex:DCM (3:1, v/v) and 4 mL Hex
2)Sample application
3)Elution with 10 mL Hex:DCM (3:1,v/v)

Aliquot 5 mL of ethylacetate = 5 g of matrix
Aliquot depends on fat content

↓ *Evaporation*
Redissolved residues in 0.5 ml isooctane

Identificaton + quantification 45 min

BFRs, PCBs and PAHs GC/TOFMS (EI)

← *syringe standards (BDE 77, ^{13}C-PCB 101, ^{13}C-PAHs)*

Abramis brama

FISH: Performance characteristics

Analytes		Level 1		Level 2	
		Rec [%]	RSD [%]	Rec [%]	RSD [%]
Mono-ortho PCBs	PCB 105	113	9	108	11
	PCB 114	99	18	100	6
	PCB 118	95	9	87	13
	PCB 123	85	14	79	7
	PCB 156	96	9	77	11
	PCB 157	91	8	95	9
	PCB 167	75	10	76	10
	PCB 189	106	16	93	15
Major PCBs	PCB 138	82	8	78	11
	PCB 153	96	5	74	10
	PCB 180	84	11	77	8
Non-ortho PCBs	PCB 77	88	7	87	6
	PCB 81	91	4	84	5
	PCB 126	77	5	74	11
	PCB 169	105	10	101	11
PBDEs	PBDE 28	82	8	87	7
	PBDE47	86	9	93	7
	PBDE 99	97	7	95	5
	PBDE 100	98	8	107	6
	PBDE 153	95	7	95	6
	PBDE 154	95	8	94	5
	PBDE 183	93	6	94	7
HBCD	all isomers	89	12	85	10
PBB	PBB 153	85	9	82	14

Analytes		Level 1		Level 2	
		Rec [%]	RSD [%]	Rec [%]	RSD [%]
EU PAHs	B[a]A	82	2	86	6
	B[a]P	97	6	96	4
	B[b]F	84	4	86	7
	B[c]Fln	76	6	85	2
	B[j]Fln	85	4	82	4
	B[k]Fln	85	5	90	4
	B[ghi]P	96	6	94	4
	Chr	89	6	91	6
	CP[cd]P	83	7	89	5
	DB[ah]A	94	6	95	5
	DB[ae]P	85	3	86	2
	DB[ah]P	83	3	86	3
	DB[ai]P	83	4	85	6
	DB[al]P	90	9	92	6
	I[cd]P	95	4	91	6
	5MeChr	79	6	78	6

Oncorhynchus mykiss

CEREAL BASED BABY FOOD: New rapid sample prep procedure for PAHs

EXTRACTION
2.5 g of extruded flour + 10 ml H_2O
10 ml **acetonitrile**

Shaking 1 min

2 g NaCl + 4 g $MgSO_4$

Shaking (1 min)
Centrifugation (5 min, 11 000 rpm)
4 mL aliquot of acetonitrile EtAc layer
Evaporation and reconstitution of sample in 1 mL hexane

Clean-up on Silicagel mini-column (1 g)

1)Conditionation with 6 mL Hex:DCM (3:1, *v/v*) and 4 mL Hex
2)Sample application
3)Elution with 10 mL Hex:DCM (3:1,*v/v*)

Evaporation
Redissolved residues in 0.25 µl of toluene containing syringe standard of ^{13}C PAHs

GC –TOFMS (PEGASUS III)

Aliquote 4 ml of acetonitrile = 1 g of matrice

CEREAL BASED BABY FOOD: PAHs

Spiked non contaminated extruded flour

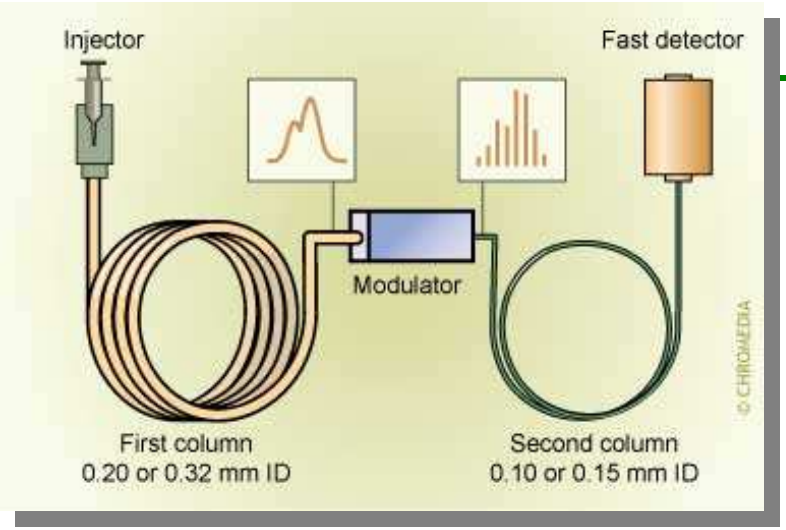
Spiked level: 0.5 µg/kg and 2 µg/kg

	Spiking level 1		Spikig level 2	
	Recovery %	RSD %	Recovery %	RSD %
benzo[*c*]fluorene	89	8	88	6
benzo[*a*]anthracene	84	4	92	3
chrysene	88	8	94	3
cyclopenta[*c,d*]pyrene	77	6	81	4
5-methylchrysene	76	9	79	5
benzo[*b*]fluoranthene	80	3	84	4
benzo[*k*]fluoranthene	78	3	79	3
benzo[*j*]fluoranthene	76	5	80	5
benzo[*a*]pyrene	82	8	86	4
dibenz[*a,h*]anthracene	79	8	82	7
indeno[1,2,3-*cd*]pyrene	84	7	86	6
benzo[*g,h,i*]perylene	79	9	82	5
dibenzo[*a,l*]pyrene	76	9	82	8
dibenzo[*a,e*]pyrene	78	8	83	7
dibenzo[*a,i*]pyrene	79	8	88	4
dibenzo[*a,h*]pyrene	81	6	83	5

Aliquote 4 ml of acetonitrile = 1 g of matrix

GCxGC-TOF MS

- High chromatographic resolution needed for complex matrix / analytes mixture (separation of isomers)
- Focusing of peaks in 2nd dimension – decrease of LODs (DL-PCBs, BFRs)



Column systems tested:

