Analysis, fate and risks of organic contaminants in river basins under water scarcity
Valencia, 7-8 February 2011

POPs by GC-MS

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Analytical methods – GC-MS

- Sampling
- Extraction
- Cleanup

Separation
- GC
- GC x GC

Identification
- LRMS (EI, NCI)
- HRMS
- IT-MS-MS
- ToF-MS

Quantification
- External Standard
- Internal Standard
- Isotopic Dilution
GC-MS components

- First use during the 1950s (Roland Gohlke and Fred McLafferty)
- Development of affordable and miniaturized computers has helped in its simplification and improvement
- Carrier gas: inert gas
- Injector: temperature is regulated to volatilize the sample once injected
- Capillary columns:
  - DIAMETER: 50 to 500 μm
  - LENGTH: 5m to 200m
  - STATIONARY PHASE: coated as a thin film (ca 0.25μm thick) on the internal surface of the tube

Split/Splitless GC-MS inlets

- Split system:
  - A constant flow of carrier gas moves through the inlet
  - A portion of the carrier gas flow acts to transport the sample into the column
  - Another portion of the carrier gas flow gets directed to purge the inlet of any sample following injection
  - Preferred when the detector is sensitive to trace amounts of analyte and there is concern about overloading the column.

- Splitless system:
  - All the samples is introduced into the column
  - Larger amount of sample is introduced to the column.
Purge and Trap GC-MS

- It is used to introduce the samples for the analysis of volatile compounds (VOCs and BTEX)
- Target analytes are extracted and mixed with water and introduced into an airtight chamber
- Nitrogen (N₂) is bubbled through the water (PURGING)
- The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber.
- The volatile compounds are drawn along a heated line onto a ‘trap’ (column of adsorbent material at ambient temperature)
- The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system

MS detection: ionization

- Electron Impact (EI)
  - The most common and standard form
  - Uses an electron beam to ionize gas-phase molecules
  - Produces “hard ionization”: creation of more fragments of low mass to charge ratio (m/z) and few molecules approaching the molecular mass unit
  - The molecular fragmentation pattern is dependant upon the electron energy applied to the system, typically 70eV
  - Exists a library of 70 eV ionization spectra

- Chemical Ionization (CI)
  - A reagent gas, typically methane or ammonia is introduced into the mass spectrometer
  - The reagent gas will interact with the analyte molecules to form ions
  - Produces “soft ionization”: a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.
  - It can be Positive Chemical Ionization (PCI): interaction with a proton exchange. This produces the species in relatively high amounts; of Negative Chemical Ionization (NCI): the reagent gas decreases the impact of the free electrons on the target analyte.
**MS Detection:**

**Guideline to select the best ionisation technique**

<table>
<thead>
<tr>
<th>Nature of sample</th>
<th>Ionisation technique</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaseous samples and volatile thermally stable substances</td>
<td>EI, CI (EI may well not show a molecular ion)</td>
<td>CO₂, NO, Solvents, PAHs, Dioxins</td>
</tr>
<tr>
<td>Small molecules (&lt;1000) which are pure and sufficiently stable and volatile to desorb from a probe</td>
<td>EI, CI (EI may well not show a molecular ion)</td>
<td>Many organic molecules</td>
</tr>
<tr>
<td>Small molecules (&lt;1500) which are not volatile or thermally stable. Molecules need to have a degree of proton affinity or potential to form a cation or an anion</td>
<td>FAB, if pure, from a probe. ESI or APCI on-line in a solvent stream, or with an LC separation if required</td>
<td>Amino acids, Carbohydrates, Lipids and many organic molecules</td>
</tr>
<tr>
<td>Small molecules as above which have been derivatised to give stable volatile products</td>
<td>EI or CI using GC on-line with MS</td>
<td>Acids as esters, Alcohols as silyl ethers</td>
</tr>
<tr>
<td>Peptides, Proteins, Oligonucleotides</td>
<td>ESI from solution or on-line with micro LC</td>
<td>Intact proteins</td>
</tr>
<tr>
<td>Proteins and Peptides and mixtures of same. Polymers</td>
<td>MALDI</td>
<td>Tryptic digests. PPG, PEG</td>
</tr>
<tr>
<td>&quot;Non-covalent&quot; interactions</td>
<td>ESI (nanoflow) with MS-MS</td>
<td>Drug-Drug or Drug-Protein interactions</td>
</tr>
</tbody>
</table>

The ability of mass spectrometer to separate two masses M₁ and M₂ is termed **RESOLUTION**. The commonest definition of R is given by:

\[ R = \frac{M}{AM} \]

(Back to Basis, VG Analytical)

The ability of analysers to measure m/z satisfactorily is quantified in the form of a parameter known as the **RESOLVING POWER**. It is defined as the mass to be measured divided by the difference in masses to be identified, \( \frac{M}{AM} \).

