

Strategies and Techniques for Identifying Target and Non-Target Compounds in Environmental Samples

NEMC 2016 August 11, 2016

Target and Non-Target Analysis

- More than 109 million organic and inorganic substances and 66 million protein and DNA sequences in the CAS data base (<u>http://www.cas.org/content/counter</u>)
- There are 45 million compounds listed in ChemSpider
 - <u>WWW.Chemspider.com</u>
- There are >750,000 MS spectra in Wiley v10 library
- There are >240,000 MS spectra in NIST 14 library
- Most analytical tests are performed on target analytes
- There are only a relatively small number of compounds analyzed routinely
 - Mainly limited because of the number of analytical standards and validated methods available

Target and Non-Target Analysis

- Target Compound Analysis
 - Stockholm POPs, PCNs, PAH, PCPPs Established methods, optimized for target compounds – Very specific and can filter out any potential unknowns

Suspect Targeted Identification

- Compounds in Industrial Products, Patents, Howard & Muir EST 2010 (~500 compounds that are Persistent, Bioacumulative, Toxic)
- Have some information to help find compounds

Untargeted Unknowns

 Other compounds and degradation products

Environ. Sci. Technol. 2006, 40, 7157–7166

Are There Other Persistent Organic Pollutants? A Challenge for Environmental Chemists[†]

DEREK C. G. MUIR*.[‡] AND PHILIP H. HOWARD[§] Water Science and Technology Directorate, Environment Canada, Burlington, Ontario, Canada, and Syracuse Research Corporation, Environmental Science Center, North Syracuse, New York



The Universal Mass Spectrometry System

Waters Xevo G2-XS Q-TOF



System Attributes

- Mass range m/z 20 4000
 Capable of analyzing most environmental contaminants in GC or LC mode
- Maximum Acquisition rate 30 Hz Capable of GCxGC experiments
- Mass Resolution 25,000 35,000 FWHM >10,000 at 10% valley. Acceptable for HRMS dioxin analysis and capable of mass defect analysis.
- Mass Accuracy < 1mDa
- Full Scan and Tandem data acquisition

Can do both Target and Non-Target analysis

Zoex ZX2 modulator



Practical aspects of a universal mass spectrometry system



- Less Power required 1/3rd of an HRMS (3-5kVA vs 13kVA)
- NO chilled water
- NO cryogens (Liq N₂, He)
- ONLY ONE backing vacuum pump
- MUCH LESS experience to operate
- ONLY ONE HOUR to reconfigure for LC or GC modes of operation

The Universal Mass Spectrometer can replace multiple instruments



Atmospheric Pressure Gas Chromatography (APGC)



GC inlet and ionization

- Corona pin initiates ionization.
- Ionization similar to processes observed for APCI in LCMS
- Positive ionization usually occurs by charge exchange with N₂^{•+}.
- H₂O and other gases can be introduced to modify the ionization process. (proton transfer e.g.)
- Negative ions may be generated by electron capture, but other mechanisms may also occur.

Corona Pin Position in APGC Source

> Positive mode: $N_2^{\bullet+} + M \rightarrow M^{\bullet+} + N_2$

Negative mode: $e^{-} + M \rightarrow M^{-}$

O₂•- + M → [M-X+O]-(where X = H, Cl, Br)

The Waters Xevo G2-XS Q-TOF mass spectrometer

Scan Modes :





Enhanced Dynamic range with a Q-TOF using a single injection?