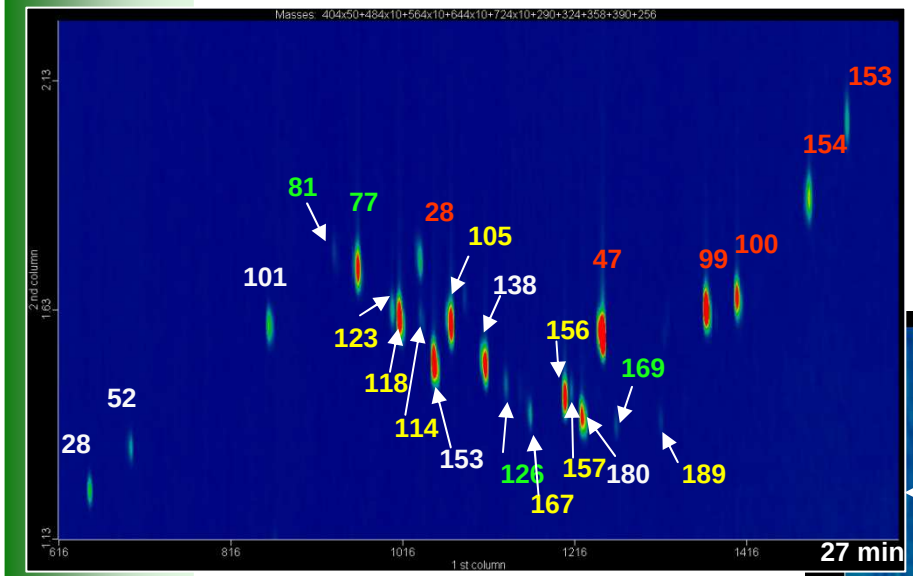
A. 1st column dimension: BPX 5 (30 m × 0.25 mm × 0.25 µm)
   2nd column dimension: BPX 50 (1 m × 0.1 mm × 0.1 µm)
B. 1st column dimension: BPX-5 (30 m × 0.25 mm × 0.25 µm)
   2nd column dimension: Rt-LC35 (1 m × 0.15 mm × 0.1 µm)
C. 1st column dimension: BPX 5 (30 m × 0.25 mm × 0.25 µm)
   2nd column dimension: HT 8 (1 m × 0.1 mm × 0.1 µm)
D. 1st column dimension: BPX 50 (30 m × 0.25 mm × 0.25 µm)
   2nd column dimension: BPX 5 (1 m × 0.1 mm × 0.1 µm)

E. 1st column dimension: BPX 50 (30 m × 0.25 mm × 0.25 µm)
   2nd column dimension: HT 8 (1 m × 0.1 mm × 0.1 µm)

GC×GC–TOF MS (EI): PCBs (m/z 256, 290, 324, 358, 390) PBDEs (m/z 404, 484, 564, 644,724)

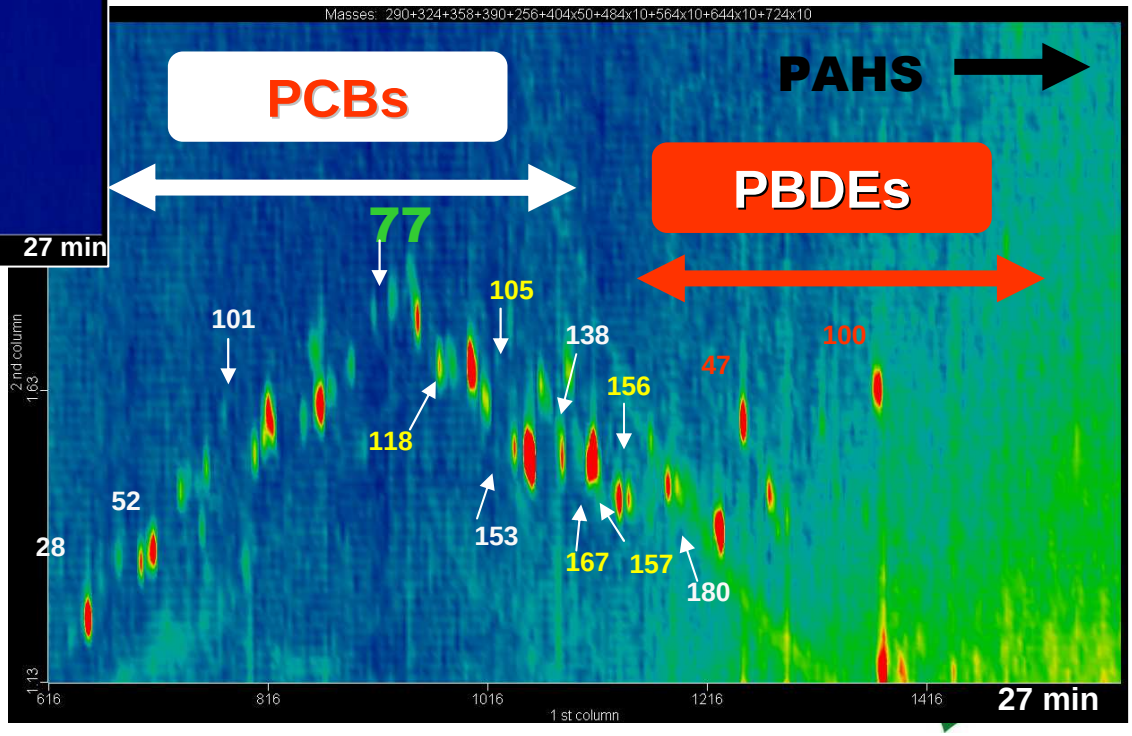
System A

standards▼



PCBs + PBDEs mixture in isooctane

- **Level 1 (PCB 77, 105, 118, 138, 156, 180) (PBDE 47) 1000 pg injected**
- **Level 2 (PCB 28, 52, 101, 123, 167) (PBDE 100, 154) 100 pg injected**
- **Level 3 (PCB 81, 114, 126, 157, 169, 189) (PBDEs 28, 99, 153, 183) 50 pg injected**



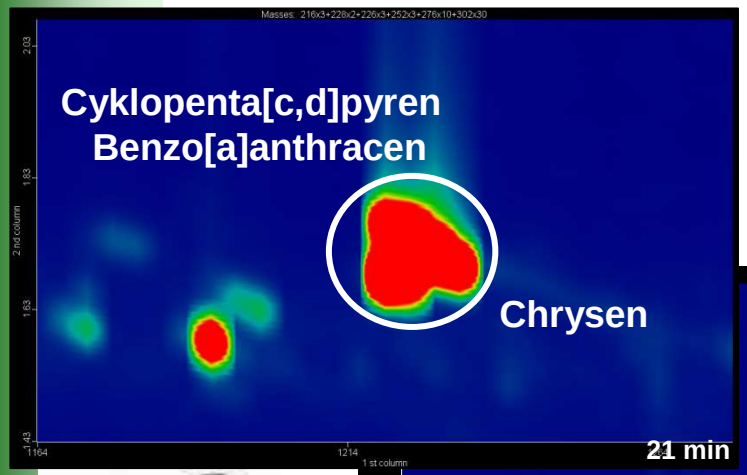
yellow – mono-ortho PCBs
green – non-ortho PCBs
white – indicator PCBs
red - PBDEs

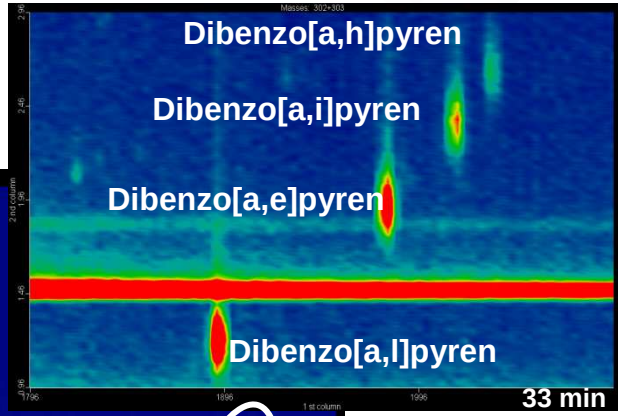
Contaminated fish fillets (Ide – *Leuciscus Abramis*)►