GC-MS/MS

- Tandem mass spectrometry (MS/MS) is a more powerful technique to quantitate low levels of target compounds in the presence of a high sample matrix background.
- Allows you to monitor the transition of precursor $\rightarrow$ product ion for each compound. This transition is compound specific.
- Can obtain structural information of unknowns.
- Ion trap, triple quadrupole, linked-scan, ICR

- The first quadrupole (Q1) is connected with a collision cell (q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed.
- Eliminates background interferences ("noise")

MS Analysis

- **Full scan analysis**
  - Records all ions within a specified mass range, need to select specific mass chromatograms for quantification with high sample matrix background
  - Can suffer interference
  - Sensitivity is decreased due to performing fewer scans per second
  - Useful in determining unknown compounds in a sample.
  - It is useful to determine the retention time and mass fragment fingerprint

- **Selected ion monitoring (SIM)**
  - Records specific ions for each target compound
  - More selective when compared with full scan, greater sensitivity
  - Detection limit is lower since the instrument is only looking at a small number of fragments during each scan
  - Matrix interferences are typically lower.

- **MRM analysis**
  - Records compound specific transitions for each target analyte
  - High selectivity, High sensitivity
### Ocs, PAHs and PBDEs: GC-MS conditions

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Ocs, PAHs</th>
<th>PBDEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC (Carlo Erba GC 8000) -MS (FISON MD 800) -EI(70 eV)</td>
<td>GC-NCI(Agilent 6890) -MS (Agilent 5973 Network)</td>
<td></td>
</tr>
</tbody>
</table>

### Temperature program

<table>
<thead>
<tr>
<th>Temperature</th>
<th>GC-MS Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C (1 min)</td>
<td>6°C/min</td>
</tr>
<tr>
<td>100°C (1 min)</td>
<td></td>
</tr>
<tr>
<td>175°C (4 min)</td>
<td>4°C/min</td>
</tr>
<tr>
<td>185°C (1 min)</td>
<td></td>
</tr>
<tr>
<td>235°C (1 min)</td>
<td>5°C/min</td>
</tr>
<tr>
<td>240°C (5 min)</td>
<td></td>
</tr>
<tr>
<td>300°C (5 min)</td>
<td>2°C/min</td>
</tr>
</tbody>
</table>

### Carrier gas

- Helium (50 cm/s)
- Methane

### Injection

- Splitless mode 48s
- Splitless mode 60s

### Injector Temp.

- 280 °C

### Transfer line Temp.

- 250 °C

### Ion source Temp.

- 200 °C
- 250°C

### Ocs chromatogram

- 1 Hexachlorobutadiene
- 2 Pentachlorobenzene
- 3 Hexachlorobenzene
- 4 4,4-DDD
- 5 2,4-DDD
- 6 4,4-DDT
**PAHs chromatogram**

1. Naphthalene
2. Octilphenol
3. Anthracene
4. Fluoranthe
5. Benzo(b)fluoranthe
6. Benzo(k)fluoranthene
7. Benzo(a)pyrene
8. Indeno(1,2,3-cd)pyrene
9. Benzo(g,h,i)perylene

**OCs chromatogram**

1. α-HCH
2. HCB-13C6
3. HCB
4. β-HCH
5. γ-HCH
6. antracè-D10
7. δ-HCH
8. heptaclor
9. aldridina
10. isodrina;
11. heptaclor-exo-epoxid
12. heptaclor-endó-epoxid
13. 2,4'-DDE
14. α-endosulfan
15. dieldrina
16. 4,4'-DDE-D8
17. 4,4'-DDT
18. 2,4'-DDD
19. endrina
20. β-endosulfan
21. 4,4'-DDD
22. 2,4'-DDT
23. endrina aldehid
24. endosulfan-sulfat
25. 4,4'-DDT-13C12
26. 4,4'-DDT

