Emerging Hyphenated and Comprehensive Multi-Dimensional Techniques for the Measurement of POPs in Food

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Mass Spectrometry Laboratory
Biological and Organic Analytical Chemistry
Persistent Organic Pollutants

9 agrochemicals:
- Aldrin
- Chlordane
- DDT
- Dieldrin
- Endrin
- Heptachlor
- HCB
- Mirex
- Toxaphene

3 industrial substances:
- PCBs
- PCDDs
- PCDFs
Target Analytes

- 2,3,7,8-substituted PCDDs (7)
- 2,3,7,8-substituted PCDFs (10)
- Non-ortho PCBs (4)
- Mono-ortho PCBs (8)
- EU marker ‘Tracer’ PCBs (6)
- ‘Biomonitoring’ PCBs (30)
- Selected OCPs (12)
- Selected PBDEs (8)

\[ \sum = 80^+ \]
Target Matrices

Contributors to human intake

- Fish
- Meat
- Milk

PCDD/Fs + NO-PCBs

- Animal feedingstuffs: 33%
- Human specimen: 35%
- Fish: 32%

Belgium 2001 (Focant et al., 2002)
QA/QC Guidelines

✓ EU Directive 1881/2006 EC
✓ EU Directive 1883/2006 EC
✓ EURACHEM Guide ‘The fitness for purpose of analytical methods’

Requirements for analytical procedures:
- Sensitivity (LOQs) - Selectivity
- Accuracy - Trueness
- Robustness - $^{13}$C-labeled IS
- Recovery rates - Separation: ‘should be sufficient’
EU Directive 1883/2006 EC

✓ Requirements for analytical procedures
  – High sensitivity: low MDLs
  – High selectivity: distinction for PCDDs, PCDFs and DL-PCBs from co-extracted and interfering compounds
  – High accuracy: provide a valid estimate of the true concentration
  – Trueness: +/- 20 % and CV< 15%
  – $^{13}$C-labeled IS: addition of PCDD/F and DL-PCB
  – Recovery rates: 60-120 % (30 - 140 %)
  – GC separation: “should be sufficient”
  – Lower - Upperbound: 20 % difference (in the range of 1 pg/g fat for food)
The Dream

Sample

Congener-specific Results

<table>
<thead>
<tr>
<th>Time</th>
<th>Cost</th>
<th>Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>€10</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Magic Box
Dreams Sometimes Come True

1886

J. G. COCHRAN & J. KRITCH.
DISH WASHING MACHINE.
No. 301,782.
Patented Oct. 30, 1886.

1970’s

1886
Area of Efforts

- Reducing manual handling
- Increasing sample throughput (# analytes)
- Increasing analytical speed
- Decreasing blank levels (improve LOQs)
- Get a better position in The Triangle
POP Analysis Skeleton

- **Extraction**
  - LLE, Soxhlet, SFE, MAE, SPME, SPE, MSPD, SBSE, PLE, …

- **Clean-up**
  - Prep. LC (Silica), HPLC, SEC (GPC), …

- **Fractionation**
  - Florisil, Alumina, Carbon, PYE, …

- **Separation**
  - GC, GC-GC, GCxGC, …

- **Measurement**
  - Sector HRMS, QISTMS\(n\), TOFMS, \(\mu\)ECD, EIAs, RBAs, …
SOLUTIONS

PHENOLEGIST
Coupling (Instrumental)

- SPME-GC-MS
- SBSE-GC-MS
- SFE-GC-MS

Limited efficiency for Dioxins

- SPE(or PLE)-Prep.LC-GC-IDMS(or RBA)

Liquids
Solids
Silica
Alumina
Carbon

Automated
ECF Integrated Approach

Samples

Automated SPE or PLE

Automated MCLC Clean-up System

Batches of 5-10 samples

Time

Cost

Problems

0.5 day

€400

0.5
Fluid Samples

- Sample homogenisation
- Spiking, proteins precipitation, ...
- **SPE (C\textsubscript{18})** extraction
- Side ‘lipid percent’ determination

Clean-up
Reverse Phase
Non-polar phase (C\textsubscript{18})
Polar solvent (MeOH, ...)

