Data visualization approaches for rapid, heuristic interpretation of highly complex mixtures of structurally and compositionally diverse chemical entities associated with pharmaceutically relevant materials

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Visualization vs Imaging

Visualization is a technique and a process of translating information and large datasets into graphic or pictorial representations in order to promote and/or clarify communication of unique features or concepts.

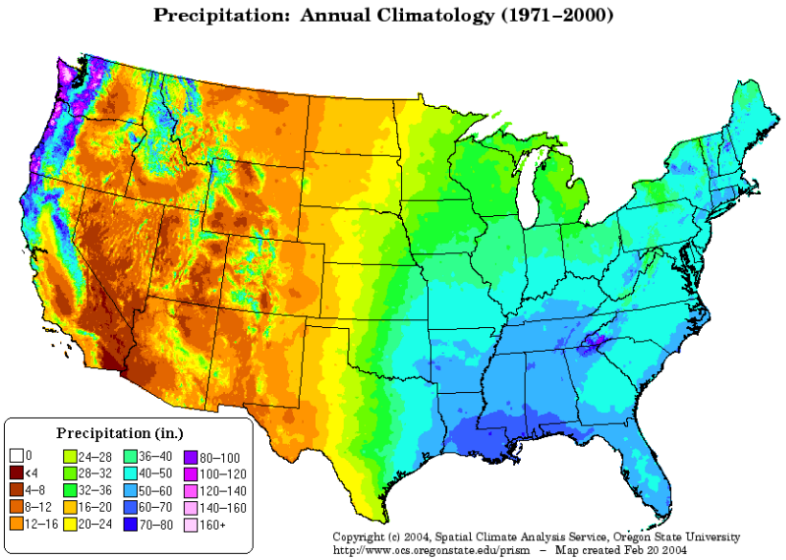
Imaging involves the capture, storage and processing of signals to produce or enhance an image or a visual representation of an object, often in 2D/3D graphical representation (e.g., pixel/voxel rendering).

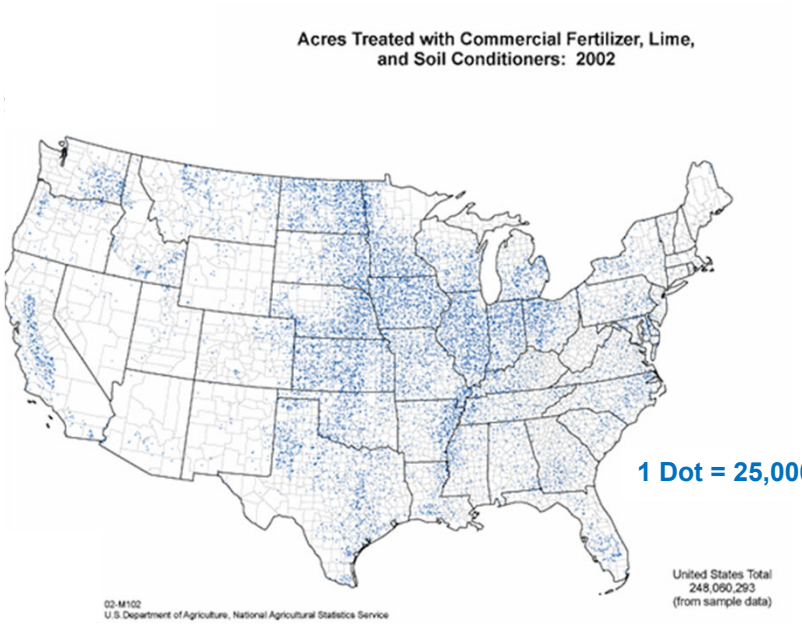
A map is an example of a visualization involving diagrammatic representation or collection of data illustrating relationships, characteristics, distribution, size, number or spatial arrangement of unique features over a given area and according to a chosen scale.

- Maps can be two-dimensional representations of three-dimensional space or can be three-dimensional.

Some examples. . .

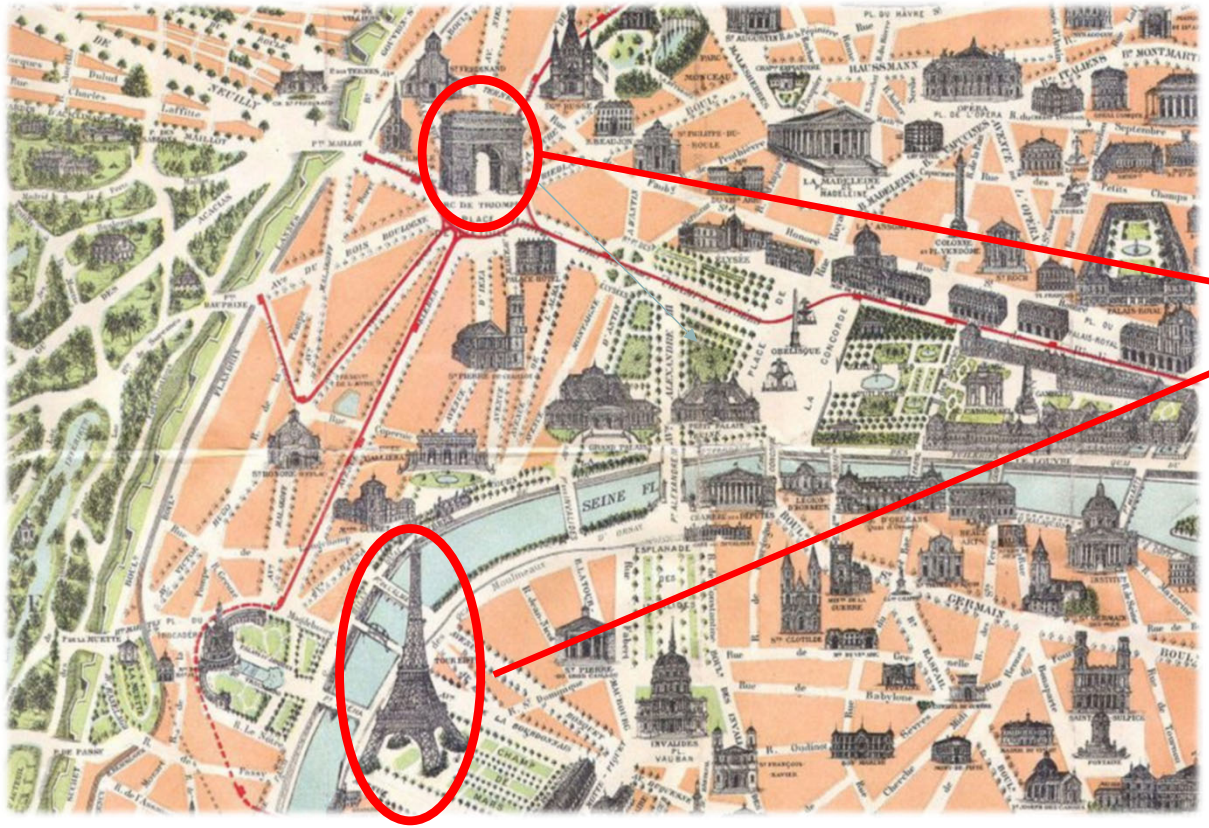
Semi-quantitative …and quantitative maps





Some examples. . .

A qualitative map: Paris, 1900



Intended to bring specific features to the viewer's attention

Complex Molecular Mixtures. . .

Contain many components across a range of concentrations

- **Petroleum products (fuels, polymers, etc.)**
- **Natural organic matter (dissolved marine organic matter)**
- **Biological materials (cell lysates, fermentation media, metabolites, etc.)**
- **Formulated drugs and pharmaceutical materials**




Complex Molecular Mixtures. . .

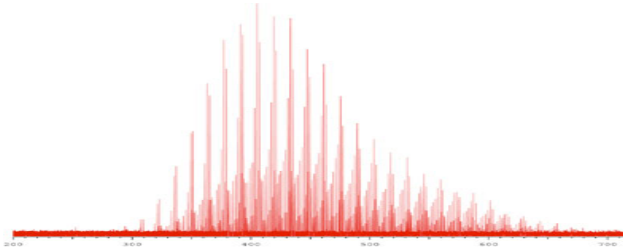
…of pharmaceutical relevance typically include:

- 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



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
- Biocides and fungicides
- Catalysts
- Heat stabilizers
- Colorants

Structural Diversity

Compositional Diversity

Extractables can also include polymer fragments, monomers, and oligomers

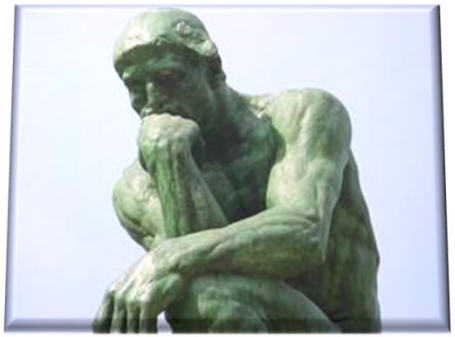
Molecular features contributing to compound diversity

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	N-3	N-2	N-1	N
	• Bulk process chemicals • Storage stabilizers	• Initiators/Catalysts • Storage stabilizers • Antioxidants • Processing aids	• Antioxidants • Processing aids • Light stabilizers • Antistatic agents	• Colorants • Processing aids • Lubricants

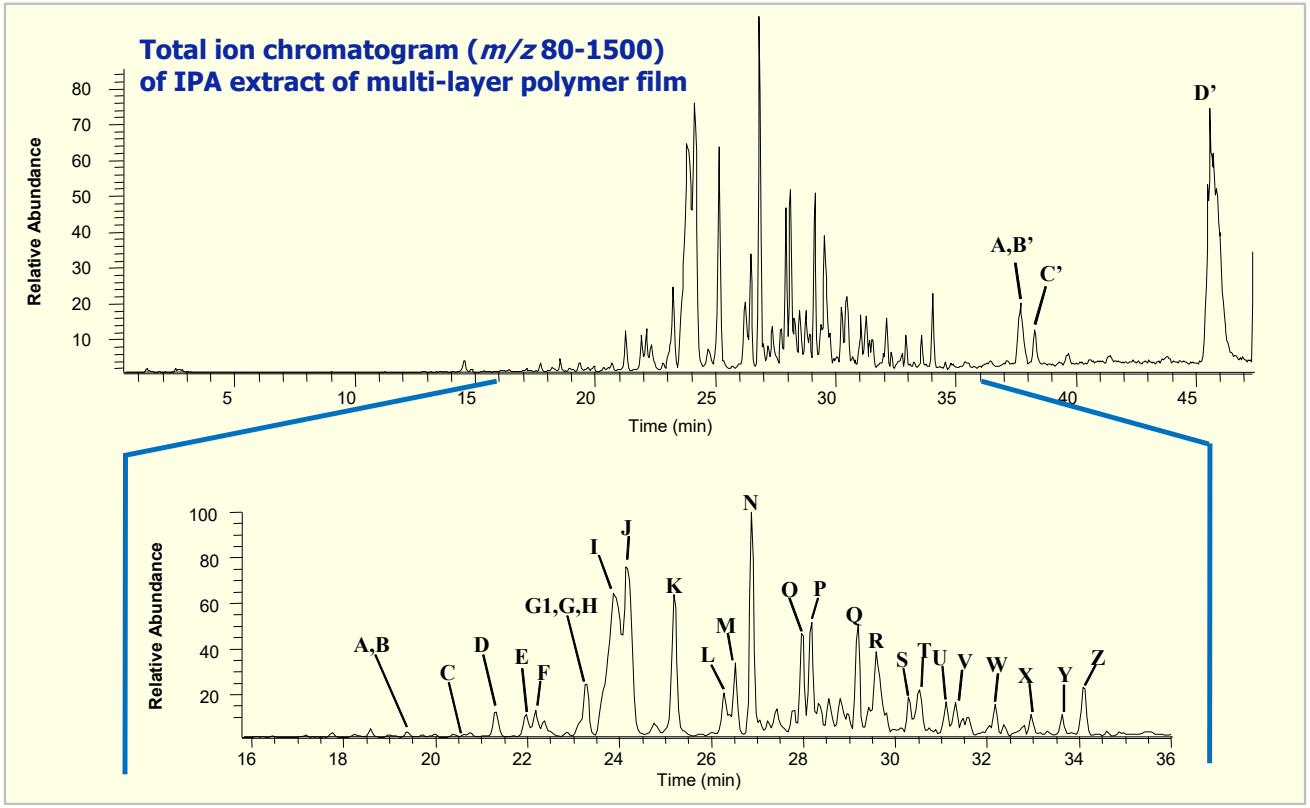
Components for
- Packaging
- Devices
- Container/closure
- Processing

May be cumulative!

Complex Molecular Mixtures. . .