**Temperature program**

- **Inject**: 280 °C
- **Transfer**: 250 °C
- **Ion source**: 200 °C
- **Column**: HP-5MS
- **1st window**: 30 m x 0.25 mm, 0.25 µm film thickness
- **Injection time**: 60 °C (1 min)
- **Temperature Increase**: 130 °C (3 °C min⁻¹)
- **Transfer**: 220 °C (10 °C min⁻¹)
- **Column**: 300 °C (5 min)
PAHs chromatogram

1: naftalè-D8
2: naftale
3: acenaftilè
4: acenaftè-D10
5: acenaftè
6: fluore
7: fenantrè-D10;
8: fenantrè
9: antracè-D10;
10: antracè
11: fluorescentè
12: pirè
13: benzo(a)antracè
14: crisè-D12
15: crisè
16: benzo(b)fluorescentè
17: benzo(k)fluorescentè
18: benzo(a)pirè;
19: perilè-D12
20: indè(1,2,3-cd)pirè
21: dibenzo(a,h)antracè
22: benzo(g,h,i)perilè

Temperature program (2)
- Injector: 280 ºC
- Transfer: 250 ºC
- Ion source: 200 ºC
- Column: HP-5MS
- 30 m x 0.25 mm
- 0.25 µm film thickness

- Temperatures
- Temperature program (2)

PBDE Analysis: GC Separation

- Melting points range from <0ºC (Br ≤ 5) to over 300ºC (deca-BDE)
- Many congeners are very thermally labile and decompose before they reach their boiling point → analytical detection very difficult
- Molecular weights range between 240 and 970 amu → relatively difficult to analyse in a single capillary column:
  - one run for mono- to hepta-BDEs
  - another run for octa- to deca-BDE
Analytical difficulties for BDE-209 analysis

- Is not stable at higher temperatures in the injector and at the CG column
- Is sensitive for degradation by UV light
- Their behaviour in the MS source is different from those of chlorinated and lower brominated compounds

Solutions for BDE-209 analysis

- Injection techniques such as pressure-pulse injection or on-column
- Use of short chromatographic column (10-15 m)

PBDE Analysis: EI- vs NCI-MS

**NCI-MS:**
Major signals for [Br].
NO STRUCTURAL INFORMATION.

**EI-MS:**
Simultaneous detection of molecular ion and fragmentation products.
STRUCTURAL INFORMATION.
### PBDE Analysis: EI- vs NCI-MS

![Graph showing EI and NCI mass spectra](image)

EI more selective than NCI

### Instrumental Detection Limits

<table>
<thead>
<tr>
<th>Type</th>
<th>NCI-MS (fg)</th>
<th>EI-MS (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono-BDEs</td>
<td>572-1716</td>
<td>0.56-0.86</td>
</tr>
<tr>
<td>Di-BDEs</td>
<td>51-154</td>
<td>0.53-1.31</td>
</tr>
<tr>
<td>Tri-BDEs</td>
<td>33-89</td>
<td>0.69-2.29</td>
</tr>
<tr>
<td>Tetra-BDEs</td>
<td>51-79</td>
<td>1.53-3.43</td>
</tr>
<tr>
<td>Penta-BDEs</td>
<td>68-134</td>
<td>3.91-21.50</td>
</tr>
<tr>
<td>Hexa-BDEs</td>
<td>54-168</td>
<td>2.55-8.19</td>
</tr>
<tr>
<td>Hepta-BDEs</td>
<td>128-209</td>
<td>13.58-32.09</td>
</tr>
</tbody>
</table>

NCI more sensitive than EI
**Advantages of GC x GC**
- $R_{GCxGC} = R_{GC} \times R_{GC}$
- Increase of signal to noise ratio
- Increase of sensitivity
- Identification by two coordinate retention times
- Identification more reliable

**Problem**
Must be coupled to a FAST detector such as:
- FID
- µECD
- ToF-MS
Application of GC x GC
PCB analysis (white-tailed eagle)


Application of GC x GC-ToF-MS
Cigarette smoke

(A) Detail of the GC×GC contour plot. The vertical line at 583 s indicates the second-dimension chromatogram, which is shown separately in (B). In (B) the horizontal lines indicate the positions where peaks were found by the deconvolution algorithm of the GC–TOF-MS software. Provisional identifications are summarized on the right-hand side of the figure. (C) The deconvoluted mass spectra of the peak at 0.24 s. (D) The corresponding library spectrum.