Selective retention of the analytes
In parallel, 2 h/batch

ECF Integrated System (liquids)

- Gauge
- Pump

Nitrogen tank

C18

Sample

Aqueous waste

Silica

Carbon

1. Hexane
2. Hexane-Dichloromethane (1:1)
3. Ethyl acetate-Toluene (1:1)
4. Toluene

1. Water
2. Acetonitrile
3. Hexane-Dichloromethane (1:1)
4. Air

1. PCDD/Fs & cPCBs

Organic waste

GC-MS

1. Hexane
2. Hexane-Dichloromethane (1:1)
3. Ethyl acetate-Toluene (1:1)
4. Toluene

1. Water
2. Acetonitrile
3. Hexane-Dichloromethane (1:1)
4. Air

1. PCDD/Fs & cPCBs
SPE for GC-MS

Extract for GC-MS

Milk
Accuracy (milk CRM)

![Graph showing accuracy of different methods for milk CRM](image)

- Soxhlet
- Manual SPE
- Integrated SPE

**Y-axis:** pg g⁻¹ milk powder

**X-axis:** Compounds:
- 2,3,7,8 TCDD
- 1,2,3,4,7,8 PCDD
- 1,2,3,6,7,8 PCDD
- 1,2,3,7,8,9 PCDD
- 2,3,7,8 TCDF
- 1,2,3,7,8 PCDF
- 2,3,4,7,8 PCDF
- 1,2,3,4,7,8 HCDF
- 1,2,3,6,7,8 HCDF
- 2,3,4,6,7,8 HCDF

Accuracy (milk CRM)
Serum QC Chart

Concentration (pgTEQ/g)

Run number

Manual SPE
Integrated SPE

PCDD/Fs
(semi)Solid Samples

- Sample homogenisation (liquid $\text{N}_2$)
- Freeze-drying (overnight)
- Grinding
- Spiking, PLE extraction
- Side ‘lipid percent’ determination

Clean-up
Pressurized Liquid Extraction

- Cryo-homogenization
- Drying (lyoph, …)
- 120°C, 1500 psi
- Hexane (others)
- Static and/or dynamic

10-30 min/sample
Several groups reported the use of \( \text{H}_2\text{SO}_4\)-Si to remove part of the lipids directly inside the extraction cell.

**BUT:**
- Restricted to few extraction solvents
- \( \text{H}_2\text{SO}_4\)-Si very ‘strong’ in conditions like 120°C/1500 psi
ECF Integrated System (solids)

On-line evaporation

100ml & nitrogen

20ml sharp pulse

100ml & nitrogen

In parallel, 1.5 h/batch (5-10)
CARP-1 CRM

PCDD/F concentrations

I-PCB concentrations

(Sample size = 4g)

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Measured</th>
<th>Trueness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUM PCDD/Fs</td>
<td>13.3</td>
<td>12.6</td>
<td>-5.3</td>
</tr>
<tr>
<td>SUM PCBs</td>
<td>60.3</td>
<td>66.2</td>
<td>9.8</td>
</tr>
<tr>
<td>SUM PCDD/Fs+PCBs</td>
<td>73.6</td>
<td>78.8</td>
<td>7.1</td>
</tr>
</tbody>
</table>

pgTEQ/g f.w.
How to Measure?

SPE(or PLE)-Prep.LC → ??

Liquids → Solids → Si, Al, C

On-line and Automated

One needs extra dimensions

Chromatography, Spectroscopy, ...

GC & MS

Sample Dimensionality ↔ System Dimensionality
Reference Method

(HR)GC-IDHRMS
PTV-LVI-GC-IDQISTMS/MS
ISO-17025 alternative method

Could we better match sample dimensionality?