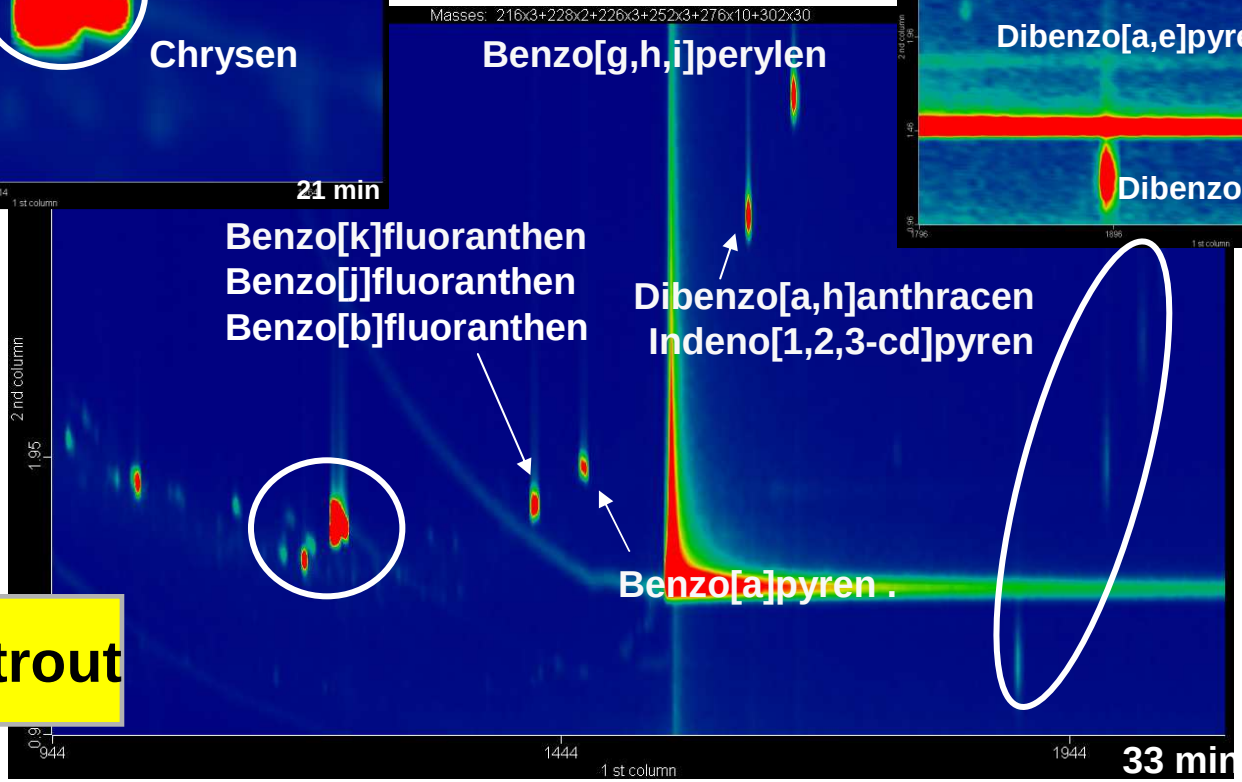
GC×GC–TOF MS (EI): PAHs **System A**

(m/z 302, 276, 252, 242, 228, 178, 202, 216)

Estimated LOD for PAHs
5 – 50 pg injected







Smoked trout

WP1b Perfluorinated compounds

WP leader: Marinella Farre (CSIC)

WP deputy: Jan Poustka (ICT Prague)

PFC analyses: Petra Hradkova (ICT Prague)

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Tasks

T.1b.1. Development of innovative and simplified protocols for analysis of PFCs in food and feed samples based on different extraction procedures followed by LC-MS and LC-MS/MS (WQU-CSIC, VSCHT).

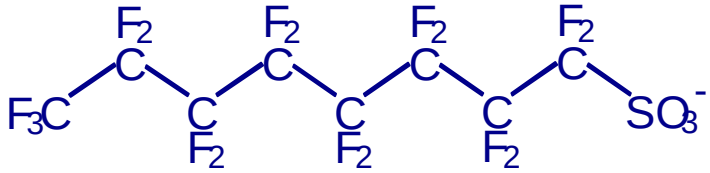
T.1b.2. Evaluation of the developed simplified mass spectrometric methods using spiked test materials and samples from daily practice (WQU-CSIC, VSCHT, Nutreco).

T.1c.3 Toxicity assessment of individual perfluorinated compounds, including PFOS, PFOA and FOSA (WQU-CSIC).

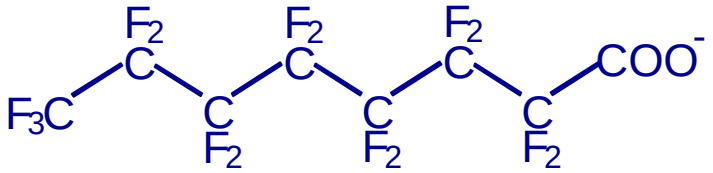
T.1b.4. Organization and evaluation of a first interlaboratory study on PFCs analysis in food and feed samples (WQU-CSIC, in collaboration with WP1b beneficiaries).

Target analytes and matrices

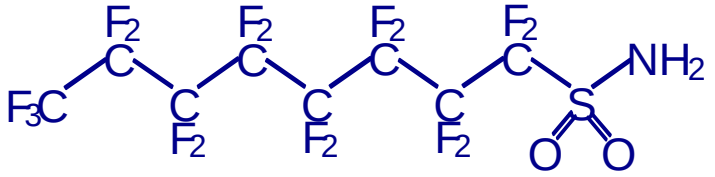
Perfluorooctanesulphonate – PFOS



Perfluorooctanoic acid – PFOA



Perfluorooctanesulphonamide – FOSA



Tested matrices

- **Animal tissues**
  (fish liver and muscles)
- **Dairy products**
  (milk; 1.5% fat content)
- **Fish feed**

Test matices - fish tissues, fish feed

2 g sample

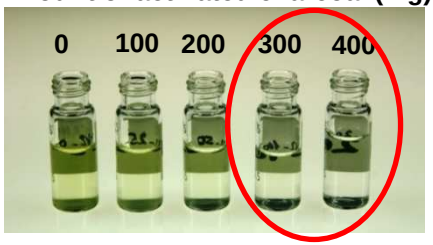
EXTRACTION (6 mL methanol)

Addition of isotopically labelled standards – $^{13}C_4$-PFOS a PFOA

CLEAN – UP (340 mg activated charcoal)

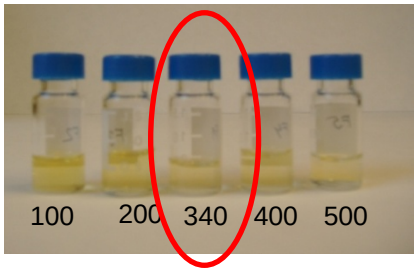
Amount of activated charcoal (mg):