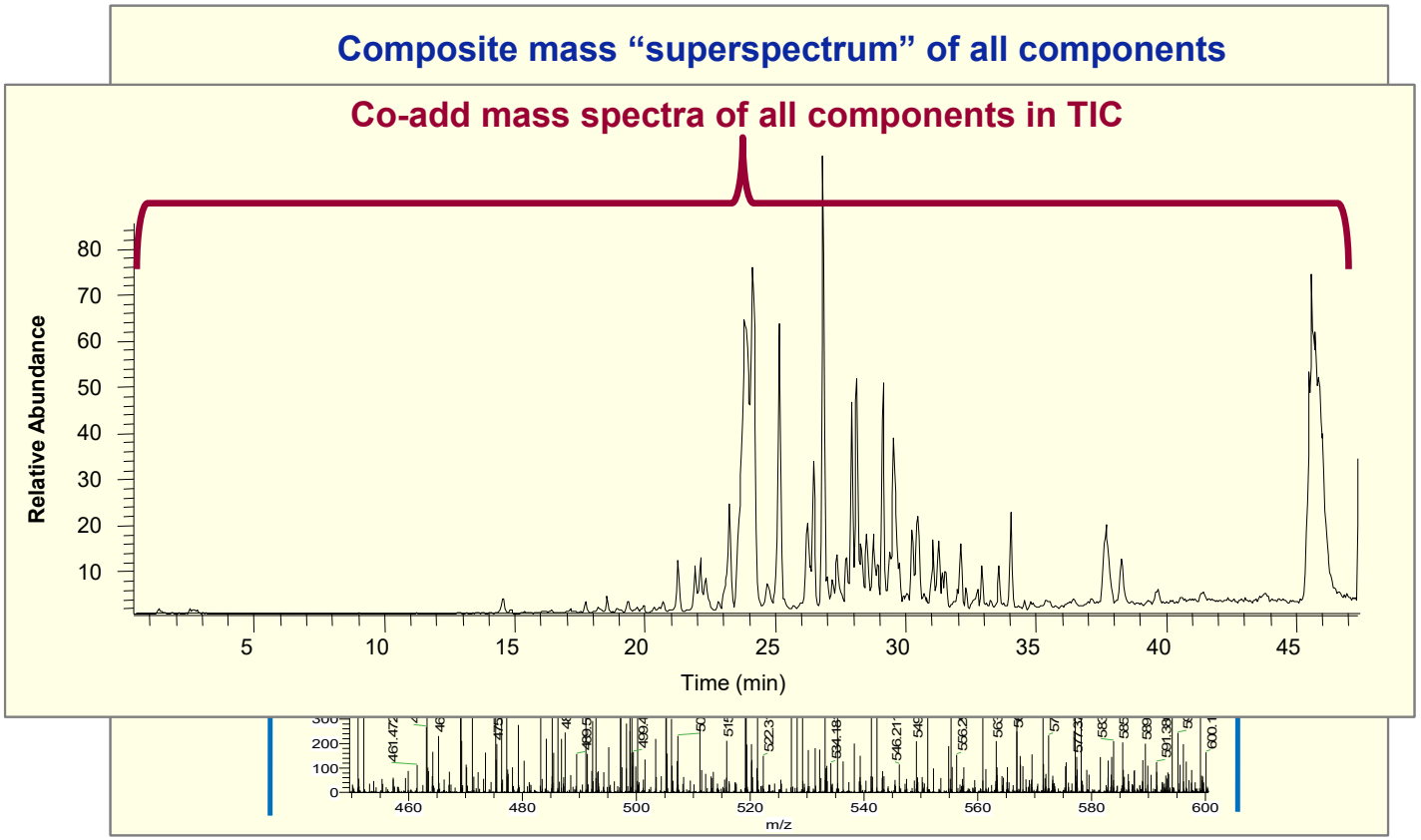
How Complex is "Complex?"

Chromatographic complexity



How Complex is "Complex?"

Mass spectral complexity



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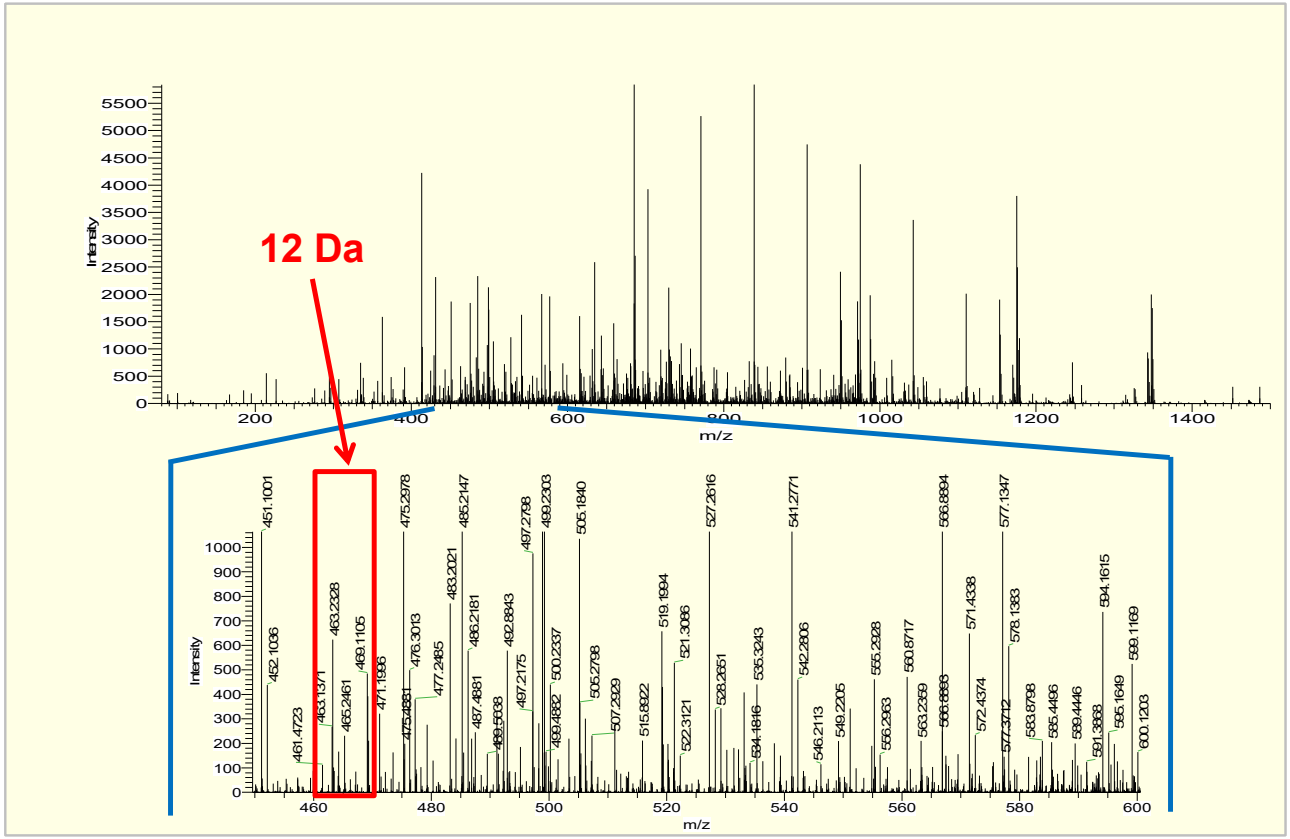
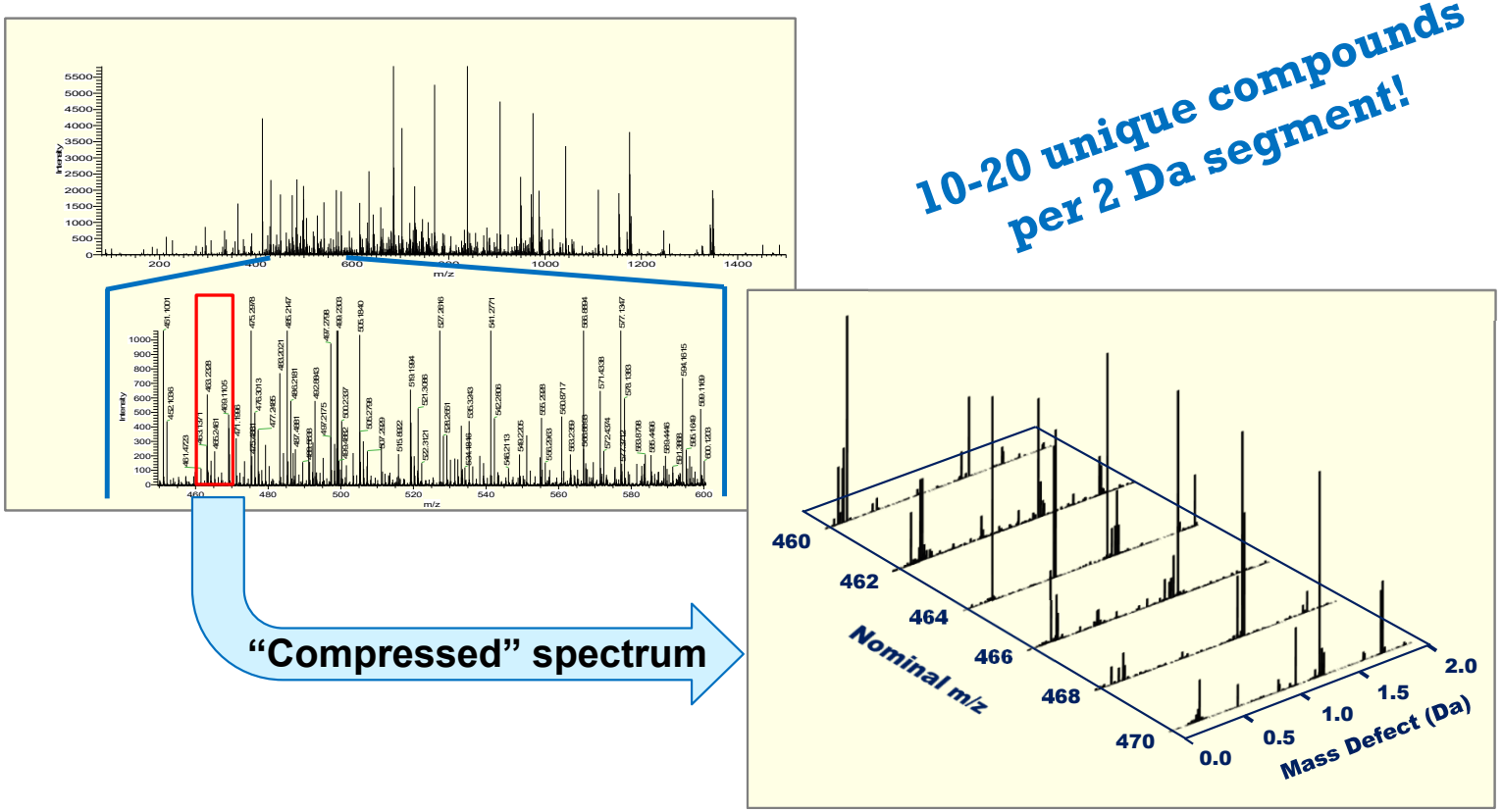
Mass spectral complexity

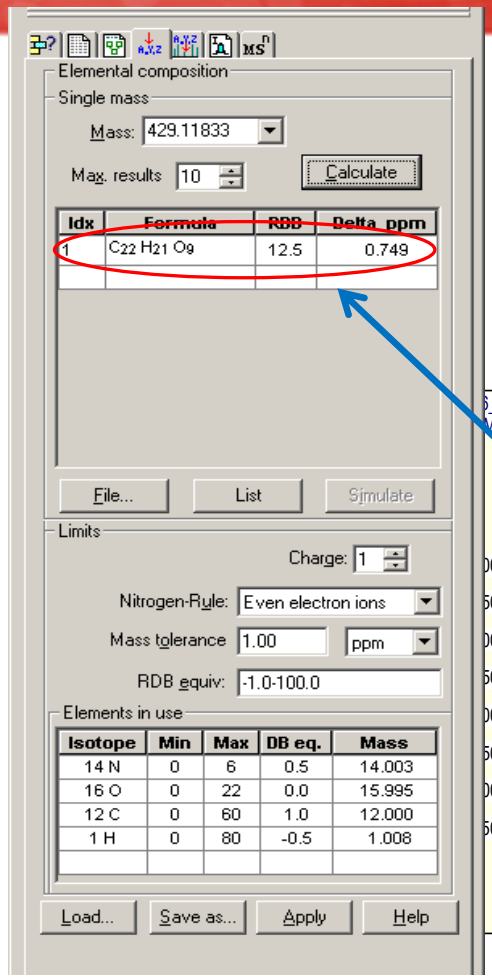


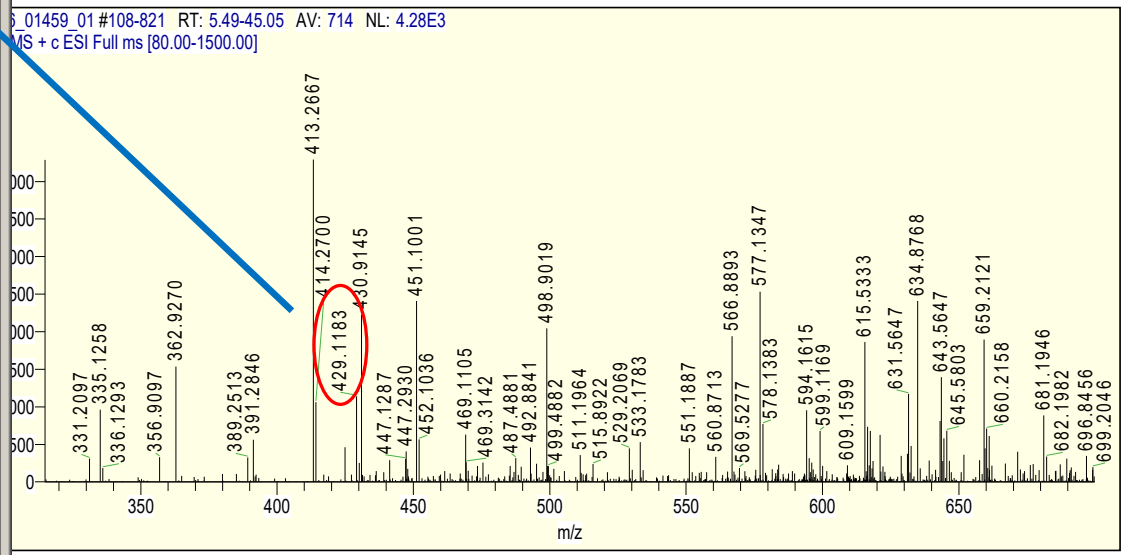
How Complex is "Complex?"