| 🗹 Expo | eriment Setup - c:\masslynx\kjj.pro\acqudb\dxf_ms_targetenh Edit Options Toolbars Functions Help | anced_lh.exp | | | | |
|---------|---|--------------|--------------------------|---------|-------------------|-----------------|
| 0 🖻 | 🗧 🔄 📝 🗙 🔺 🔻 💊 LockSpray 🔰 🏁 Method Ex | vents | | | | |
| 2 | MS 🛛 📝 MSMS 🔤 🛃 PID Product 🛛 🛃 PID Neutral | | MS ^E Centroid | Tof-MRM | 🖉 Fast DDA | T |
| Total R | un Time: 40.00 \leftrightarrow | ſ | , | | 30mins | |
| No. | Information | | | Time | | |
| 1 T | of MS, Time 0.00 to 40.00, Mass 100.00 to 1000.00 API+ | | | | | |
| 2 T | of MS, Time 0.00 to 40.00, Mass 100.00 to 1000.00 API+ | | | | | |
| 3 Т | of MS, Time 10.00 to 19.50, Mass 50.00 to 500.00 API+ | | | | | - |
| 4 T | of MS, Time 19.50 to 22.50, Mass 50.00 to 500.00 API+ | | | | See. | |
| 5 T | of MS, Time 22.50 to 25.00, Mass 50.00 to 600.00 API+ | | | | | |
| 6 T | of MS, Time 25.00 to 29.00, Mass 50.00 to 650.00 API+ | | | | | |
| 7 T | of MS, Time 29.00 to 40.00, Mass 50.00 to 1000.00 API+ | | | | | |
| 6 | | | | | LockSpray: Enable | d LockMass: APG |

Can use a combination of full scan, target enhancement and MS^E functions

- Target enhancement functions to monitor molecular ions
- Two full scan functions at low and high collision energy for screening



Instrument sensitivity (APGC) - full scan vs MRM

Wellington Dioxin Sensitivity Mix



Organtini et al., Anal. Chem. (2015) 87 (15), 7902-7908



The Mass Spectrometry of Polychlorinated Dibenzo-p-Dioxins

J. Ronald Hass† and Marlin D. Friesen‡

Environmental Biology and Chemistry Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA

Michael K. Hoffman

Washington University Medical School, St Louis, Missouri, USA

The negative ion chemical ionization mass spectra of polychlorinated dibenzo-p-dioxins using oxygen, methane and methane/oxygen are reported together with their methane positive ion chemical ionization mass spectra and conventional electron impact spectra. The methane/oxygen negative ion chemical ionization mass spectra proved to be the most useful of the negative ion spectra for structure determination.



ORGANIC MASS SPECTROMETRY, VOL. 14, NO. 1, 1979 9

Differentiation of TCDD isomers by GC-APCI⁻



Reactions with O₂ improves selectivity



Analysis of PBDDs is often performed using a short, thin film GC column.
 APCI⁻ improves separation between key TCDD and TBDD isomers.

Comprehensive two-dimensional gas chromatography

Schematic of a GC \times GC





Second Dimension Modulation





PCB Standard by GC×GC-ECD Orthogonal Elution







GC×GC can enhance sensitivity





APGC reduces interferences and GC×GC can resolve them





Goal : a routine, Targeted and Non-targeted analysis



(2) Software deconvolution of both target and non-target compounds.



(1) Mass defect filtering reveals halogenated ions



(3) Interpretation of full-scan high resolution mass spectra leads to structure proposal :



Target and non-target analysis in a single injection



The TEQ is increased 10x when 2,3,7,8-TBDD/F are included!

Identifying Unknown Compounds

- Compounds must be separated from bulk matrix and other interfering compounds or isomers – GC, GCxGC or LC is used to attempt to get a clean mass spectrum
- Use tandem mass spectrometry (MS/MS) or high resolution mass Spectrometry (HRMS) to confirm elemental composition, structure and functional groups
- Requires extensive investigative work background knowledge from patents, manufacturers, e.g. INCHEM (International Programme on Chemical Safety – <u>www.inchem.org</u>, ChemSpider, <u>www.chemspider.com</u>
- No previous information identify by first principals
 - RDBE, Element Restriction Rule, Nitrogen Rule, LEWIS and SENIOR (valence) Rules, Isotope Patterns, H/C check, Hetroatom/Carbon (<1.3) and data bases (CAS, ChemSpider)
- Synthesize compound as authentic standard for confirmation

Kind et al, BMC Bioimformatics, (2007), <u>8</u>, 105 Godfrey et al, Anal Bioanal Chem, (2012) <u>404</u>, 1159







Plastimet

- ✤ 400+ tonnes of polyvinylchloride stored on site burned.
- ✤ The fire burned for 3 days before it was finally extinguished.
- ✤ Nearby residents were very concerned about their health.
- ✤ Several firefighters developed serious health problems.
- Chrome plating on some fire engines came off in the months following the fire due to atmospheric HCI.





