Overview of Use of GC-HRMS for the Analysis of Dioxins

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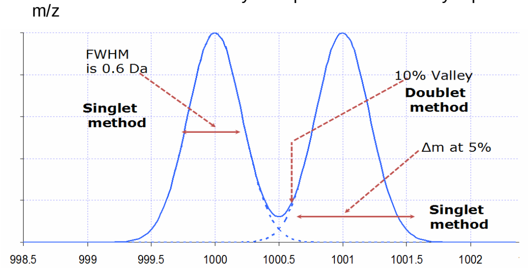
Overview

- Introduction to High Resolution Mass Spectrometry (HRMS)
  - Resolution Theory
- Comparison of GC-HRMS to other GC-MS techniques
- Dioxins: What are they? Why Analyse for them?
- Sample Prep and Clean-Up
- Analysis of Dioxins using GC-HRMS
- Reporting of Results (TEQ)

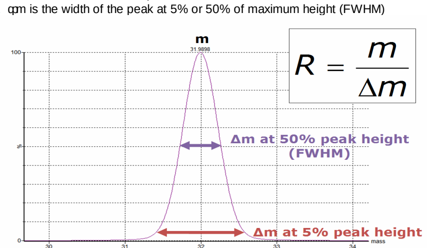
Resolution Theory

- Mass Resolution: The ability to separate ions of nearly equal m/z



Resolving Power

- Mass Resolution: The ability to separate ions of nearly equal m/z
  - m is the m/z centroid of a peak
  - Δm is the width of the peak at 5% or 50% of maximum height (FWHM)



Why the need for HR-MS?

- Molecular ions of Nitrogen, Carbon Monoxide, and Ethene => Isobaric Ions
- All with same nominal mass of 28 => Potential Interferences

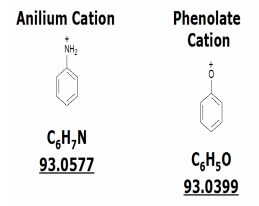
N_2^+ → 28.00559 u

CO^+ → 27.99437 u

$C_2H_4^+$ → 28.03075 u

What Resolution is Required to Separate 2 Species?

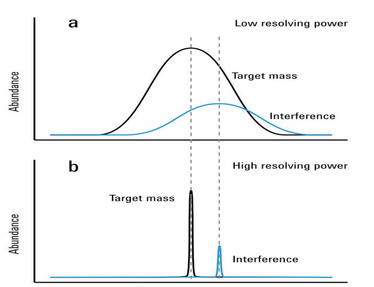
- Resolution: Ability of MS to distinguish between different ions



$$R = \frac{m}{\Delta m}$$

$$R = \frac{93.0339}{93.0577 - 93.0339} = 3909$$

Comparison of Low and High Resolution MS



High Resolution Mass Spectrometry (HRMS) – Comparison of Resolution

- Standard GC-MS can resolve masses 1 amu apart
- GC-MS/MS (e.g. Triple Quad) can resolve masses ~ 1000 amu apart
- GC-HRMS increases resolution to 10,000 amu
  - Increased sensitivity means that 0.5 fg of 2,3,7,8-TCDD on-column can be detected

High Resolution and Accurate Mass

- High Res and accurate mass measurements are closely related
  - ÅGood mass accuracy depends on sufficiently resolved peaks (i.e. high mass resolution)
  - ÅInadequate resolution impinges significantly on ability to discern the presence of an interference
- External Mass Calibration: Several peaks of well-known m/z values evenly distributed over mass range of interest
  - ÅSupplied by mass reference compound, e.g. PFK

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GC-HRMS Instrumentation

- Fitted with large magnet
- 6 to 8 foot flight tube
- Dual magnetic and electrostatic sectors
  - Å Sequential electric and magnetic fields, in conjunction with various correcting lenses
  - Å Magnetic Sectors => Mass Filtering
  - Å Electrostatic Sectors => Energy Focussing
  - Å Combined sector MS designed such that ions of different energies, but same mass, re-converge at the collector
- This configuration increases resolution to 10,000 amu; i.e. can separate molecules that have same retention time, but have masses 0.0001 amu apart
- Wide dynamic range
- Suitable for use with isotope dilution techniques with ^{13}C-labelled standards, which aid accuracy/precision

