What do Petroleomics, Jet Fuel and Pharmaceuticals Have in Common?

Visualization and Characterization of Complex Mixtures of Extractables/Leachables and Other Pharmaceutically Relevant Compounds using High Resolution 2-D and 3-D Mass Mapping

Douglas Kiehl[*], Scott Campbell[†], David Stranz[†]
George Maydwell[†] and Diane Paskiet[‡]

[*]Eli Lilly and Company, Indianapolis, IN, USA
[†]Sierra Analytics, Inc., Modesto, CA, USA
[‡]West Pharmaceutical Services, Inc., Exton, PA, USA

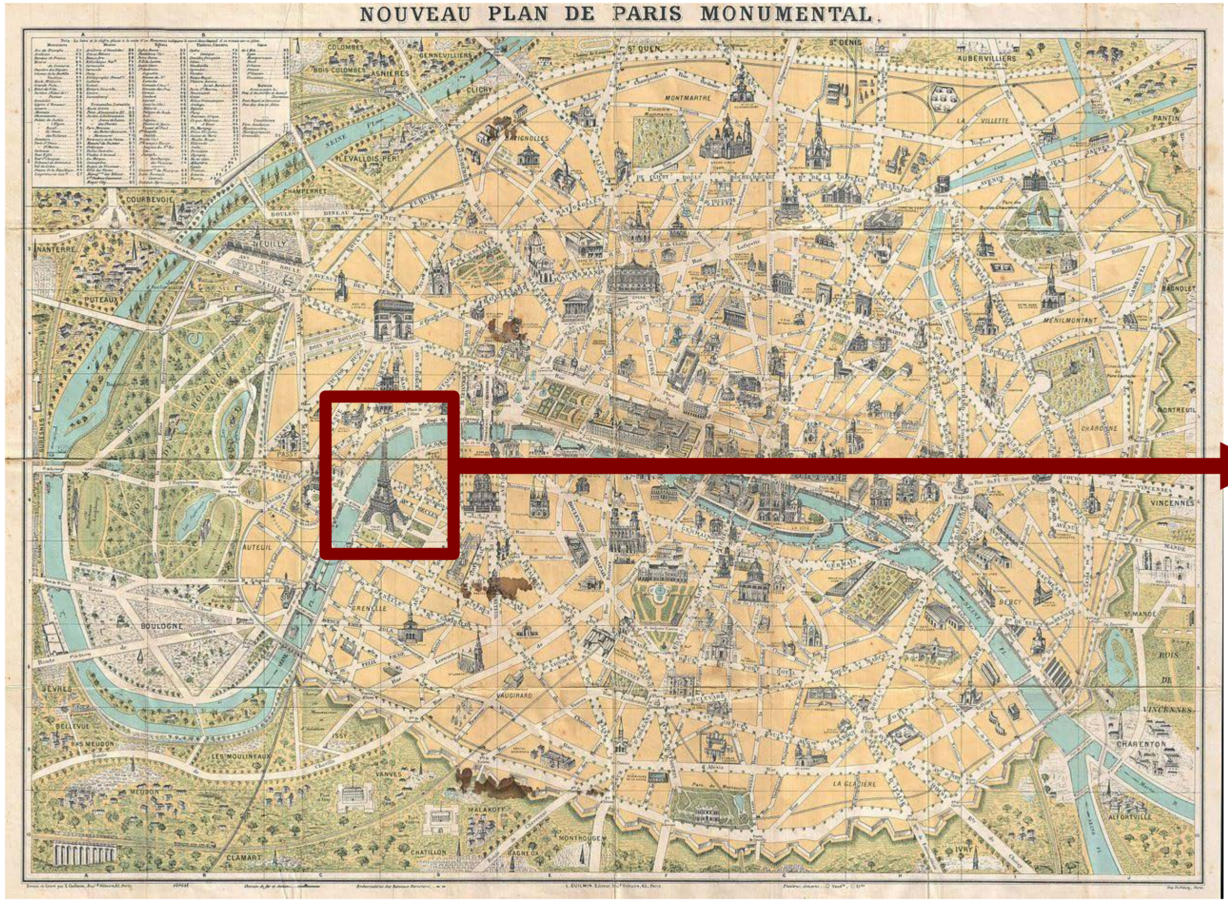
A Map is. . .

A map is a diagrammatic representation or collection of data illustrating relationships, characteristics, distribution, size, number or spatial arrangement of unique features over a given area and according to a chosen scale.

- Maps may contain highly resolved details.
- Maps can be two-dimensional representations of three-dimensional space, or can be three-dimensional.

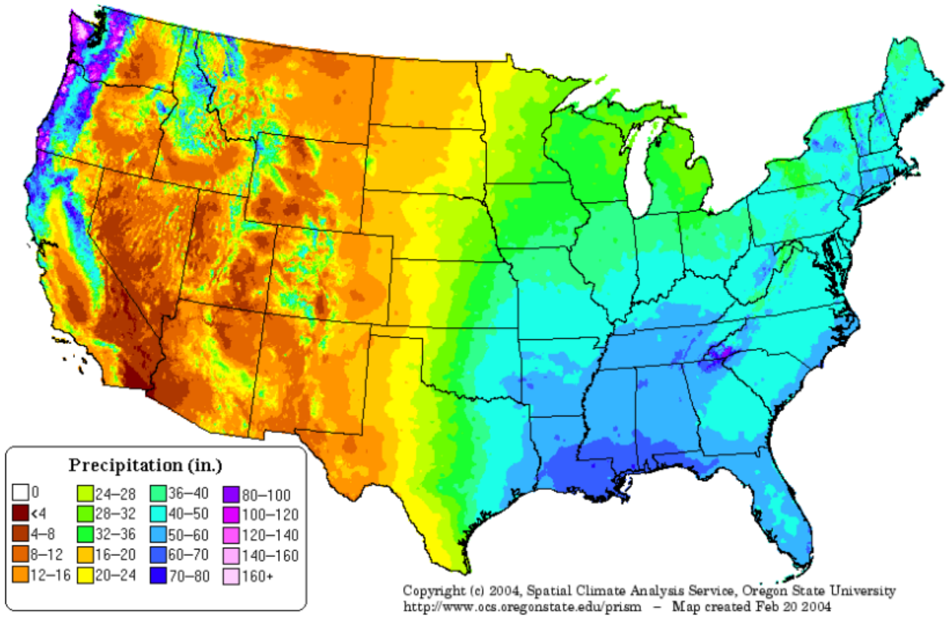
Some examples. . .

A qualitative map: Paris, 1890

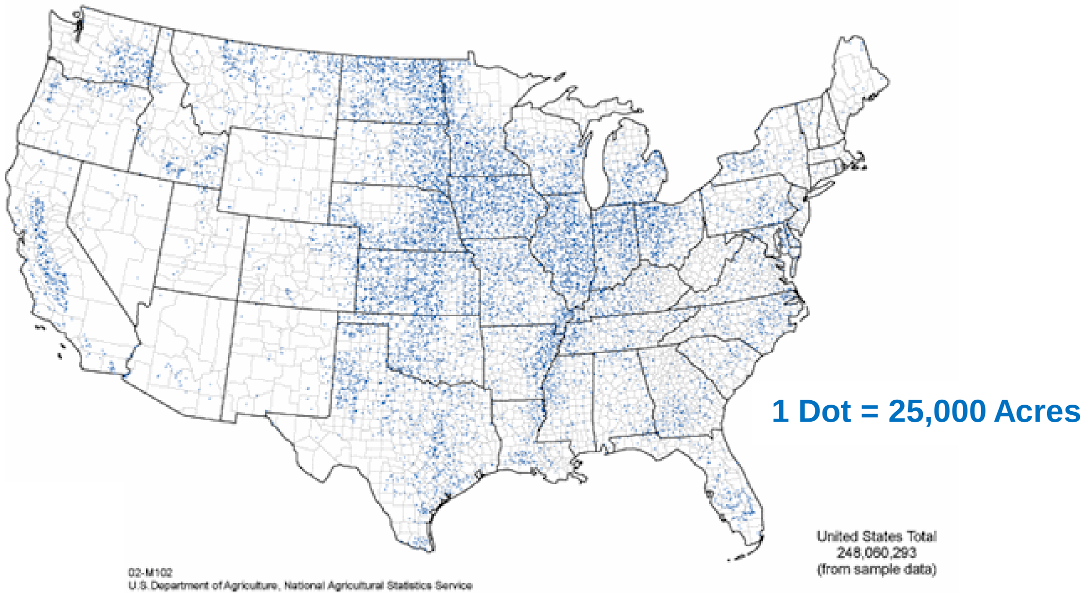


Semi-quantitative …and quantitative maps

Precipitation: Annual Climatology (1971–2000)



Acres Treated with Commercial Fertilizer, Lime, and Soil Conditioners: 2002



Complex Molecular Mixtures. . .

Contain many components across a range of concentrations

- Petroleum products
- Natural organic matter (NOM; humic and fulvic acids)
- Marine and riverine dissolved organic matter (DOM)
- Microbial-derived DOM
- Biological materials (cell lysates, metabolites, etc.)
- Formulated drugs and pharmaceutical materials

Complex Molecular Mixtures. . .

…of pharmaceutical relevance typically include:

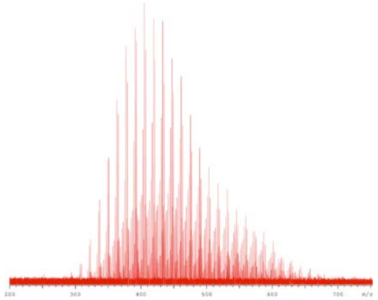
- **Extractables (and leachables)**
- **Related substances in starting materials, intermediates, API and products**
- **Process reaction impurities, reagents and their reaction products**
- **Excipients and drug-excipient interaction products**
- **Degradation products**
- **Surfactants**

Samples frequently contain multiple compounds from multiple categories!!!

Complex Molecular Mixtures. . .

- Present unique challenges to the analyst
  - Multiple, diverse components at various concentrations
  - Abundant additives and formulation components can complicate the search for analytes present at low levels
  - Higher MW analytes = many elemental composition possibilities
  - Timing for results delivery lengthens with sample complexity

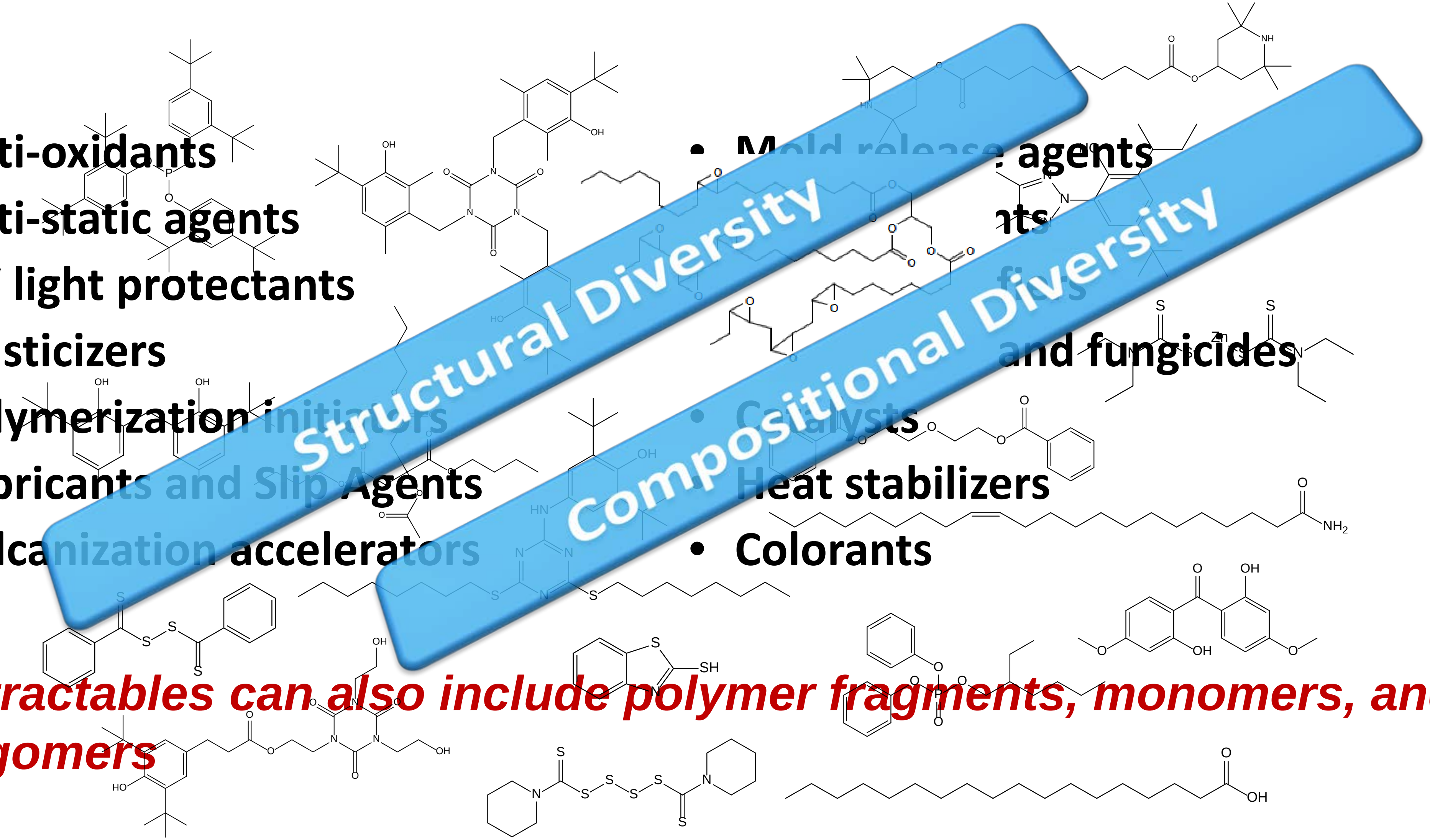
- Require advanced technologies and innovative tools
  - Deep expertise
  - Advanced instrumentation
  - Data reduction and visualization techniques



Additives and Processing Aids in Plastic and Rubber Materials

- Anti-oxidants
- Anti-static agents
- UV light protectants
- Plasticizers
- Polymerization initiators
- Lubricants and Slip Agents
- Vulcanization accelerators

- Mold release agents
- ...nts
- ...fiers
- ...and fungicides
- Catalysts
- Heat stabilizers
- Colorants



Extractables can also include polymer fragments, monomers, and oligomers

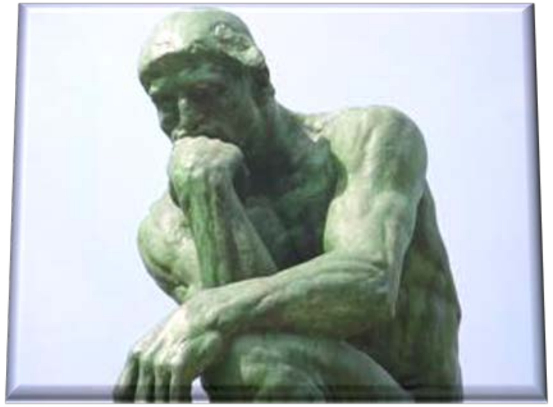
Molecular features contributing to compound diversity

Structural diversity

- *Degree of unsaturation (rings/double bond equivalents)*
- *Substituents*
- *Functional groups*

Isomeric diversity

- *Structural isomers*
- *Positional isomers*
- *Geometric isomers*
- *Stereoisomers*



Compositional diversity

- *Heteroatom content and type*
- *Isotopic contributions*

Polymer Supply Chain for Pharmaceutically Relevant Materials

Converters

Natural Raw Material → Monomer → Polymer → Masterbatcher → End Products & Suppliers

N-4 – Natural Raw Material

N-3 – Monomer
- Bulk process chemicals
- Storage stabilizers

N-2 – Polymer
- Initiators/Catalysts
- Storage stabilizers
- Antioxidants
- Processing aids

N-1 – Masterbatcher
- Antioxidants
- Processing aids
- Light stabilizers
- Antistatic agents

N – End Products & Suppliers
- Colorants
- Processing aids
- Lubricants

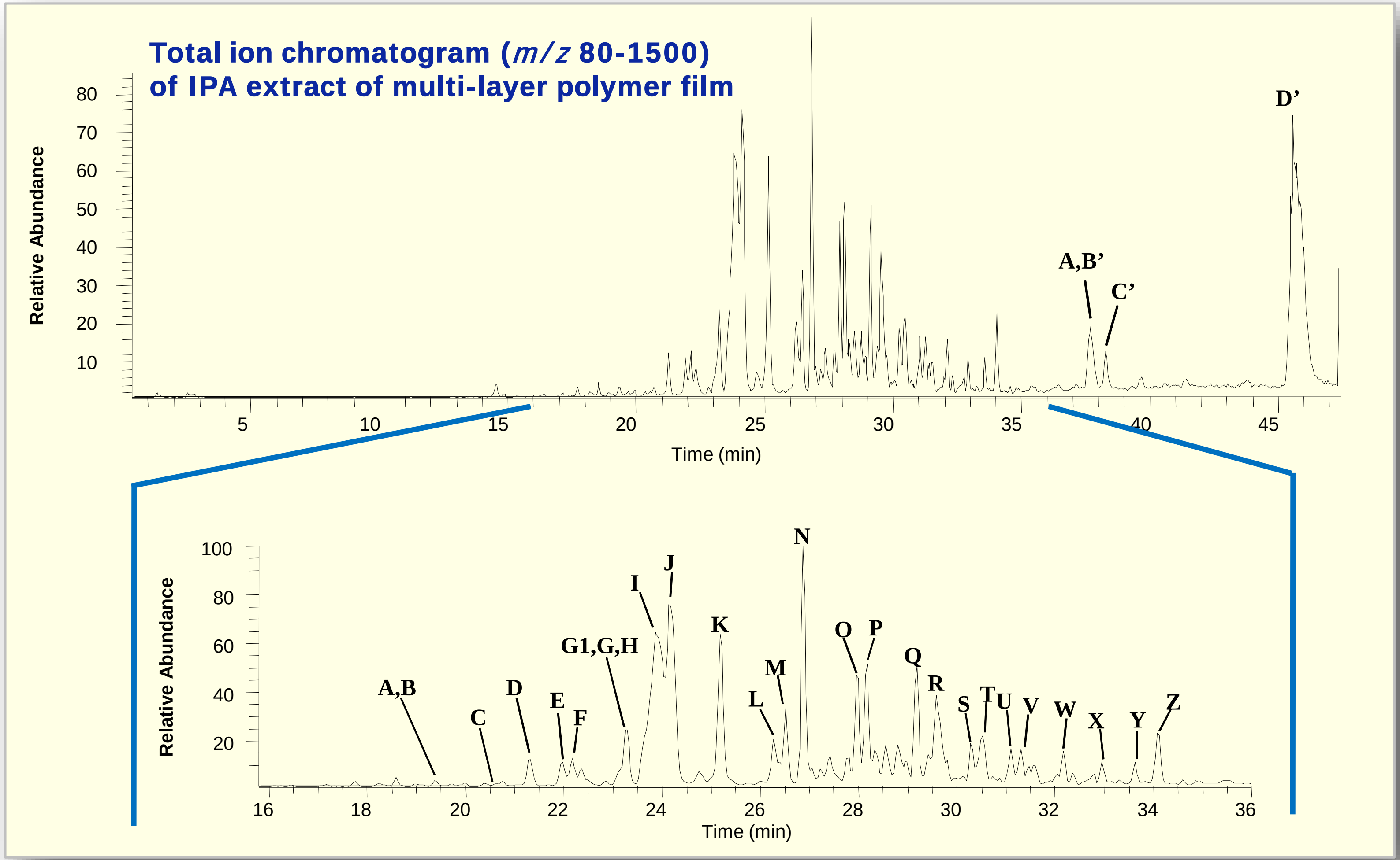
Components for
- Packaging
- Devices
- Container/closure
- Processing

May be cumulative!