How can one interpret such a *mass* of data ?

One option is to perform a "Kendrick mass analysis"

- In this procedure, the mass of CH₂ is defined as 14.000 Da, instead of the IUPAC mass of 14.01565 Da. [Kendrick mass = IUPAC mass x (14 / 14.01565)]
- In a Kendrick mass analysis, the Kendrick mass defect is plotted as function of nominal Kendrick mass for ions observed in a mass spectrum



Kendrick Mass

The Kendrick mass defect plot of the Plastimet sample The second band of peaks corresponds to halogenated ions



28

This plot displays dioxins, furans and many more CI/Br species



29

Tentative structure proposals are based on accurate masses



Electronics Recycling Plant Dust Sample Extract



Mass defect filtering simplifies the GC(×GC) chromatogram







Electronics Recycling Plant Dust Sample Extract



Electronics Recycling Plant Dust Sample Extract





Halogenated Flame Retardants



Brominated Flame Retardants

- Polybrominated diphenyl ethers (PBDEs)
- Polybrominated biphenyls (PBBs)
- Hexabromocyclododecane (HBCD)
- · "Replacements"

Chlorinated Flame Retardants

- Mirex (dechlorane)
- Dechlorane Plus®
- Dechloranes

Discovery of Dechlorane Plus (DP) - UU

DP: flame retardant, substitute of non-agricultural Mirex



- Reported in Great Lakes air, biota and sediment (Hoh et al., 2006)
- Manufactured by OxyChem in Niagara Falls, NY began before 1970s
- Used in plastic coatings of cables, wires and television electrical connectors and carpeting
- Present in sediments from Lake Ontario 60-fold higher than Lake Erie
- A HPV chemical, 1–10 million pounds/year


Unknown GC-HRMS Peaks







World Health Organization

Geneva, 1984

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA 44



MIREX

Dechlorane 602

Dechlorane 603

Dechlorane 604

NRC, 1978). Recently, non-agricultural mirex has been replaced in part by compounds such as Dechlorane plus, Dechlorane 4070, 510, 602, 603, and 604, all of which have similiar fire retardent properties. No recent consumption data for mirex in nonagricultural applications could be obtained.

Candidate Dechloranes

Dechlorane 602

- Added to Fiberglass/Nylon-6
- Used in high voltage electrical cabling with dechlorane 604

Dechlorane 603

Dechlorane 604

- Listed as in Molykote® AS-810 silicone grease
- High voltage cable









Confirmation of Suspected HFRs

- Background information suggested that some of the m/z = 272 peaks would be the Dechlorane 600 compounds
- Analyzed sample from Niagara River Bar Niagara River depositional area to get highest possible levels and attempt to observed a full mass spectrum with molecular ion
- Cl isotope ratios were correct
- Obtain synthesized pure standards of the proposed compounds
- Confirm GC retention times and mass spectra of standards are the same as analytes of interest



Parent lons in Standards / Sample





Dechlorination of Dechlorane 602







Potential for Long-Range Transport

Dec602 and β -Cl11Dec602 in Arctic polar bears





FTMS data obtained from a Lake Ontario Lake trout



Jobst et al., Anal. Bioanal. Chem., <u>405</u>, 3289 (2013)



The mass defect plot shows both known and unknown compounds



45

The unknowns are analogues of the flame retardant Dechlorane 604!



Jobst et al., Anal. Bioanal. Chem., <u>405</u>, 3289 (2013)



Synthesis of Standards



Shen et al., Environ Sci & Technol, 48, 11170 (2014)



Dec604 Analogues



Shen et al., Environ Sci & Technol, 48, 11170 (2014)



Naphthenic acids









Z = -6

- Naphthenic acids (NAs) = Carboxylic acids with at least one saturated ring.
- Can contain heteroatoms in their structure (O, S, N).
- Can represent up to a 4% (w/w) of crude oil.