Fish liver:



Centrifugation
(10000rpm; 5 min)
FILTRATION
(0.2 µm centrifuge filter; 5000 rpm; 2 min)

Fish feed:



IDENTIFICATION & QUANTIFICATION LC-MS/MS

Matrix matched standards (150; 30; 15; 3 µg/kg)
$^{13}C_4$ std – 30 µg/kg sample

MILK

2 mL sample +
2 mL 0.1 M formic acid

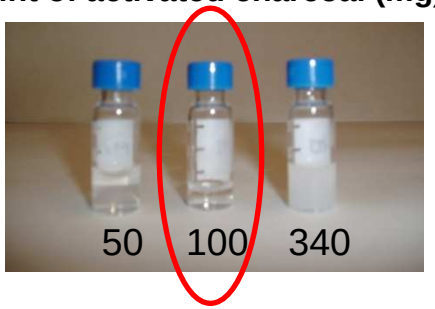
EXTRACTION
(6 mL methanol)

Addition of isotopically labelled standards
– $^{13}C_4$-PFOS a PFOA

vortex

CLEAN – UP
(100 mg activated charcoal)

Amount of activated charcoal (mg):



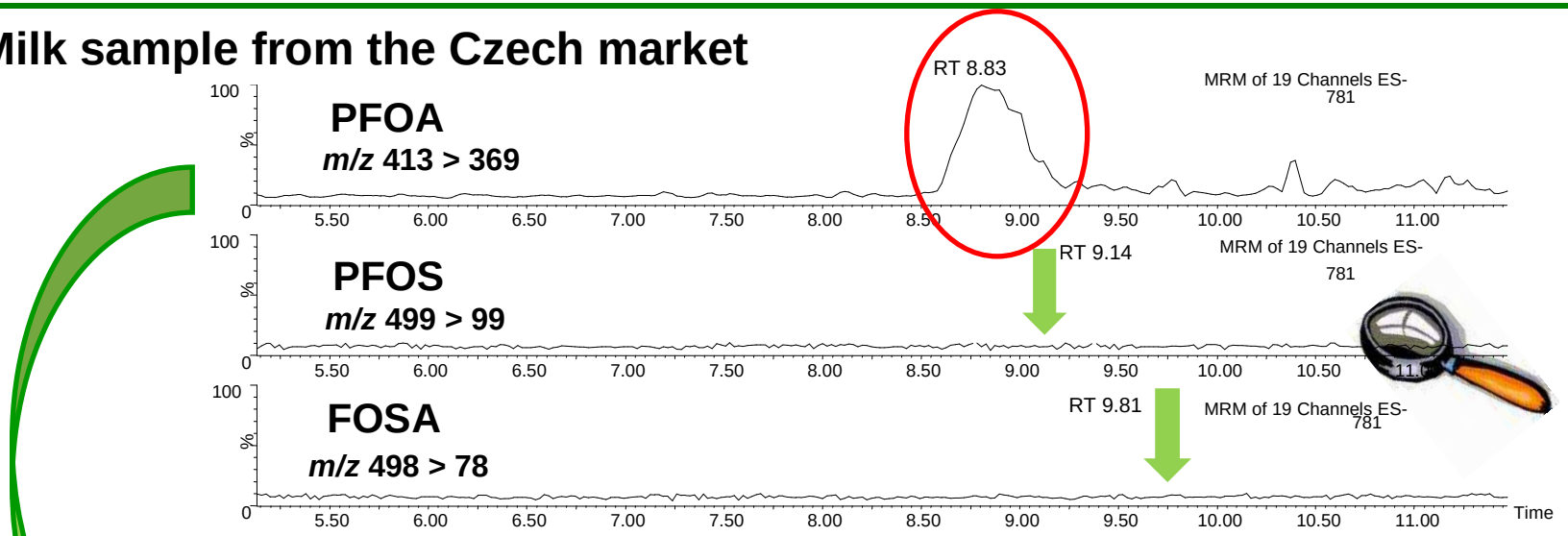
Centrifugation
(10000rpm; 5 min)
FILTRATION
(0.2 µm centrifuge filter; 5000 rpm; 2 min)

IDENTIFICATION & QUANTIFICATION
LC-MS/MS

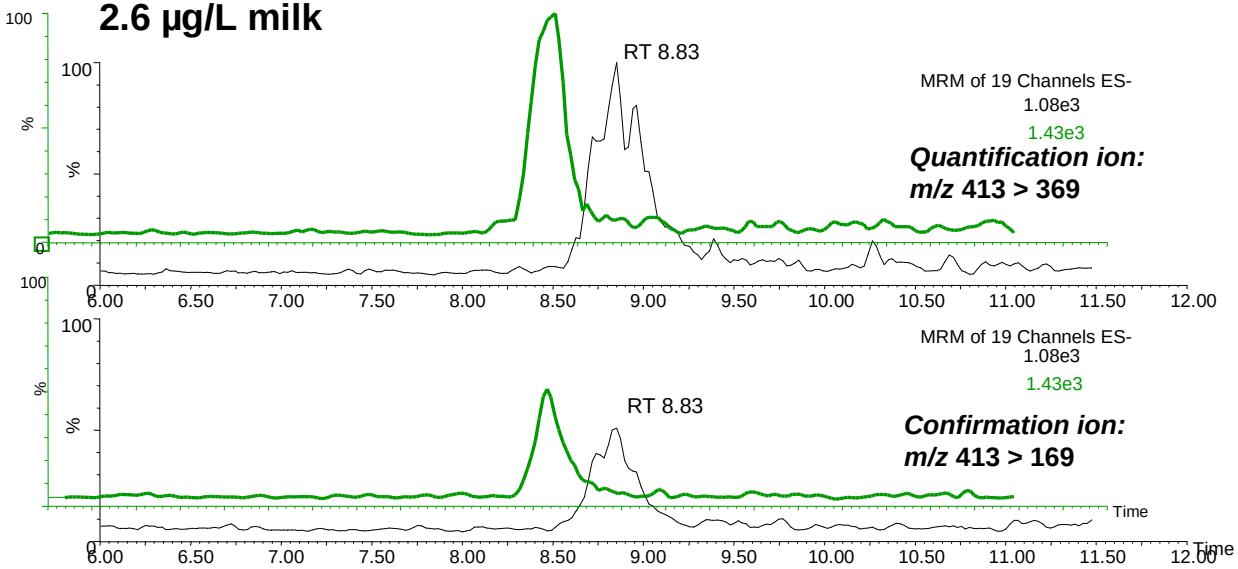
Matrix matched standards (150; 30; 15; 3 µg/kg)
$^{13}C_4$ std – 30 µg/kg sample

MILK

Milk sample from the Czech market



PFOA – incurred residues:
2.6 µg/L milk



Matrix matched standard – 3 µg/L milk; real sample

Performance characteristics

	MILK*			FISH TISSUE[a]			FISH FEED		
	PFOS	PFOA	FOSA	PFOS	PFOA	FOSA	PFOS	PFOA	FOSA
Recovery (n=5; %)	92	91	114	80	89	104	90	95	98
RSD (n=5; %)	9	6	4	24	32	4	4	3	4
LOD (µg/kg)	0.5	0.5	0.3	0.6	0.6	0.3	0.6	0.6	0.3
LOQ (µg/kg)	2	2	1.5	2	2	1.5	2	2	1.5