Complex Molecular Mixtures. . .

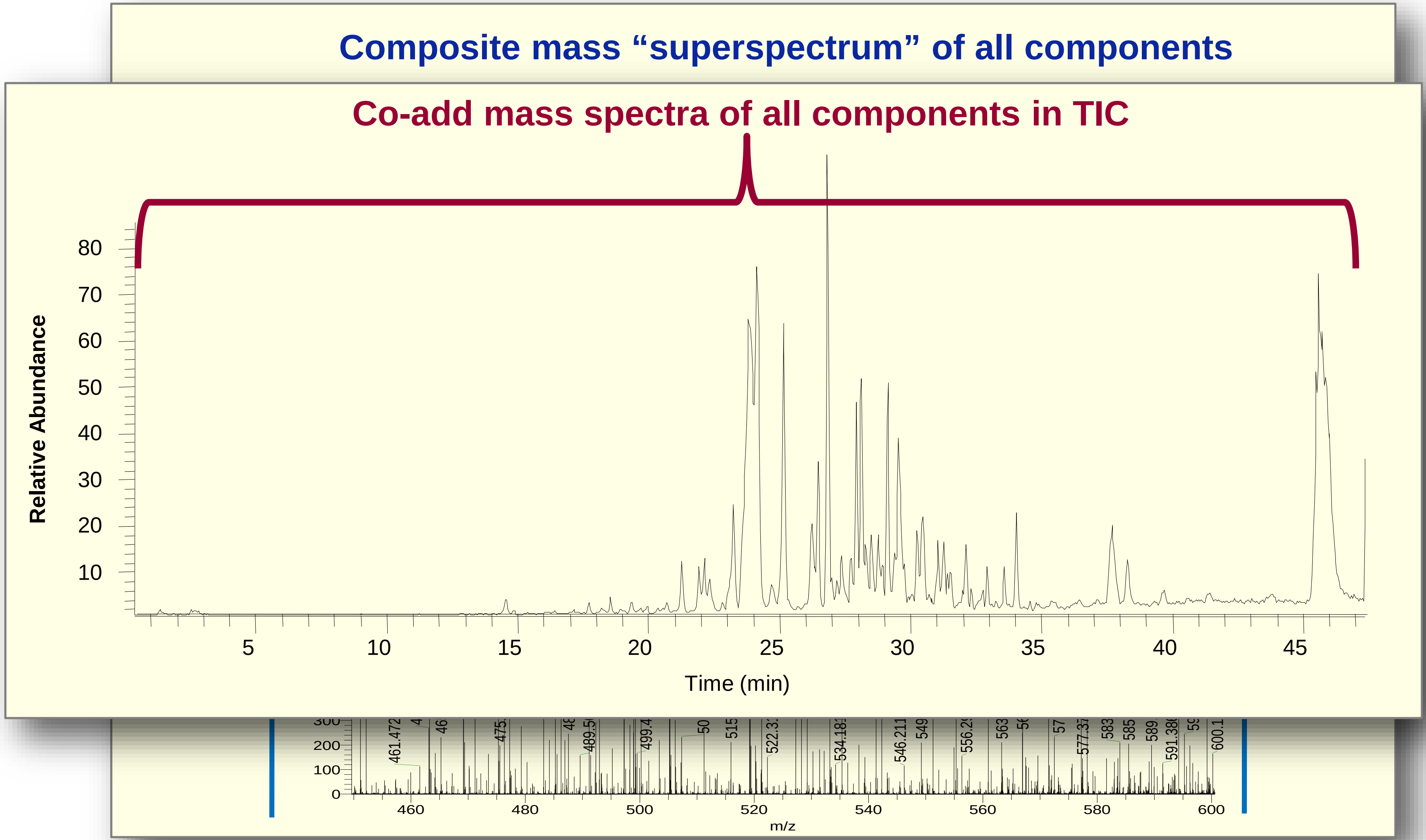
How Complex is "Complex?"

Chromatographic complexity



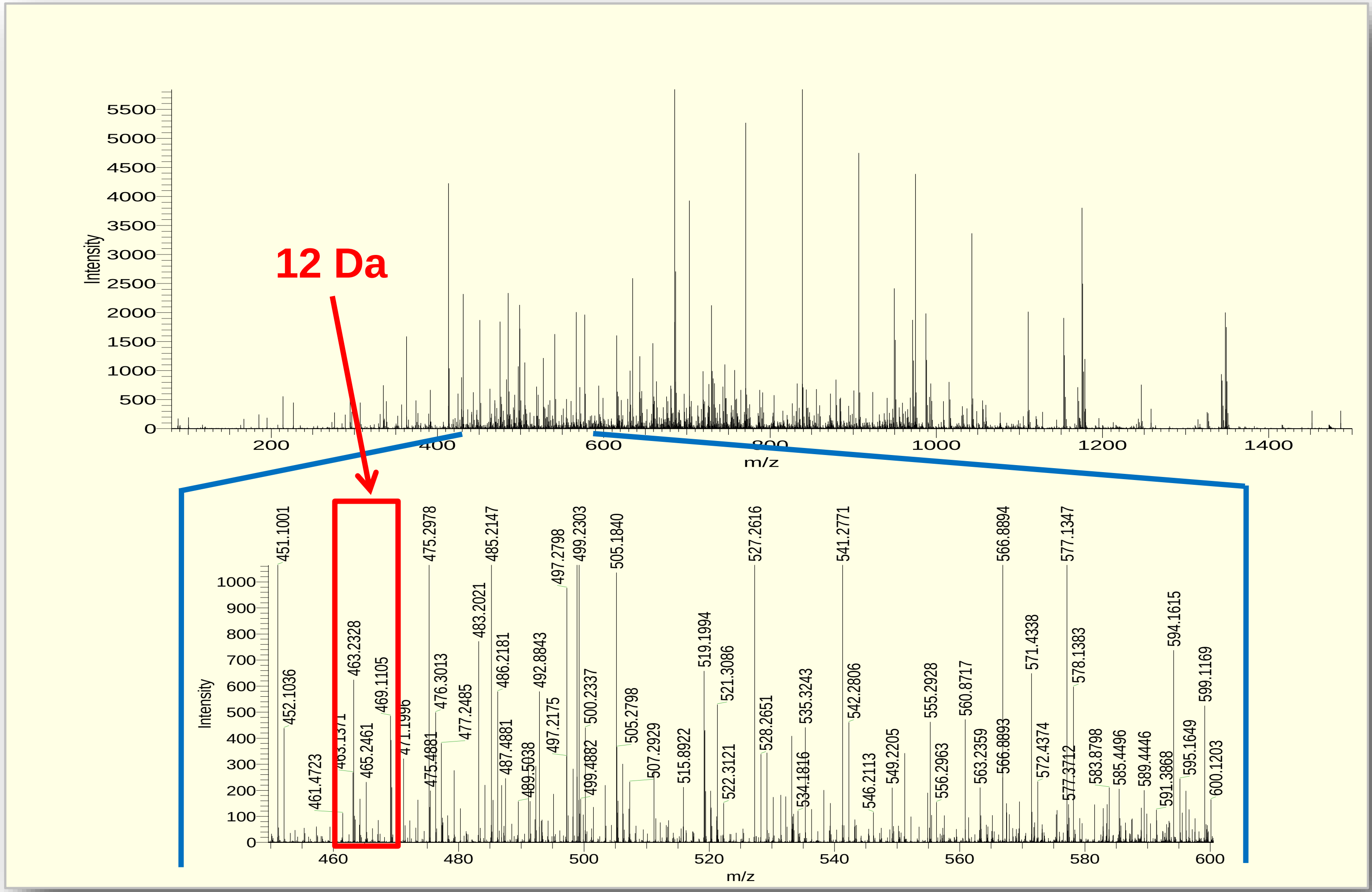
How Complex is “Complex?”

Mass spectral complexity



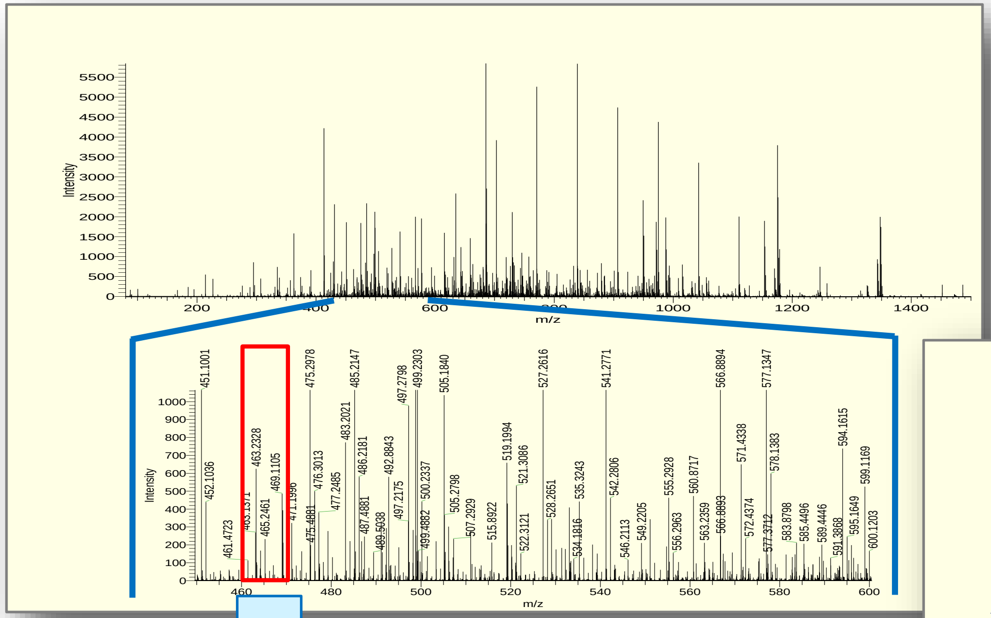
How Complex is “Complex?”

Mass spectral complexity



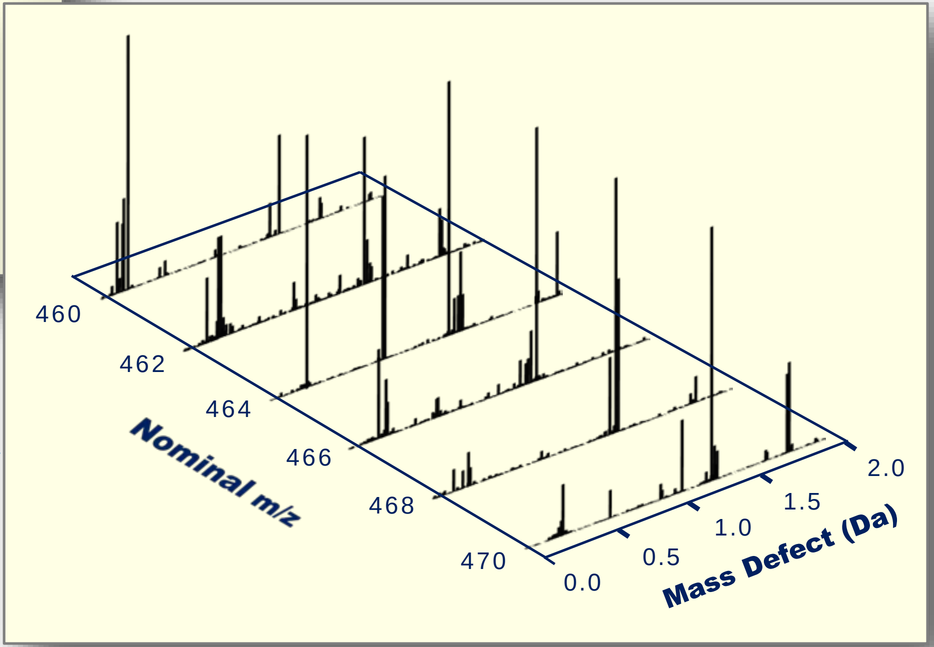
How Complex is “Complex?”

Mass spectral complexity

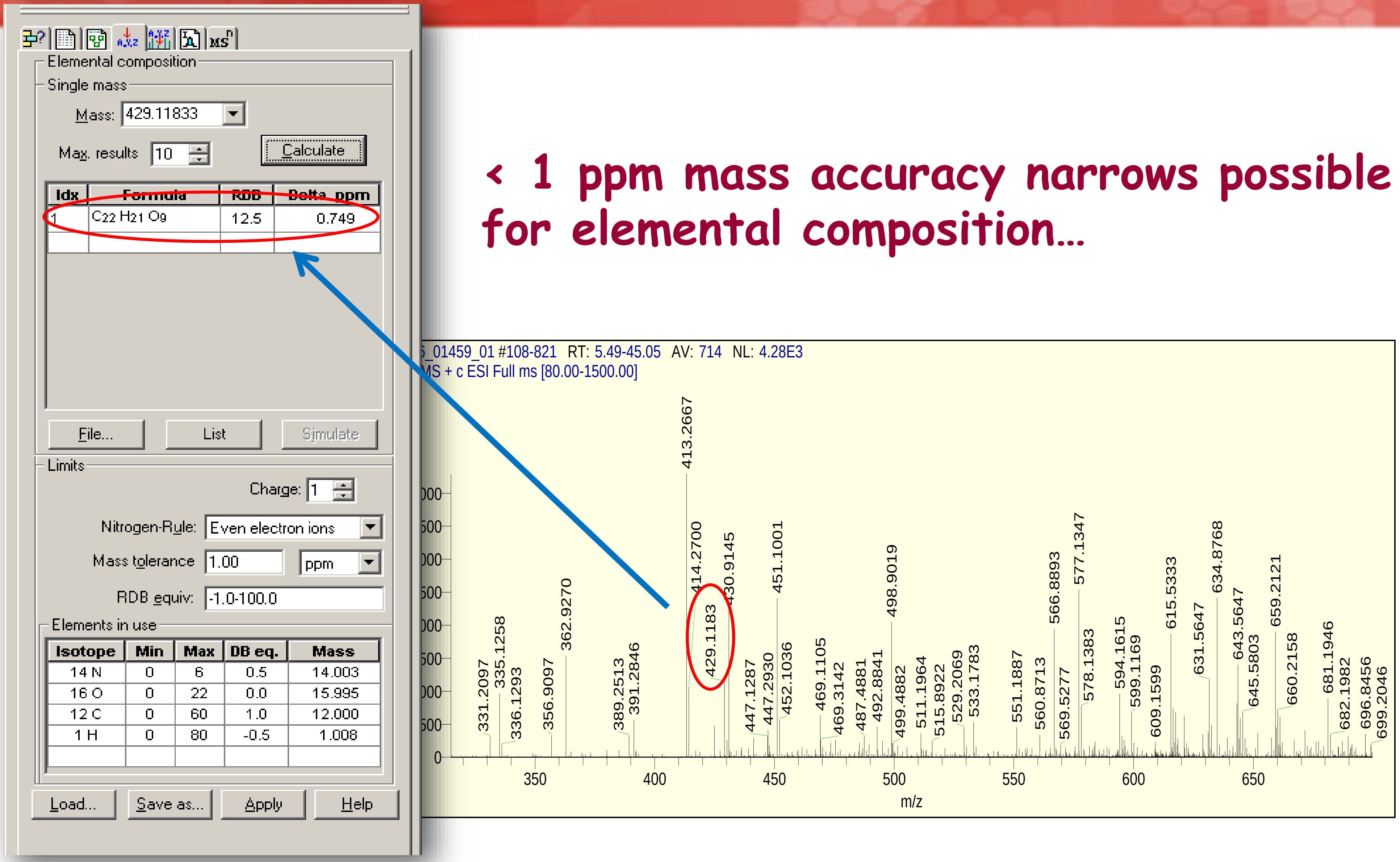


10-20 unique compounds per 2 Da segment!