Mass spectral complexity



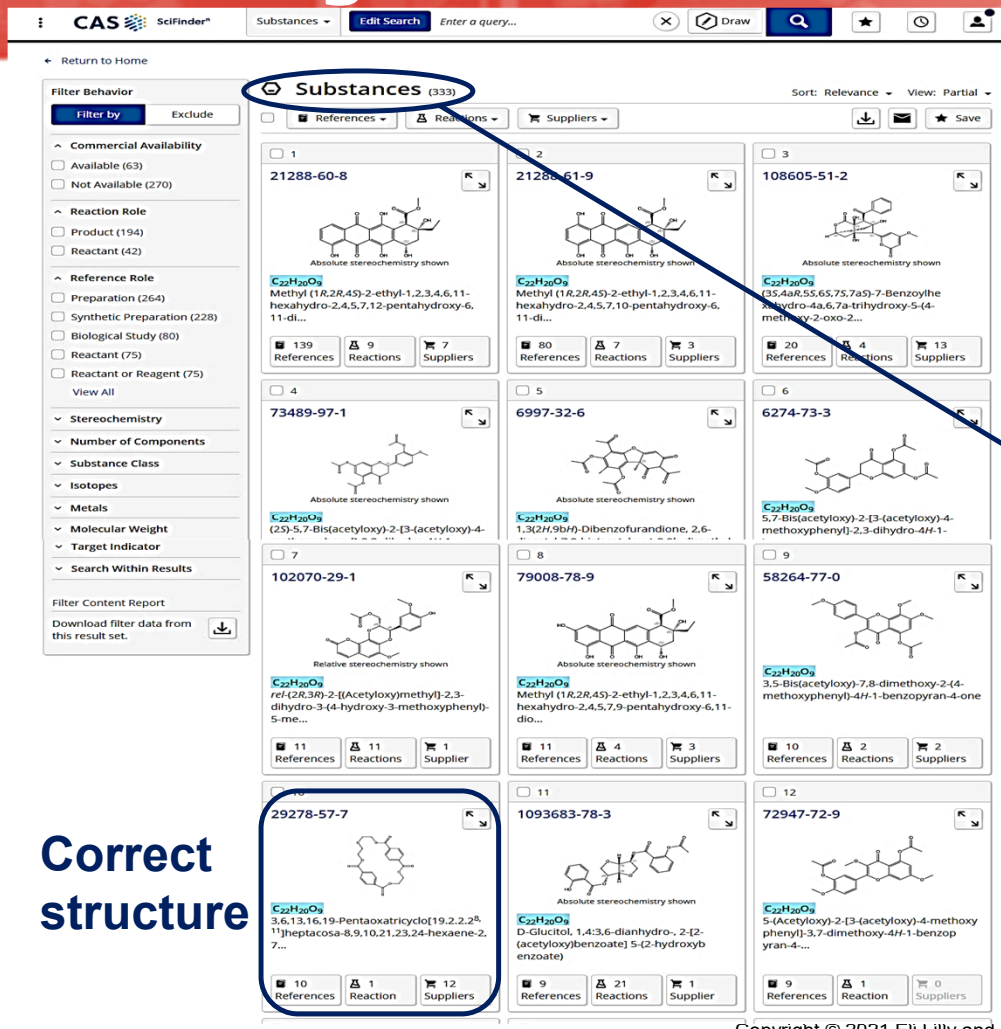
To Extol the Virtues of Accurate Mass Measurement…





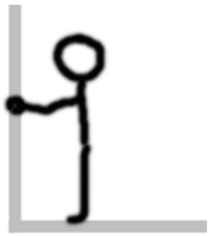
<1 ppm mass accuracy narrows possible choices for elemental composition…

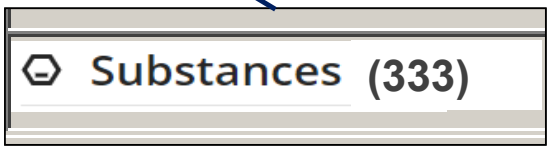
Correlating Structure with Formula



Correct structure

...but many structures are possible...





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

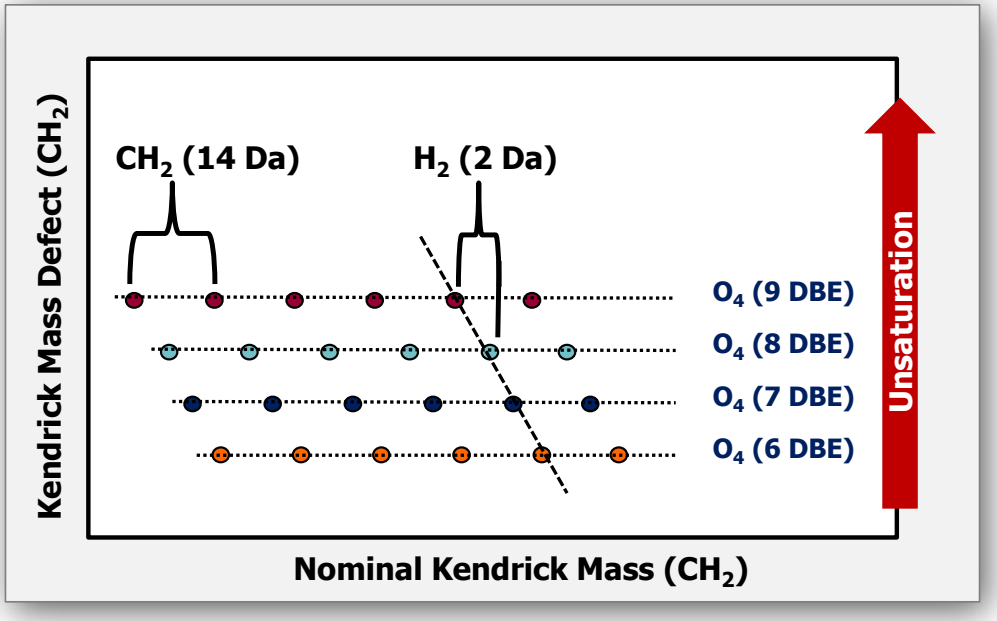
Some Tools for Visualizing Complex Molecular Mixtures: Kendrick Mass Defect Diagram

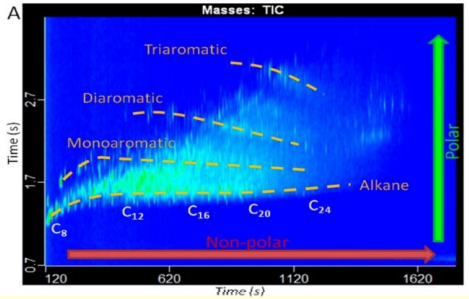
Kendrick mass defect diagram (2D or 3D modified)

– 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)

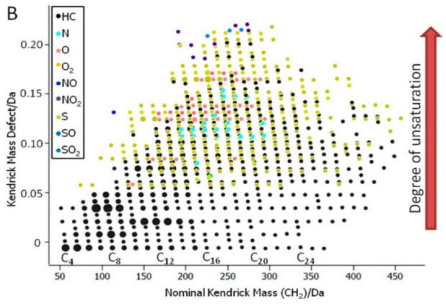
– Sorts compounds into homologous series by compound "class" (numbers of O, N, other heteroatom)

– Compound type (rings and double bonds) and degree of alkylation (CH_2 groups)





GC x GC ToFMS, heavy crude oil



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

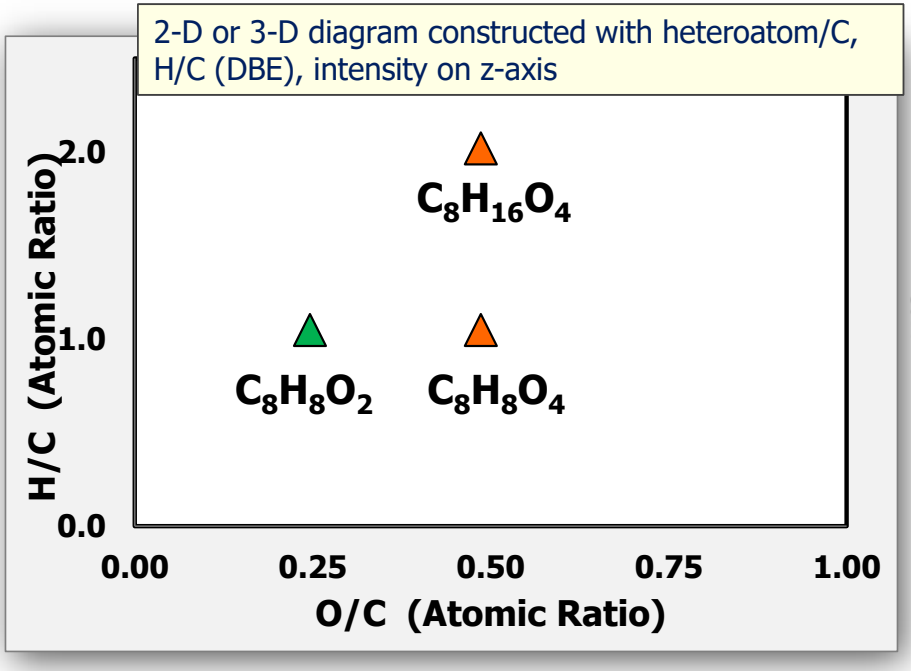
Some Tools for Visualizing Complex Molecular Mixtures: van Krevelen Diagram

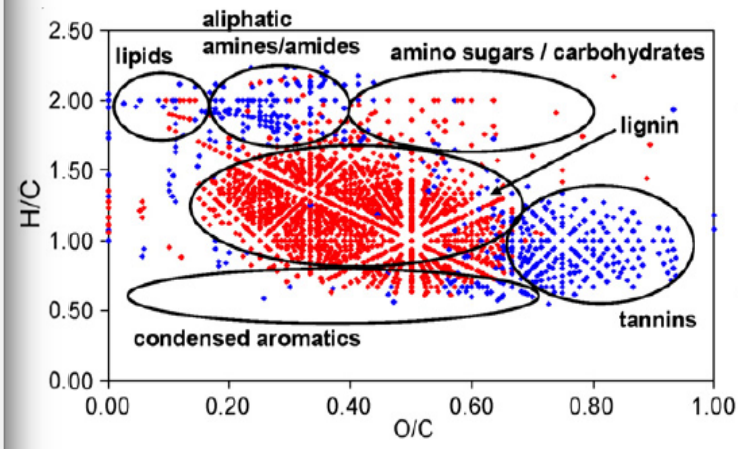
van Krevelen diagram (2D or 3D modified)

– Projects elemental composition according to H/C and heteroatom/C atomic ratios

– Compounds are classified according to degree of saturation, alkylation, heteroatoms, dehydration, deamination

– Compositional differences are amplified permitting classification, prediction of composition and origin