*µg/L; [a] – level 3 µg/kg)

Alternative analytes determination

- Fish tissues (liver, muscles)
- Performance characteristics measured on HP 1200 coupled with Applied biosystem/MDS SCIEX 5500 QTRAP®

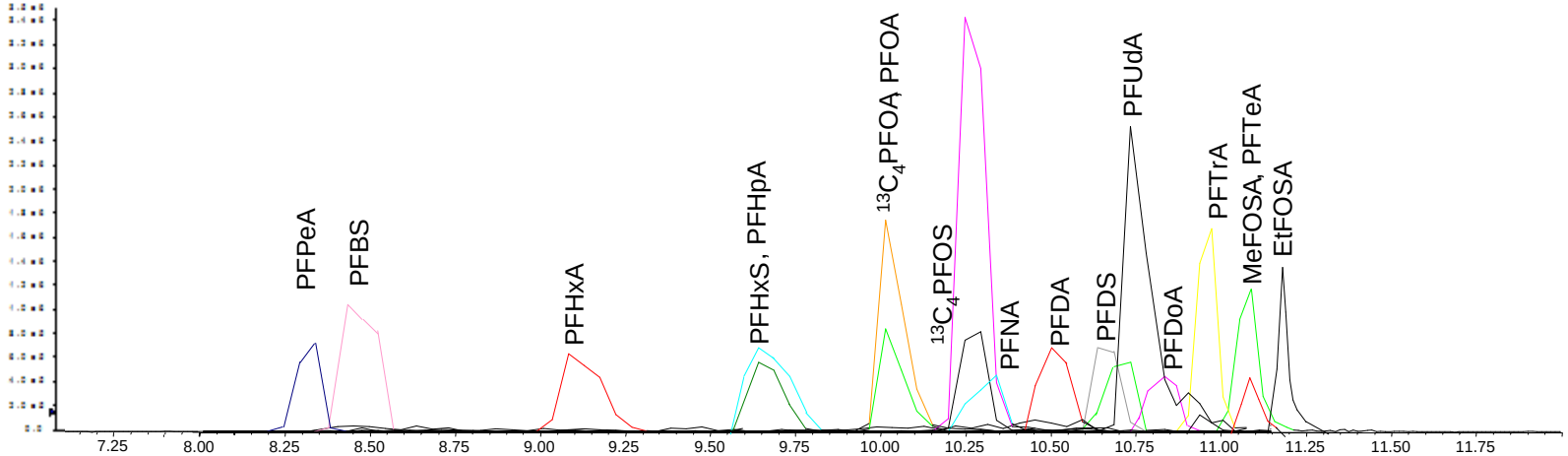
	PFOS	PFOA	FOSA
LOD (µg/kg)	0.2	0.2	0.1
LOQ (µg/kg)	0.6	0.6	0.3

Vrije Universiteit amsterdam
Institute for Environmental Studies

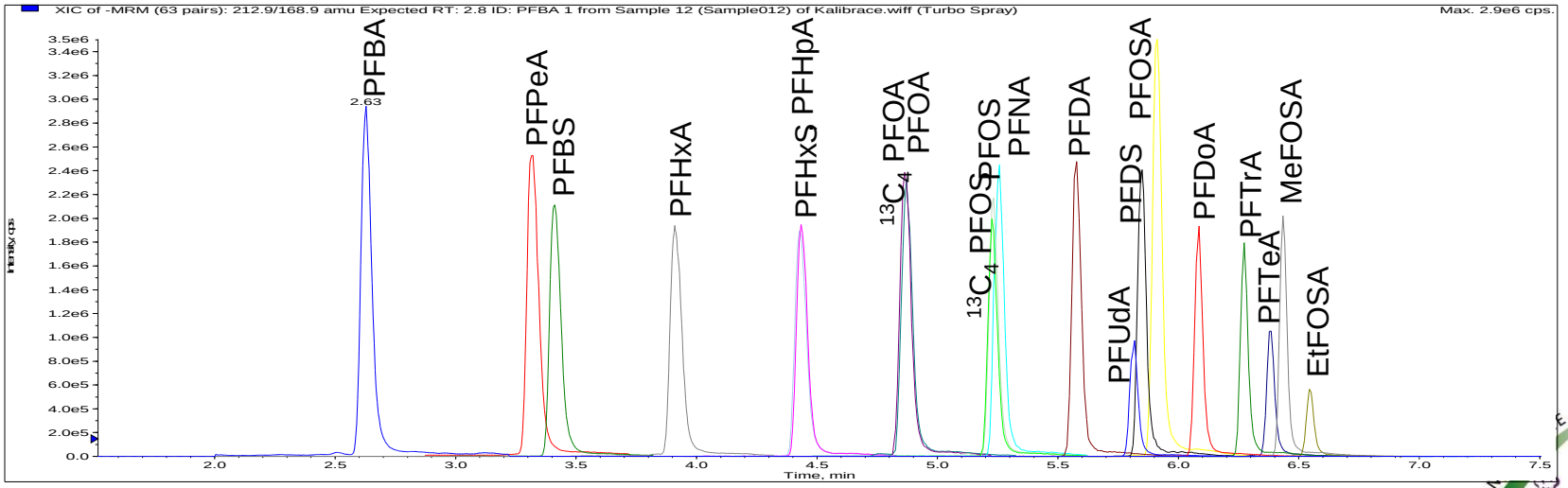
norman

LC-MS/MS chromatograms of PFCs

Alliance 2695, Quattro Premier XE (Waters)



HP 1200 and Applied biosystem/MDS SCIEX 5500 QTRAP®



Matrix matched standard – 30 µg/kg

WP1c Pesticides

WP leader: Hans Mol

RIKILT, Institute of Food Safety, Wageningen, Nl

WP deputy leader: *M. Pilar Marco*

CSIC, Barcelona, Spain

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Tasks

T.1c.1. Development of immunoreagents (haptens,conjugates and antibodies) (CSIC)

T.1c.2. Production of test materials spiked and incurred with paraquat/diquat (CSL)

T.1c.3. Development of simplified sample preparation methods for paraquat/diquat in potato and cereal (CVUA, CSL)

T.1c.4. Development of electrochemical immunosensor assays and in-house validation (CSIC)

T.1c.5. Critical comparison of the new assay with the mass spectrometric reference method (CSL, CVUA, CSIC)