“Compressed” spectrum



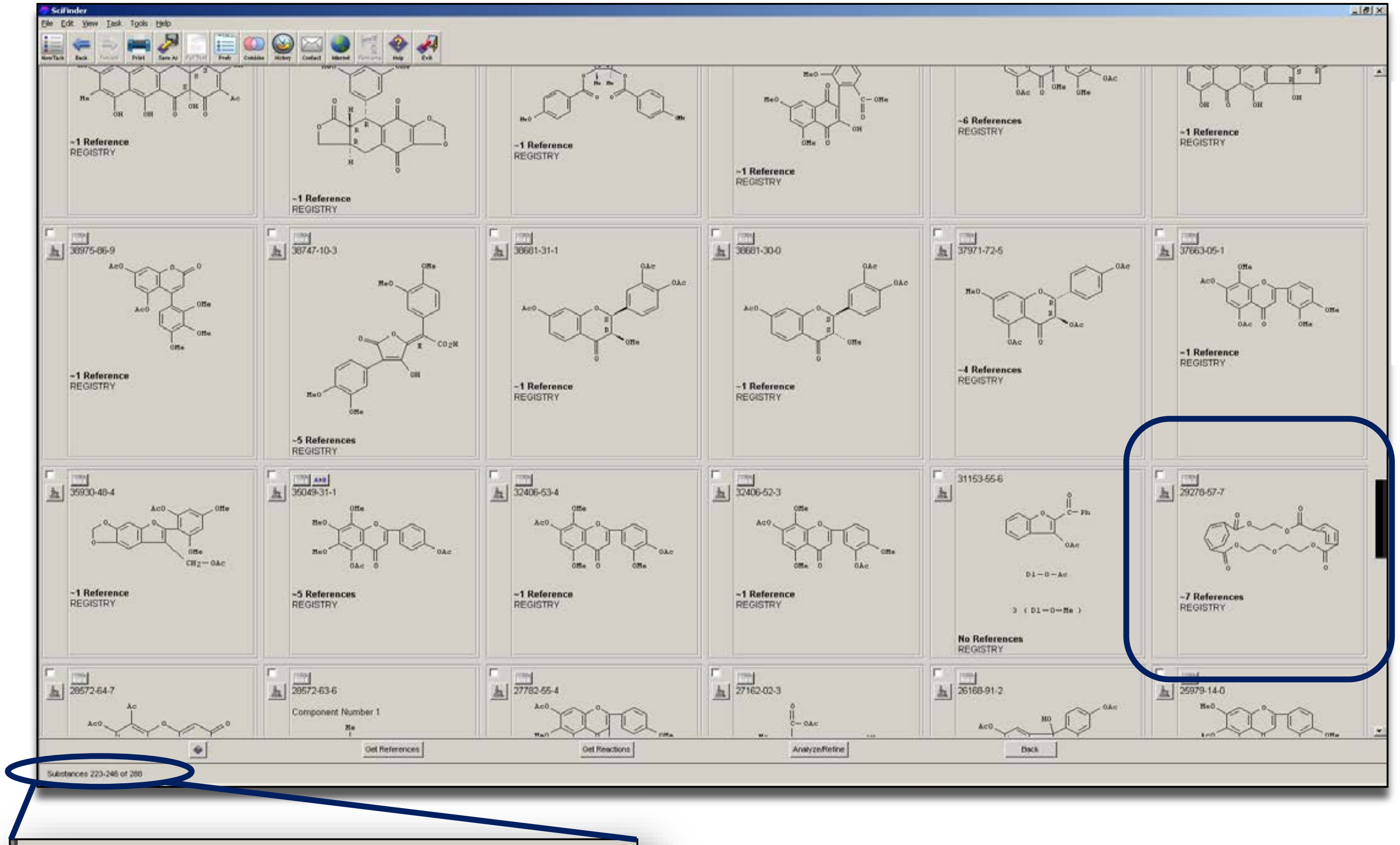
To Extol the Virtues of Accurate Mass Measurement…

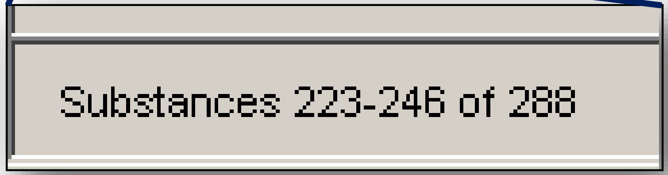


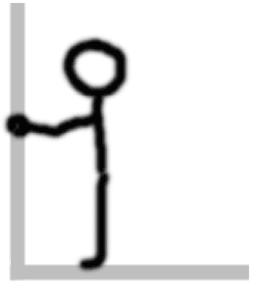
< 1 ppm mass accuracy narrows possible choices for elemental composition…

Correlating Structure with Formula

…but many structures are still possible…





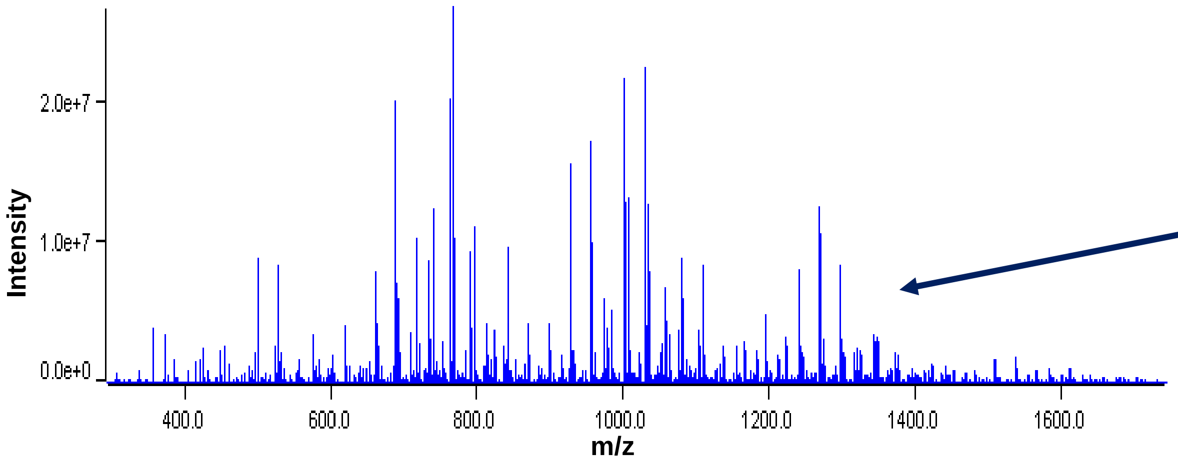


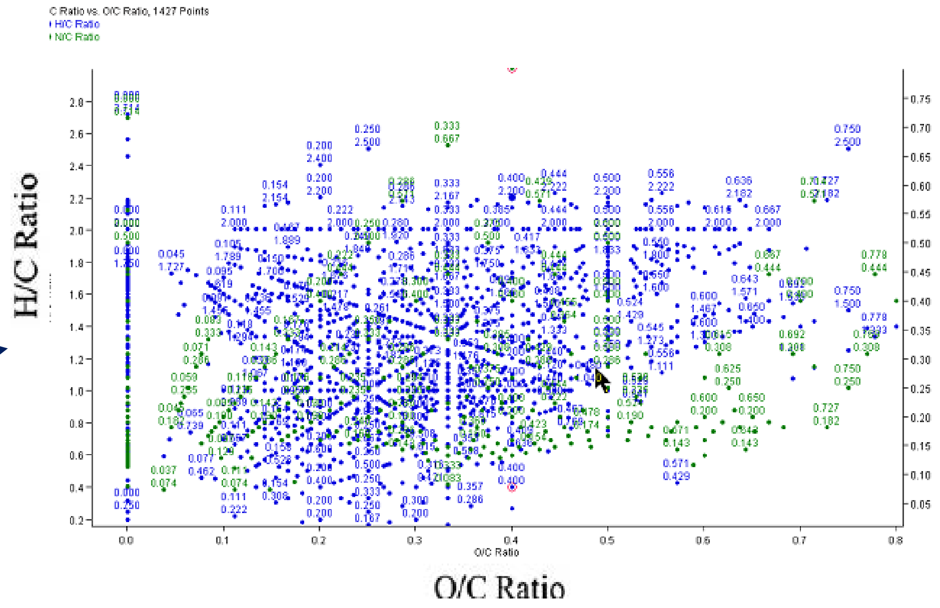
Correct structure

(All $C_{22}H_{20}O_9$!!)

A Mass Map is. . .

A mass map is a two-dimensional or three-dimensional graphic representation of mass spectrometry (m/z) data revealing unique features of chemical entities by their relative position, distribution, spatial arrangement and abundance according to a chosen scale.





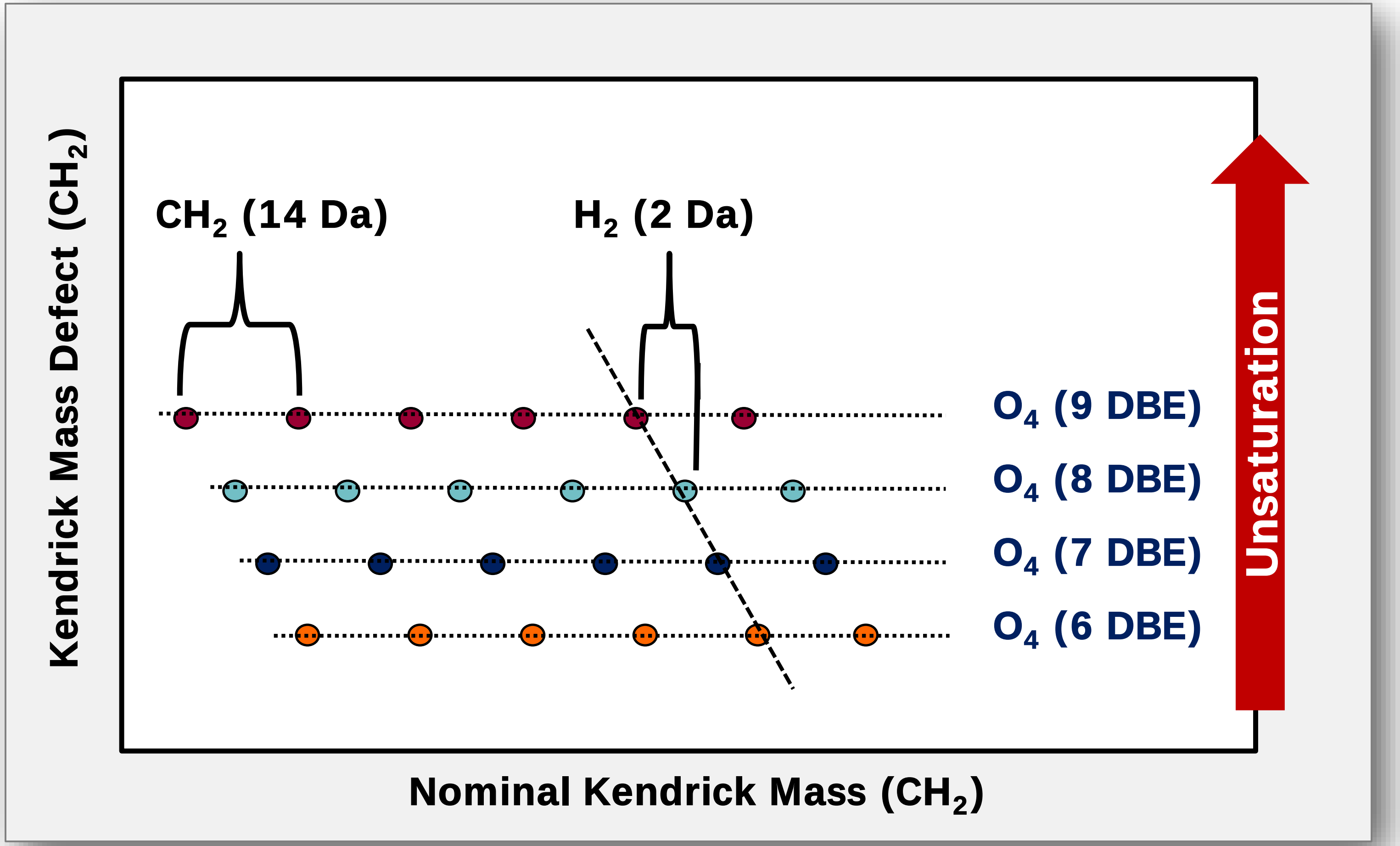
Visualizing Complex Molecular Mixture Data

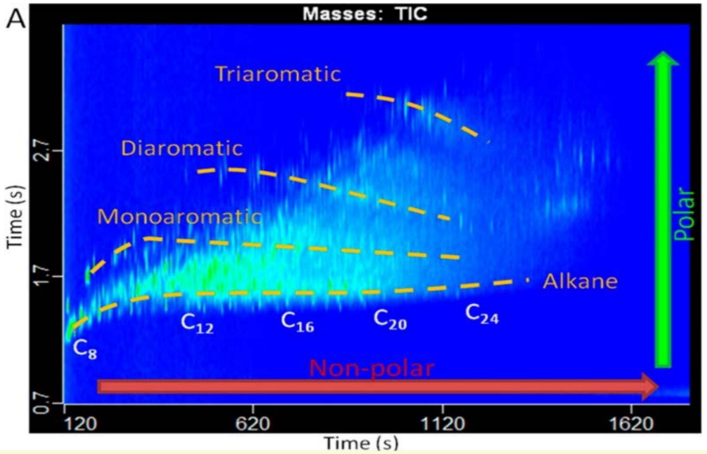
- **Kendrick mass defect diagram**
  - Rescales IUPAC mass scale (based on 12C mass = 12.000 00 Da) to “Kendrick” mass scale for CH2 (e.g. converts CH2 mass from 14.015 65 to 14.000 00 Da)
  - Sorts compounds into homologous series by compound “class” (numbers of O, N, other heteroatom)
  - Compound “type” (rings and double bonds)
  - Degree of alkylation (number of CH_2 groups)

- **The van Krevelen diagram**
  - Projects elemental composition according to H/C and heteroatom/C atomic ratios
  - Compounds are classified according to degree of saturation, alkylation, heteroatom inclusion, dehydration, deamination
  - Compositional differences are amplified permitting classification, prediction of composition and origin

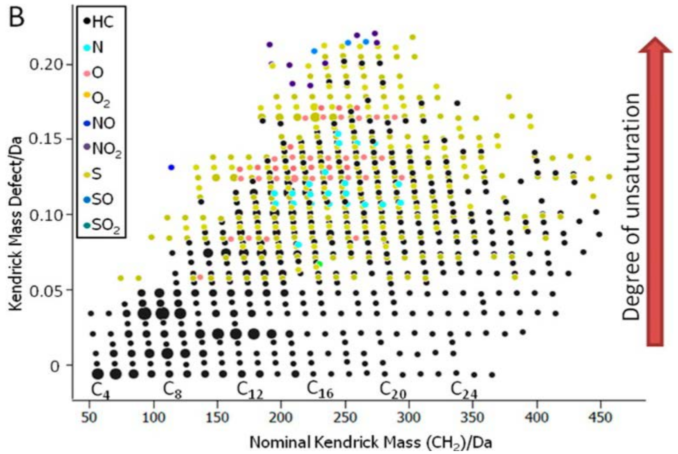
Kendrick Mass Defect Diagram

Kendrick Mass Defect Diagram (for CH_2)





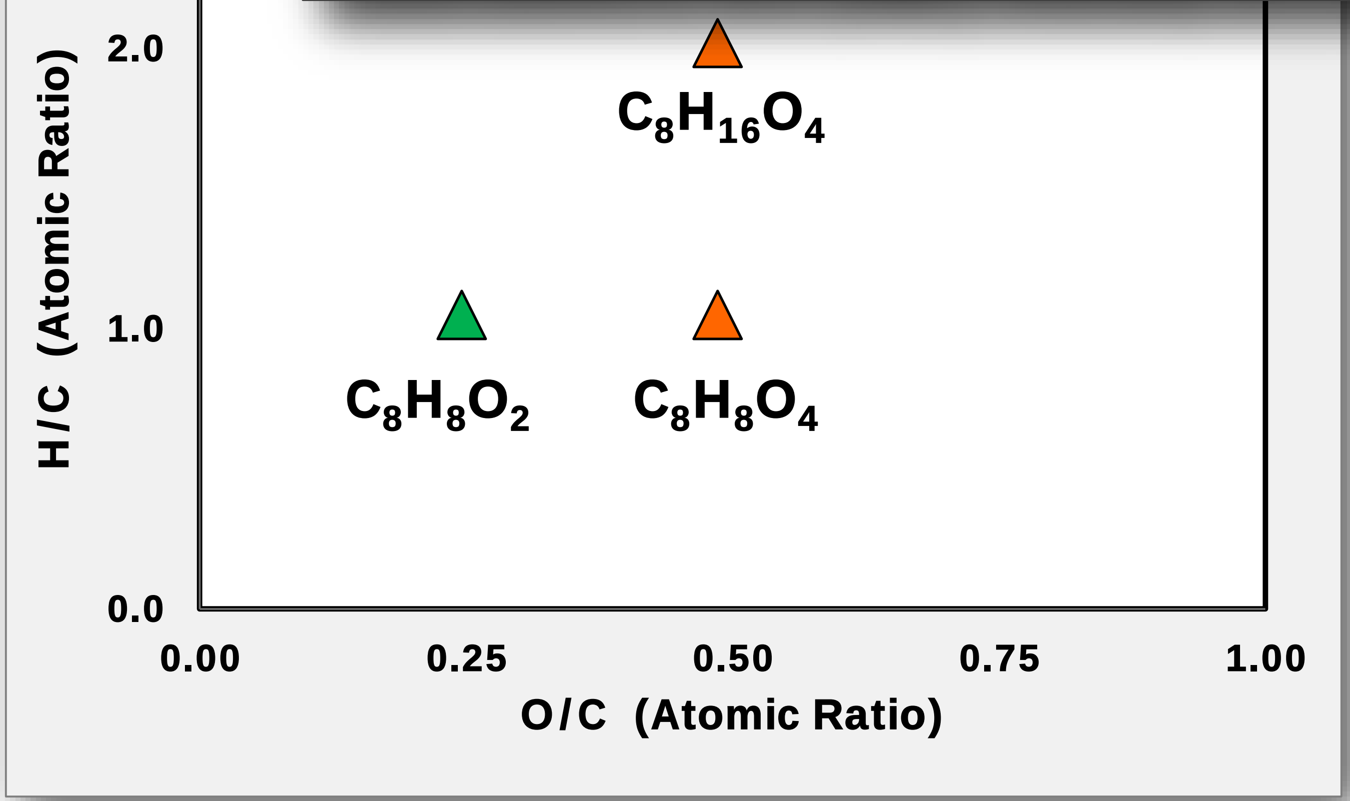
GC x GC ToFMS, heavy crude oil

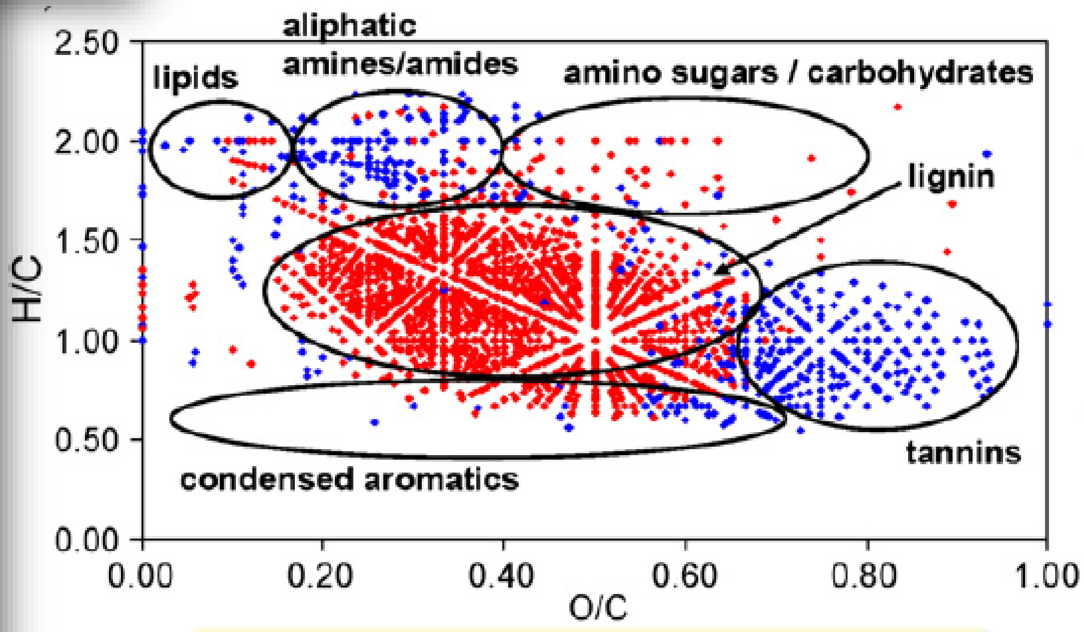


Jonathan D. Byer; Kevin Siek; Karl Jobst; *Anal. Chem.* **2016**, 88, 6101-6104.