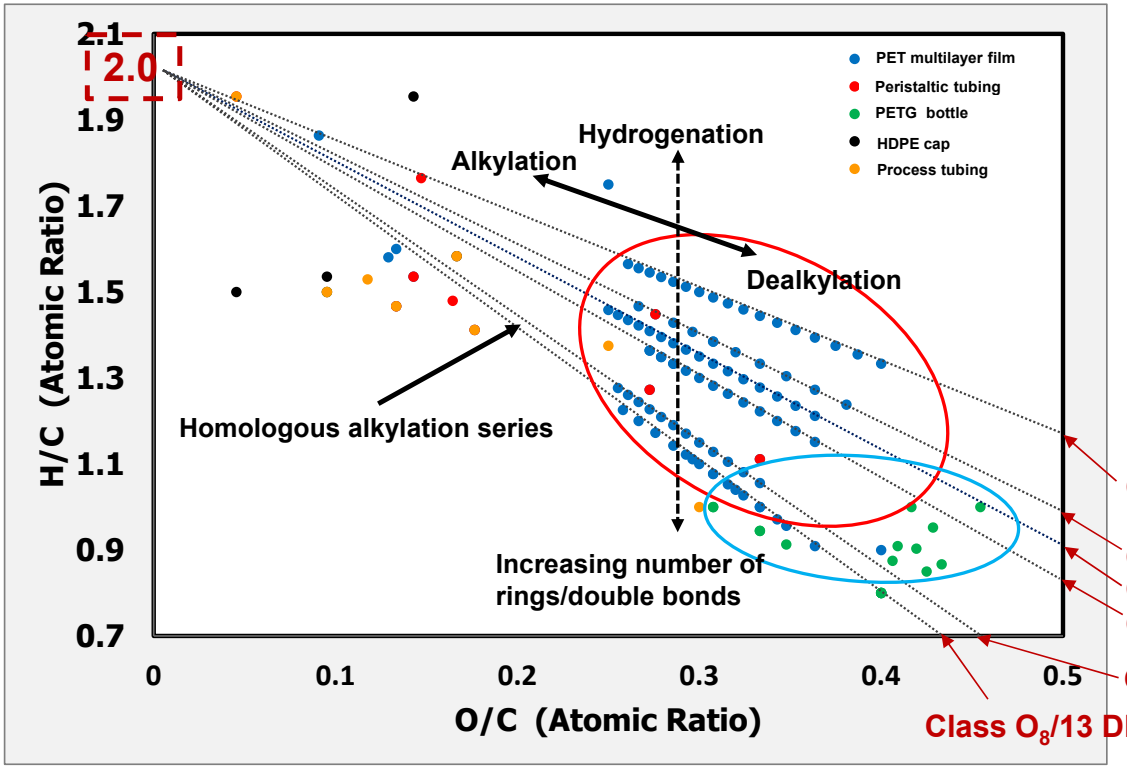


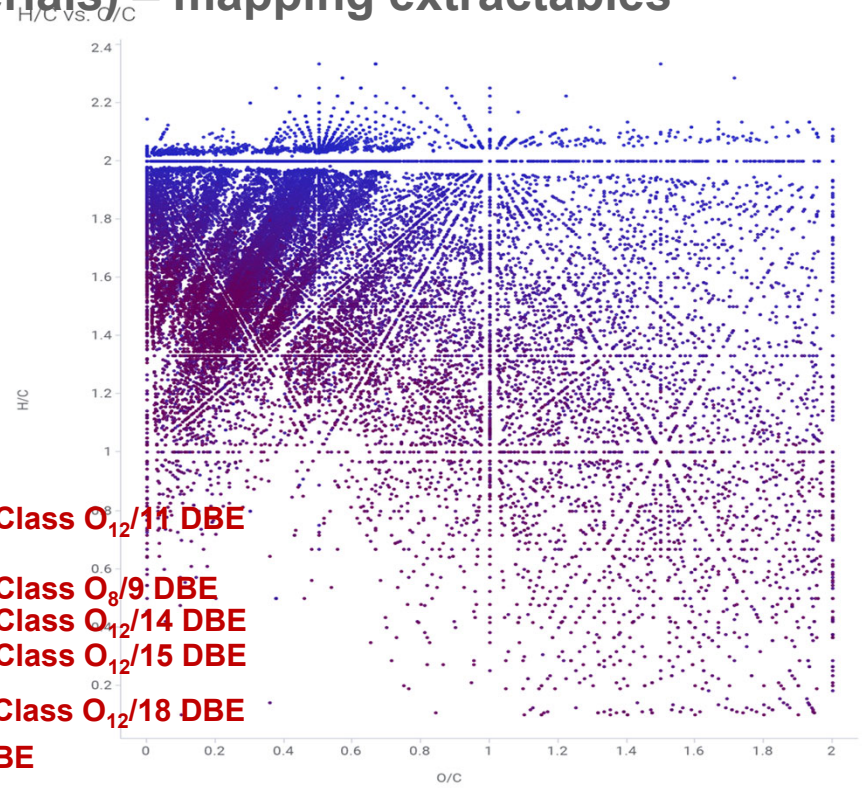
Dissolved organic matter

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

van Krevelen Diagram: Visualizing Relationships

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



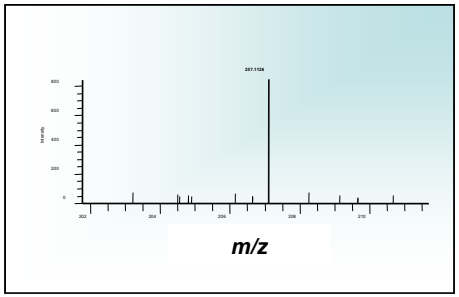


For any compound series $C_{c\text{-}n}H_{h\text{-}2n}O_o$: $y = (h\text{-}2n)/c\text{-}2n$ and $x = o/(c\text{-}n)$ ⇨ $y=2+(h\text{-}2c)/ox$

Spectral Map Libraries

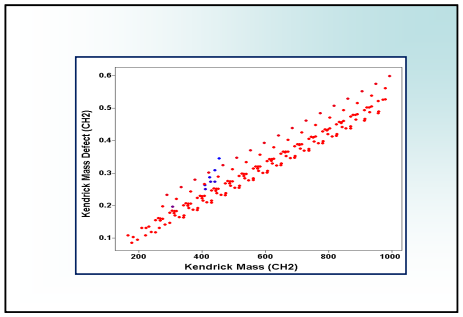
- **A traditional discrete spectral library is a collection of *individual* spectra representing individual chemical entities**

Each library entry represents a single, ***unique compound***



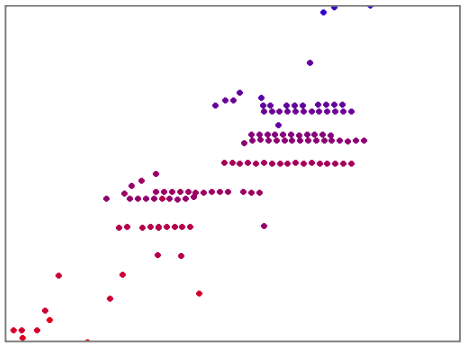
- **A spectral map library is a collection of *sets* of spectra representing visualizations of complex mixtures of structurally and compositionally diverse chemical entities**

Each library entry represents a ***unique extractables profile*** corresponding to a component or material

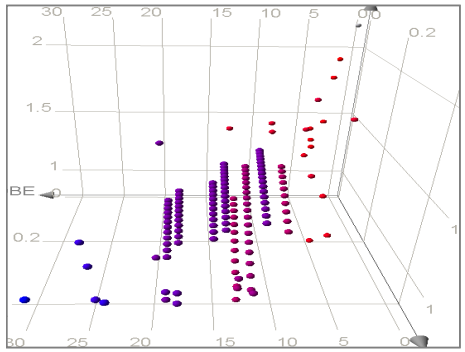


Spectral Map Libraries

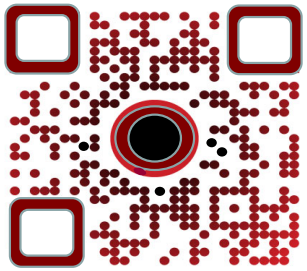
Standardized visualizations saved as QR Codes represent unique, highly complex mixture and material "fingerprints" that can be compared both qualitatively and quantitatively