- Separate injections
- Different GC phases
  - PCDD/Fs [RTX-5MS]
  - PCBs [HT-8]
  - PBDEs [STX-500]
Dimensionality

- GC-µECD
- GCxGC-µECD
- GCxGC-TOFMS
GC\times GC

Comprehensive two-dimensional gas chromatography

Peak capacity enhancer
“Two-dimensional (2D) separations are those techniques in which a sample is subject to two independent (orthogonal) displacement processes”

Definition of ‘Comprehensive’

A two-dimensional separation can be called comprehensive if:

1. **Every part** of the sample is subjected to two independent separative displacements.
   - **Orthogonality rule**

2. The separation (resolution) obtained in the first dimension is **preserved** throughout the process.
   - **Conservation rule**
Orthogonality Rule

Volatility

Polarity
Conservation Rule

OK

KO
Classical GC

Peak capacity = 50 peaks

30m x 0.25mm x 0.25µm
GCxGC

 Injector

 1\textsuperscript{st} dimension

 Column 1
 30m x 0.25mm x 0.25\textmu m

 Detector

 2\textsuperscript{nd} dimension

 Column 2
 2m x 0.25mm x 0.25\textmu m
Peak capacity = $^{1D_{nc}} x ^{2D_{nc}}$

= $50 \times 19 = 950$ peaks

$[t_{TOT \, GCxGC} = t_{TOT \, classical \, GC}]$
Instrumental Setup

Splitless

1D RTX-500 40m
0.18mm ID x 0.11µm df

LN$_2$-Quad-jets Modulator

1Tt$_R$ $\sim$ 50 min

2D BPX-50 1.5m
0.10mm ID x 0.10µm df

PM = 4 sec
Detector

1st dimension

LN₂ supplier

2nd dimension

1st Oven

2nd Oven

Releasing

Injector
Refocusing
Injection into second dimension
Modulation Process

Sampling rate 0.25 Hz

1st Dim $t_R$

2nd Dim $t_R$

4 seconds

Each ‘slice’ is a separate second dimension chromatogram
Signal at the Detector

Signal

Time

1520 1522 1524 1526 1528 1530 1532 1534 1536
0 2 0 2 0 2 0 2 0

4 sec

Signal

Time
Displaying the Data

1st Dim $t_R$

2nd Dim $t_R$ (4 sec)

Signal

3D Plot

Contour Plot
Why TOFMS?

Scanning MS
(HRMS, QISTLRMS²)

Full Scan
– Comprehensive mass analysis
– Slow

SIM
– Selective mass analysis
– Fast

Non-scanning MS

TOF
– Comprehensive mass analysis
– Fast

✓ Simultaneous sampling
✓ Ion ratio remains constant across peaks
✓ Spectral continuity

Deconvolution
Specific Identification

Ideal Mass Analyzer?

Time Compression

200 ms peak

10 scans/s

200 scans/s

✓ TOF up to 500 scans per sec
✓ Scanning quad 2-5 scans per sec...
Coelution Solving Power

Coelution

GCxGC

↑ Peak capacity

Chromatographic resolution

Separation

TOFMS

Deconvolution

Analytical resolution
Symbiotic Relation

- TOFMS is the detector of choice to describe narrow 2D GCxGC peaks

&

- GCxGC zone compression enhances TOFMS sensitivity
Comprehensive Multidimensionality

1\textsuperscript{st} Dim. GC  \rightarrow  2\textsuperscript{nd} Dim. GC  \rightarrow  3\textsuperscript{rd} Dim. TOFMS

- 1\textsuperscript{st} Dim. GC: GC
- 2\textsuperscript{nd} Dim. GC: GC
- 3\textsuperscript{rd} Dim. TOFMS: TOFMS

- Thermal Modulation
- Ionisation Modulation

- Volatility
- Polarity
- Mass

- Time scale: Minutes, Seconds, Miliseconds

Orthogonality
PCDD/PCDFs and Planar-PCBs
209 PCBs (HT-8/BPX-50 Set)

196 congeners separated in 150 min
The 3 Dimensions are Needed
Isotope Dilution ($^{13}\text{C}_{12}$-Labels)