WP objectives and tasks

A

Simplified MS screening tool
Dithiocarbamates
Intact veg/fruits

DART-MS

INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

DESI-MS

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

B

Immunosensor
Paraquat/diquat
Potato/Barley

Test material

CSL

Extraction protocol

Chemisches und Veterinäruntersuchungsamt Stuttgart

Immunoreagents
Immunosensor

CSIC

CONFIDENCE

Dithiocarbamates

DART-TOF-MS

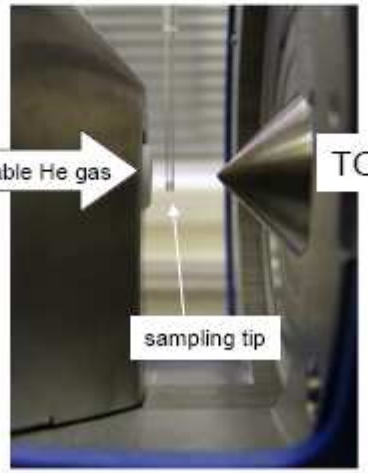
- Principle / set up

1. tubes with sample solution

2. Immerse sampling tip
Into solution

3. Position between
DART gun and MS

4. Detect



2007/57/EC

COMMISSION DIRECTIVE 2007/57/EC

of 17 September 2007

amending certain Annexes to Council Directives 76/895/EEC, 86/362/EEC, 86/363/EEC and 90/642/EEC as regards maximum residue levels for dithiocarbamates

(Text with EEA relevance)

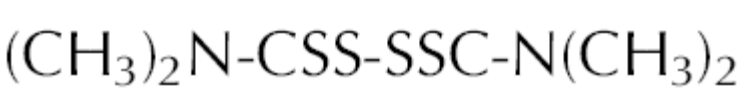
Groups and examples of individual products to which the MRLs apply	Dithiocarbamates, expressed as CS_2, including maneb, mancozeb, metiram, propineb, thiram and ziram (1), (2)	Propineb (expressed as propilendiammine) (3)	Thiram (expressed as thiram) (3)	Ziram (expressed as ziram) (3)
Others	0,05 (*)			
(iii) **POME FRUIT**	5 (ma, mz, me, pr, t, z)	0,3		
Apples			5	0,1 (*)
Pears			5	1
Quinces				
Others			0,1 (*)	0,1 (*)

mg/kg

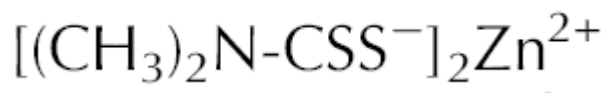
Sample preparation

- **First considerations for the determination of thiram and ziram in fruits (pears) using DART–TOFMS**
  - Both compounds soluble in acetonitrile (this information not available in literature!)
  - Possibility to use acetonitrile for sample extraction (QuEChERS approach)
  - Internal standard needed for reliable quantification

Thiram



Ziram

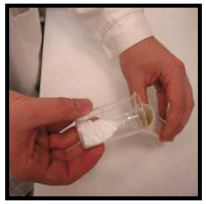


Sample preparation

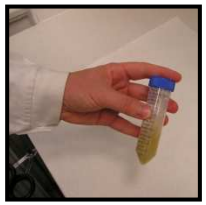
- **QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)**

(1) 

10 g sample (pears)
10 mL acetonitrile
Shaking – 1 min

(2) 

4 g $MgSO_4$ + 1 g NaCl

(3) 

Shaking – 1 min

(4) 

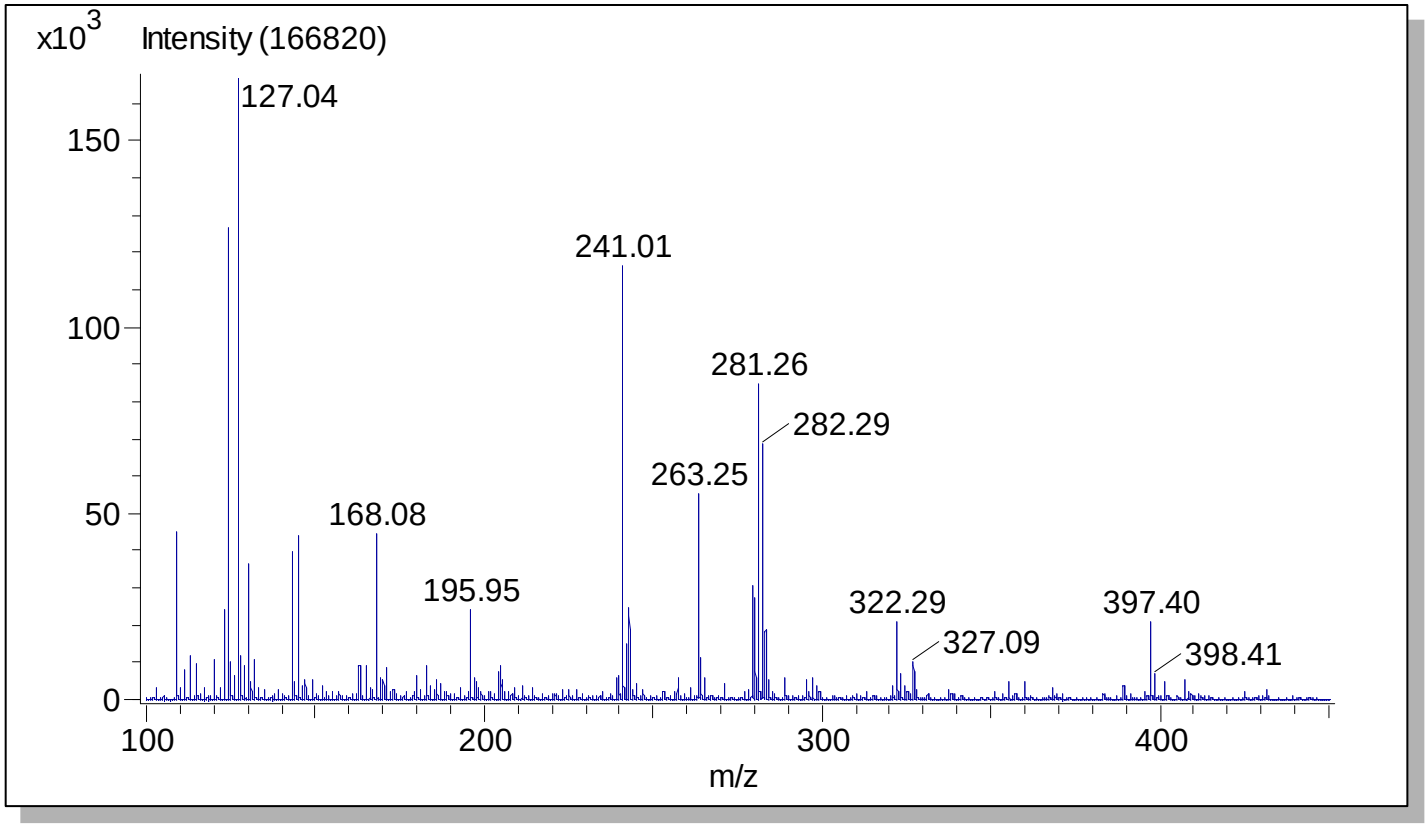
Centrifugation – 5 min, 11,000 rpm

(5) 