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Primary uses for GC-HRMS

- Ultra-trace confirmatory analysis:
  - Å Dioxins
  - Å PCBs
  - Å BFRs
  - Å PAHs
  - Å Sports Doping/Toxicology
  - Å Petrochemicals ï Biomarkers and Metabolites
  - Å Metabolomics in Agriculture

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GC-HRMS Instrumentation in State Lab

Thermo Scientific - DFS

Waters - Autospec

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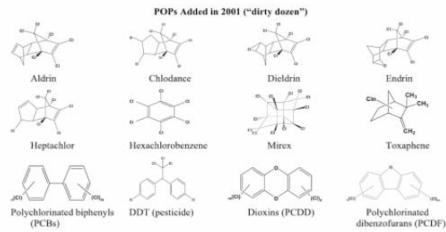
Persistent Organic Pollutants (POPs)

- POPs are bioaccumulative and toxic chemicals
  - Resistant to environmental degradation
  - Prone to long-range transport
  - Potential to elicit adverse effects on environmental and human health
  - Elimination/Restriction of production and use of POPs
  - Stockholm Convention on Persistent Organic Pollutants

Stockholm Convention on Persistent Organic Pollutants

- Elimination/Restriction of production and use of POPs
- Global Treaty: Stockholm Convention => Adopted in 2001
- Aim: To protect humans and environment => Particularly against unintentional release



EU Legislation

- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
- Commission Regulation EU 1259/2011 setting maximum levels of dioxins and PCBs in food
- Commission Regulation EU 277/2012 setting maximum levels of dioxins and PCBs in feed
- Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs

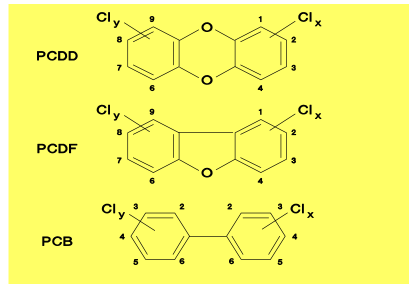
Dioxins - Formation

- Dioxins are Organo-Halogen compounds formed during:
  - **Incomplete combustion**
  - **Metal smelting**
  - **Paper pulp bleaching with chlorine**
  - **Manufacture of certain chlorophenol chemicals**

Dioxins

- Polychlorinated dibenzo-p-dioxins (PCDDs)
- Polychlorinated dibenzofurans (PCDFs)
- PCBs (Polychlorinated biphenyls)
- PCDDs and PCDFs have 210 possible congeners
  - **Different numbers and positioning of chlorine atoms**
  - **17 congeners with higher toxic potential**
  - **PCBs (Dioxin-like): 18 congeners with relatively high toxicity**

Dioxins/PCBs - Structure



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Dioxins – Risk to Humans

Viktor Yushchenko, former President of Ukraine, in 2003, and 2004, after Dioxin poisoning

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Analysis of Dioxins in State Laboratory

- **3 Principal Steps**
- **1 – Extraction of Fat from Sample**
- **2 – Purification/Clean-Up**
- **3 – Confirmatory Analysis by GC-HRMS**

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Extraction

- Lipophilic => Accumulate in Fatty Tissue
- Elimination of water/moisture during sample prep
- Extraction methods based on isolation of lipids from sample matrix
- Various methods, including Soxhlet extraction
- Pressurised Liquid Extraction => ASE (Accelerated Solvent Extraction)
  - Uses solvents at high pressures and temperatures
  - Automated, Increased efficiency
  - Shorter extraction time

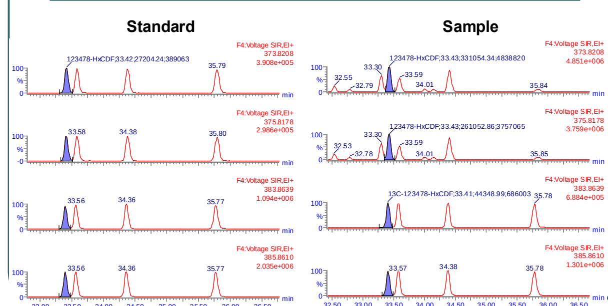
Sample Clean-Up

- Matrix components can be coextracted with target analytes
- E.g. Lipids, Sulphur
- Can cause interference/co-elution on the GC-MS
- Lipds can reside in GC parts and affect sensitivity
- PowerPrep^{TM}: Automated and integrated cleanup system
  - Disposable Columns
  - Acidified Silica => Digestion of Fat
  - Alumina and Carbon/Celite => Further clean-up and fractionation of analytes
    - Fraction A: Mono-ortho PCBs and indicator PCBs
    - Fraction B: Dioxins and non-ortho PCBs