Area = 1+2+3+4 ($^{12}\text{C}$ & $^{13}\text{C}$)
<table>
<thead>
<tr>
<th>Standard</th>
<th>Area</th>
<th>Certified Concentration</th>
<th>Calculated Concentration</th>
<th>Response Factor</th>
<th>Expected Ion Ratio</th>
<th>Calculated Ion Ratio</th>
<th>Ion Ratio Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC-07:1</td>
<td>6078</td>
<td>5</td>
<td>4.8</td>
<td>0.97</td>
<td>0.654</td>
<td>0.615</td>
<td>Passed</td>
</tr>
<tr>
<td>CDC-08:1</td>
<td>9121</td>
<td>7.5</td>
<td>7.5</td>
<td>1.01</td>
<td>0.654</td>
<td>0.643</td>
<td>Passed</td>
</tr>
<tr>
<td>CDC-06:1</td>
<td>3005</td>
<td>2</td>
<td>2.0</td>
<td>1.01</td>
<td>0.654</td>
<td>0.717</td>
<td>Passed</td>
</tr>
<tr>
<td>CDC-05:1</td>
<td>1420</td>
<td>1</td>
<td>1.0</td>
<td>0.98</td>
<td>0.654</td>
<td>0.663</td>
<td>Passed</td>
</tr>
<tr>
<td>CDC-04:1</td>
<td>218</td>
<td>0.5</td>
<td>0.5</td>
<td>0.99</td>
<td>0.654</td>
<td>0.612</td>
<td>Passed</td>
</tr>
<tr>
<td>CDC-03:1</td>
<td>379</td>
<td>0.2</td>
<td>0.3</td>
<td>1.59</td>
<td>0.654</td>
<td>0.649</td>
<td>Passed</td>
</tr>
</tbody>
</table>
Sensitivity

1 µl injected SL

50 pg $^{13}$C-2,3,7,8-TCDF

Quan S/N = 10
Area = 350

0.20 pg $^{12}$C-2,3,7,8-TCDF
Quality Control Charts

(A) BDE-47

(B) BDE-100

(C) CB-153
‘Cleaned’ Fish Extract

CB-80 recovery std (0.5 ng)
Tracer PCBs in Fish

1.5g sample size

RSD GCxGC-TOFMS: 1-14%
RSD GC-HRMS: 3-5%
RSD GC-MS/MS: 2-5%

N = 6
Non- and Mono-ortho PCBs in Fish

N = 6

15g sample size

RSD GCxGC-TOFMS: 2-17% (33%)
RSD GC-HRMS: 4-8% (34%)
RSD GC-MSMS: 6-22%
PCDD/Fs in Fish

- N = 6
- 15g sample size

RSD GCxGC-TOFMS: 12-60% (95%)
RSD GC-HRMS: 4-32% (64%)
RSD GC-MSMS: 5-30% (44%)

Bar chart showing the concentrations of various PCDD/F congeners in fish samples, with error bars indicating the relative standard deviation (RSD) for each method.
RIC

Labels (~85pg)

Natives (~1pg)

1,2,3,7,8-PeCDF

PM = 4s
‘Cleaned’ Milk Extract

Analytes of interest

Matrix interferences
Non- and Mono-ortho PCBs in Milk

RSD GCxGC-TOFMS: 5-20% (46%)
RSD GC-HRMS: 2-10%
RSD GC-MSMS: 7-15%

120g sample size

N = 6
PCDD/Fs in Milk

120g sample size

RSD GCxGC-TOFMS: 9-55% (75%)
RSD GC-HRMS: 7-14% (34%)
RSD GC-MSMS: 5-30%
Mono-ortho PCBs in Pork

30g sample size

RSD GCxGC-TOFMS: 4-22%
RSD GC-HRMS: 3-17%
RSD GC-MS/MS: 9-18%
PCDD/Fs in Pork

30g sample size

RSD GCxGC-TOFMS: 15-71%
RSD GC-HRMS: 4-16%
RSD GC-MS/MS: 8-35%
Comparison in terms of TEQs

- Use of physico-chemical congener-specific data and WHO-1998 TEFs to estimate the sum of PCDD/F and PCB TEQs.