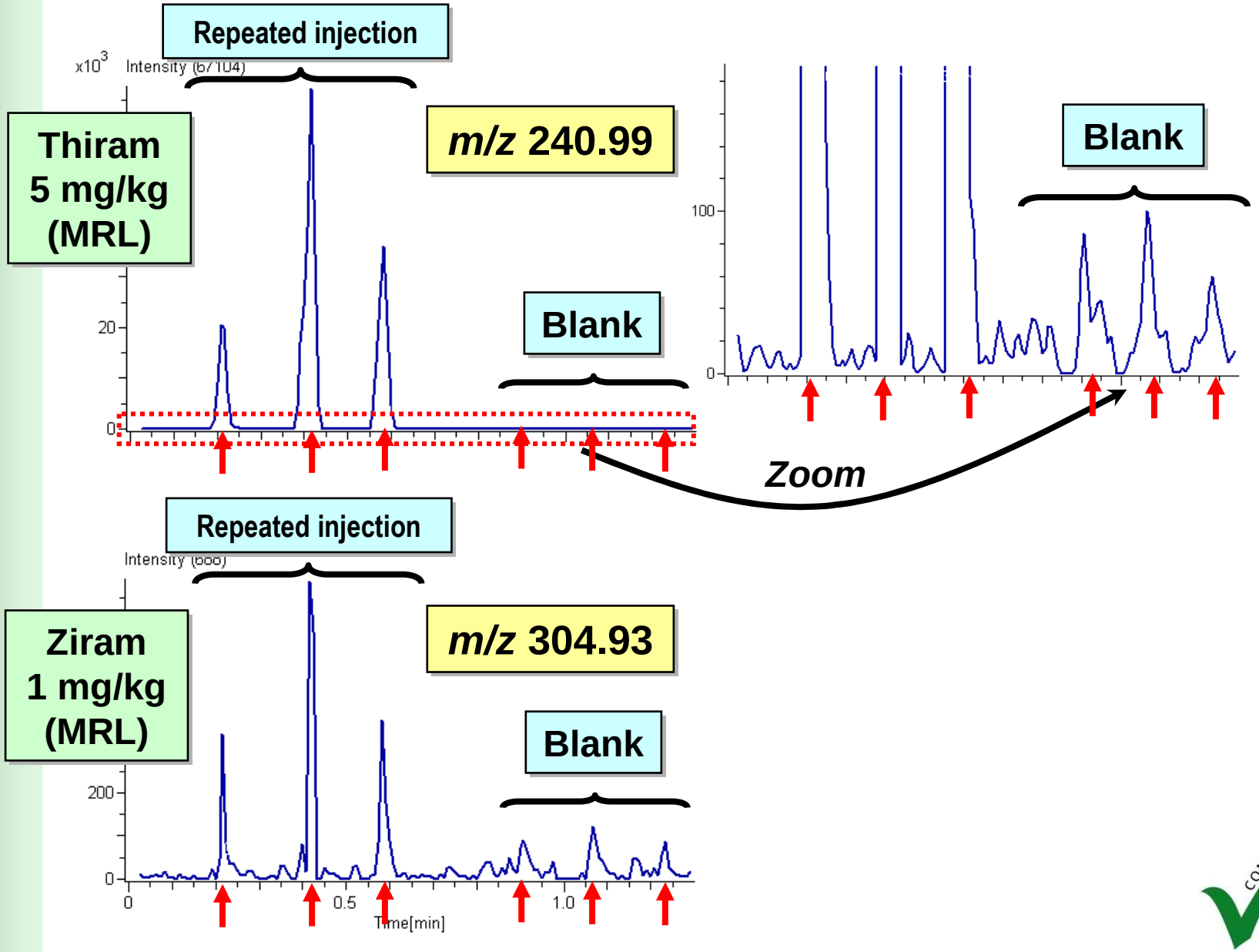
DART–TOFMS analysis

Activities and achievements

- **DART–TOFMS spectrum of matrix-matched standard (pear extract)**



Activities and achievements



Activities and achievements

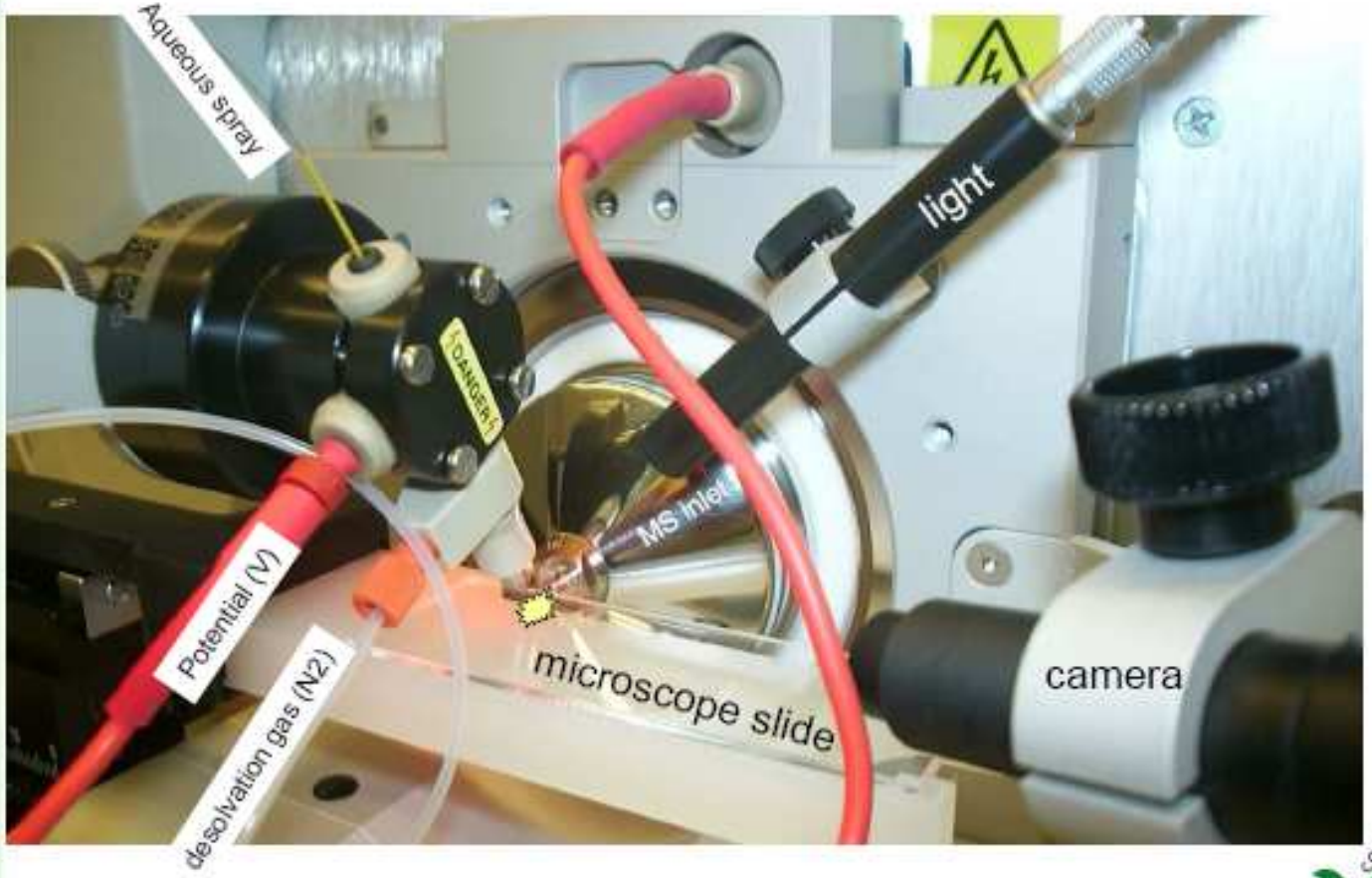
- **Validation study**
- **Matrix: pears**