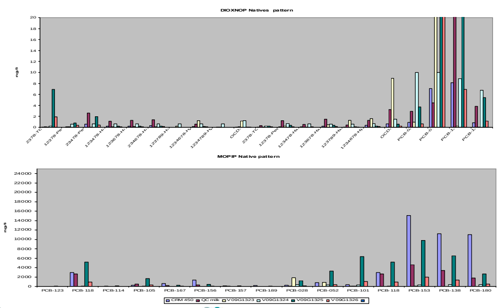
Confirmatory Analysis by GC-HRMS

- Isotope Dilution Method (Spiking of a known amount of enriched isotope to a sample)
- Injection on GC-HRMS
  - Fraction A: 2 µl using PTV on splitless mode
  - Fraction B: 60-80 µl using PTV on solvent mode
- GC column: Agilent DB-5MS, 60m x 0.25mm x 0.25µm
- Ionisation: EI
- Identification of congeners through ion abundance ratio between 2 monitored product ions (compared to predicted values)

Confirmatory Analysis by GC-HRMS



Typical Levels in Feed Samples



Reporting – TEF Concept

- TCDD is considered most toxic
- Other dioxin congeners are assigned toxicity ratings on a scale of 0 to 1, where TCDD = 1
- Toxicity rating = Toxic Equivalence Factor (TEF)
- Observed concentrations of each congener are then multiplied by the relevant TEF, and added to get a total TEQ value
- Compared to limits laid down in EU legislation

$$TEQ = \sum_{i=1}^{N} C_i \times TEF_i$$

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TEF Values for Dioxin Congeners

Congener	TEF value	Congener	TEF value
Dibenzo-p-dioxins ('PCDDs')		**'Dioxin-like' PCBs Non-ortho PCBs and Mono-ortho PCBs**	
2,3,7,8-TCDD	1		
1,2,3,7,8-PeCDD	1	*Non-ortho PCBs*	
1,2,3,4,7,8-HxCDD	0,1	PCB 77	0,0001
1,2,3,6,7,8-HxCDD	0,1	PCB 81	0,0003
1,2,3,7,8,9-HxCDD	0,1	PCB 126	0,1
1,2,3,4,6,7,8-HpCDD	0,01	PCB 169	0,03
OCDD	0,0003		
Dibenzofurans ('PCDFs')		*Mono-ortho PCBs*	
2,3,7,8-TCDF	0,1	PCB 105	0,00003
1,2,3,7,8-PeCDF	0,03	PCB 114	0,00003
2,3,4,7,8-PeCDF	0,3	PCB 118	0,00003
1,2,3,4,7,8-HxCDF	0,1	PCB 123	0,00003
1,2,3,6,7,8-HxCDF	0,1	PCB 156	0,00003
1,2,3,7,8,9-HxCDF	0,1	PCB 157	0,00003
2,3,4,6,7,8-HxCDF	0,1	PCB 167	0,00003
1,2,3,4,6,7,8-HpCDF	0,01	PCB 189	0,00003
1,2,3,4,7,8,9-HpCDF	0,01		
OCDF	0,0003		

Abbreviations used: 'T' = tetra; 'Pe' = penta; 'Hx' = hexa; 'Hp' = hepta; 'O' = octa; 'CDD' = chlorodibenzodioxin; 'CDF' = chlorodibenzofuran; 'CB' = chlorobiphenyl.

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Future Work in the State Lab

- Confirmatory Analysis of Brominated Flame Retardants (BFRs)
- Use of APGC-MS/MS as a screening method for Dioxins/PCBs
  - Prior to confirmatory analysis by GC-HRMS if required

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