van Krevelen Diagram

2-D or 3-D diagram constructed with heteroatom/C, H/C (DBE), intensity on z-axis



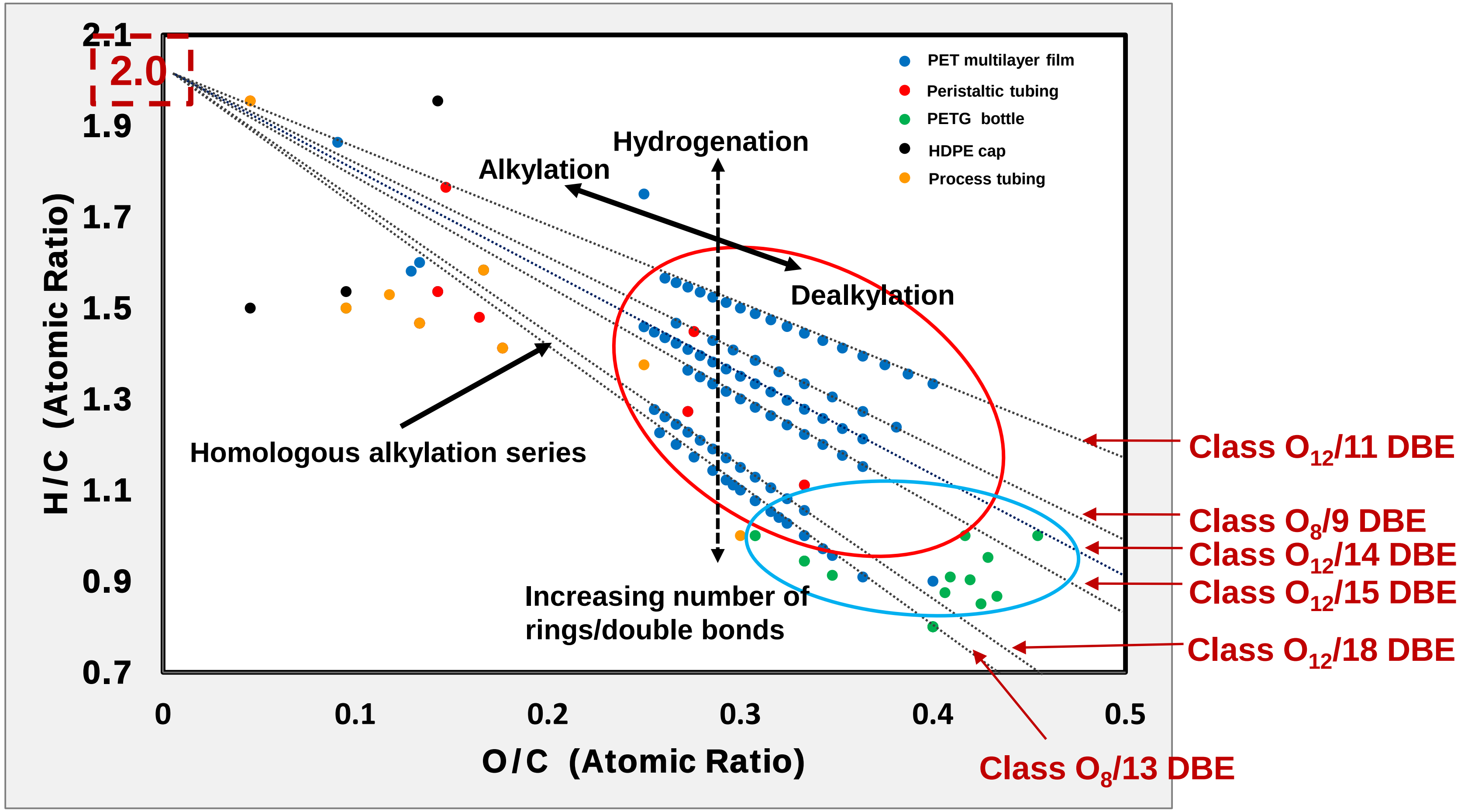


Dissolved organic matter

R.L. Sleighter, P.G. Hatcher / Marine Chemistry 110 (2008) 140–152

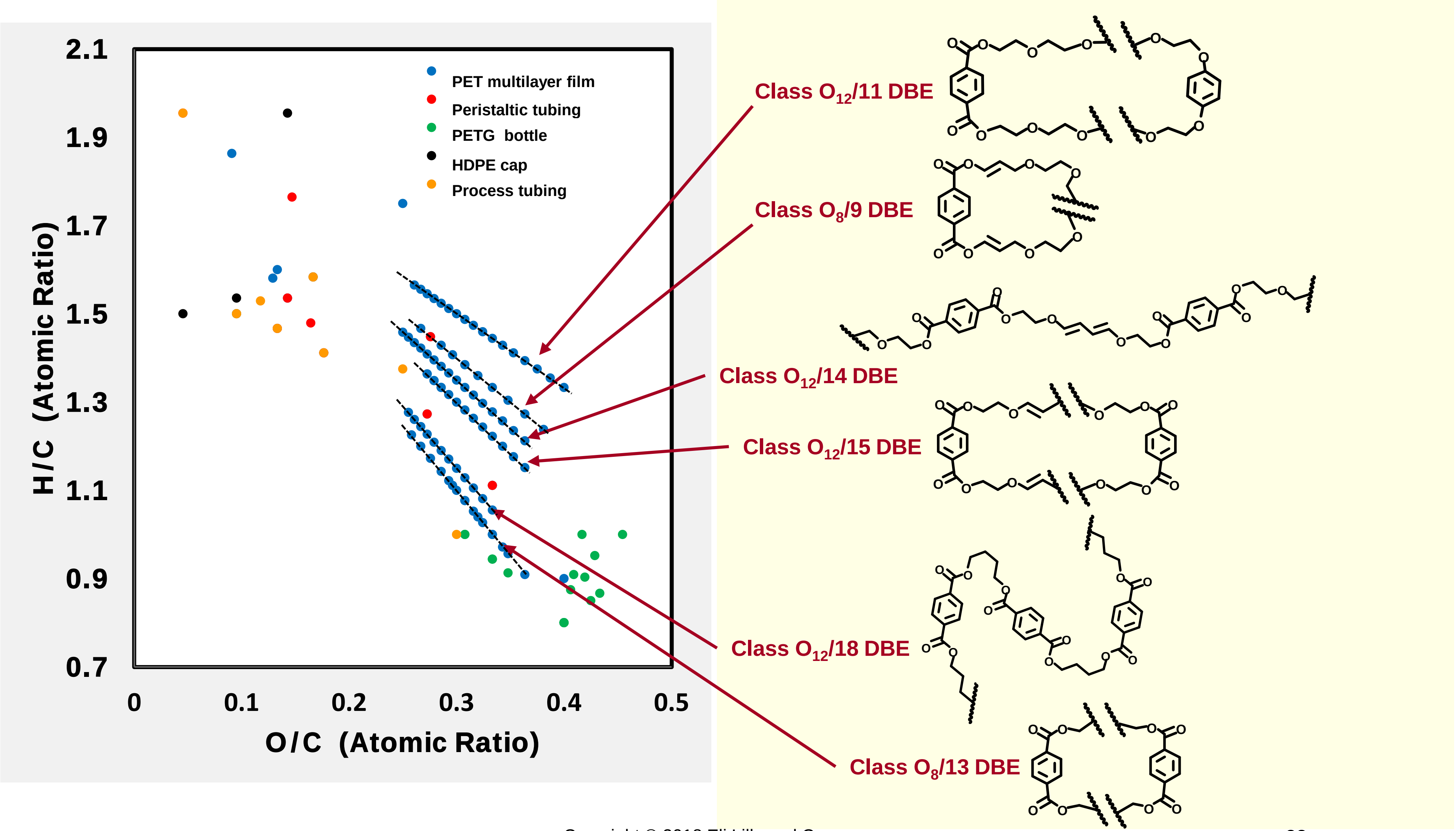
van Krevelen Diagram

FT-ICR LC-MS analysis of IPA extracts (5 materials) – mapping extractables



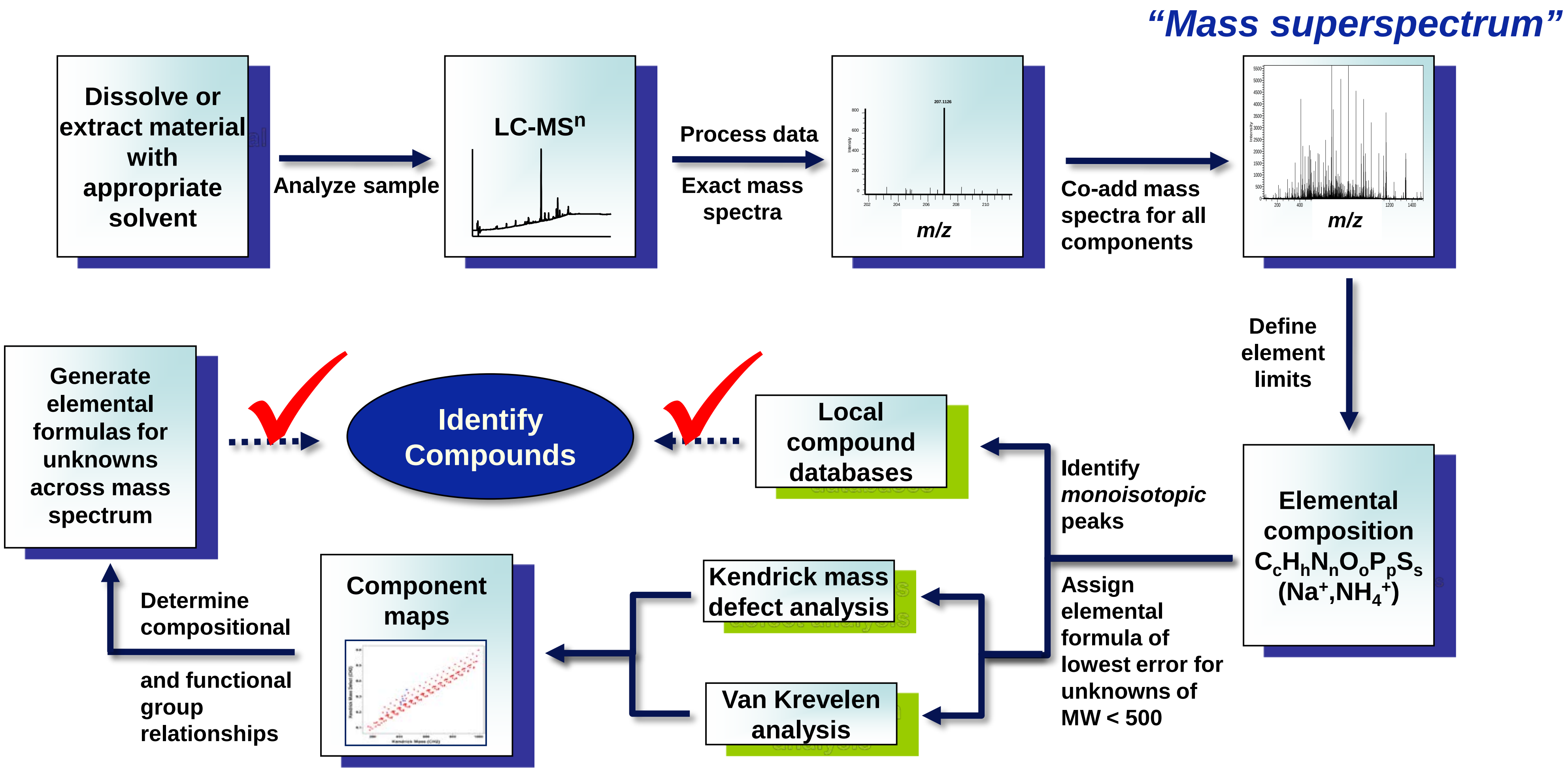
For any compound series $C_{c-n}H_{h-2n}O_o$: $y = (h-2n)/c-2n$ and $x = o/(c-n)$ ⇨ $y=2+(h-2c)/ox$

van Krevelen Diagram



23

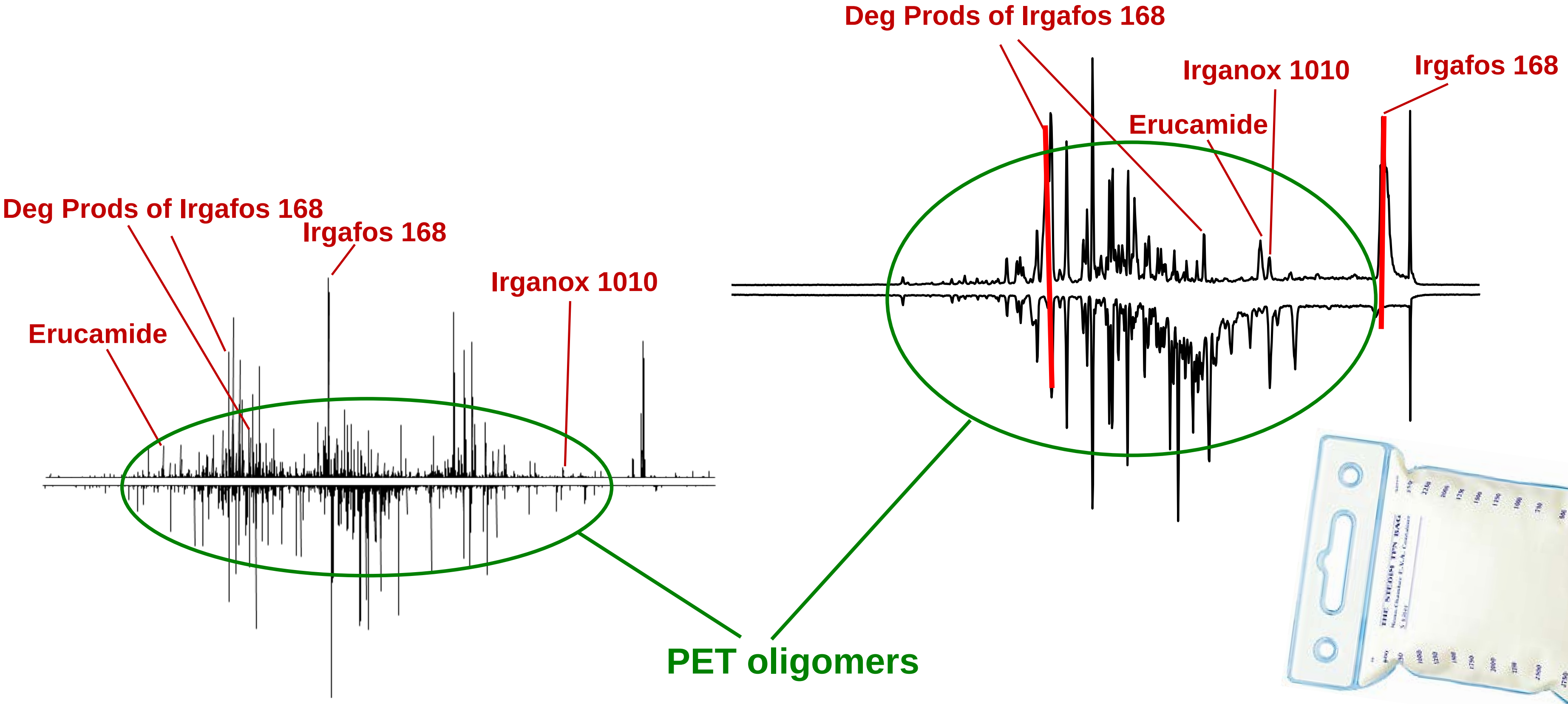
LC-MS Analytical Workflow for Extractables Assessment



24

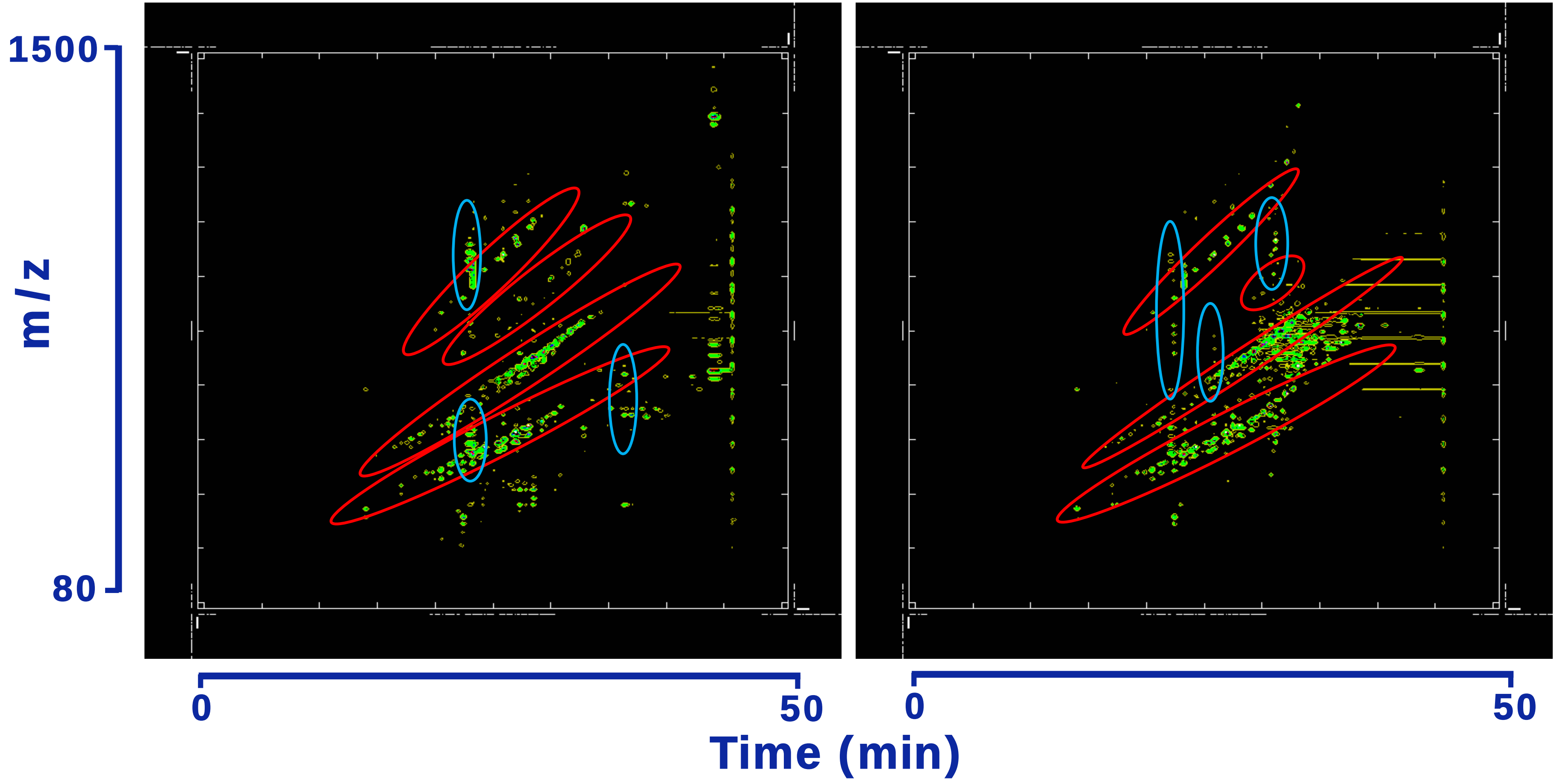
Mass Mapping in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