2-D

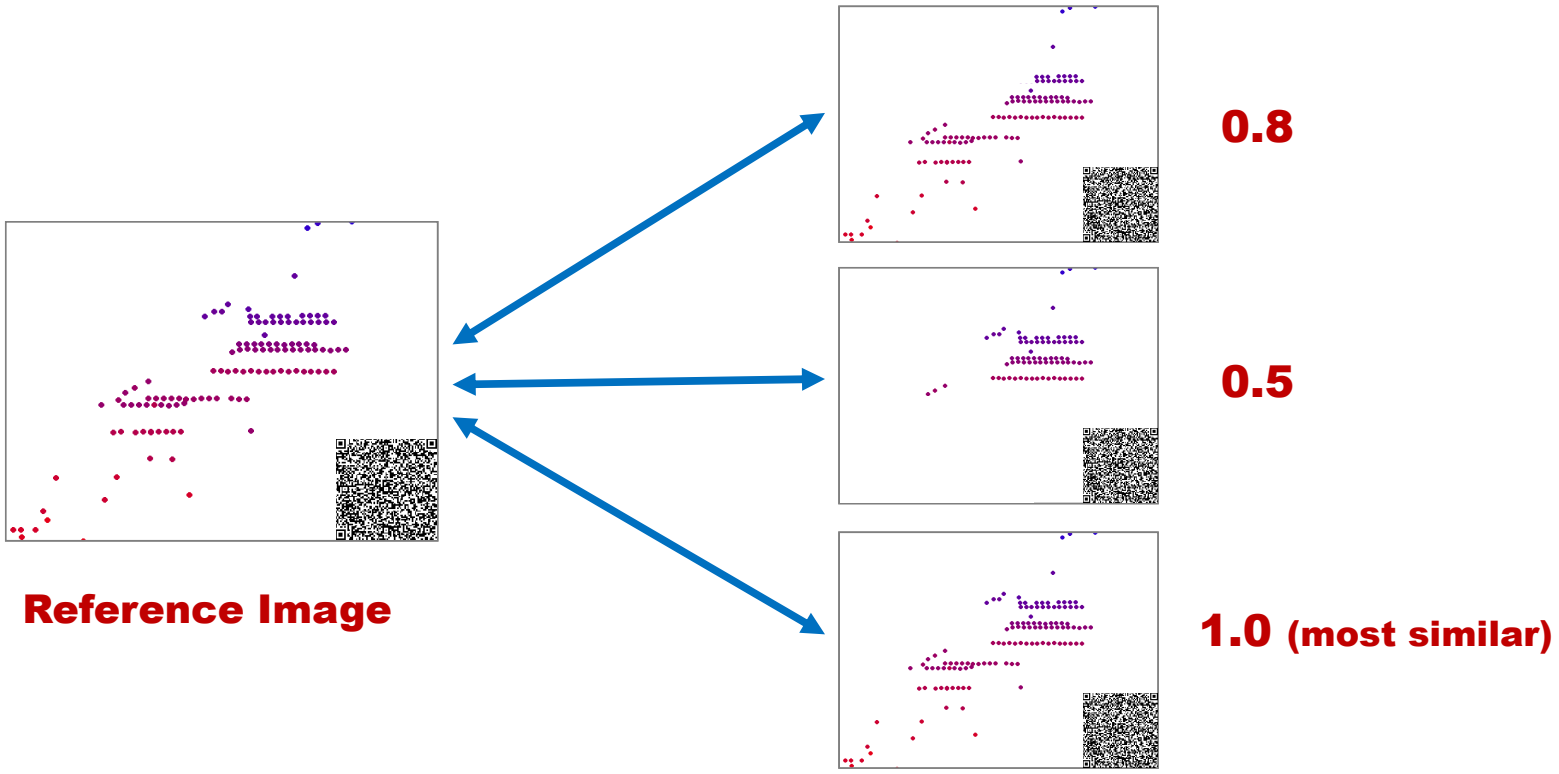


3-D



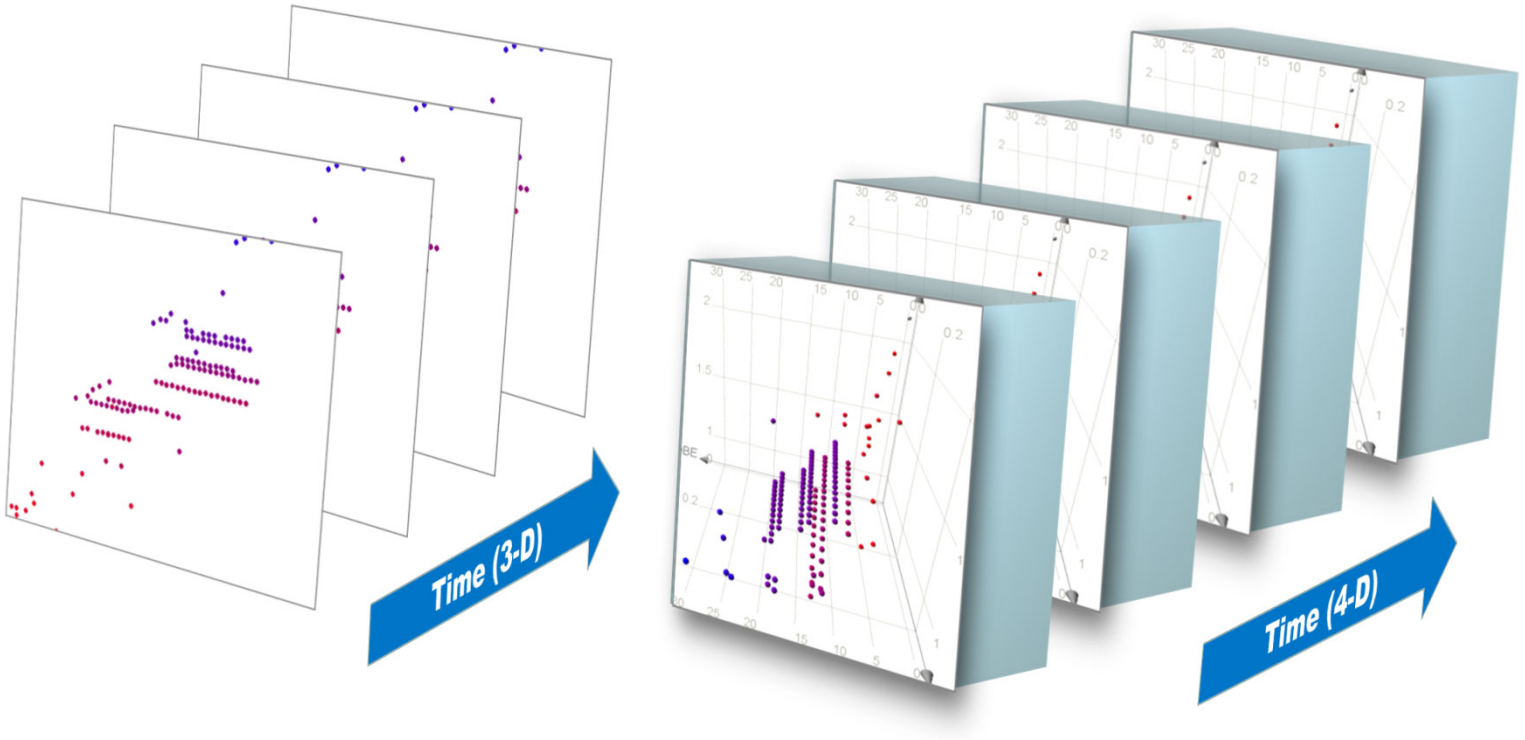
Spectral Map Libraries

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

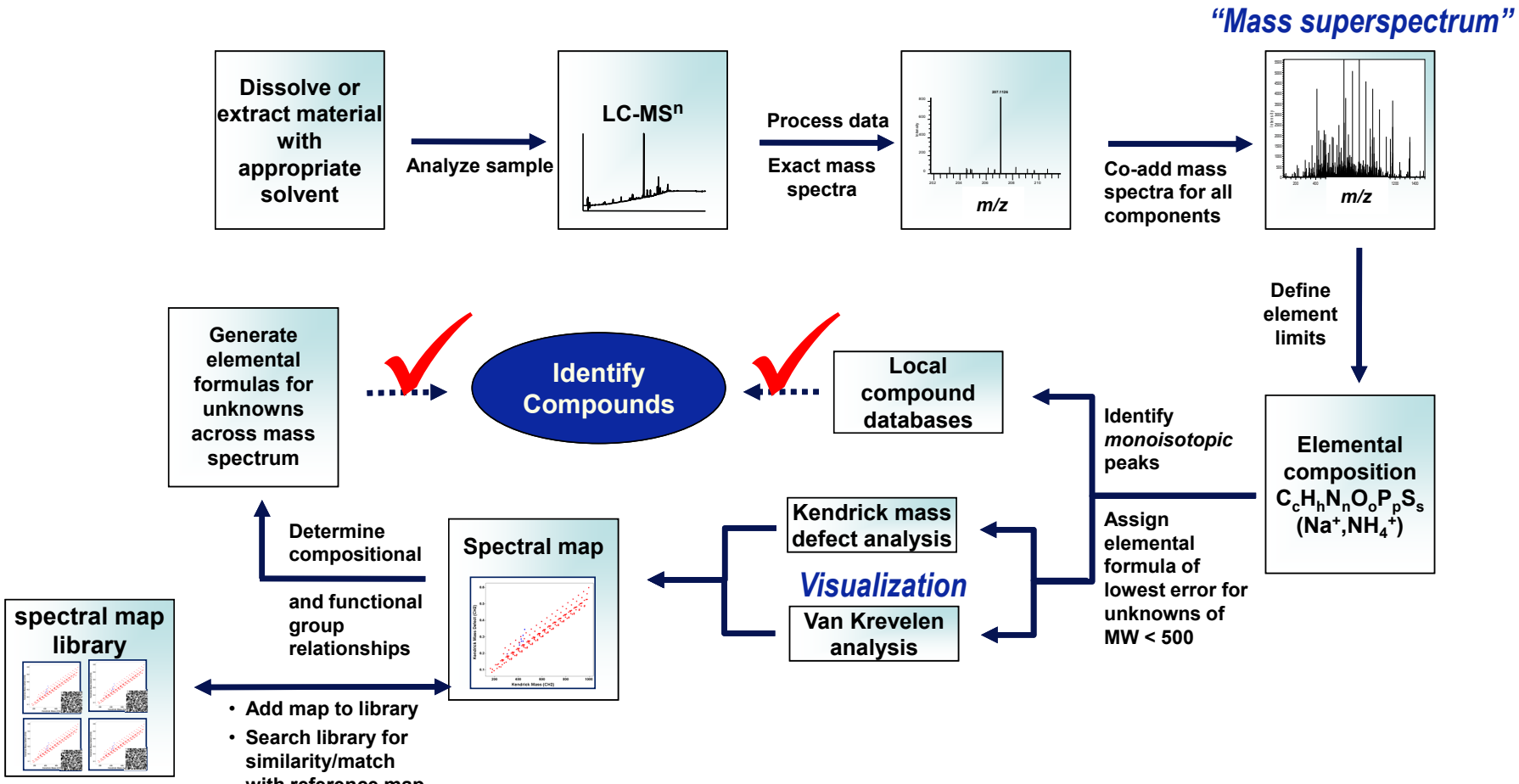


Spectral Map Libraries

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

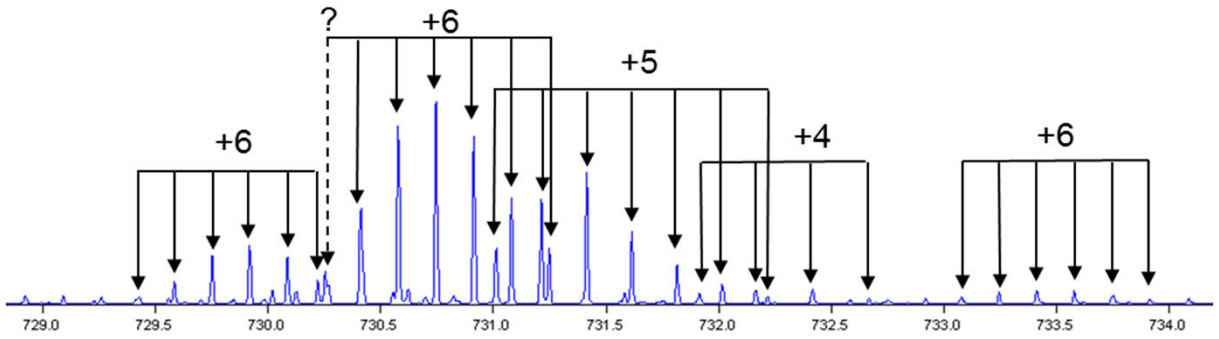


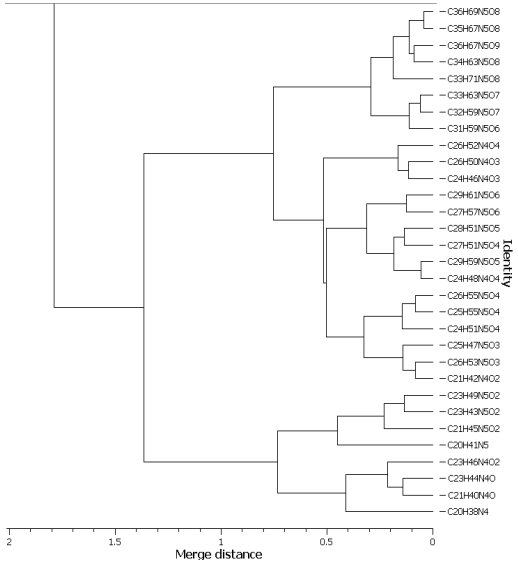
LC-MS Analytical Workflow for Extractables Assessment



Difference Correlation and Self-Calibrating Mass Maps

- 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



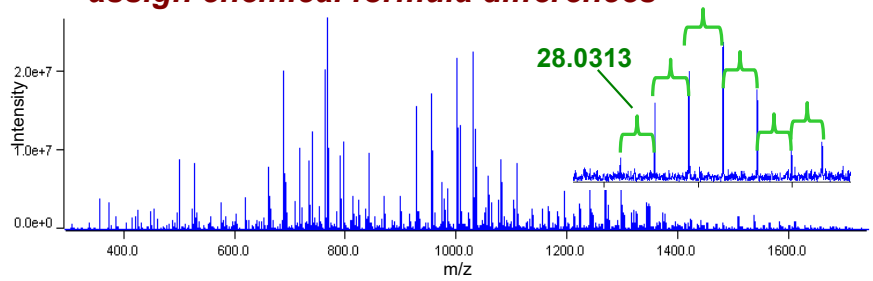


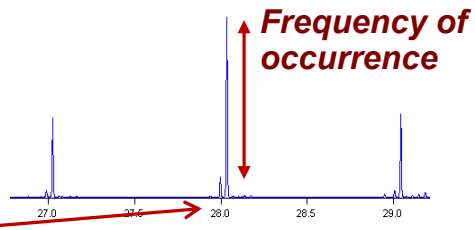
Difference Correlation and Self-Calibrating Mass Maps