- Parallel measurement of sample toxicities using the DR-CALUX assay on sub-samples.
Bottom: PCDD/F TEQ
Top: PCB TEQ
Comparison in terms of Cost

Based on 1,000 samples/year over 5 years (including employment, purchases, paying-off, maintenance, licensing, etc…)

<table>
<thead>
<tr>
<th></th>
<th>GC-IDHRMS</th>
<th>GCxGC-IDTOFMS</th>
<th>GC-IDQISTMS/MS</th>
<th>DR-CALUX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scientist employment</td>
<td>23%</td>
<td>35%</td>
<td>35%</td>
<td>36%</td>
</tr>
<tr>
<td>Extraction</td>
<td>11%</td>
<td>8%</td>
<td>11%</td>
<td>7%</td>
</tr>
<tr>
<td>Clean-up</td>
<td>28%</td>
<td>27%</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>Measurement</td>
<td>38%</td>
<td>30%</td>
<td>21%</td>
<td>8%</td>
</tr>
<tr>
<td>Licensing and royalties</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20%</td>
</tr>
<tr>
<td>Cost per sample (Euros)</td>
<td>500</td>
<td>500</td>
<td>420</td>
<td>270</td>
</tr>
</tbody>
</table>
Take Home Message #1

✓ GCxGC-TOFMS using ID works fine.
✓ QA/QC criteria of ‘reference method’ can be applied PLUS 2 $t_R$ available for specificity.
✓ TOFMS is a robust instrument.
✓ Integration of peaks requires more work.
✓ A sensitivity improvement is still welcome.
✓ MS-based alternative methods not to be assimilated to biological screening methods.
Take Home Message #2

Real life Pyramid

Political, economical, …

Selectivity

Speed
(Low cost)

Trend

GC-QISTLRMS²

GC-TOFMS

GC-HRMS

GCxGC-TOFMS

Sensitivity

EIAs, RBAs
Multi-Group Analyte Measurement

GCxGC-IDTOFMS

1 analyte/45min
0.8 analyte/min
One single injection
Source tracking (pattern recognition)
Metabolite Screening

Focant, Sjödin, Patterson Jr., *unpublished data.*
Sensitivity Backup

GC$xGC-IDHRMS

Sectors

R$>$10,000

Selective MS
Low data density
Target analytes
GCxGC-HR(sector)MS

Cryogenic zone compression on sector HRMS

- Attogram (10^{-18}g)
  detection level…

313ag 2,3,7,8-TCDD
(S/N=890)

- Not much ions in
the MS anymore…

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Number of moles</th>
<th>Number of molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 microgram (µg) or 10^{-6} g</td>
<td>3 nanomoles or 3.10^{-9}</td>
<td>2,000,000,000,000,000 (2.10^{15})</td>
</tr>
<tr>
<td>1 nanogram (ng) or 10^{-9} g</td>
<td>3 picomoles or 3.10^{-12}</td>
<td>2,000,000,000,000 (2.10^{12})</td>
</tr>
<tr>
<td>1 picogram (pg) or 10^{-12} g</td>
<td>3 femtomoles or 3.10^{-15}</td>
<td>2,000,000,000 (2.10^{9})</td>
</tr>
<tr>
<td>1 femtogram (fg) or 10^{-15} g</td>
<td>3 attomoles or 3.10^{-18}</td>
<td>2,000,000 (2.10^{6})</td>
</tr>
<tr>
<td>1 attogram (ag) or 10^{-18} g</td>
<td>3 zeptomoles or 3.10^{-21}</td>
<td>2,000 (2.10^{3})</td>
</tr>
<tr>
<td>1 zeptogram (zg) or 10^{-21} g</td>
<td>3 yaktomoles or 3.10^{-24}</td>
<td>2 (2.10^{0})</td>
</tr>
<tr>
<td>1 yaktogram (yg) or 10^{-24} g</td>
<td>Phantom moles</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitivity not a Dimension...

Low level sample

Sample preparation

GCxGC-IDHRMS (Selected Descriptors)

2,3,7,8-TCDD...

Report with NO ‘not detected’

GC-IDHRMS (Multi-Group)

17 PCDD/Fs + NO-PCBs
Thanks to

- D. Patterson Jr., W. Turner, A. Sjödin and Lab. staff at CDC
- G. Eppe, C. Pirard, M.-L. Scippo and Lab. staff in Liège
- Fluid Management Systems Inc.
- Leco Corp.
Related papers (i)


Related papers (iii)


Related papers (iv)


Related papers (v)