FT-ICR LC-MS total ion chromatograms (right) and mass spectra (left) of IPA extracts of multi-layer biopharmaceutical bags from different sources



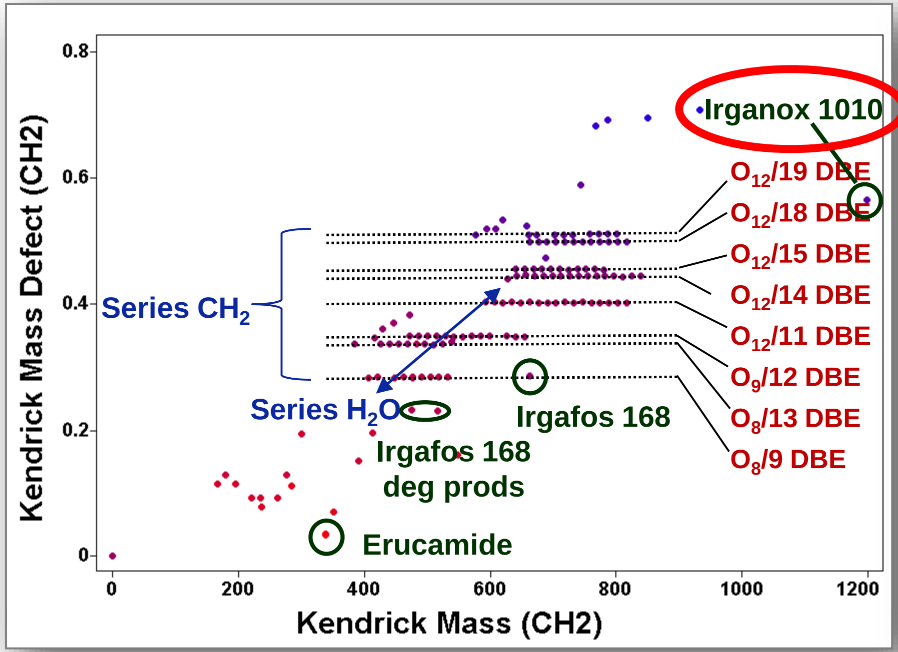
Mass Mapping in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

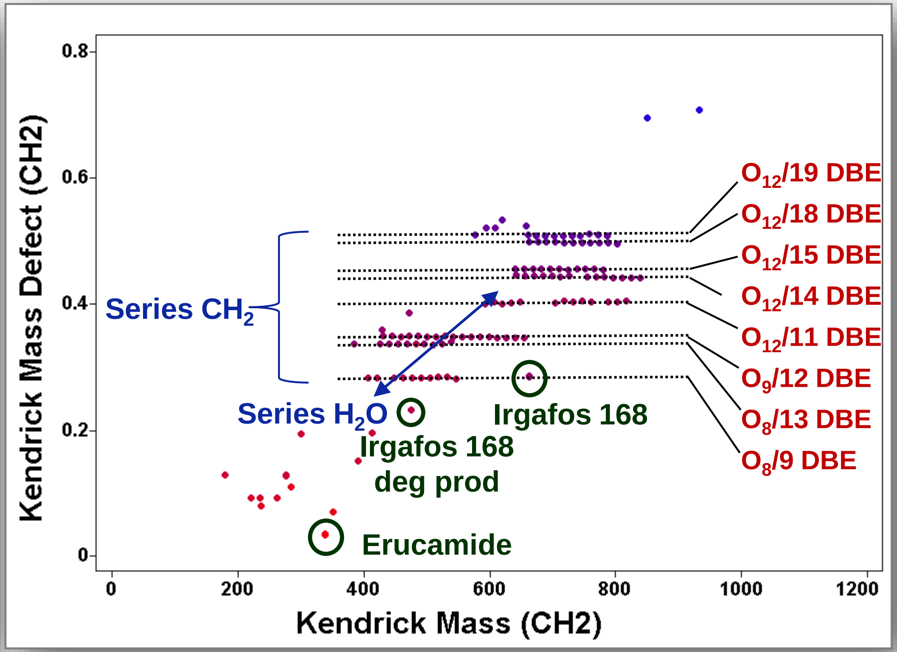
Comparative FT-ICR LC-MS contour maps



Mass Mapping in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

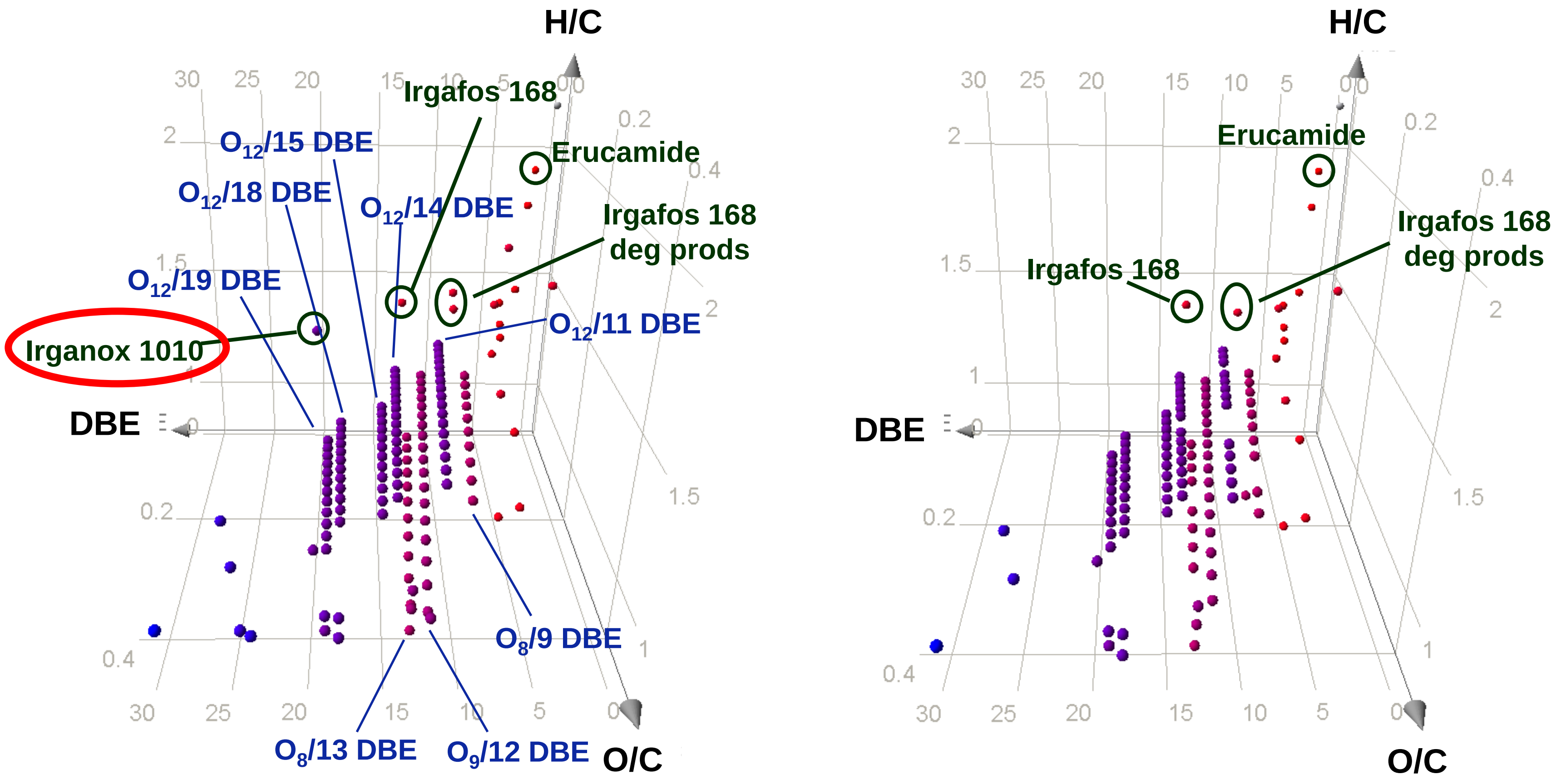
Comparative Kendrick mass defect diagrams





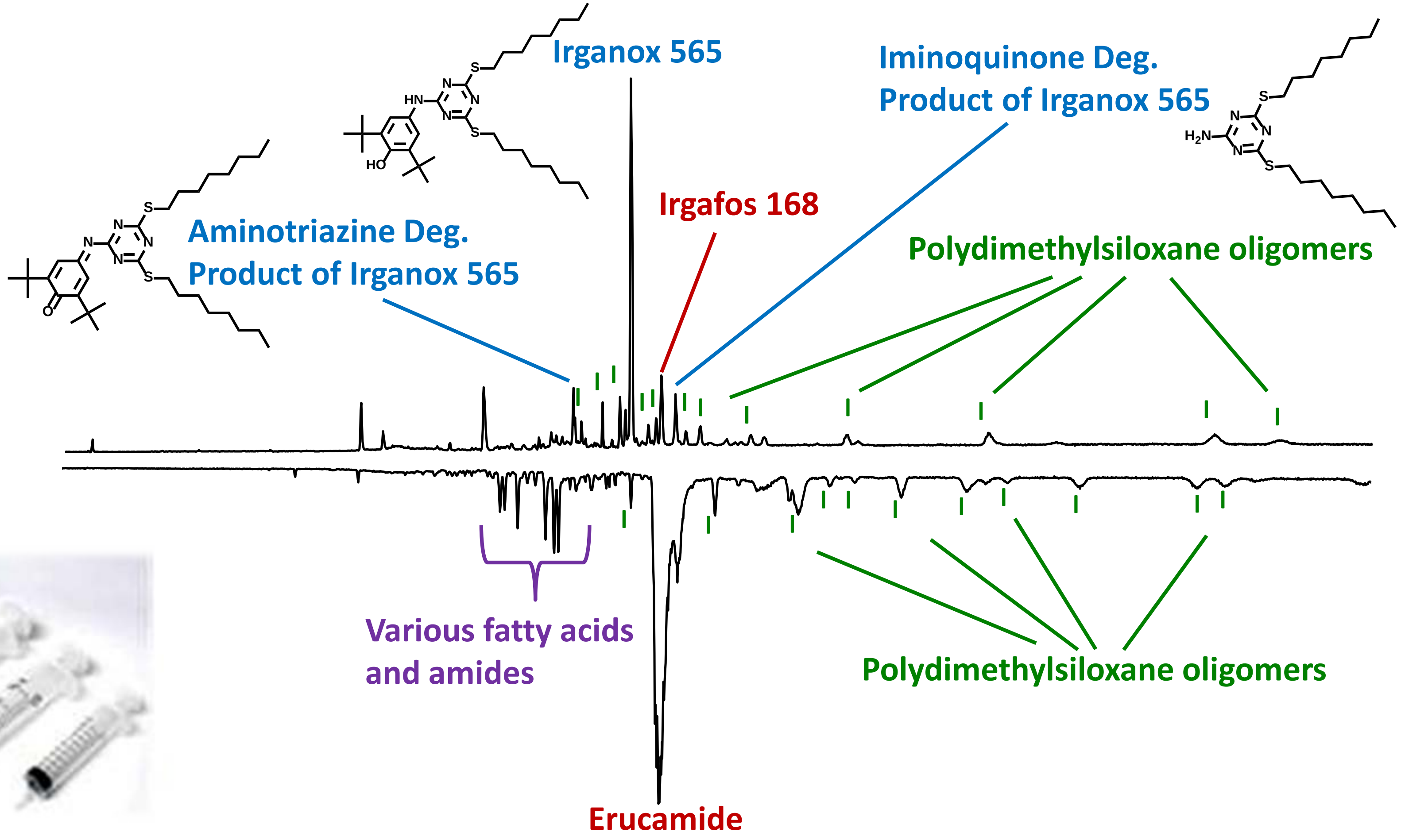
Mass Mapping in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

Comparative 3D van Krevelen diagrams



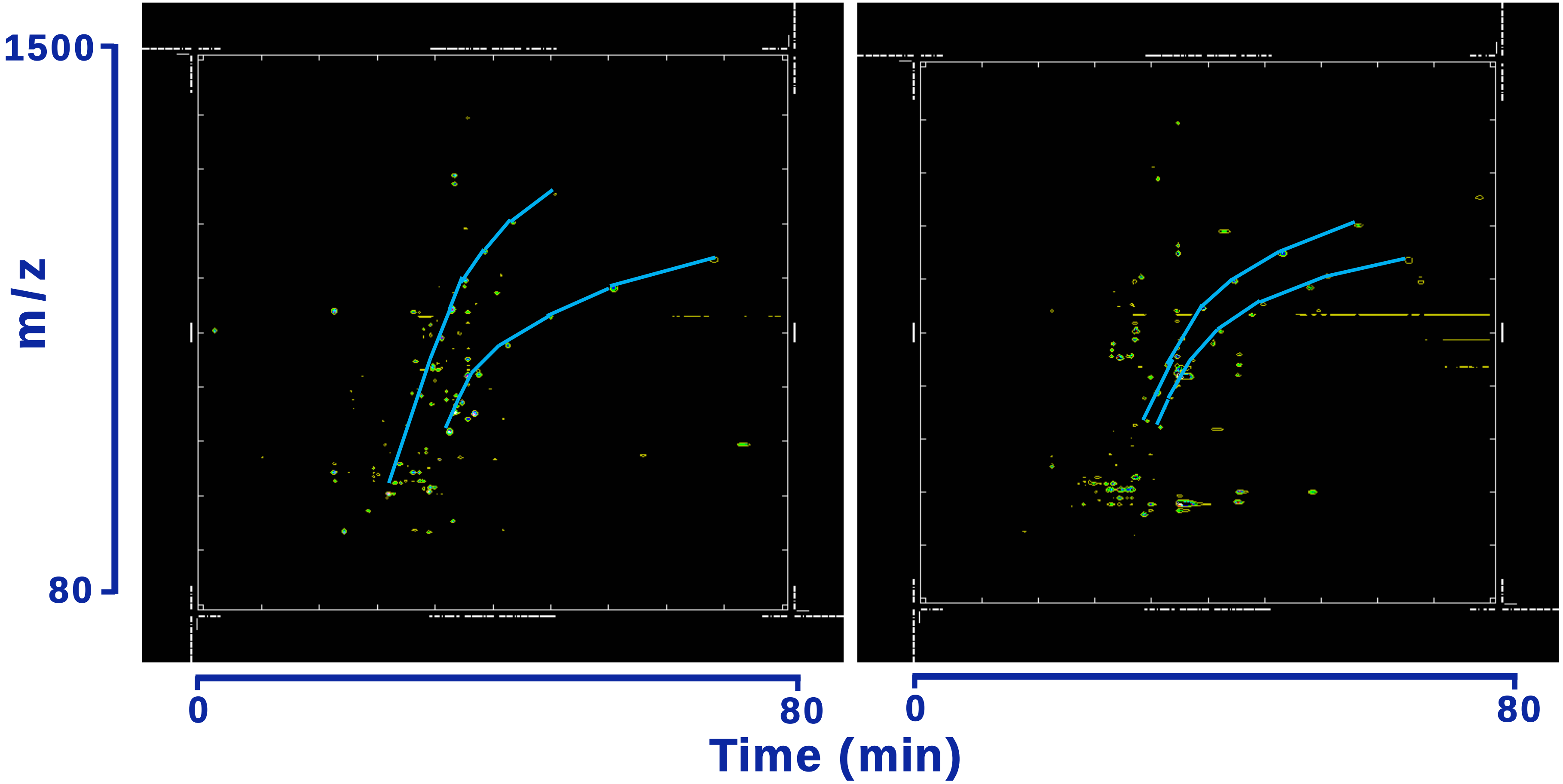
Mass Mapping in Practice: Polypropylene Syringe Extractables

FT-ICR LC-MS total ion chromatograms (m/z 80-1500) of combined CH_2Cl_2 extracts of polypropylene syringes from two different sources



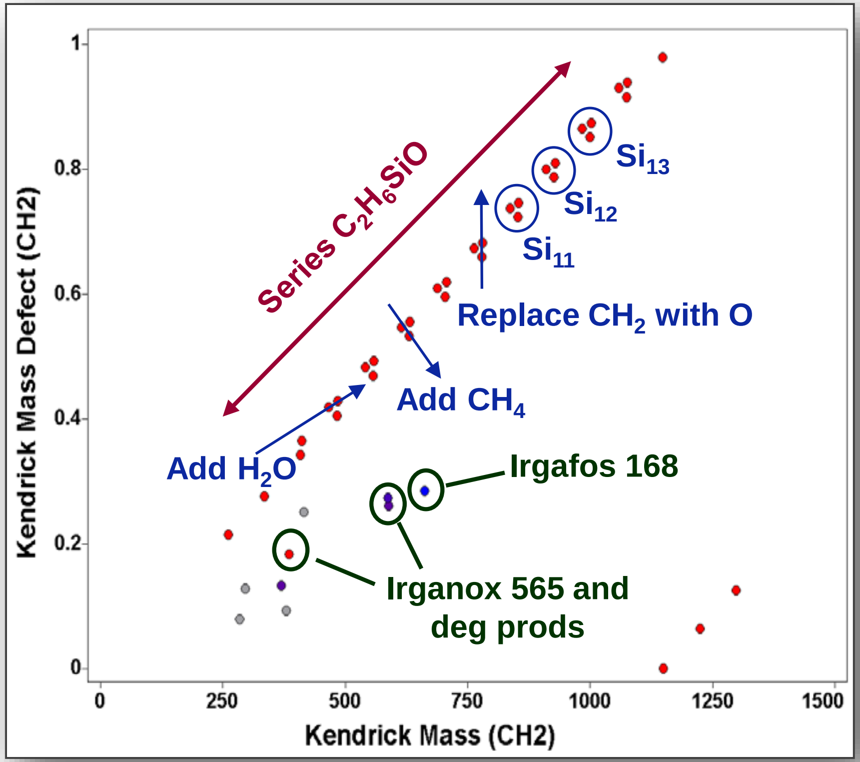
Mass Mapping in Practice: Polypropylene Syringe Extractables