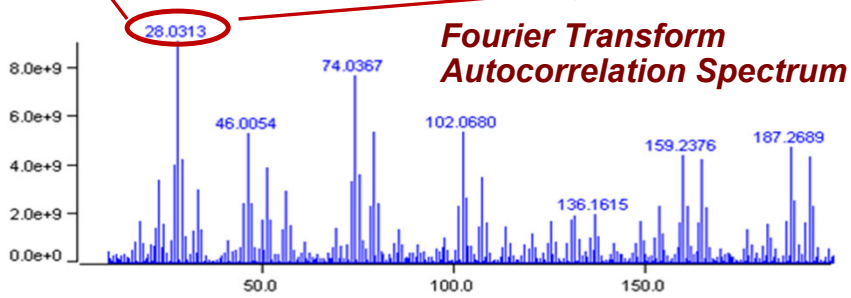
accurate m/z *difference* correlation

	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



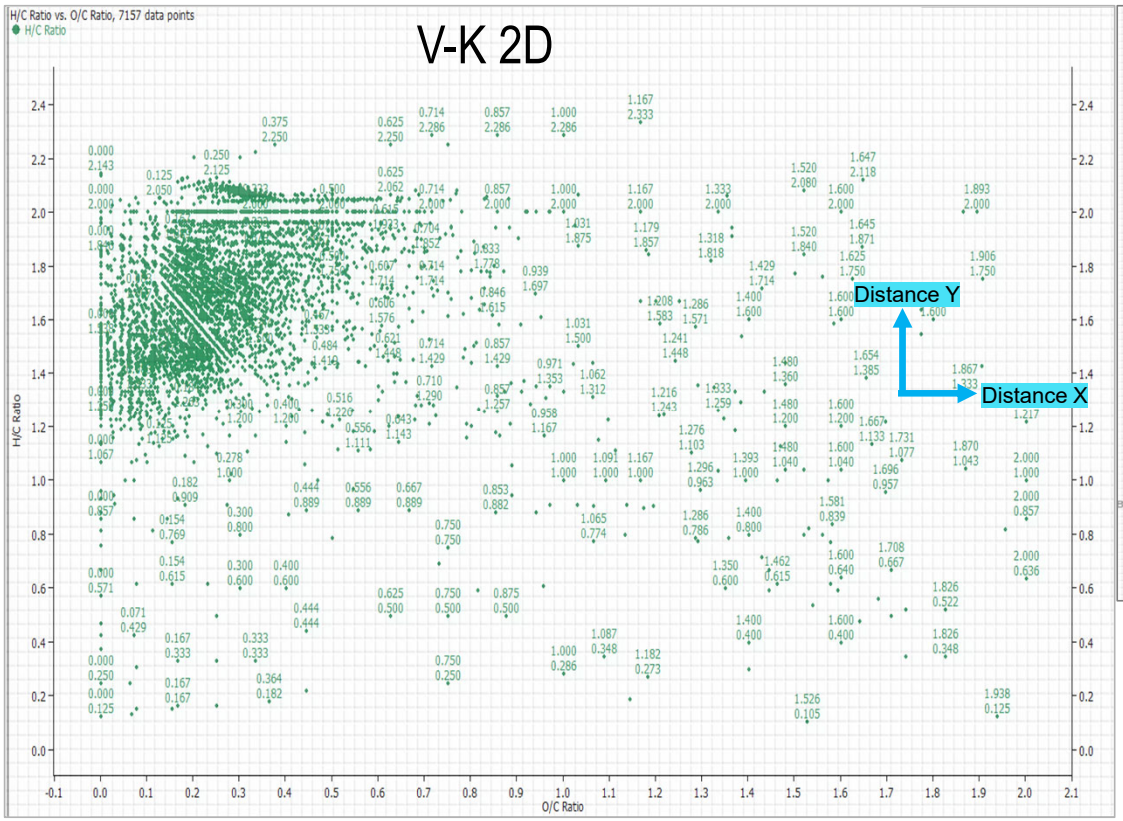


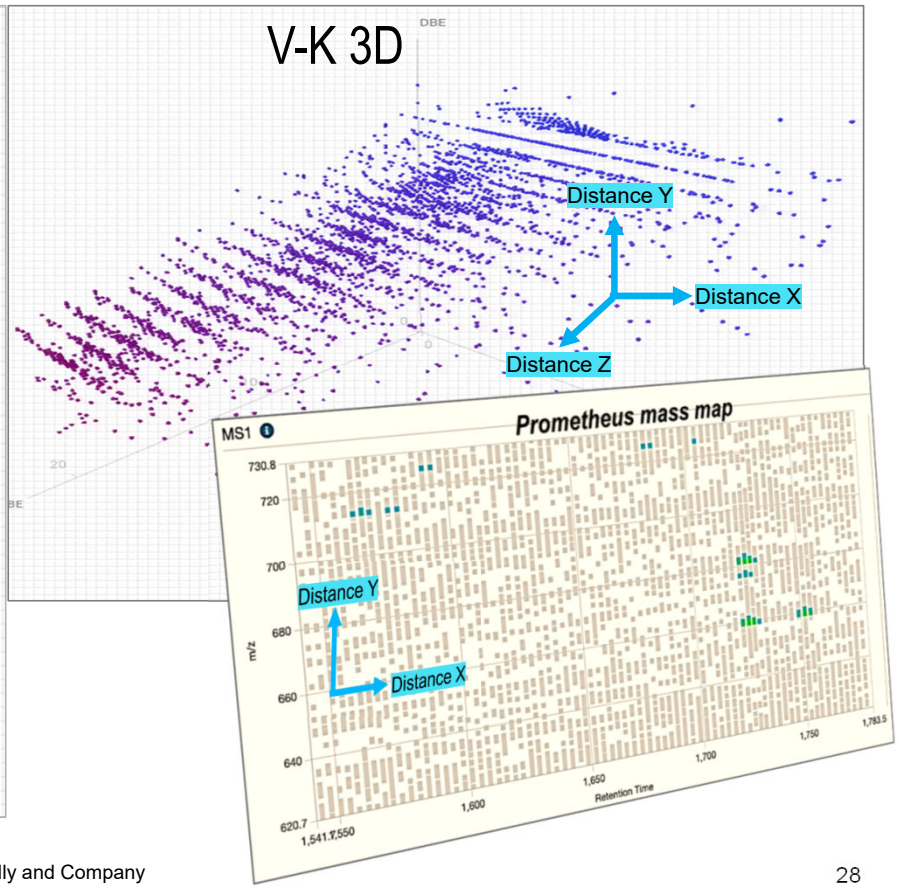


Basis for a *self-calibrating* mass map

Difference Correlation and Self-Calibrating Mass Maps

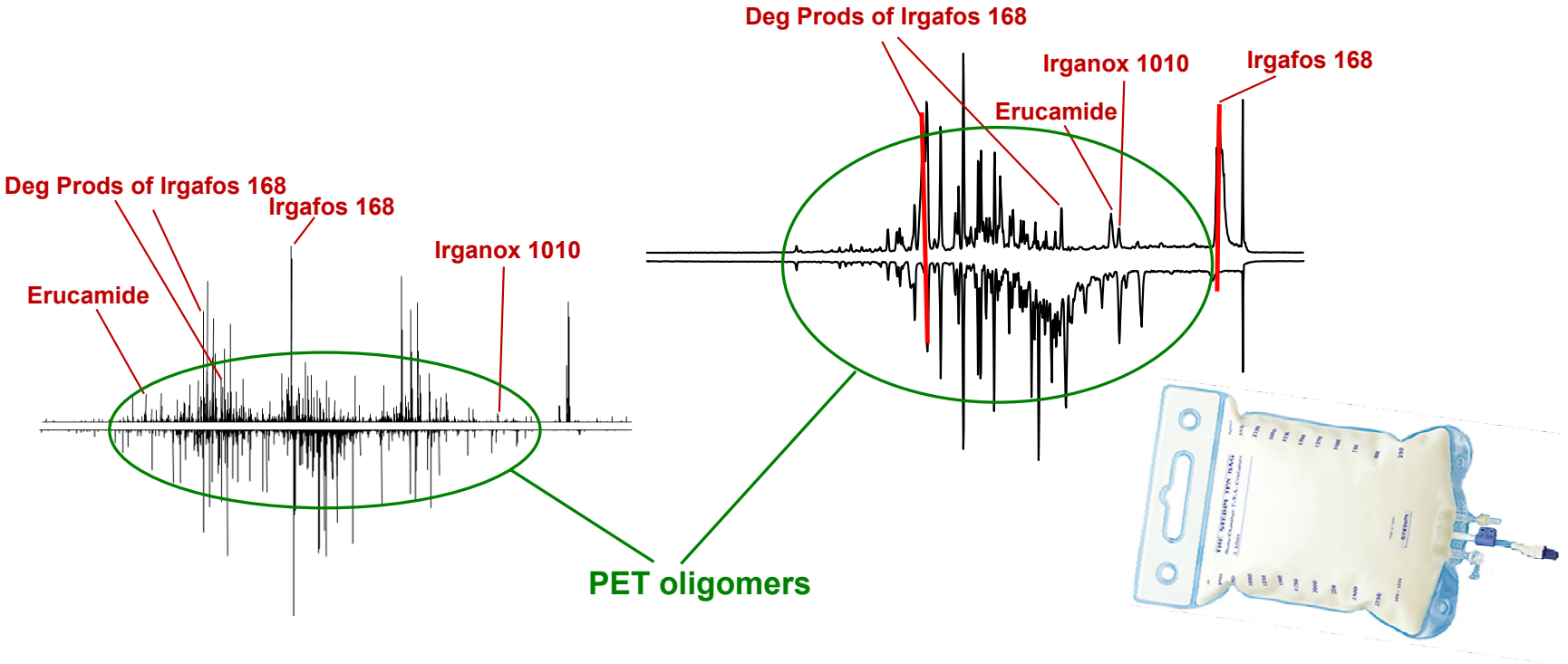
N-dimensional difference correlation and self-calibrating mass maps



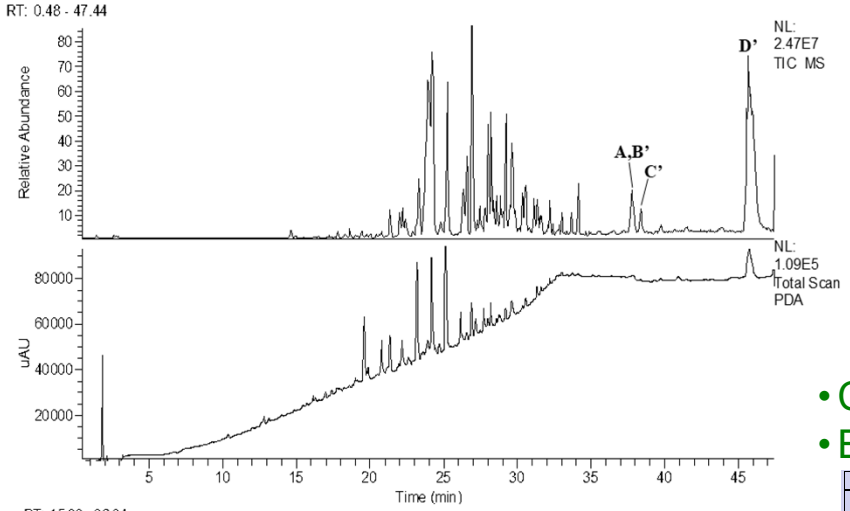


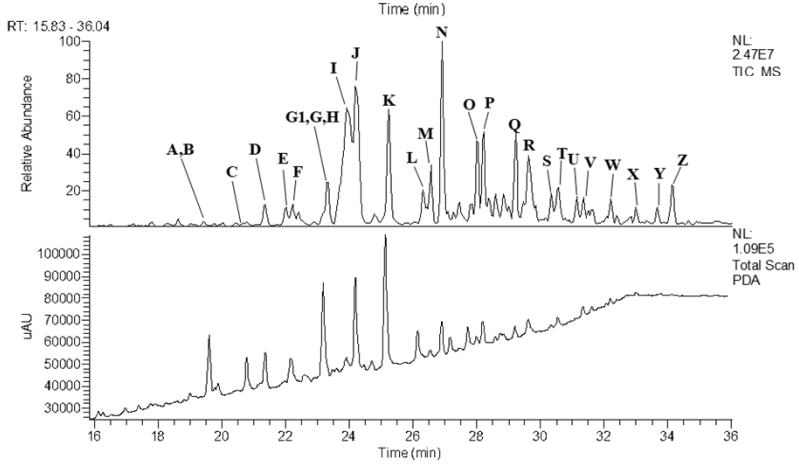
Visualization in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

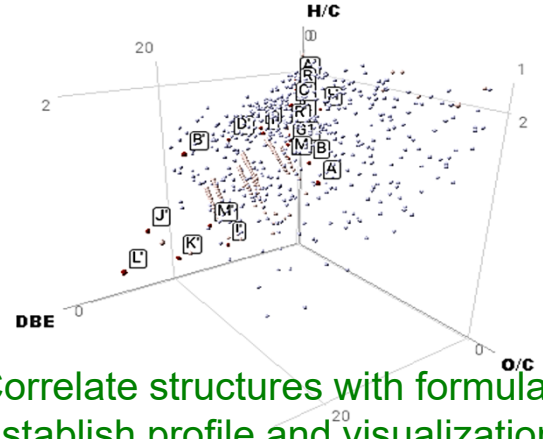
FT-ICR LC-MS total ion chromatograms (right) and mass spectra (left) of IPA extracts of multi-layer biopharmaceutical bags from different sources



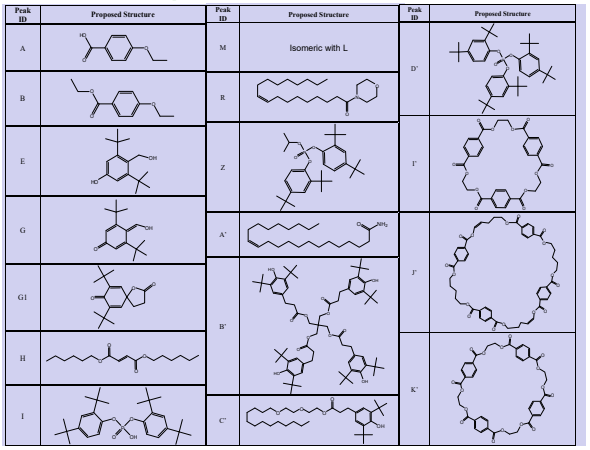
Visualization in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