Ortiz at al, Analytical Chemistry, <u>86,</u> 7666 (2014)

Analysis of Naphthenic acids



50

FTICR Instrumentation



"The smallest mass difference commonly encountered in broadband mass spectra of fossil fuels or peptides is 3.4 mDa, corresponding to the difference between ¹²C₃ and ³²SH₄.



Figure Obtained from: Purcell et al. J. Am. Soc. Mass Spectrom. (2007) 18: 1265

Only FT-ICR and Orbitrap mass spectrometers offer the requisite mass resolution (\$\$\$)

Macondo Crude Oil (NIST SRM 2779) – GC×GC-QTOF

- Exact Mass (Elemental Composition) can be determined at low resolution if peaks are resolved
- Chromatography can be used to resolve ions of similar mass



JD Byer, K Siek, KJ Jobst Anal Chem – Re-submitted 2016



Chromatographic Separation of Isobaric Doublets (C_3/SH_4)



Figure Obtained from: Barrow et al. J. Am. Soc. Mass Spectrom. (2015) 26: 1508



Dave Bowman (McMaster University)

Resolved MS peaks do not need Ultra-high resolution to determine exact mass



Extracted ion chromatogram *m/z* 246 +/- 0.5



GC × GC–ToF MS: Scripting Filters



Development of the Scripting Filter



Stream sediment samples



Stream sediment samples



Stream sediment samples



Identification of potential dechlorane analogs



Identification of potential dechlorane analogs



nalogs



















Untargeted analysis: Unknowns in Tuna New CI-Br class of compounds





C₉H₆OBr₃Cl confirmed by FT-ICR MS



Untargeted analysis: Unknowns New CI-Br class of compounds



Pub. No.: US 2008/0221230Al Pub. Date: Sep. 11, 2008



ChemSpider Candidates

Untargeted analysis: Unknowns New CI-Br class of compounds


Identification of halogenated flame retardants by $\text{GC} \times \text{GC-HRTOF}$



From dust to data : GC×GC of house dust

House Dust (NIST SRM 2585) analyzed by APGC GC×GC-QTOF



Known and unknown contaminants are easy to spot



M. Ubukata et al., J. Chrom. A, 1395 (2015) 152



EI Library Searching of the Unknown





Identifying unknowns using APGC



proposals from ChemSpider.com



Identifying unknowns using APGC

| M/Z | Elemental Composition |
|---------|--|
| 424.900 | C ₁₂ H ₉ Cl ₆ O ₃ N ⁺ (M ⁺) |
| 409.799 | $C_{12}H_6CI_5O_3N^+$ (M-CH ₃ ⁺) |
| 396.903 | $C_{12}H_9Cl_6O_2N^+$ (M-CO ⁺) |
| 359.926 | $C_{12}H_9Cl_5O_2N^+$ (M-COCl ⁺) |
| 260.884 | C ₇ H ₂ Cl ₅ ⁺ |



Review of mass spectrum suggests a revised structure proposal



Summary

Current technology allows simultaneous target and non-target analysis

- Atmospheric pressure ionization can be coupled to almost any inlet type (LC, GC, GC × GC) so that a wide range of target and non-target chemical compounds can be separated and analyzed.
- Hybrid mass analyzers: both high resolution (elemental composition information, mass defect filtering) and tandem MS (structure diagnostic fragmentation)
- Chromatography is still very important : isomers that can exhibit very different toxicities and need to be separated.

There are still many challenges for non-target analysis

- How can we efficiently **extract** and **identify** every compound in a sample? Data sets, especially when using GCxGC, are enormous!
- Libraries (NIST, ChemSpider) are helpful, but not all environmental contaminants have library entries (e.g. impurities and degradation products) and **there are many inaccuracies**.
- We need new software tools that can screen huge data sets, automatically interpret mass spectra and predict structures and toxicities.



Co-Authors/Collaborators/Contributors





Thank you for your attention!

www.IMSC2016.ca Toronto, Ontario, Canada August 21-26 2016

8th MDC Workshop Toronto, Ontario, Canada January 5 & 6, 2017, www.multidimensionalchromatography.com/