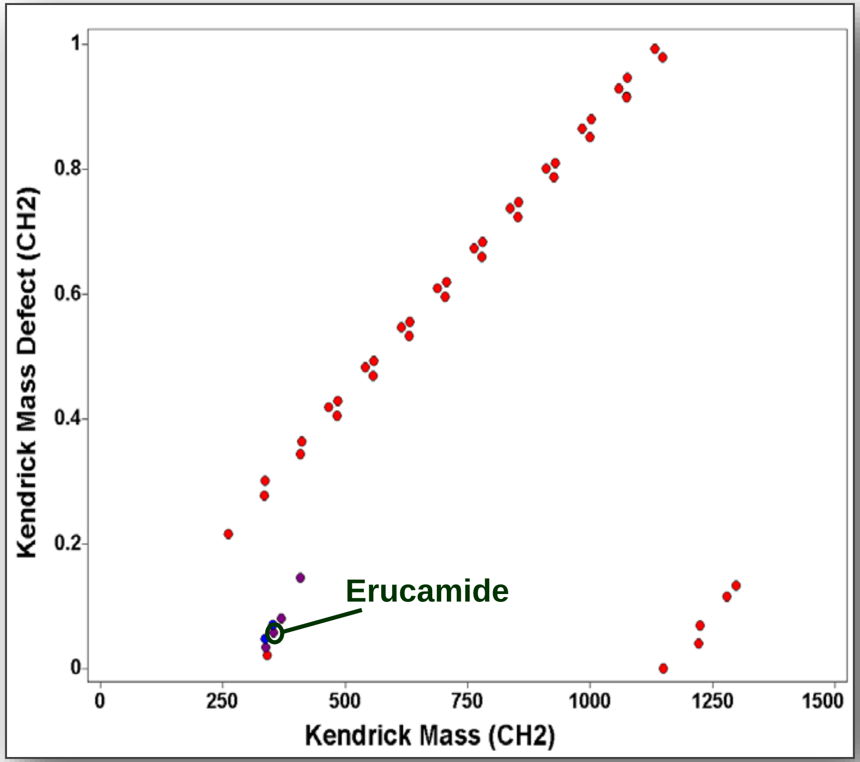
Comparative FT-ICR LC-MS contour maps



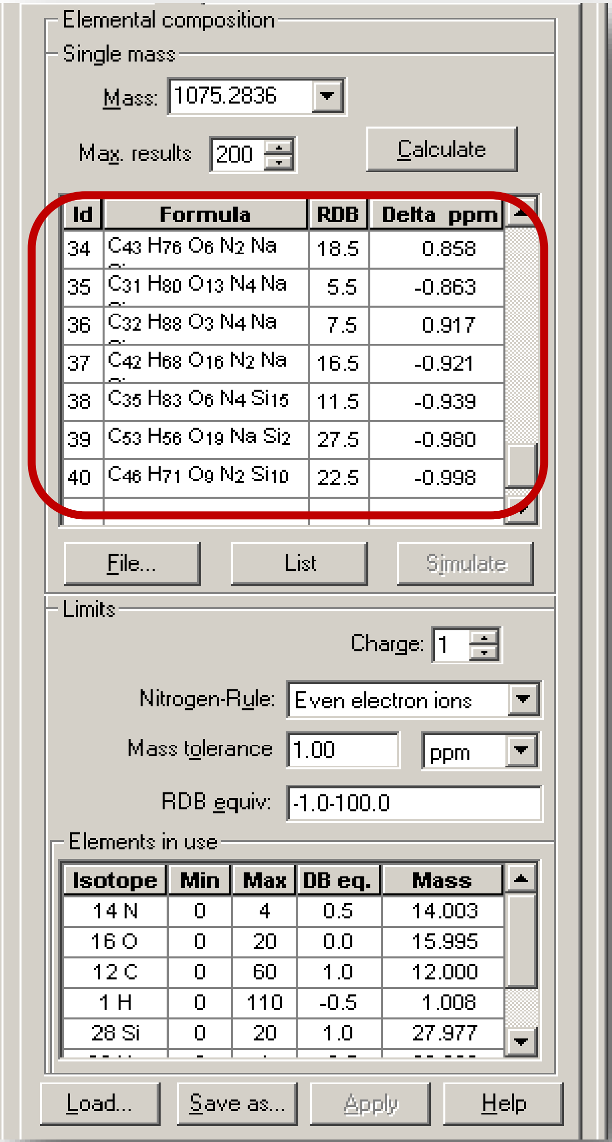
Mass Mapping in Practice: Polypropylene Syringe Extractables

Comparative Kendrick mass defect diagrams

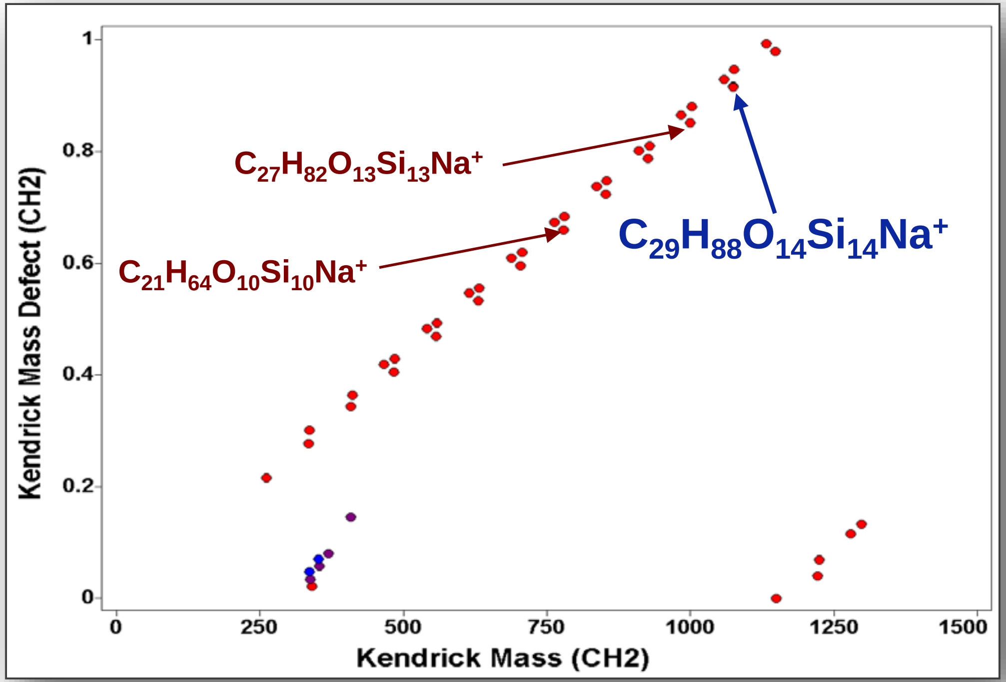




Mass Mapping in Practice: Polypropylene Syringe Extractables

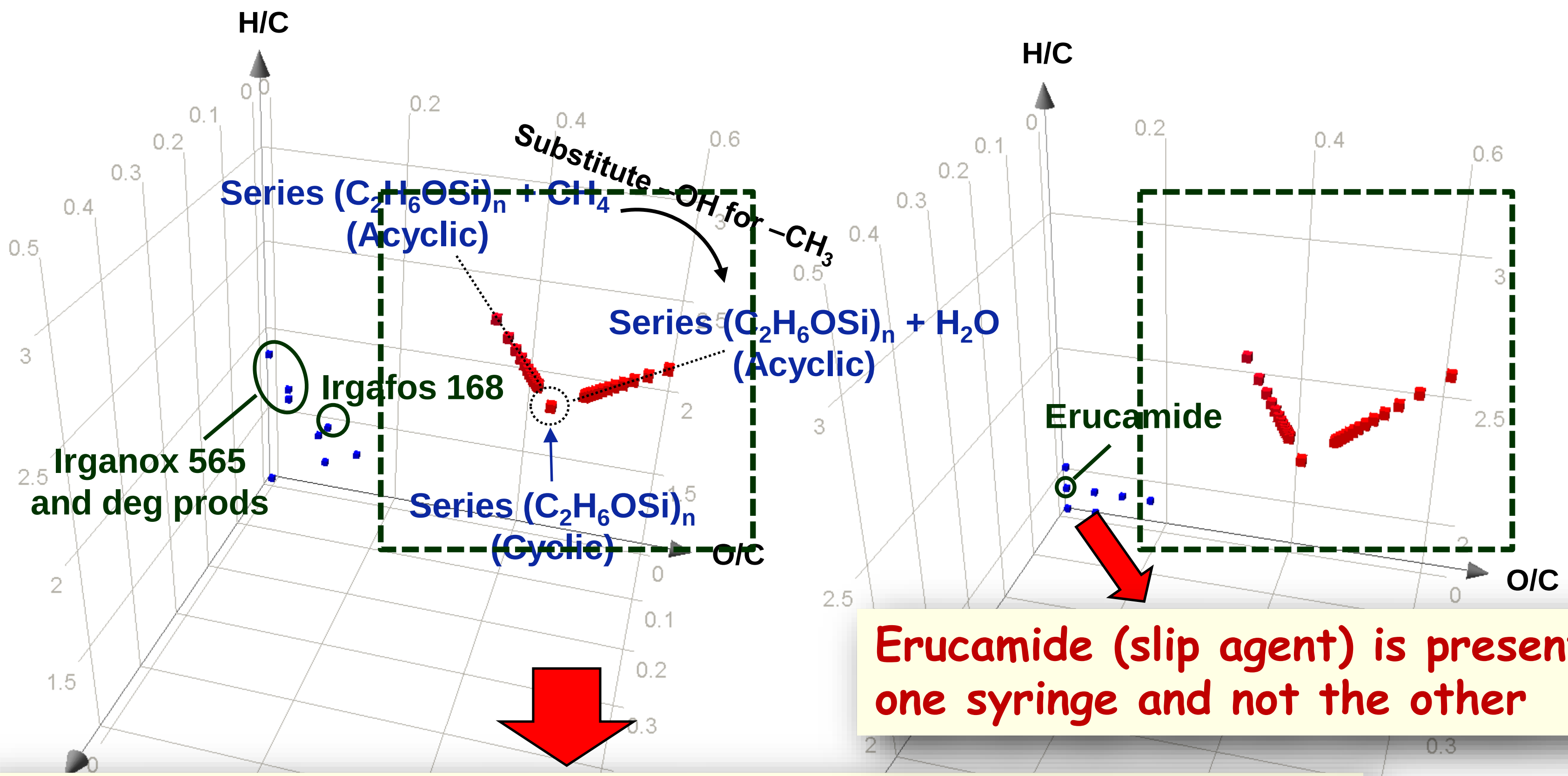


40 possible elemental formulae for m/z 1075.2836 at 1 ppm; but by locating any other known formula in series on the plot, Kendrick defect mapping can determine the correct assignment ($C_{29}H_{88}O_{14}Si_{14}Na^+$)



Mass Mapping in Practice: Polypropylene Syringe Extractables

Comparative 3D van Krevelen diagrams

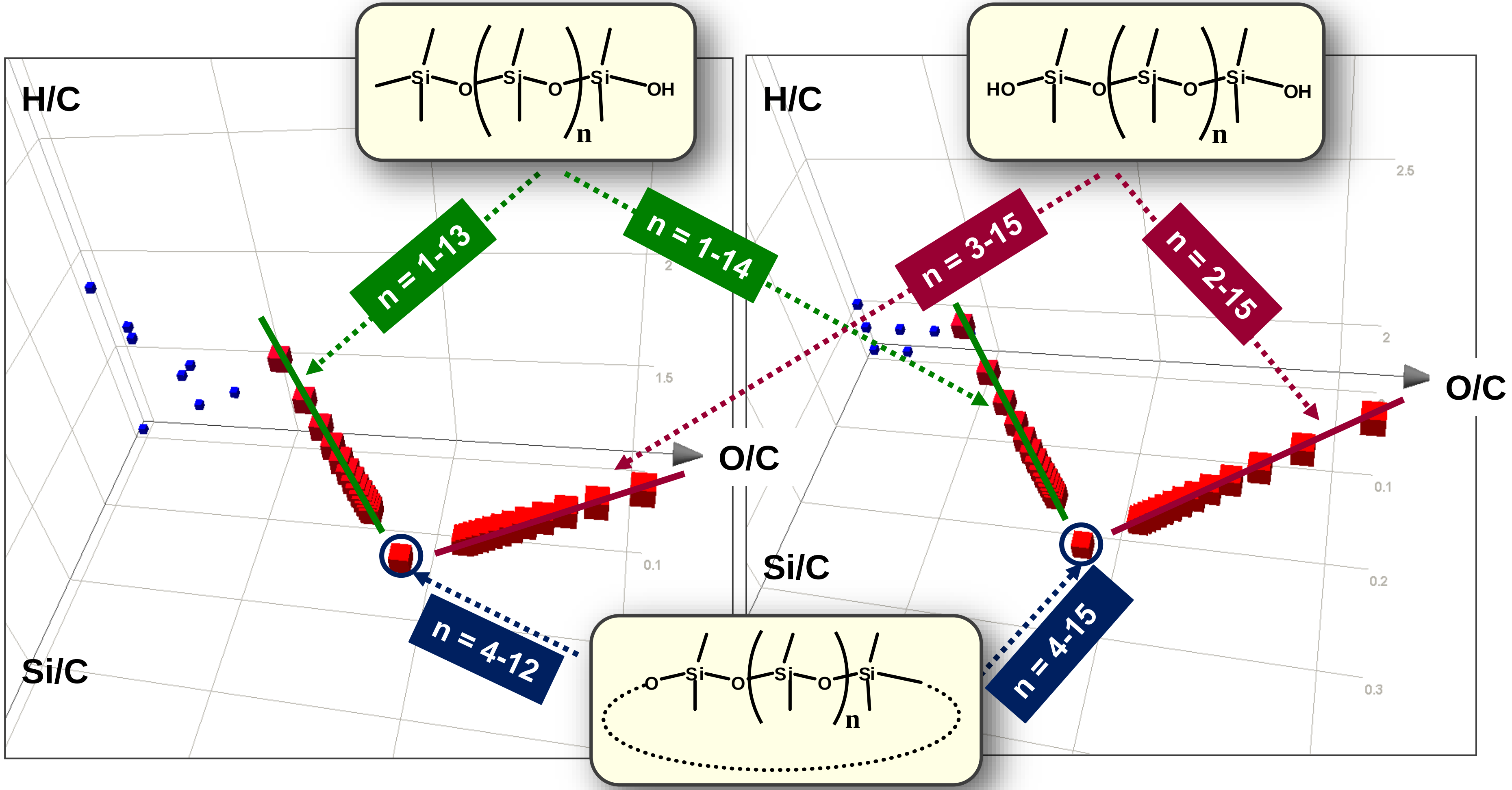


Erucamide (slip agent) is present in one syringe and not the other

Three distinct series of polydimethylsiloxane are clearly visible, and distribution is similar in both syringe extracts

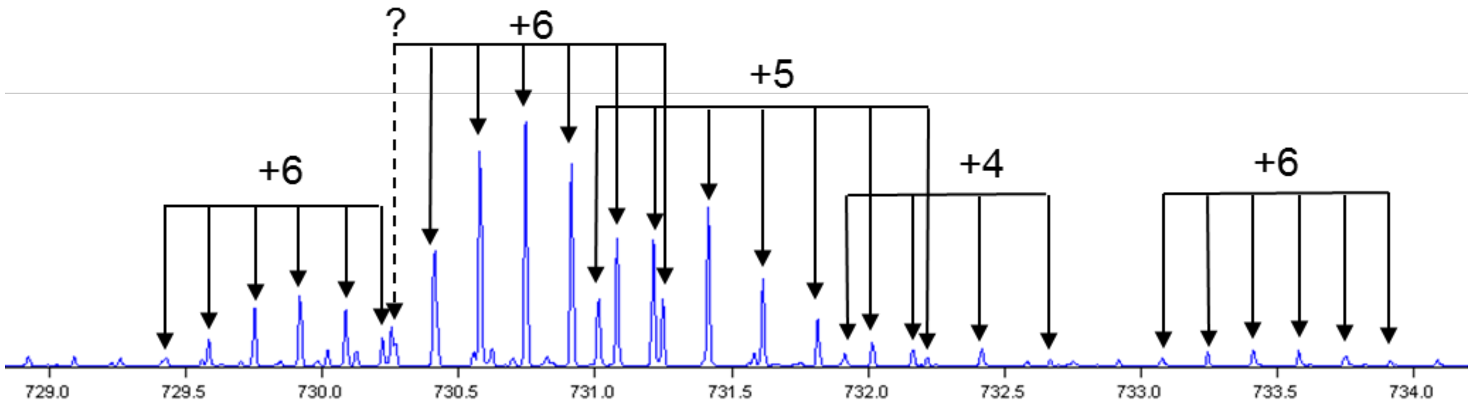
Mass Mapping in Practice: Polypropylene Syringe Extractables

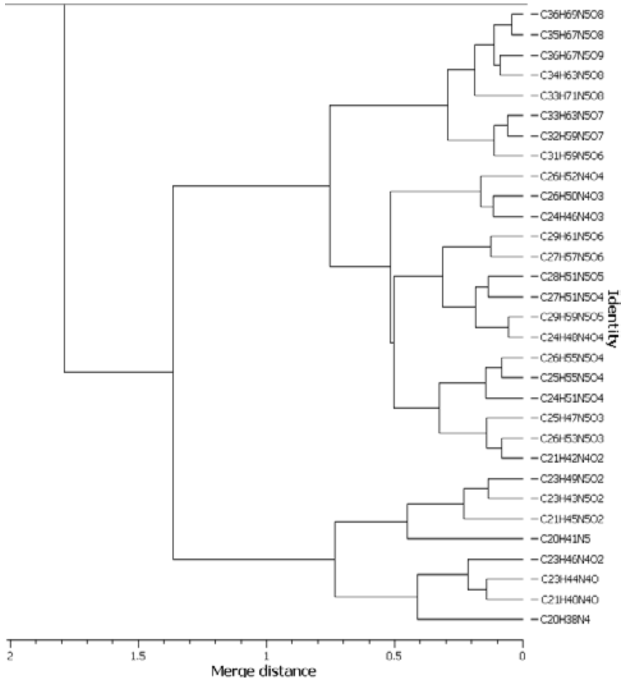
Comparative 3D van Krevelen diagrams (expanded)



Several Novel Approaches Examined, One Example: Determining Chemical Formula Relationships

- Detect monoisotopic peaks
- Determine charge state
- Generate an ***autocorrelation spectrum*** using Fourier transform methods
- Autocorrelation spectrum has peaks corresponding to ***m/z differences*** source spectrum
- Apply Kendrick mass defect methods to classify peaks based on formula relationships
- Group related compositions using hierarchical clustering or automated van Krevelen plot analysis