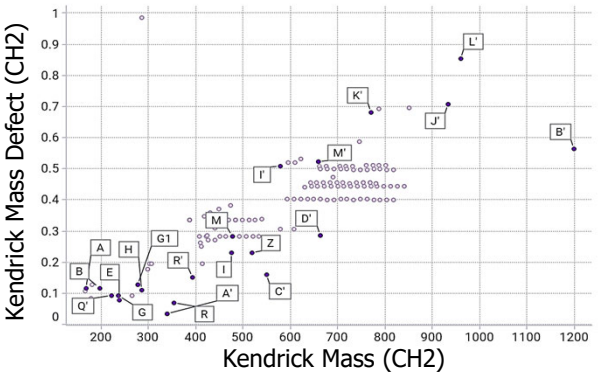


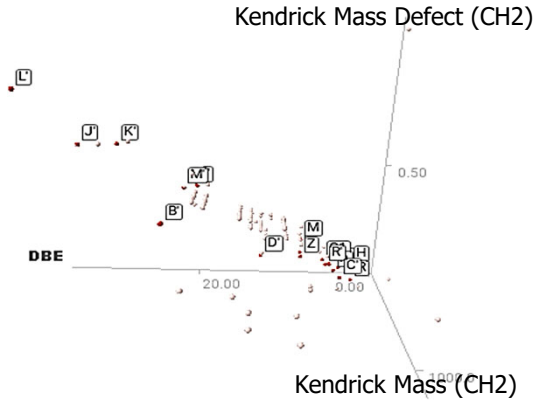




- Correlate structures with formulas
- Establish profile and visualizations

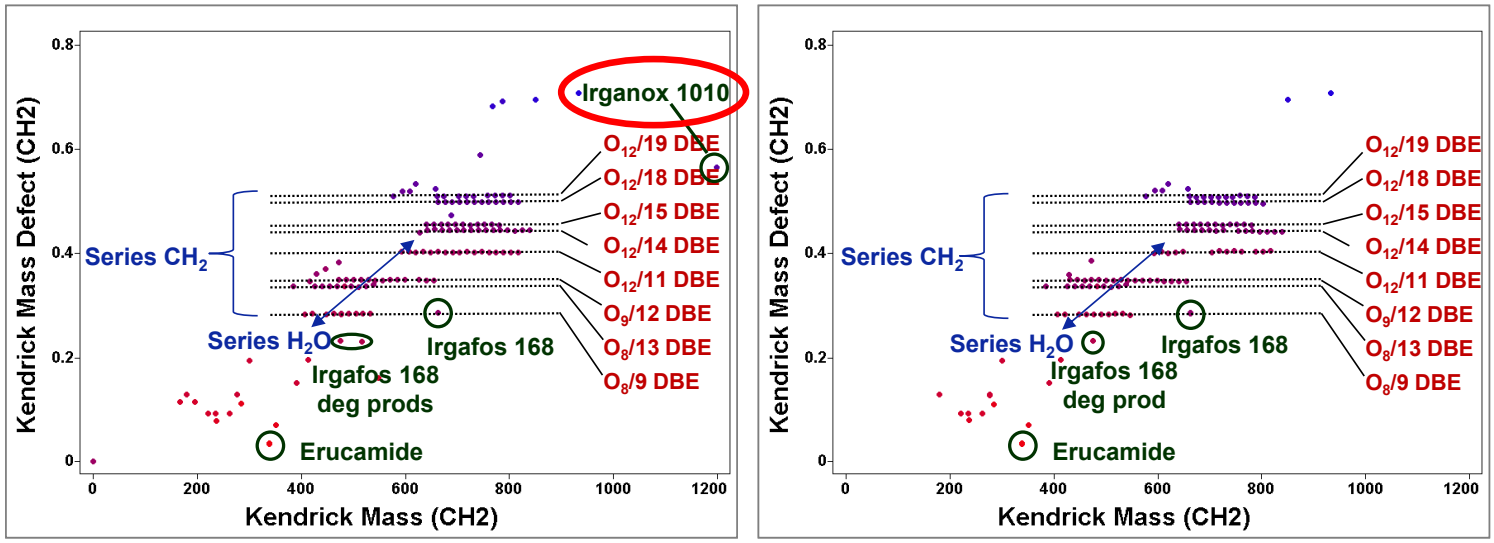






Visualization in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

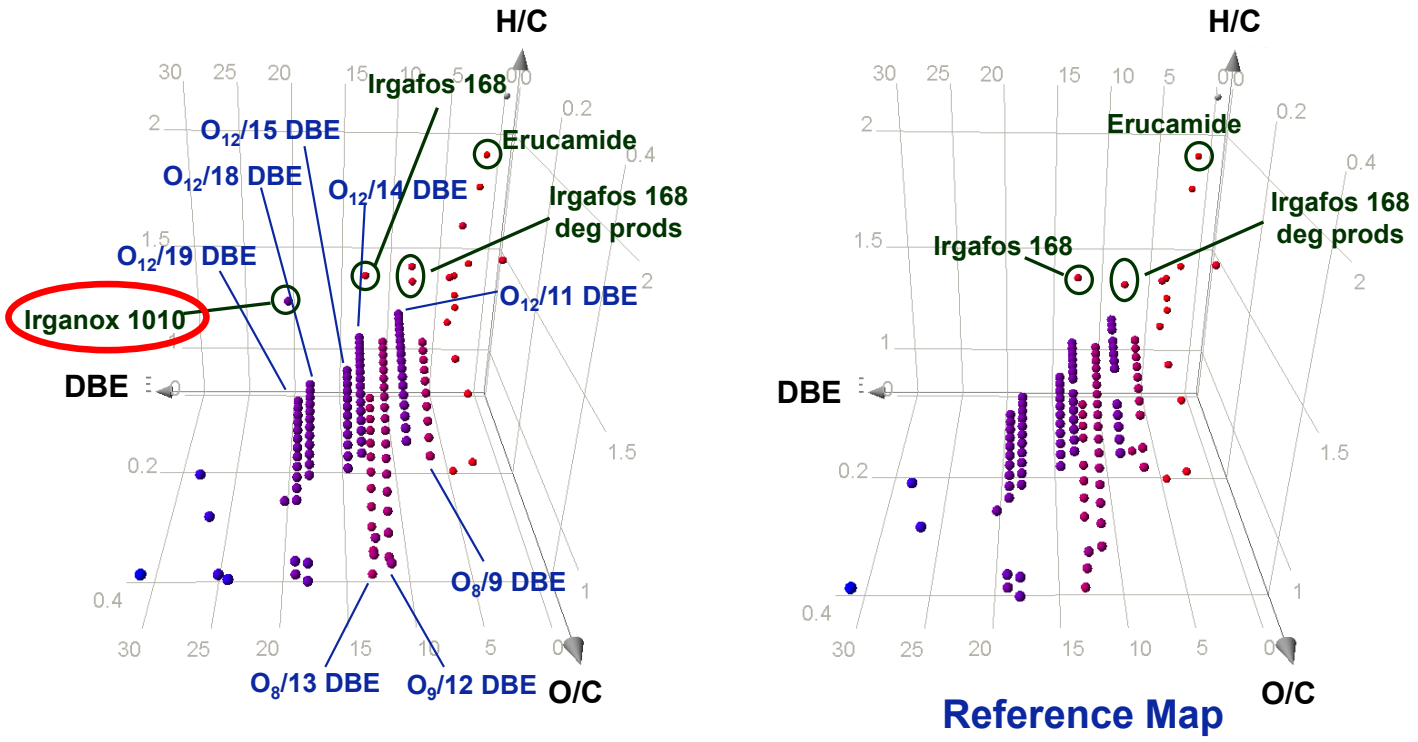
Comparative Kendrick mass defect diagrams



Reference Map

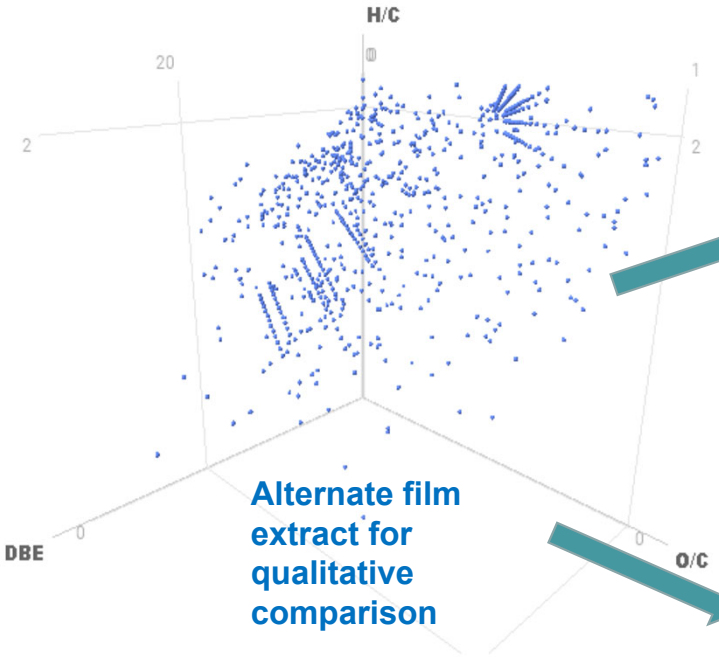
Visualization in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

Comparative 3D van Krevelen diagrams

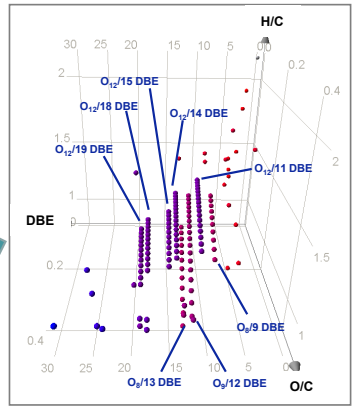


Visualization in Practice: Multi-layer Film Biopharmaceutical Bag Extractables

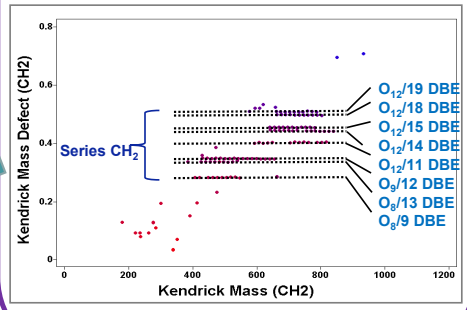
Visualized similarity and anomaly detection

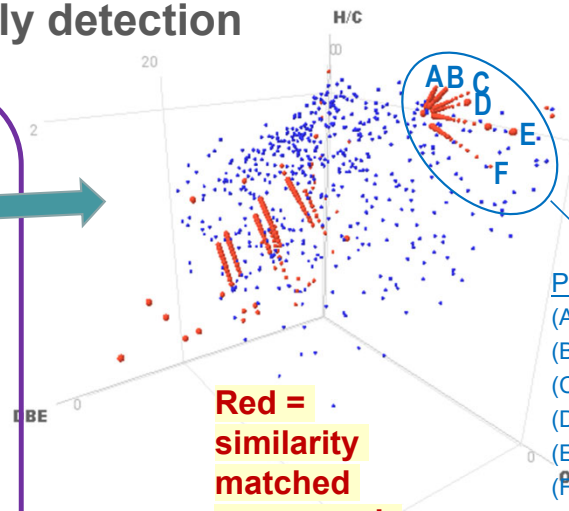


Alternate film extract for qualitative comparison



Library spectral maps

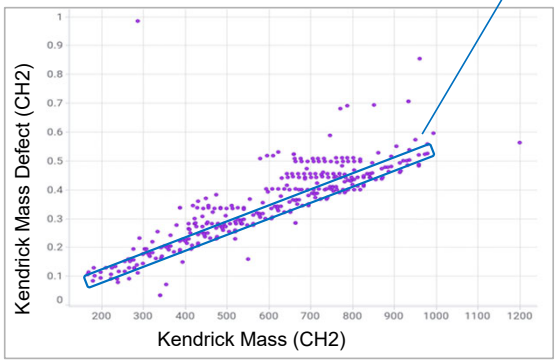




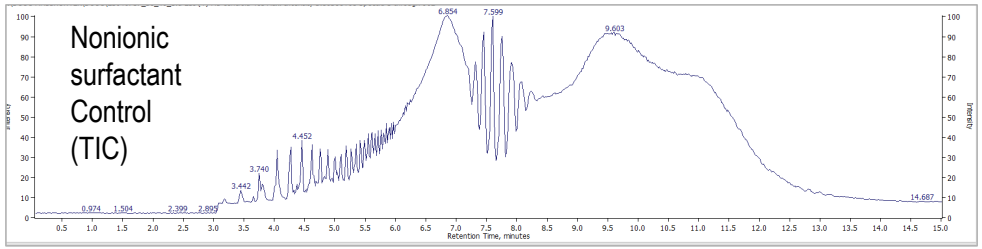
Red = similarity matched compounds

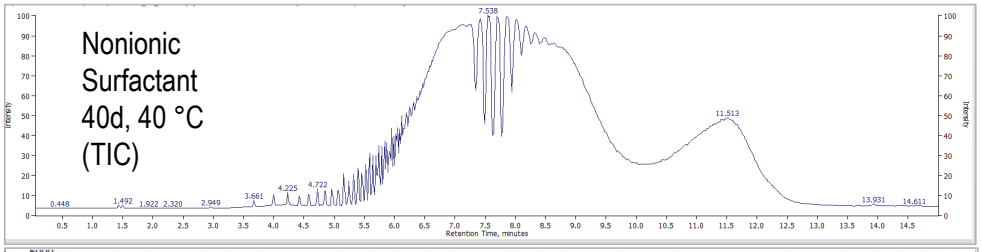
PEG series

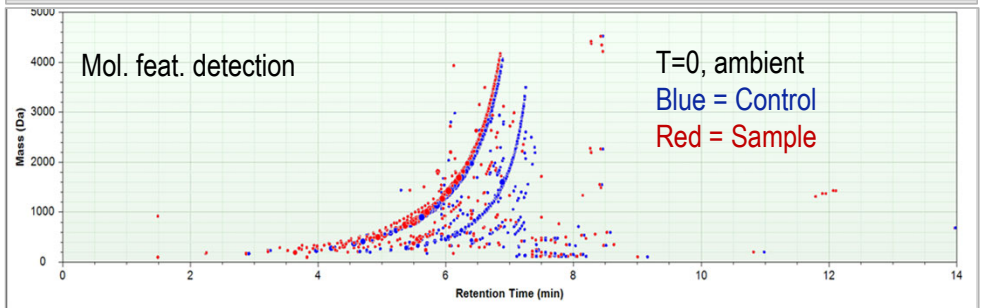
(A) $[(C_2H_4O)_n + H_2O + CH_2 + H]^+$
(B) $[(C_2H_4O)_n + H_2O + H]^+$
(C) $[(C_2H_4O)_n + CH_2O_2 + H]^+$
(D) $[(C_2H_4O)_n + O_2 + H, Na]^+$
(E) $[(C_2H_4O)_n + O_2 + H, NH_4]^+$
(F) $[(C_2H_4O)_n + C_2H_2O_4 + Na]^+$

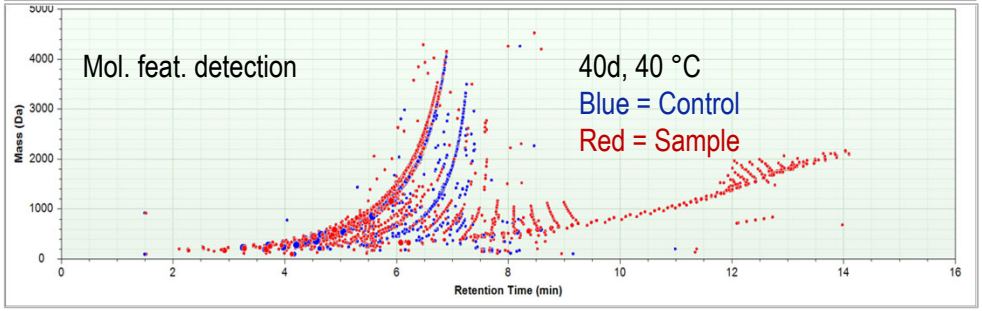


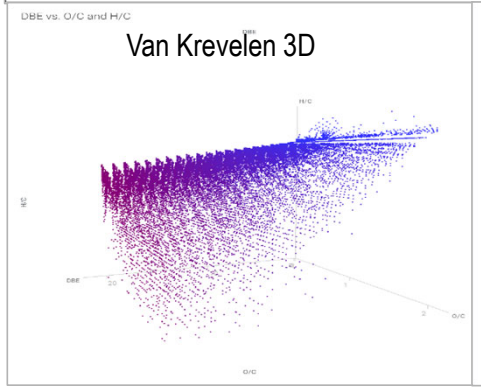
Nonionic Surfactants: Comparative Control and Stress Degradation Samples