Analyte	MRL (mg/kg)	Spike level (mg/kg)	Recovery (%)	RSD (%)
Thiram	**5**	**5**	**85.2**	**6.7**
Ziram	**1**	**1**	**82.7**	**8.9**

*) *Note: Quantification performed using matrix-matched standards with internal standard (TPP) correction*

DESI

- DESI-MS/MS (LXQ; linear ion trap)



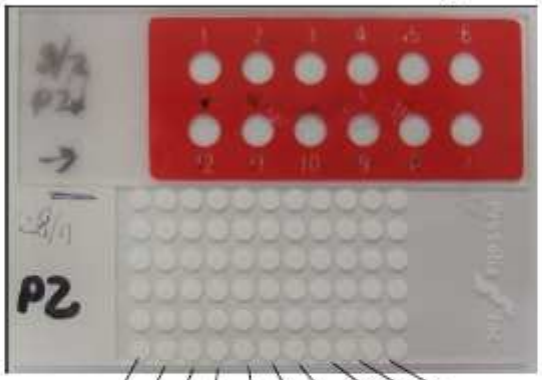
Dithiocarbamates

DESI feasibility

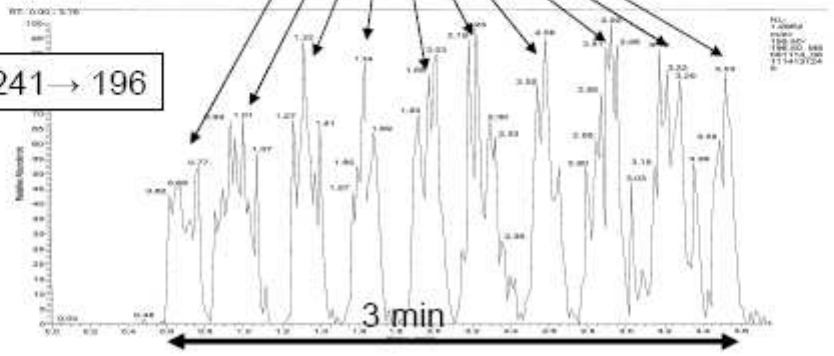
- DESI-MS/MS (LTQ; linear ion trap)

  - direct detection from surface without any sample preparation

Thiram



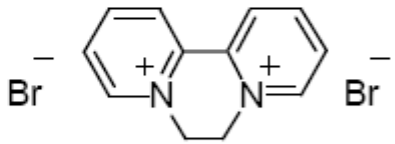
MS/MS 241→ 196



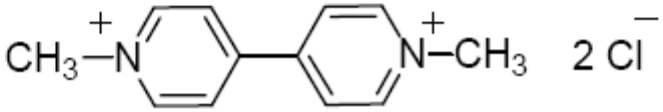
Paraquat and Diquat

- Extraction/chromatographic behavior deviates from typical MRM conditions
- World-wide extensively used as herbicides/desiccants

- Diquat: default/LOD MRL = 0.05 mg/kg most crops
  tolerance for: maize (1), millet (1), oats (2), barley (10 mg/kg)



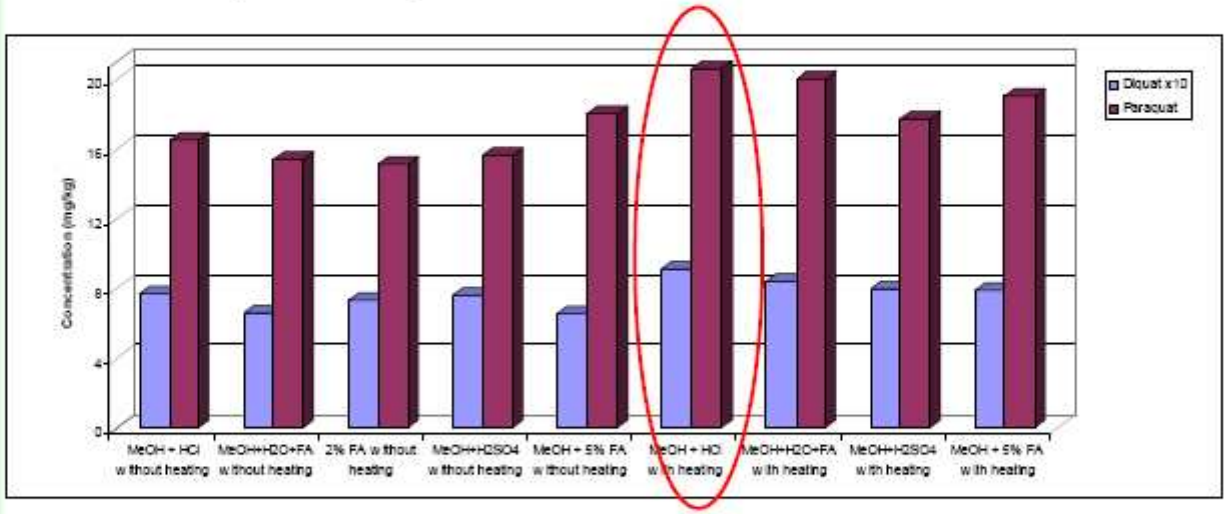
- Paraquat: highly toxic (ARfD 0.005 mg/kg bw);
  default/LOD MRL = 0.02 mg/kg most crops
  Banned in EU since 2008



Paraquat & Diquat

Extraction protocol*

- Simplified and compatible with immunosensor
- Developed using incurred materials



methanol/water/HCl (80°C) 15 min + dilution
to replace 6h H_2SO_4 reflux method from industry

*Poster Diana Ströher Kolberg

Thank you for your attention.

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Test material for PAHs – smoked trout

	Mean	R.S.D.%
Benzo[*c*]fluorene	16.25	7
Benz[*a*]anthracene	60.6	4
Chrysene	45.86	5
cyclopenta[*c,d*]pyrene	42.63	4
5-Methylchrysene	2.67	6
Benzo[*b*]fluoranthene	13.81	4
Benzo[*k*]fluoranthene	7.84	4
Benzo[*j*]fluoranthene	6.58	6
Benzo[*a*]pyrene	18.21	4
Dibenzo[*a,h*]anthracene	0.55	7
Indeno[1,2,3-*cd*]pyrene	6.83	8
Benzo[*g,h,i*]perylene	6.69	5
Dibenzo[*a,l*]pyren	0.39	7
Dibenzo[*a,e*]pyren	0.48	9
Dibenzo[*a,i*]pyren	0.32	6
Dibenzo[*a,h*]pyren	< 0.3	-

TIC – smoked trout