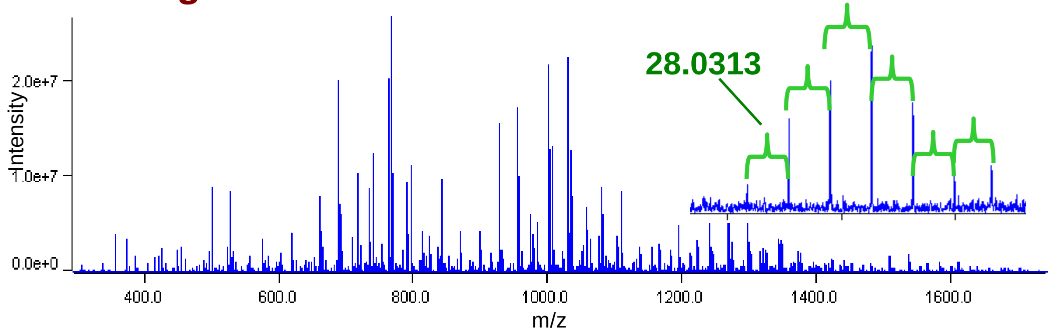


Several Novel Approaches Examined, One Example: Determining Chemical Formula Relationships

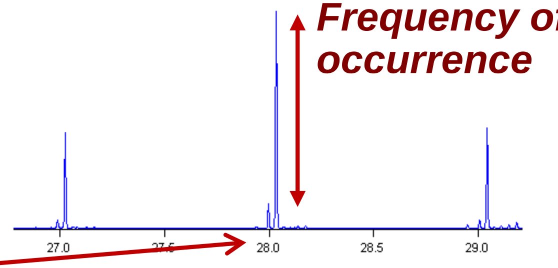
accurate m/z *difference* correlation

	Formula	.oss	Calc Mass	Expt Mass	Mono Inty	Abs Error	mDa Error	ppm Error
1	H2O		18.0106	18.0106	675963072	0.000055	0.06	3.06
2	CO		27.9949	27.9949	988126208	0.000013	-0.01	-0.47
3	C2H4		28.0313	28.0313	8958516224	0.000010	-0.01	-0.37
4	CH2O2		46.0055	46.0054	5211857920	0.000071	-0.07	-1.55
5	C3H4O		56.0262	56.0261	1176259584	0.000088	-0.09	-1.57
6	C3H6O2		74.0368	74.0367	7576710656	0.000065	-0.07	-0.88
7	C5H8O		84.0575	84.0574	799050624	0.000101	-0.10	-1.20
8	C4H6O3		102.0317	102.0316	552958464	0.000080	-0.08	-0.79
9	C5H10O2		102.0681	102.0680	5281296384	0.000079	-0.08	-0.78
10	C4H8O4		120.0423	120.0421	1193746816	0.000126	-0.13	-1.05
11	C6H10O3		130.0630	130.0629	842935104	0.000121	-0.12	-0.93
12	C7H14O2		130.0994	130.0993	1729864064	0.000091	-0.09	-0.70
13	C8H14O3		158.0943	158.0941	606668480	0.000169	-0.17	-1.07

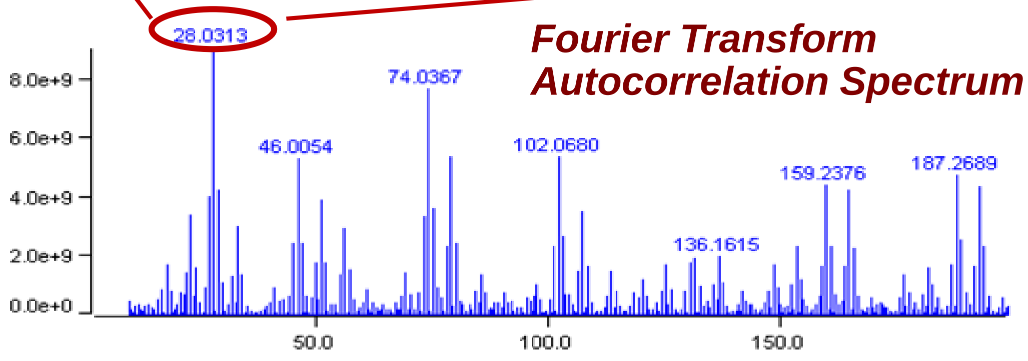
Identify monoisotopic peaks, charge state and assign chemical formula differences



Frequency of occurrence



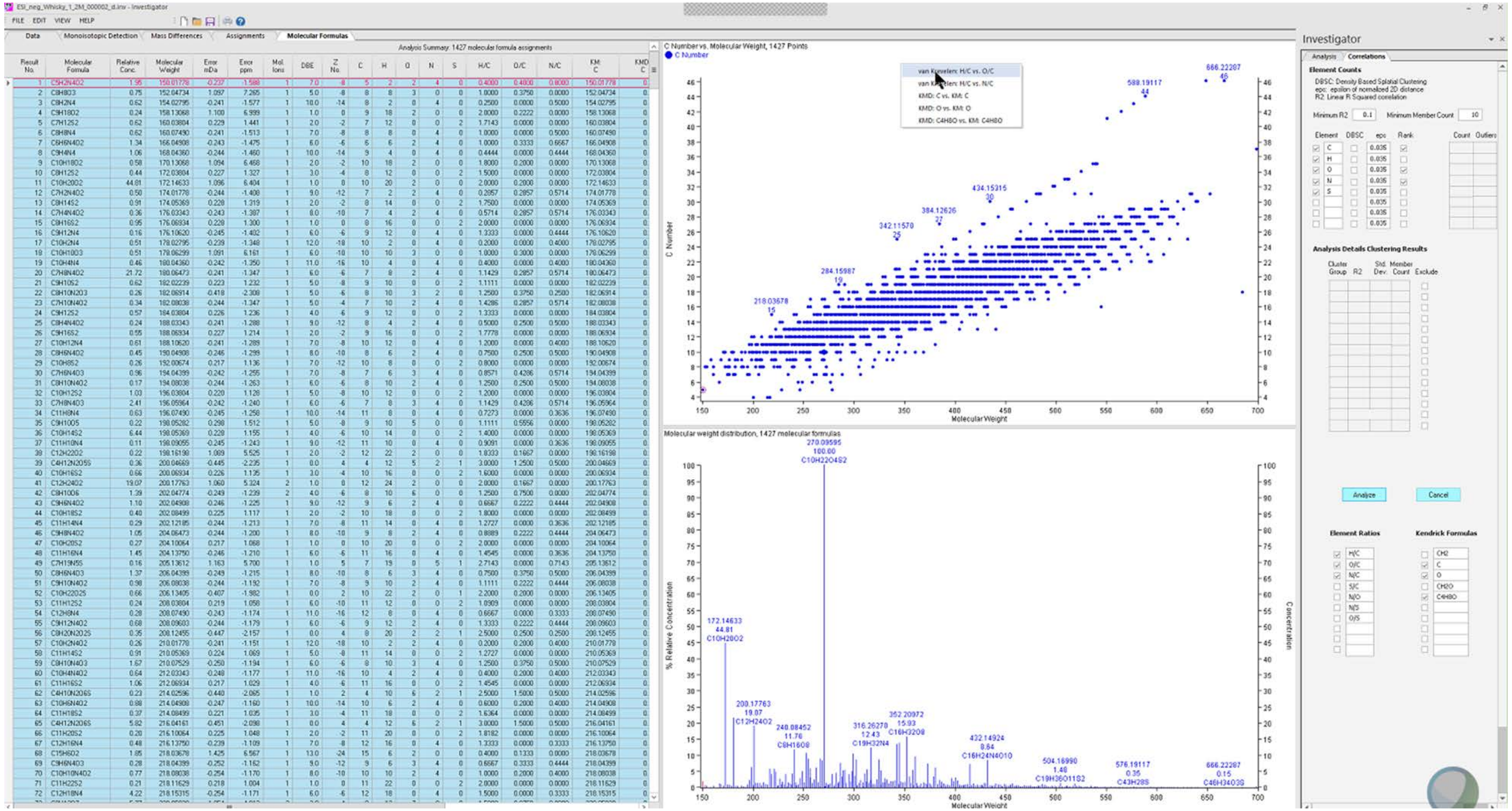
Fourier Transform Autocorrelation Spectrum



Basis for a *self-calibrating* mass map

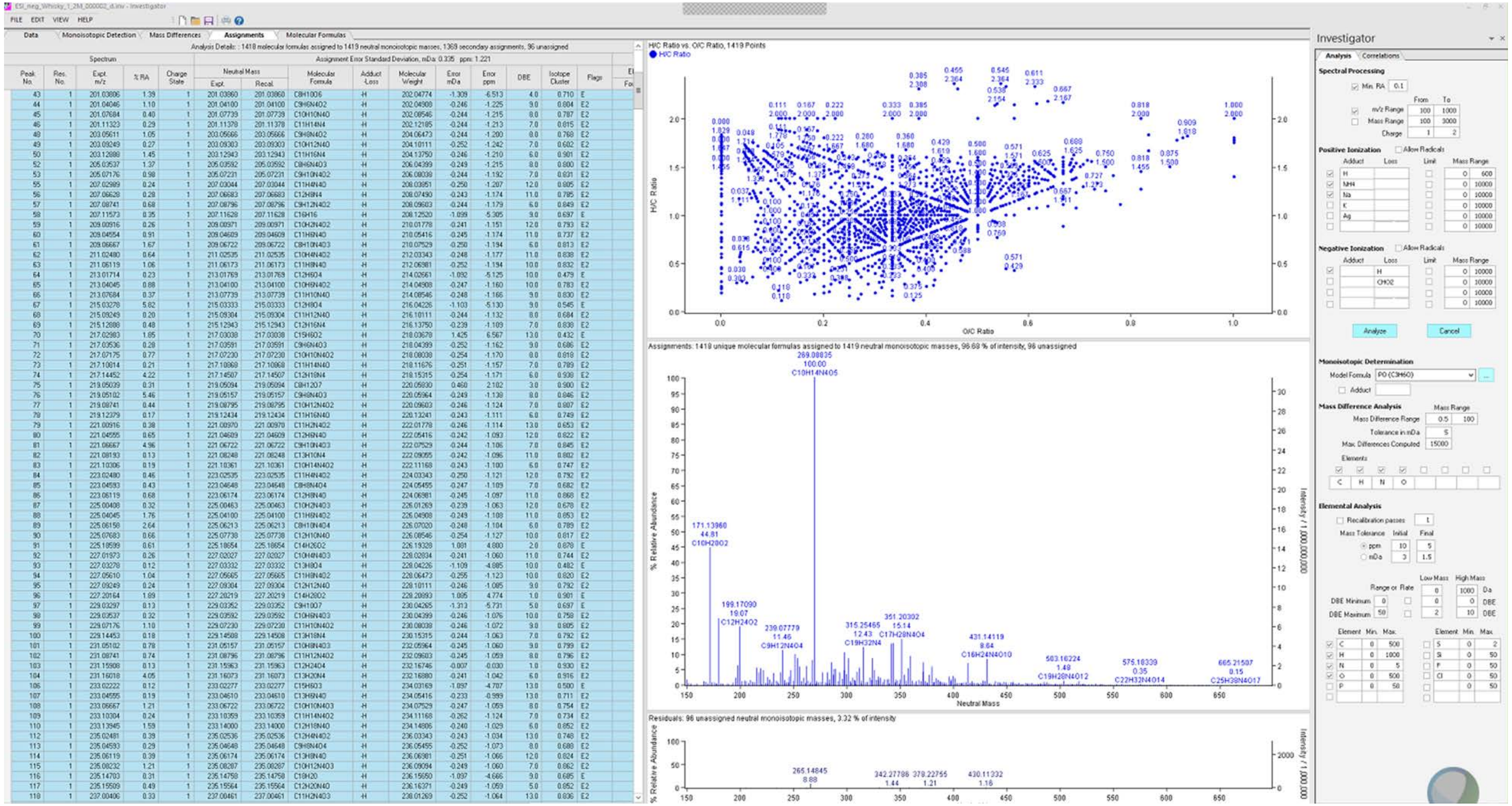
EMMA (E&L Mass Mapping Assessment) with *Investigator*

Autocorrelation and determining chemical relationships using Kendrick Mass Defect analysis

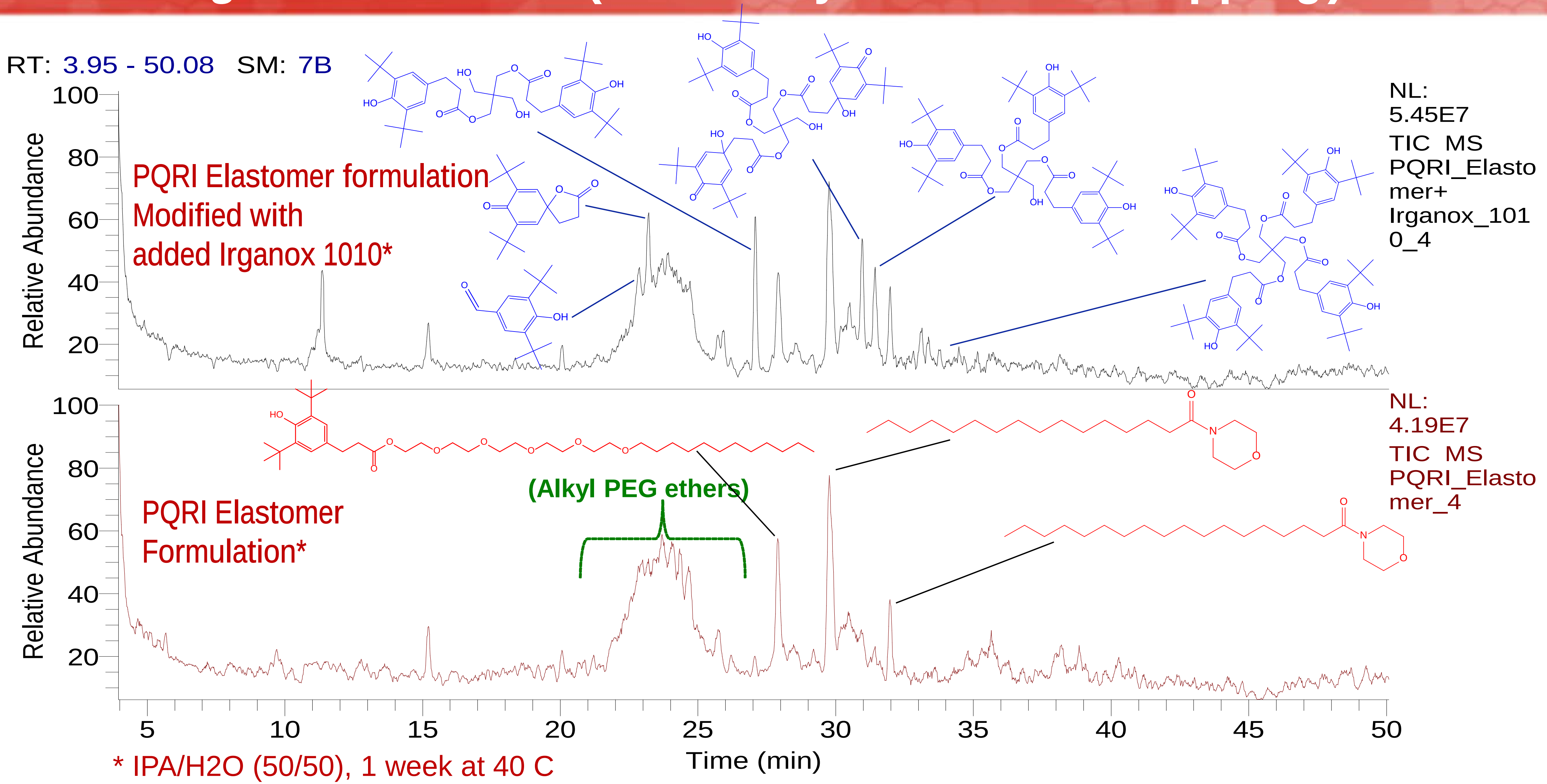


EMMA (E&L Mass Mapping Assessment) with *Investigator*

Composition and formula generation with van Krevelen plots



EMMA (E&L Mass Mapping Assessment) with *Investigator* and *SAM* (Similarity Attributes Mapping)

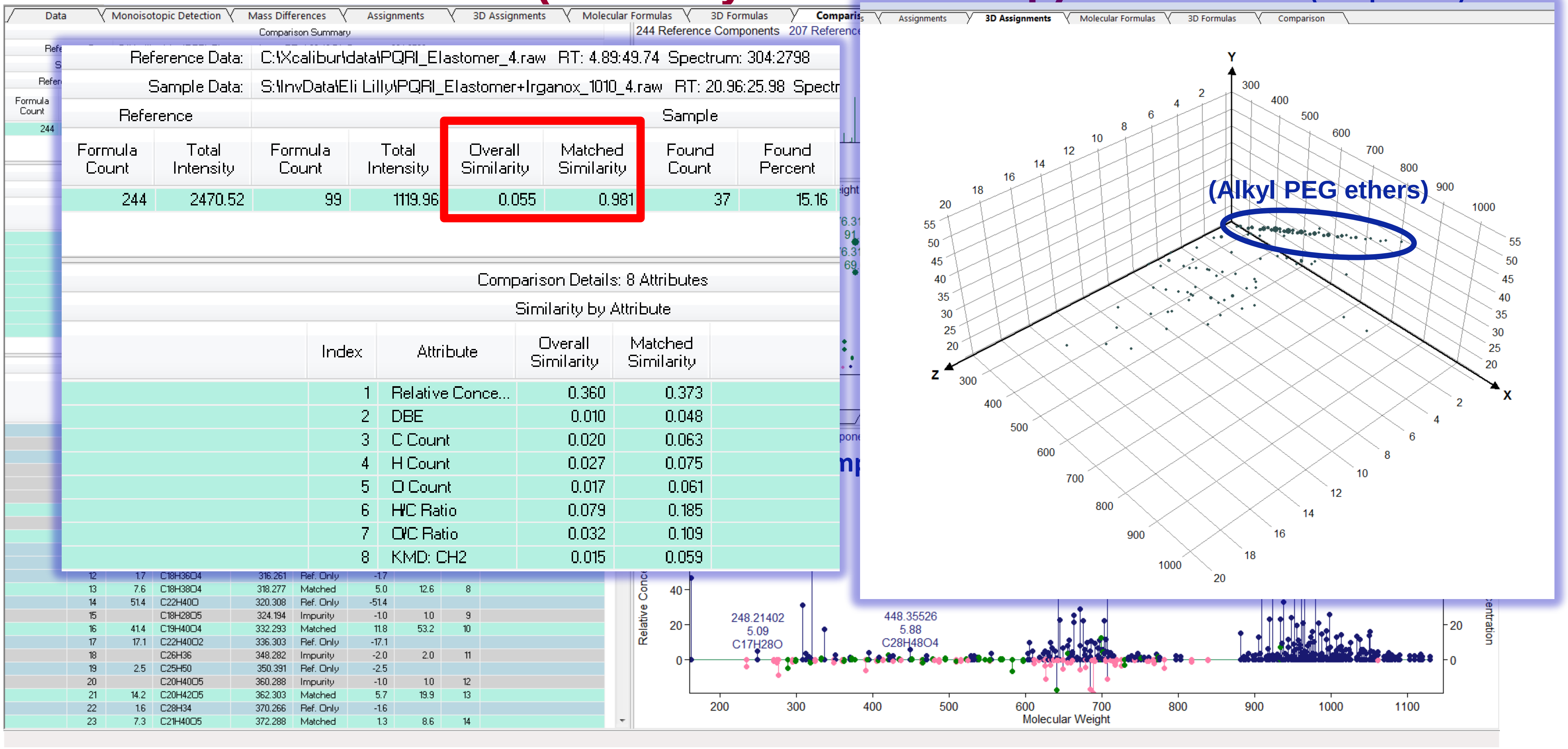


* IPA/H2O (50/50), 1 week at 40 C

EMMA (E&L Mass Mapping Assessment) with *Investigator* and *SAM* (Similarity Attributes Mapping)