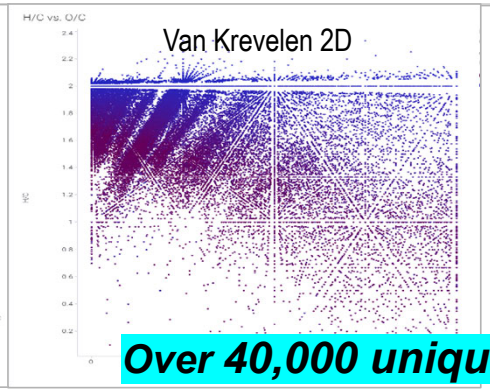


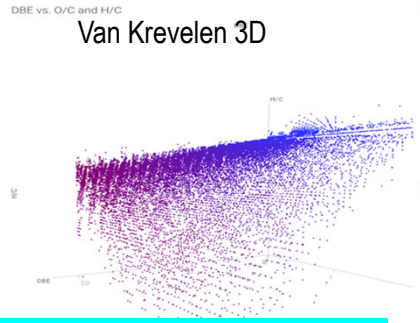


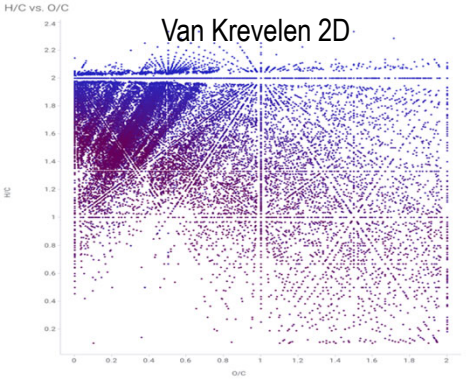






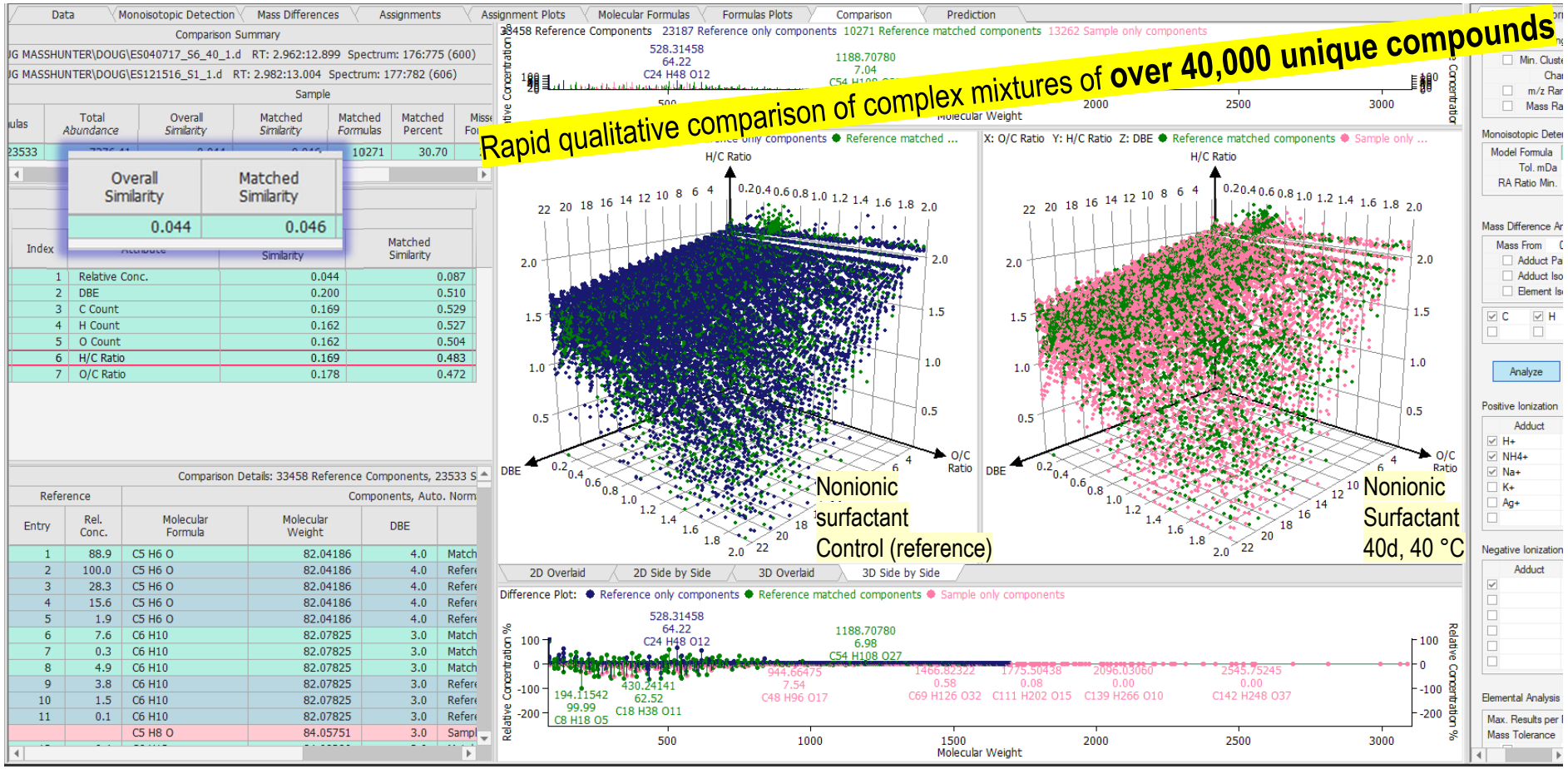




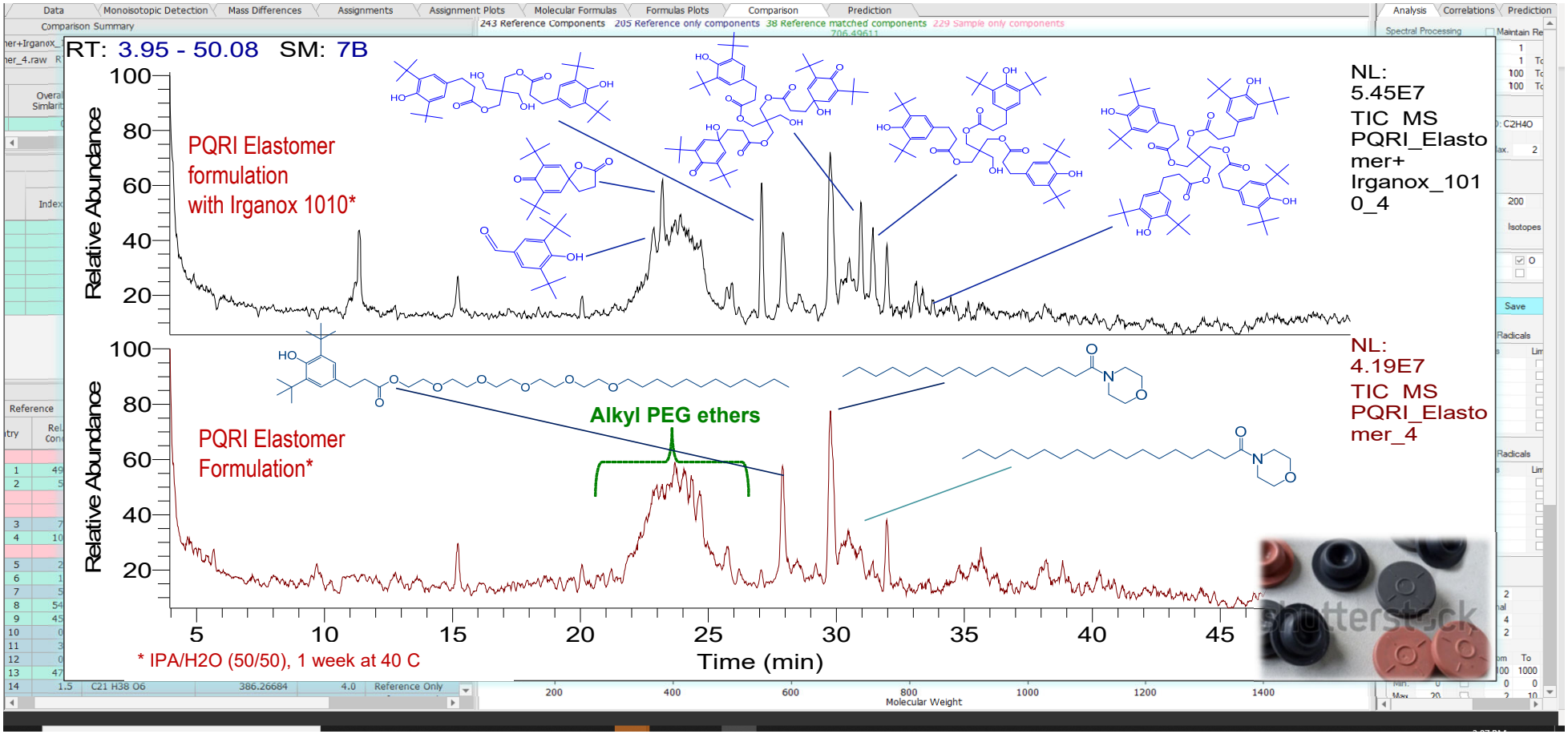


Over 40,000 unique compounds detected

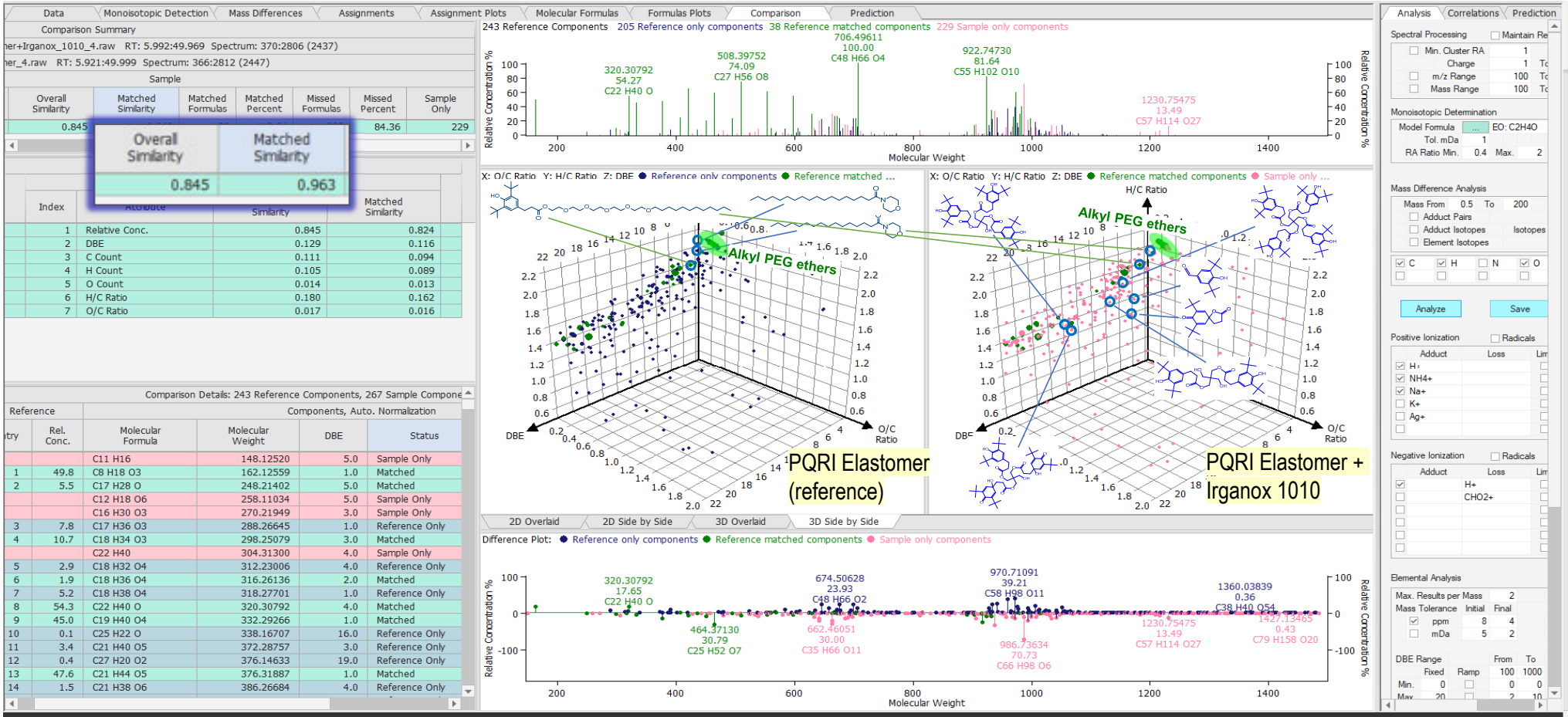
Nonionic Surfactants: Similarity Index and Anomaly Detection



Elastomers: Similarity Index and Anomaly Detection



Elastomers: Similarity Index and Anomaly Detection



In Conclusion, Visualization Approaches Enable…

- 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
- N-dimensional self-calibrating mass maps based on mass difference correlation
- Rapid and comprehensive pairwise and batch comparison with an index of similarity and anomaly detection
  - Change management
  - Failure mode analysis
  - Material characterization and selection

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Thank you!

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