SAM (Similarity Attributes Map)

Blue = reference (comparator) mixture



Reference Data:	C:\Xcalibur\data\PQRI_Elastomer_4.raw RT: 4.89:49.74 Spectrum: 304:2798
Sample Data:	S:\InvData\Eli Lilly\PQRI_Elastomer+Irganox_1010_4.raw RT: 20.96:25.98 Spectr

Reference		Sample					
Formula Count	Total Intensity	Formula Count	Total Intensity	Overall Similarity	Matched Similarity	Found Count	Found Percent
244	2470.52	99	1119.96	0.055	0.981	37	15.16

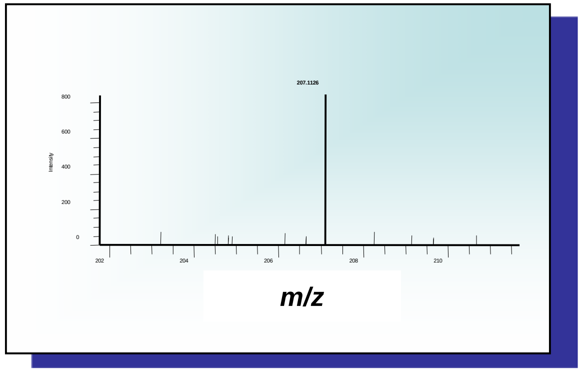
Comparison Details: 8 Attributes

Similarity by Attribute

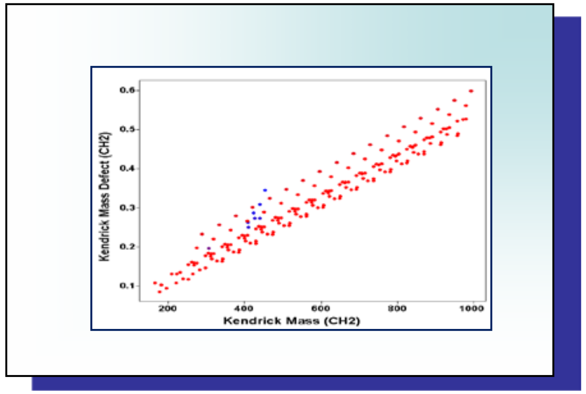
Index	Attribute	Overall Similarity	Matched Similarity
1	Relative Conce...	0.360	0.373
2	DBE	0.010	0.048
3	C Count	0.020	0.063
4	H Count	0.027	0.075
5	O Count	0.017	0.061
6	H/C Ratio	0.079	0.185
7	O/C Ratio	0.032	0.109
8	KMD: CH2	0.015	0.059

Mass Mapping and Libraries…

- **A traditional spectral library is a collection of *individual* spectra representing individual chemical entities**

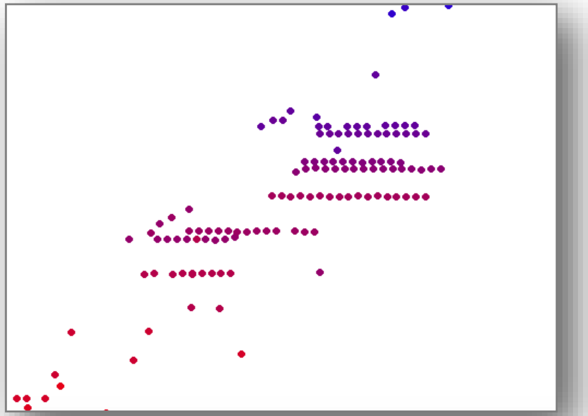


- **A mass mapping library is a collection of *sets* of spectra representing complex mixtures of structurally and compositionally diverse chemical entities, characteristic of a unique and complex material profile (e.g. elastomer or plastic fingerprint)**

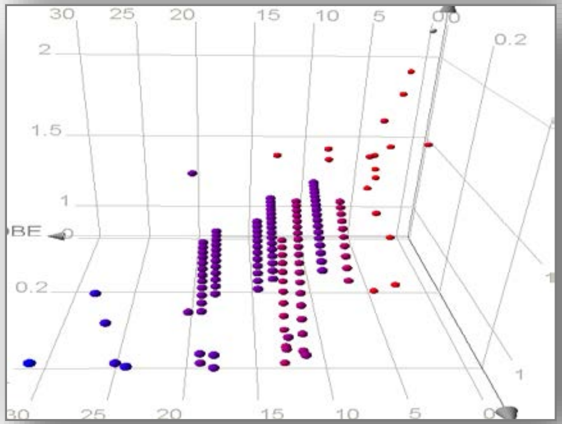


Mass Mapping and Libraries…

Standardized scannable images (e.g. “QR Codes”) represent unique, highly complex mixture and material “fingerprints” that can be compared both qualitatively and quantitatively



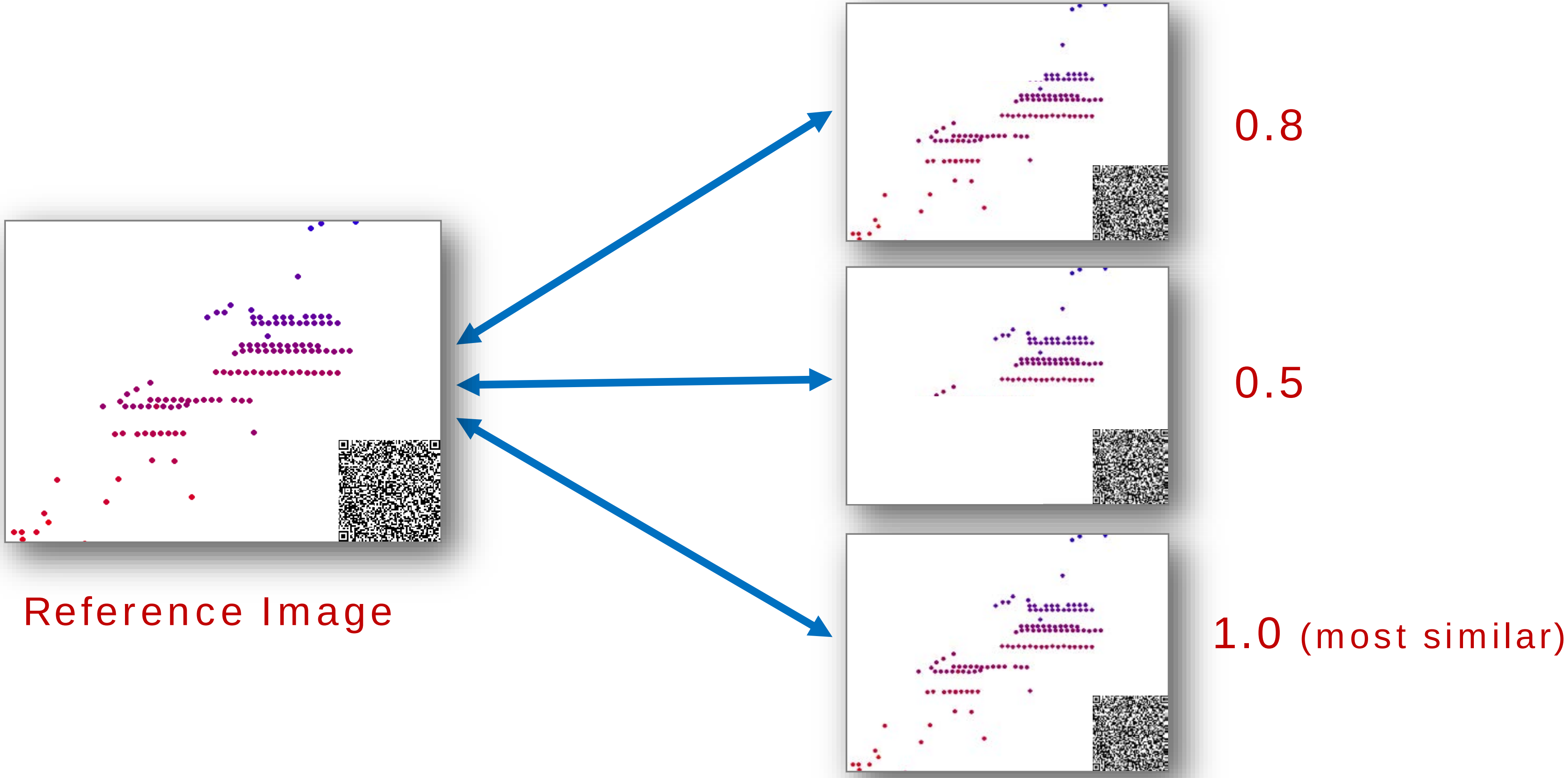
2-D



3-D

Mass Mapping and Libraries…

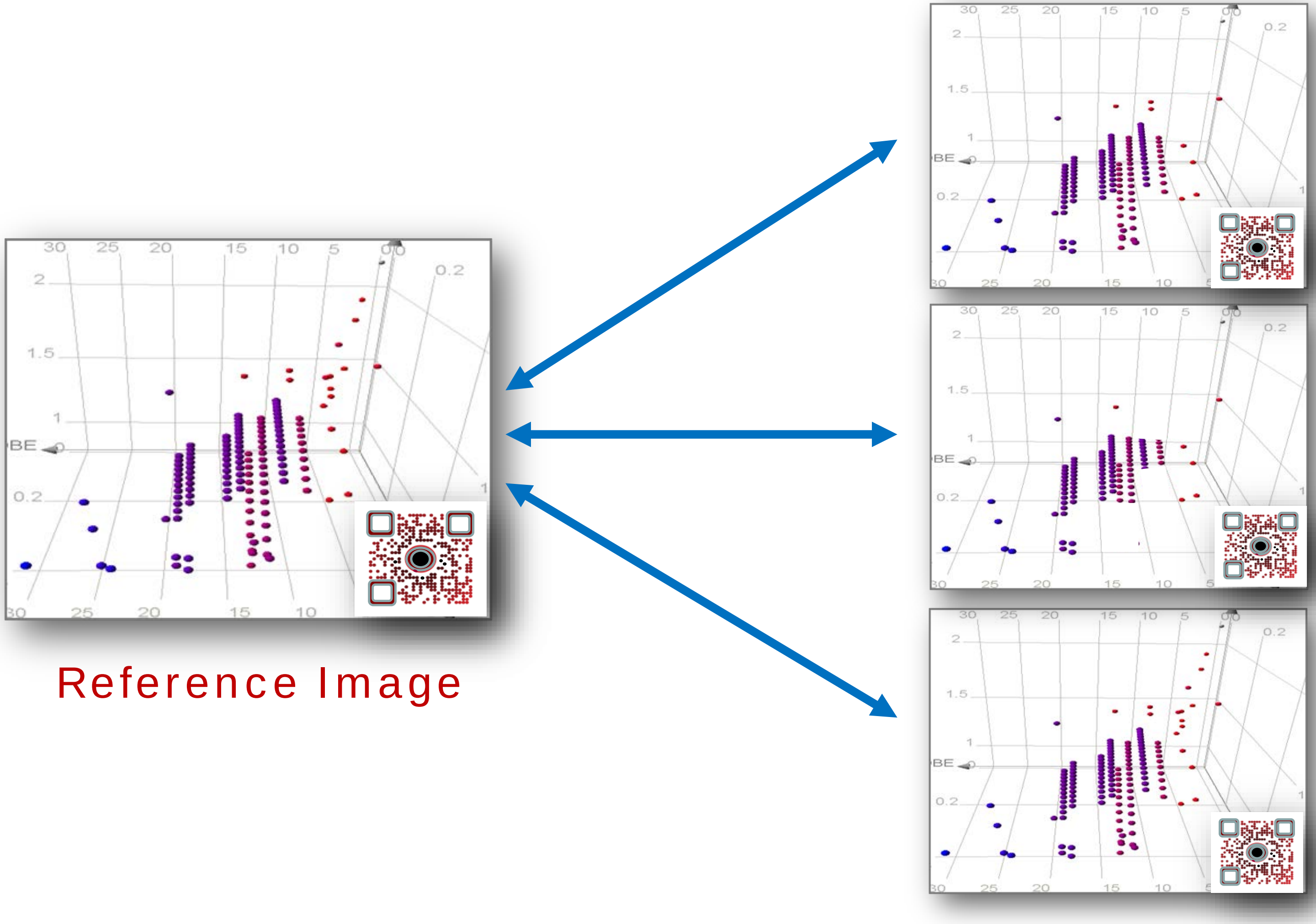
Pairwise or batch comparison with a reference material and index of similarity or dissimilarity



Reference Image

Mass Mapping and Libraries…

Likewise, 3-D…



Reference Image

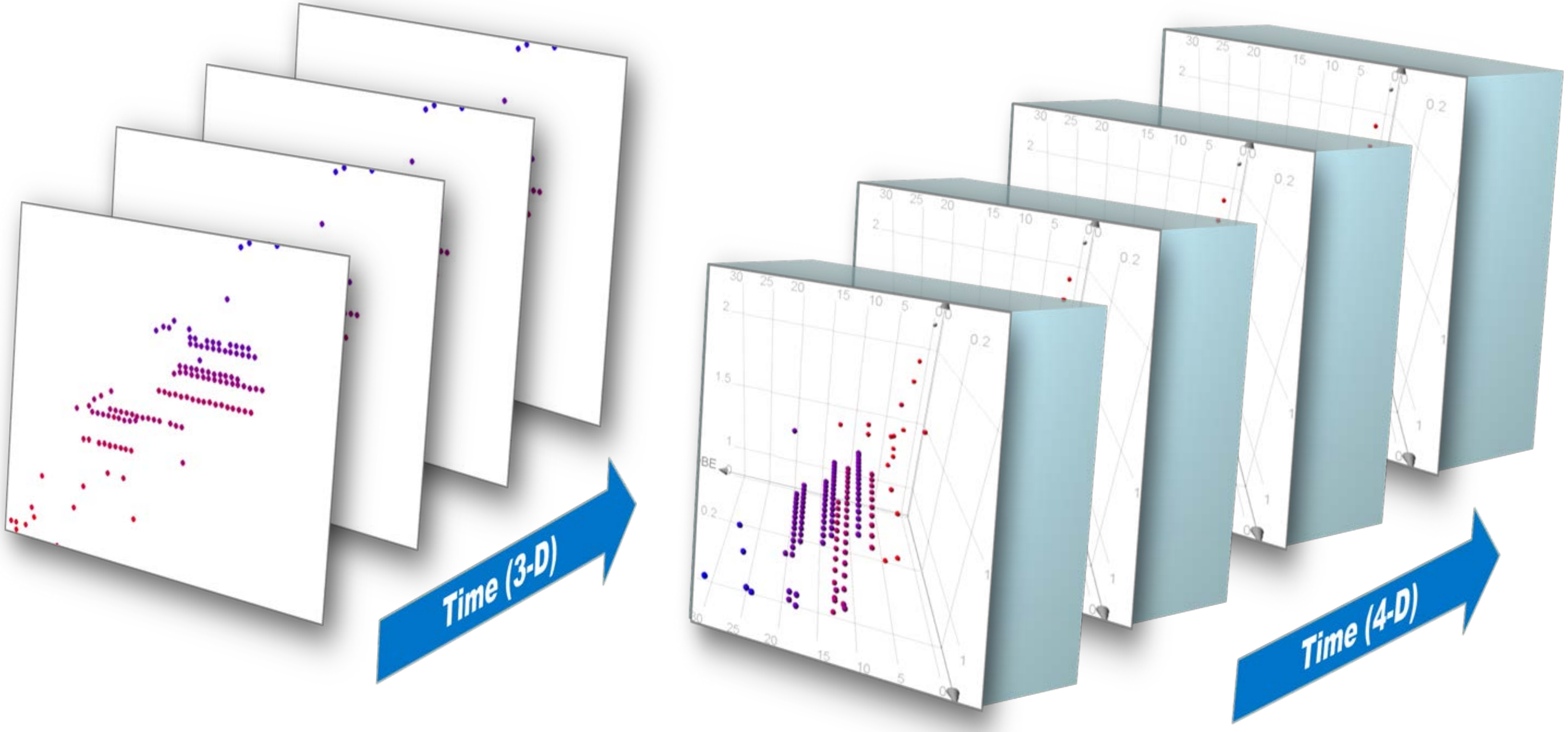
0.8

0.5

1.0 (most similar)

Mass Mapping and Libraries…

High resolution digitized image grouping to evaluate change over time (pixelated 2-D or voxelated 3-D image)



In Conclusion, Mass Mapping Enables…

- Simple, intuitive approach to enabling rapid “at-a-glance” heuristic assessment and visualization of highly complex mixtures of structurally and compositionally diverse chemical entities
- Graphically sorting complex mixture components by series or class based on structural and compositional features
- Confident assignment of elemental composition to higher masses with initial assignment of elemental composition to a limited number of lower MW components
- Assembly of N-dimensional self-calibrating mass maps based on mass differences
- Rapid and comprehensive pairwise and batch comparison with an index of similarity or dissimilarity

  - Change management
  - Failure mode analysis

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In memory of Brian McCarry, Ph.D. (1946-2013)
Friend and Collaborator

Thank you!

Assumptions

- ✓ Positive and negative ion mass spectra were acquired, but in all cases the positive ion mass spectra were much more rich in information
- ✓ Elemental compositions can be assigned typically with < 1 ppm error depending on position in mass range
- ✓ Most organic compounds have even mass (depending on N content), so the majority of peaks represent $[M+H]^+$ or $[M+Na]^+$ ions and are therefore observed at odd m/z values
- ✓ The majority of peaks at even m/z are ^{13}C isotope peaks of molecules with peaks at odd masses.   However, some PEG series are uniquely $[M+NH_4]^+$ adducts and are observed at even m/z
- ✓ The majority of peaks observed at odd m/z are singly charged ions, confirmed by ensuring that corresponding ^{13}C isotope peaks are present at unit mass difference
- ✓ The primary peaks observed and processed do not have two ^{13}C atoms